and 27, $P<0.001$). Moreover, in MCAC group, nuclei were significantly more elongated, more irregular with more heterogenous chromatin as compared to the MC.
Conclusions: Strict morphologic criteria of MC coupled with the nuclear features (size, shape, and chromatin texture) facilitate discrimination between rare mucin producing tumors of the salivary glands. Different clinical behavior emphasizes the importance of differentiating MC from MCAC and mucin-rich SDC.

Hematopathology

994 Megakaryocytic Nuclear Phospho-STAT5 Staining in Non-CML Myeloproliferative Disorders Correlates with JAK2 V617F

S Aboudola, H Szpurka, G Murugesan, JP Maciejewski, RR Tubbs, NL Prescott, MA Verbic, ED Hsi. Cleveland Clinic Foundation, Cleveland, OH.
Background: The diagnosis and classification of chronic myeloproliferative disorders (MPDs) is often challenging. Although attempts at finding reliable diagnostic immunophenotypic markers have been made, no such markers exist. The novel activating JAK2 V617F is found in many non-CML MPDs and in only rare cases of other myeloid disorders such as myelodysplastic syndrome (MDS) or overlap MDS/MPD. Activated JAK2 phosphorylates STAT5 (p-STAT5), resulting in translocation to the nucleus, where it promotes cell survival and proliferation. We used immunohistochemistery (IHC) to examine the diagnostic utility of activated p-STAT5 (nuclear p-STAT5) in non-CML MPDs.
Design: We examined bone marrow trephine biopsies (BM) from 44 patients with non-CML MPD (16 polycythemia vera [PV], 15 essential thrombocythemia [ET], and 13 chronic idiopathic myelofibrosis [CIMF]) for nuclear p-STAT5 expression by IHC. We similarly examined 6 cases of MDS and 11 cases of MDS/MPD overlap syndrome. Our control group included 20 BMs submitted for lymphoma staging and other non-myeloid disorders. Positive p-STAT5 staining was defined as nuclear staining in > 10% of megakaryocytes (nMEG p-STAT5). JAK V617F status was determined by allele specific PCR or LightTyper assay.
Results: The results are summarized in Table 1. In the control group nMEG p-STAT5 was observed in 2/20 patients; interestingly, both were receiving growth factor support (EPO and G-CSF), which is known to activate STAT5. When excluding growth factor therapy, IHC for nMEG pSTAT5 was 100% sensitive and 90% specific for JAK2 V617F. Similarly, when growth factor therapy is excluded, nMEG pSTAT5 was 86% sensitive and 100% specific for the diagnosis of non-CML MPD in our patient population (PV, ET and CIMF) compared to the control group.

Disease Type	JAK2 V617F		Wild Type JAK2	
	p-STAT5 (+)	p-STAT5 (-)	p-STAT5 (+)	p-STAT5 (-)
Control (20)	0	0	2	18
MDS (6)	0	0	0	6
MDS/MPD (11)	1	0	2	8
PV (16)	14	0	0	2
ET (15)	11	0	2	2
CIMF (13)	11	0	0	2

Table 1

Conclusions: nMEG pSTAT5 expression in V617F MPDs appears to reflect JAK2 activation. In the absence of growth factor therapy, it strongly suggests the diagnosis of a myeloid malignancy and should prompt confirmatory molecular testing for JAK2 V617F. Finally, the presence of JAK2 V617F and nMEG pSTAT5 in some cases of MDS/MPD overlap syndrome suggests closer biologic similarity of such cases to MPDs than to MDS.

995 Extranodal Diffuse Large B Cell Lymphoma of Cutaneous Follicular Lymphoma Type

F Aigner, D Korol, E Levi, N Probst-Hensch, H Moch, M Kurrer. University Hospital, Zurich, Switzerland; University of Zurich, Zurich, Switzerland; John D Dingell VAMC and Wayne State University, Detroit, MI.
Background: Cutaneous follicular lymphoma (cFL) and marginal zone lymphoma (MZL) are distinct WHO/EORTC clinico-pathological entities of cutaneous lymphomas carrying an excellent prognosis. MZL is well recognized to occur in other extranodal locations. The aim of the study was to investigate, whether diffuse large B cell lymphomas (DLBCL) of cFL type could also be identified in other extranodal localizations and whether it could also be associated with a similar distinct clinical course.
Design: Among 1500 consecutive lymphoma diagnoses on file in the Department of Pathology of the University Hospital Zurich (from 1992 to 2004) 111 cases of non cutaneous non gastrointestinal DLBCL were retrieved and reviewed. 10 cases were associated with HIV positivity, 1 case with immunosuppression after organ transplantation, 55 cases had documented previous nodal disease or documented other nodal disease at presentation, 5 cases represented Burkitt lymphoma or blastoid mantle cell lymphoma, and 4 cases had no paraffin material left for analysis. The 36 remaining cases were analyzed for nuclear morphology and immunohistochemical profile and classified according to WHO/EORTC criteria into DLBCL of cFL type (centrocytoid, BCL-6+ CD10-/+ BCL-2- IRF-4-) or in DLBCL of non cFL type (centroblastic or immunoblastic, BCL-6+/- CD10+/- BCL-2+ IRF-4+).
Results: Lymphomas that matched the WHO/EORTC criteria for DLBCL of cFL type by morphology, immunohistochemical profile and clinical course could be identified. One case showed a localized extranodal extracutaneous recurrence with a follicular growth pattern 9 years after primary diagnosis and local radiation therapy, 1 patient was free of disease 8 years after surgical excision alone. 8/36 cases were classified as DLBCL of cFL type, 28/36 cases were classified in analogy to DLBCL of non cFL type. 1/8 patients (13%) died of lymphoma in the former group, 4/28 patients (14%) died of lymphoma in the latter group (median follow up 60 months).
Conclusions: In analogy to extranodal MZL the data suggest that DLBCL of cFL type as defined by WHO/EORTC criteria does occur in other extranodal extracutaneous localizations. Limited stage extranodal lymphoma can be associated with an excellent disease specific survival. No difference in survival was found between cases that matched the WHO/EORTC criteria for cFL and DLBCL cases of non cFL type.

996 Differential Expression of Cyclin Dependent Kinase 1 (CKS-1) in Small Cell and Blastoid Variant Mantle Cell Lymphoma

N Akyurek, GZ Rassidakis, K Giaslakiotis, RJ Knoblock, LJ Medeiros. The University of Texas MD Anderson Cancer Center, Houston, TX.
Background: Mantle cell lymphoma (MCL) is a distinct type of B-cell lymphoma characterized by the t(11;14)(q13;q32). Most cases of MCL are composed of small lymphocytes, but a subset of cases is composed of larger or more immature cells, known as blastoid variant. Blastoid MCL is associated with a higher proliferation rate, more aggressive clinical course, and commonly has genetic alterations affecting the cell cycle in addition to the t(11;14). Cyclin dependent kinase (CDK) subunit-1 (CKS-1) is essential for the ubiquitination and subsequent degradation of p27, a universal CDK inhibitor.
Design: We analyzed CKS-1 and p27 expression in four MCL cell lines including Mino, SP53, Jeko and Z-138 using Western blot analysis after subcellular fractionation. CKS1 and p27 expression and localization was further assessed in two of these cell lines (Mino, SP53) by immunohistochemistry in paraffin-embedded cell blocks. CKS1 and p27 expression was also assessed in 51 MCL tumors (35 small cell, 16 blastoid) using immunohistochemical methods and correlated with proliferation index (MIB1) and apoptotic rate (TUNEL) of these neoplasms.
Results: High levels of CKS-1 were detected in the Mino, SP-53 and Jeko cells. CKS1 was predominantly cytoplasmic. In MCL tumors, using a 10% cutoff, 10 of 35 (28.6%) small cell tumors versus 14 of 16 (87.5%) blastoid tumors were positive for CKS-1 (p=0.0002). Analyzed as a continuous variable, the percentage of CKS-1 positive tumor cells also significantly correlated with blastoid variant (p=0.001). p27 levels were low in the Mino and SP53 cell lines. Using a 25% cutoff, p27 was expressed in 12 of 51 (23.5%) mantle cell lymphoma tumors. However, there was no correlation between p27 expression levels and histologic type (small cell vs. blastoid) of mantle cell lymphoma. Proliferation rate, as assessed by Ki-67 expression, was higher in blastoid variant than in small cell tumors and was inversely associated with p27 levels in the small cell but not in the blastoid MCL group. In the small cell MCL group, progression-free survival did not differ significantly in patients with CKS1+ versus CKS1- tumors. However, in the blastoid MCL group, 2 patients with CKS1- tumors did not relapse or progress.
Conclusions: We conclude that CKS-1 is commonly over-expressed in mantle cell lymphoma cell lines and tumors, particularly in the blastoid variant. CKS-1 overexpression appears to be a manifestation of cell cycle dysregulation in mantle cell lymphoma.

997 Differential Expression of Smac/DIABLO in B-Cell Non-Hodgkin Lymphomas (NHL)

N Akyurek, Y Ren, GZ Rassidakis, LJ Medeiros. MD Anderson Cancer Center, Houston, TX.
Background: The pathogenesis of B-cell NHL may be regulated by apoptotic mechanisms. Low-grade lymphomas (e.g. CLL/SLL) are characterized by slow accumulation of mature B cells, suggesting that these diseases have defects in the regulation of apoptosis. By contrast, high-grade lymphomas are highly proliferative and can be associated with either low (e.g. pre-B LBL) or high levels of apoptosis (e.g. Burkitt lymphoma). Caspases, the central component of the apoptotic machinery, can be inhibited by members of the inhibitor of apoptosis protein (IAP) family. Other proteins can negatively regulate IAPs, one of which is Smac/DIABLO. In this study, we assessed for expression of Smac/DIABLO in B-cell lymphoma cell lines and primary B-NHL tumors.
Design: The study group included 187 B-NHL tumors and 10 B-NHL cell lines: 3 MCL, 2 DLBCL, 2 Burkitt, 2 pre-B LBL, and 1 primary effusion lymphoma (PEL). Expression of Smac/DIABLO was determined by immunohistochemistry and Western blot analysis using a polyclonal antibody (Imgenex, San Diego, CA). For immunostaining, tumors were analyzed using either tissue microarrays or full tissue sections.
Results: Smac/DIABLO was positive in 7 of 10 cell lines (negative in 1 DLBCL, 1 Burkitt, and 1 PEL). Immunostaining in tumors showed a variable pattern of expression in different NHL types (see table). In particular, both CLL/SLL and Burkitt showed no staining of Smac/DIABLO. By contrast, over half of follicular lymphomas (mostly high-grade), MCL, DLBCL, and pre-B-LBL showed moderate to strong expression, with the strongest intensity and highest frequency of expression being in pre-B LBL (68%).
Conclusions: We conclude that Smac/DIABLO is differentially expressed in B-NHLs, suggesting that different apoptotic mechanisms are involved in the pathogenesis of these diseases. The lack of Smac/DIABLO immunostaining in CLL/SLL supports the notion that this neoplasm is characterized by defective apoptosis. By contrast, the high apoptotic rate in Burkitt lymphoma is most likely regulated by mechanisms different from the IAP-Smac/DIABLO pathway. Smac/DIABLO may be a potential target for therapy in tumors that are most often positive, such as pre-B LBL.

Expression of Smac/DIABLO Proteins in B-cell NHL

Lymphoma Type	No.	Smac/DIABLO
Follicular lymphoma	30	19 (63%)
Mantle cell lymphoma (MCL)	38	20 (53%)
CLL/SLL	9	0
Lymphoplasmacytic lymphoma (LPL)	7	2 (29%)
N-marginal zone lymphoma (MZL)	7	3 (43%)
E-MZL	10	1 (10%)
SMZL	8	3 (38%)
Diffuse large B-cell lymphoma (DLBCL)	38	20 (53%)
Burkitt	12	0
Pre-B lymphoblastic lymphoma (LBL)	28	19 (68%)

998 Primary Diffuse Large B-Cell Testicular Lymphoma Belong to the Non Germinal Center B-Cell–Like Subgroup: Immunohistochemical Study of 18 Cases

MA Al-Abbadi, EM Hattab, MS Tarawneh, SS Amr, T Ulbright, A Orazi. Wayne State Universty, Detroit, MI; Indiana University, Indianapolis, IN; University of Jordan, Amman, Jordan; Dhahran Medical Center, Dhahran, Saudi Arabia.

Background: The most common type of primary testicular lymphoma is diffuse large B-cell type (DLBCL) which generally exhibits aggressive clinical behavior. DLBCL can be further subclassified into two major prognostic categories: a germinal center B-cell-like (GCB) and non-germinal center B-cell-like (non-GCB). Such distinction is made possible using the immunohistochemical expression of CD 10, bcl-6 and MUM1. The aim of this study was to stratify PTL of the DLBCL type according to this scheme and evaluate the potential prognostic implications of such categorization.

Design: Controlled standard immunohistochemical stains for CD 10, bcl-6 and MUM1 were performed on 18 well-documented cases of PTL-DLBCL from 4 medical centers. Subclassification was carried out as previously described where CD 10 and/or bcl-6 positivity and negativity for MUM1 was considered GCB type and lack of expression for CD 10 with MUM1 expression a non-GCB type. The proliferative activity was determined using immunostaining for Ki-67 antibody.

Results: Of the 18 cases examined, 17 (94%) were found to belong to the non-GCB type. One case (6%) had a CD 10 +ve, bcl-6 –ve and MUM1 –ve immunophenotype and was classified as GCB type. This particular case belonged to a 38-year-old African-American patient who was HIV +ve. In 1 case, material to perform CD 10 was not available, however, this case had a previous bcl-6 –ve/MUM1+ve immunoprofile and therefore was labeled a non-GCB type. In another case, material to perform MUM1 was not available but it had a CD 10-ve /bcl-6 +ve profile and was also classified as non-GCB type. Only 1 case of the non-GCB group had MUM1-ve profile. All the cases expressed high proliferative activity (> than 50% of cells with +ve Ki-67 staining).

Conclusions: Most (94%) primary testicular lymphomas of the diffuse large B-cell type belong to the non-GCB subgroup. All the cases had high proliferative activity. These findings provide insight into the generally aggressive behavior and poor prognosis exhibited by primary testicular large cell lymphomas. One case that belonged to the GCB group occurred in an HIV +ve patient. The frequency of this finding in HIV+ve patients with DLBCL type remains to be determined.

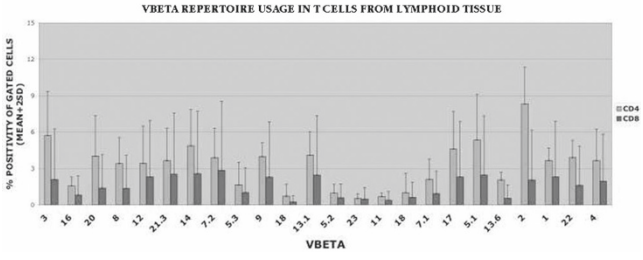
999 Flow Cytometric Assessment of T-Cell Clonality in Bone Marrow, Peripheral Blood and Lymphoid Tissue Using TCR-Vbeta Expression Analysis

SZ Al-Quran, DM Cardona, RC Braylan, TC Netzel, Y Li. Univ of Florida, Gainesville, FL.

Background: Although flow cytometry (FCM) has successfully been used in the assessment of B-cell clonality, its application to the detection of T-cell clonality has been limited. FCM analysis of the Vβ repertoire of the T cell receptor (TCR) can be a useful approach for detecting clonally expanded T-cells since these cells are expected to uniformly express a single Vβ subfamily. This analysis is simple, rapid and quantitative, and has already demonstrated utility in the detection of T-cell lymphoproliferative disorders (TLPD), mostly in blood.

Design: Retrospectively, we reviewed the results of TCR-Vβ analysis in 63 samples from 53 patients received in our laboratory between 9/2002-9/2004. Samples included 33 bone marrows (BM), 16 peripheral bloods (PB), 9 lymphoid tissues (LT) and 5 body fluids (BF). The data were correlated with morphologic observations, and with molecular results and clinical outcomes, when available. We established control reference ranges for the various Vβ subfamilies using 10 samples each of BM, PB and LT without evidence of T cell lymphoproliferative disease.

Results: The fraction of CD4 or CD8 T-cells expressing any subfamily of the Vβ repertoire in the control samples did not exceed 12% (see figure).



T-cell clonality was demonstrated by FCM in 29 cases (20 BM, 4 PB, 3 LT and 2 BF). Based on histologic, immunophenotypic, molecular and clinical findings, 18 cases were diagnosed as TLPD. In the remaining cases, patients have not developed TLPD.

Conclusions: FCM analysis of TCR-Vβ expression is a useful technique in the detection of T-cell clonality in a variety of specimens, including solid lymphoid tissue. Although clonal T cell expansions can be found in patients with no overt TLPD, this diagnosis can be made in the appropriate morphological and clinical setting. The technique is able to detect T-cell clonal expansions within heterogeneous cell populations (partial involvement by T cell neoplasia) and should be particularly useful for monitoring treatment.

1000 Transcription Factor GATA-3 Expression Correlates with Recurrence in Diffuse Large B Cell Lymphoma

AJ Ali, CE Sheehan, T Nazeer, JS Ross, A Hayner-Buchan. Albany Medical College, Albany, NY.

Background: GATA-3, a zinc finger transcription factor, plays a crucial role in early T cell development and commitment of T helper type 2 cells. Limited previous studies have shown an expression of T cell transcription factors, such as GATA-3, to explain the production of various cytokines by the neoplastic cells of Classical Hodgkin Lymphoma. Little information is available on the expression of T cell transcription factors in non-Hodgkin Lymphoma. This study was undertaken to further investigate the clinical and pathologic significance of GATA-3 in diffuse large B cell lymphoma (DLBCL).

Design: Formalin-fixed paraffin-embedded tissue sections from 51 cases were immunostained by an automated method on the Xmatrx (BioGenex, San Ramon, CA) using anti-human GATA-3 (Santa Cruz Biotechnology, Santa Cruz, CA). Nuclear and cytoplasmic immunoreactivity were scored based on intensity and distribution of GATA-3 expression by two pathologists, and the results were correlated against clinicopathologic variables.

Results: Cytoplasmic expression of GATA-3 was positive in 40/51 (78%) and negative in 11/51 (22%) cases of DLBCL. Nuclear expression of GATA-3 was positive in 20/51 (39%) and negative in 31/51 (61%) cases of DLBCL. Lack of cytoplasmic expression of GATA-3 correlated with recurrence (p=0.05), while an increased expression of nuclear GATA-3 correlated with recurrence (p=0.03). GATA-3 immunoreactivity did not correlate with advanced stage or survival.

Conclusions: Sub-cellular localization of GATA-3 expression in DLBCL is associated with disease outcome. The increased risk of recurrence associated with decreased cytoplasmic and increased nuclear expression of GATA-3 suggests that GATA-3 may play a role in the tumor biology of DLBCL and warrants further study.

1001 Chemokine Receptor CXCR4 (Fusin) Is a Poor Prognostic Factor in Diffuse Large B-Cell Lymphoma

MS Almiski, F Khanani, O Basturk, VN Adsay, MO Kurrer, E Levi. Harper Hospital, Wayne State University, Detroit, MI; University Hospital, Zurich, Switzerland; John D Dingell VAMC, Detroit, MI.

Background: Stromal cell-derived factor-1 (CXCL12/SDF-1) is a chemokine involved in development and trafficking of B cells and hematopoietic progenitors These responses are mediated by its specific receptor, CXCR4 (fusin). In an animal model, inhibition of the CXCR4 signaling by neutralizing antibodies prevents lymphoma growth. CXCR4 has also been implicated in Epstein-Barr virus mediated lymphomagenesis. In this study we evaluated the possible prognostic significance of CXCR4 expression and its relation to subtypes diffuse large B-cell lymphoma.

Design: A tissue array containing 94 cases of diffuse large B-cell lymphoma was utilized. The followup data were also available. CXCR4 staining was performed utilizing Santa Cruz (fusin (H-118): sc-9046) antibodies. In addition, expression of CD10, bcl2, bcl6, MUM1, pAkt, PAK1 were evaluated and correlated with CXCR4 expression. The cases showing at least 25% staining of tumor cells were considered positive. Only the cytoplasmic and membrane staining were taken into consideration during evaluation.

Results: In normal lymphoid tissues CXCR4 had a weak diffuse staining in the germinal centers, while in the interfollicular areas there were scattered staining lymphocytes. Among the diffuse large B-cell lymphomas, 56 of 94 (59%) cases had staining for CXCR4. Expression of CXCR4 was a bad prognostic factor in both univariate and multivariate analysis (p= 0.028). Expression of CXCR4 had no correlation with CD10, bcl-6, or bcl-2. An association with activated B-cell vs. germinal center phenotype could not be made.

Conclusions: CXCR4 expression is a bad prognostic factor in diffuse large B-cell lymphoma. This effect appears to be independent of the subtype of the diffuse large B-cell lymphoma, since no correlations with MUM1, bcl6 or CD10 expression were observed. Targeting the CXCR4 receptors has a good potential for lymphoma treatment.

1002 Atypical Blast Morphology at Relapse of Acute Promyelocytic Leukemia

H Alvarez, VD Dayton, MM Dolan, TP Singleton. University of Minnesota, Minneapolis, MN.

Background: Availability of all trans-retinoic acid (ATRA) to treat acute promyelocytic leukemia has increased the importance of morphologic identification of this particular type of acute myeloid leukemia so that cytogenetic and molecular testing can be performed for confirmation of t(15;17) and PML/RARA rearrangement. Prior studies have characterized the morphologic features of leukemic promyelocytes at initial diagnosis. We now describe an atypical morphology at relapse.

Design: The electronic pathology database was searched from 1999 to 2005 for relapsed acute promyelocytic leukemias with t(15;17) and with peripheral blood or bone marrow smears available at diagnosis and at relapse. Bone marrow smears were counted to categorize 100 leukemic blasts and promyelocytes using the following criteria: type 1 myeloblasts (no cytoplasmic granules or Auer rods), type 2 myeloblasts (1 to 15 cytoplasmic azurophilic granules or an Auer rod), and promyelocytes (more than 15 cytoplasmic granules). These cell types were further subdivided based on nuclear outline (regular, round, or oval versus irregular, reniform, bilobed, or folded), cytoplasmic granules in promyelocytes (hypergranular versus microgranular), and presence or absence of Auer rods in type 2 myeloblasts and promyelocytes.

Results: Six patients had relapsed acute promyelocytic leukemia with peripheral blood or bone marrow smears available at diagnosis and at relapse. All cases had documented t(15;17) at diagnosis and relapse, and all patients had been treated with ATRA. Neoplastic hypergranular or microgranular promyelocytes predominated at

initial diagnosis in all patients (6 of 6). Most patients (4 of 6) had increased myeloblasts at relapse (30% to 90% of leukemic cells) compared to the initial diagnosis (0% to 10% of leukemic cells).

Conclusions: Relapsed acute promyelocytic leukemia with t(15;17) often has atypical morphology with numerous myeloblasts. These morphologic features at relapse may overlap with other types of acute myeloid leukemias. It is important to recognize this atypical morphology, particularly if available clinical history is limited, because specific therapy may be directed at relapsed acute promyelocytic leukemia.

1003 Protein Profiling of DLBCL Identifies OCT-2, STAT-1 and p53 as Independent Variables for Specific Death

VP Andrade, D Cubero, JHTG Fregnani, FA Soares. Hospital do Cancer, São Paulo, Brazil.

Background: The Diffuse Large B cell Lymphoma (DLBCL) has been recently reshaped using molecular profiling and can be assigned in two categories regarding prognosis, activated B-cell like (ABC) and germinal center B-cell like (GCB). This molecular classification is based on lymphocyte differentiation antigens MUM1, bcl6 and CD10. Our study was designed to evaluate the protein profile to subtype DLBCL and compare with clinical variants and patient survival.

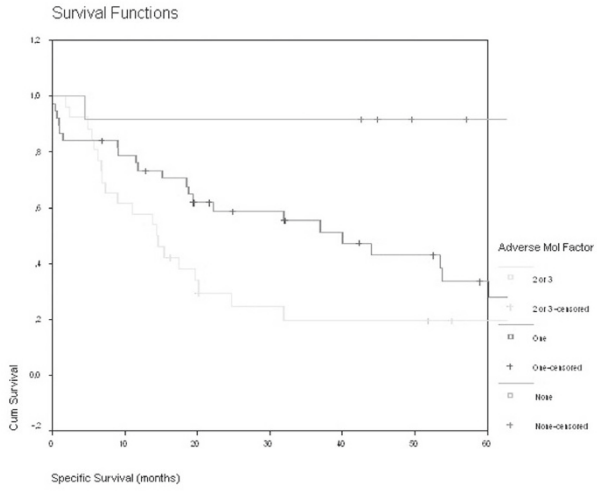
Design: We studied 109 adult nodal DLBCL without previous treatment biopsied from 1980 to 2001 using TMA. Sections were analyzed in triplicate to generate a representative score of intensity and proportion of positive cells. Forty proteins from different categories related to cell cycle, apoptosis and B-cell differentiation were studied by IHC. Statistics included univariate analysis using Kaplan-Meier curves and multivariate analysis by COX regression model using stepwise forward selection.

Results: ABC and GCB phenotype groups showed no difference in specific deaths curves. The following variants were statistically significant by univariate analysis: stage (St), International Prognostic Index (IPI), complete remission (CR), number of chemotherapeutic cycles, chemotherapeutic program completed, Cyclin B1, p53, NF-kB (c-rel), OCT-2 and STAT-1. The COX regression model identified St, CR, p53, OCT-2 and STAT-1 as independent variables (Table). p53 positive and OCT-2 or STAT-1 negative were considered adverse molecular factors. The number of adverse molecular factors influenced the survival curves (log rank=0,018; Graph). Stage III/IV patients with no adverse molecular factor showed 100% five-year cumulative survival compared to 24.7% when one or more of those factors were present (log rank=0,011).

Conclusions: OCT-2, STAT-1 and p53 were highly associated with outcome in DLBCL in COX regression model, especially in advanced disease. ABC and GCB phenotypes failed to show any clinical relevance.

Independent Variables for Specific Death Identified by COX Regression Model

Variables	Significance	Hazard Ratio
Stage I	.010	
Stage II	.021	7.737
Stage III	.003	12.926
Stage IV	.002	14.424
p53 (+)	.016	3.099
OCT-2 (-)	.001	4.356
STAT-1 (-)	.008	2.633



1004 Sézary Syndrome Cells Typically Express CD158k/KIR3DL2 Which Allows for Their Identification by Flow Cytometry

DW Bahler, L Hartung, GM Bowen, EC Vonderheid. University of Utah, Salt Lake City, UT; ARUP Institute of Pathology, Salt Lake City, UT; Johns Hopkin's Medical Institute, Baltimore, MD.

Background: Accurate enumeration of Sézary cells in peripheral blood specimens is necessary for diagnosing Sézary syndrome (SS) and monitoring treatment responses. Because assessment of Sézary cell numbers by morphology alone can be difficult, flow cytometry immunophenotypic analysis is often employed. However, the reported immunophenotypic features of Sézary cells that allow for discrimination from normal T-cells are variable, not present in all cases, sometimes found in reactive T-cell populations, or subtle and difficult to identify.

Design: Peripheral blood lymphocytes from 33 cases of SS were evaluated for expression of killer cell immunoglobulin-like MHC receptors (KIR) CD158a, CD158b, CD158e, CD158i, and CD158k by multiparameter flow cytometry using monoclonal antibodies EB6, GL183, FES172, Z27, and Q66. KIR are normally expressed by NK cells, but have also been recently identified on small populations of normal T-cells and some T-cell neoplasms, including European SS cases. All patients were from the U.S. and satisfied International Society for Cutaneous Lymphoma criteria for SS.

Results: A variety of pan-T-cell antigenic abnormalities were observed, with 15/33 cases showing dim or negative CD2, 8/33 having dim CD3, 5/33 showing brighter than normal CD5, 3/33 showing dim CD5, and 21/33 cases being CD7 negative. 3 cases did not show pan-T cell abnormalities, and 5 cases only showed loss of CD7. Uniform positive expression of CD158k on Sézary cells was observed in 32/33 cases. Expression of other KIR evaluated was present in only three cases, one of which was uniformly positive for CD158a, and two others which showed partial CD158b positivity.

Conclusions: CD158k, also known as KIR3D2L or P140, is uniformly expressed by Séezary cells in almost all cases. Since CD158k is expressed by only rare normal peripheral blood T-cells, CD158k appears to be an excellent marker to help identify and quantitate Sézary cells by flow cytometry. Enumeration of Sézary cells using CD158k should be especially helpful for cases that do not have aberrancies of pan-T-cell antigens and for cases that only show loss of CD7. The high frequency of CD158k expression contrasts with other KIR, which are only infrequently expressed by Sézary cells, and other T-cell neoplasms, that express CD158k much less frequently. These findings suggest that CD158k plays an important and perhaps unique role in the pathogenesis of SS.

1005 EBV Negative Clonal B-Cell Proliferations/Lymphomas in PTCL/AILT

O Balagué, L Colomo, A Martinez, A García, E Rosselló, M Martinez, E Campo. Hospital Clinic, University of Barcelona, Barcelona, Spain; Hospital Dr Peset University of Valencia, Valencia, Spain; University of Mendoza, Mendoza, Argentina.

Background: Oligoclonal and monoclonal B cell populations have been described in PTCL/AITL as secondary EBV driven B cell proliferations that may evolve to a true B-cell lymphoma. EBV negative B-cell proliferations associated with T-cell lymphomas are uncommon and have not been previously well characterized. We report six cases of EBV negative B-cell clonal proliferations/lymphomas developing in the setting of PTCL/AILT.

Design: Eleven biopsies from six patients with concomitant PTCL and B-cell clonal proliferations/lymphomas were reviewed. EBV infection was examined by immunohistochemistry (IHC) for LMP-1 and ZEBRA (BZLF1) , by in situ hybridization (ISH) for EBER1,2 and viral DNA by PCR. HHV-8, HHV-6 and CMV viral infections were also examined by IHC. IgH and TCRγ gene rearrangements were studied by PCR. Glyceraldehide 3-phosphate dehydrogenase(GPDH) ISH was used to asses mRNA viability in all tissues.

Results: T-cell tumors were classified as PTCL, NOS in four cases and AITL in two cases. One patient had a diagnosis of nodal atypical T-cell hyperplasia prior to the development of the T cell tumor. Composite B and T proliferations presented in the skin (3 cases) or lymph nodes (3 cases). The B-cell component was classified as atypical plasma cell proliferation in two cases, clonal large B cell proliferation in one case, small B cell lymphoma with plasmacytic differentiation in two cases, and anaplastic plasmacytoma in one case. All cases showed Ig light chain restriction (3κ and 3λ). Molecular studies demostrated clonal IgH and TCRγ gene rearrangements in the four cases examined. CMV and HHV-8 infection were negative in all cases. Two cases had scattered EBER or very occasional ZEBRA positive bystander B-cells. Two patients with follow up died 12 and 20 months after the diagnosis. A sequential lymph node biopsy performed in two patients showed only the T-cell lymphoma without morphological or molecular evidence of the B-cell component.

Conclusions: EBV negative clonal B-cell proliferations in patients with T-cell tumors may present with a spectrum of plasma cell differentiation ranging from atypical plasma cell proliferation to overt tumors with plasmacytic/plasmablastic features. The lack of common viral infection suggests that other mechanisms may promote the clonal B-cell expansion.

1006 Monoclonal Gammopathy of Undetermined Significance (MGUS) with Atypical Pathologic Features: Clinical Relevance and Immunohistochemical Correlation

NA Beaman, JA Vos. Brooke Army Medical Center, Ft Sam Houston, TX.

Background: MGUS typically shows mature plasma cell morphology and lacks definitive immunohistochemical (IHC) evidence of light chain restriction. Occasional cases, however, show atypical morphologic features, yet fall short of WHO criteria for plasma cell myeloma. The prognostic significance of these atypical morphologic and IHC features requires further study.

Design: 26 cases meeting the WHO criteria for MGUS were studied. All cases were primary diagnoses and had clinical follow-up (mean=53 months). A chart review, morphologic assessment, 500-cell differential count and IHC evaluation (CD138, CD56, cyclin D1, kappa, and lambda) were performed. Cases were divided into those with and those without atypia, and further subcategorized according to clinical outcome (stable vs progressing) based on laboratory, radiologic, and clinical assessment.

Results: Plasma cell atypia was found in 15 of 26 (58%) of cases. Whereas five of these atypical cases had eventual disease progression, only two cases progressed in the cytologically normal group. No significant differences were seen with respect to age, sex or percent of marrow plasma cells. The presence of abnormal multinucleation and Dutcher bodies, while infrequent, were seen only in cases with clinical progression. Plasma cell expression of CD56 was seen in 6 of 15 atypical cases (40%) versus 2 of 11

cases (13%) in the normal group. Cyclin D1 immunoreactivity and CD56/cyclin D1 coexpression were exclusively seen in the atypical group (40% and 20% of atypical cases, respectively). Plasma cell clusters were more often seen in the atypical group (80% vs 36%) and when present, were associated with CD56, cyclin D1, and light chain restriction. Aberrant IHC immunoreactivity (CD56 and/or cyclin D1) was seen in 71% of cases that clinically progressed, compared to 32% of stable cases. Aberrant IHC expression specifically in plasma cell clusters was demonstrated in all cases that progressed, versus 25% of cases that remained stable.

Conclusions: Atypical morphologic features strongly correlated with IHC expression of CD56 and cyclin D1 and, to a lesser extent, with clinical course. Among cases that showed clinical progression, aberrant IHC expression was frequently seen. In these cases, plasma cell clusters seen on core biopsy more commonly showed light chain restriction, CD56, and cyclin D1 immunoreactivity. These IHC stains, in combination with plasma cell morphology, provide valuable prognostic data and should be considered in the routine evaluation of MGUS.

1007 Prognostic Significance of Immunophenotype in 225 Adults with Acute Lymphoblastic Leukemia

A Beri, J Young, QL Yi, H Chang. University Health Network, University of Toronto, Toronto, ON, Canada.

Background: Acute lymphoblastic leukemia (ALL) is a heterogeneous group of diseases characterized by malignant proliferation of immature lymphoid cells. Reports vary as to the prognostic significance of the immunophenotype of precursor B- and precursor T-cell ALL. The variability may in part be attributable to differences in patient populations, methodology, markers studied and treatment.

Design: We reviewed our institutional experience of 225 adult ALL patients immunophenotyped between 1991-2004 by multiparameter flow cytometry and correlated the immunophenotype of the diagnostic marrow or blood samples with the clinical and biological parameters and treatment outcomes.

Results: The 174 (77%) patients with precursor B-ALL had a median age of 41 years (range, 17-83) while the 51 (23%) patients with precursor T-ALL had median age of 30 years (range, 17-80). The T-ALL patients were younger (p=0.0065) and more often male (p=0.0316) than the B-ALL patients and also tended to have a better complete response (CR) rate (p=0.073), but both B- and T-ALLs had similar WBC counts, disease free (DFS) and overall survivals (OS). One or more myeloid-associated antigens were expressed by 45% of the B-ALLs and 35% of the T-ALLs but CR, DFS and OS were similar for patients with or without myeloid-associated antigens. In B-ALL, the CD34+, CD10+ phenotype was significantly associated with the Philadelphia chromosome (p<0.001). 48% of the B-ALLs expressed CD20 and had a lower CR rate (57% vs. 78%, p=0.032) than CD20 negative patients. The CD20+ B-ALLs also had a shorter OS (17 vs. 30 months), but the difference was not statistically significant (p=0.113). 36% of the T-ALLs expressed CD10 and had a longer OS than CD10 negative T-ALLs, but the difference was not significant (p=0.15).

Conclusions: In this series, expression of myeloid-associated antigens "lineage infidelity" did not influence the complete response rate, disease free, or overall survivals of B- or T-ALL. In patients with B-ALL, CD20 expression was an adverse prognostic factor for CR rate and OS. CD34+, CD10+ B-ALLs were more likely to harbor a Philadelphia chromosome. In patients with T-ALL, CD10 expression was associated with lengthened overall survival but the difference was not statistically significant.

1008 Transcription Factor Pax-5 Is Consistently Absent in Plasma Cell Neoplasms

MK Bianco, JD Krochmal, MA Vasef. University of Iowa Hospitals and Clinics, Iowa City, IA.

Background: The transcription factors, Pax-5, Oct1, Oct2, Bob.1 and PU.1, have been shown to play a significant role in B-cell differentiation. The expression profiling of the transcription factors, Pax-5, Oct1, Oct2, Bob.1, and PU.1, has not been adequately examined in plasma cell neoplasms.

Design: We performed immunohistochemistry on paraffin tissue sections from ten cases of previously characterized plasma cell neoplasms from the files at the Department of Pathology at the University of Iowa. The expression profiling of the transcription factors were compared to the other World Health Organization (WHO) subtypes of B-cell lymphomas.

Results: The majority of the cases expressed the three transcription factors Oct1 (100%), Oct2 (90%), and Bob.1 (50%). Only a rare case focally expressed PU.1. In contrast to WHO categories of B-cell lymphomas, such as follicular lymphoma, chronic lymphocytic leukemia, marginal zone B-cell lymphoma that consistently express Pax-5, none of the plasma cell tumors in this study expressed Pax-5 (0%).

Conclusions: Pax-5 is consistently absent in plasma cell tumors. Inclusion of Pax-5 antibody may prove useful in separating an occasional case of immunoblastic variant of diffuse large B-cell lymphoma with overlap morphology and similar immunophenotype from plasma cell tumor.

1009 Detection of CD4, CD25, and CD152: A New Role for Flow Cytometry in the Diagnosis of Hodgkin Lymphoma?

DS Bosler, VK Douglas-Nikitin, MD Smith. William Beaumont Hospital, Royal Oak, MI.

Background: The utility of flow cytometry is limited in Hodgkin lymphoma (HL) due to the paucity and fragility of Hodgkin cells. Investigation of the background reactive T-cells, however has shown increased CD25 expression and an increased CD4:CD8 ratio in HL vs. other lymphomas and benign lymph nodes, albeit with questionable sensitivity and specificity. More recently, CD4+, CD25+ T-cells in HL have been shown to coexpress anti-cytotoxic T lymphocyte-associated antigen 4 (CTLA-4 or CD152), which appears to play a role in regulatory T-cell functions. The aim of this study was to determine whether coexpression of CD4, CD25 and CD152 are increased in HL compared with benign lymph nodes and non-Hodgkin lymphomas (NHL).

Design: Cases were run on Beckman Coulter XL by four-color flow cytometry and analyzed using WinList software. Lymphoma panels included CD3, CD4, CD8, CD25 and intracellular CD152. Routine H&E was performed with immunohistochemistry as necessary. 83 lymph node biopsies submitted for flow cytometric and morphologic analysis were studied. 10 cases of classical HL were diagnosed by morphology. 49 benign cases and 24 NHL cases were identified. Thresholds for positivity were set for each case using isotype controls. Coexpression of CD4/CD25/CD152 was indirectly determined by gating on CD25 positive lymphocytes.

Results: Levels of expression were obtained as a percentage of lymphocytes. There was no significant difference in % CD8+ T-cells (p=0.6435) or CD4:CD8 ratio (p=0.4356) comparing HL vs. all other categories. The % CD4+ was significantly higher in HL vs. NHL (p<0.0001) but not benign cases (p=0.1428). The % CD4+/CD25+, CD4+/CD152+ and CD4+/CD25+/CD152+ were significantly higher in HL vs. NHL (p=0.0021, p<0.0001, p<0.0001, respectively) and vs. benign cases (all p<0.0001). The CD152+:CD152- ratio on CD4+/CD25+ lymphocytes had a sensitivity and specificity of 80% and 92%, respectively in distinguishing HL from benign cases (cutoff ratio =3.0).

Conclusions: Our results show that flow cytometry can be useful in distinguishing HL from NHL and benign cases. Specifically, coexpression of CD4 with CD25 and/or CD152 is significantly increased on HL cases, while the combination of CD4 and CD8 alone is relatively unreliable. Also, the CD152+:CD152- ratio on CD4+/CD25+ lymphocytes has very good sensitivity and specificity in identifying HL cases. Although further studies are necessary, our findings suggest that these markers may be useful in diagnostically equivocal cases.

1010 Technical Validation and Adaptation of the InVivoScribe TCR-gamma and IgH Gene Rearrangement Assays for Detection of Clonal Lymphoid Proliferations

GC Bullock, KA Siegrist, DM Haverstick, MS Mahadevan, LM Silverman, JB Cousar. University of Virginia Health System, Charlottesville, VA.

Background: Until recently, PCR-based clonality assays for the detection of rearranged T-cell receptor gamma chain (TCRG) and immunoglobulin heavy chain (IgH) gene segments used non-standardized, lab-specific PCR primer sets and methods, making proficiency testing and lab performance assessments difficult. The objective of the present study was to develop and technically validate a method for lymphocyte clonality assessment using a commercially available, FDA-approved standardized set of multiplex PCR primers.

Design: Method development included determination of optimal DNA extraction and fragment analysis techniques and the integration of the InVivoScribe™ multiplex PCR primers. The InVivoScribe™ primer sets were developed and standardized by the BioMed-2 group (van Dongen JJ., et. al. Leukemia. 17:2257-317, 2003). Our method relies on a modification of the Qiagen QIAamp™ DNA purification system and capillary electrophoresis using the ABI™ system. Specimen types used for validation included formalin-fixed/paraffin-embedded as well as fresh tissues from skin, G.I., lymph node, liver, marrow and blood. These specimens represented a variety of B- and T-cell malignancies.

Results: The validation set included 33 cases with an unequivocal diagnosis of B- or T-cell lymphoma. Twelve of thirteen cases of B-cell lymphoma demonstrated clonal IgH rearrangements (92% concordant). Seventeen of twenty cases of T-cell lymphoma demonstrated clonal TCRG rearrangements (85% concordance). These results are similar to the detection rates reported by BioMed-2 (ie. 92% for the IgH primer sets and 89% for the TCRG primer sets). Additional validation studies showed that the lowest limit of detection, under ideal circumstances, was less than a 1% clonal population in a polyclonal lymphocyte background. Interpretive guidelines were developed and further refined with experience. Dominant fluorescent peaks were considered clonal if >3 times the height of the polyclonal background and borderline if between 2-3. A minimum peak height of >500 arbitrary units is desired to exclude background fluorescence in samples with a limited number of lymphocytes.

Conclusions: We conclude that our T- and B-cell clonality assays, using the InVivoScribe™ primer sets are technically valid. Method development and validation are an essential part of implementing commercially available primer sets and periodic assessment of interpretive guidelines is also essential.

1011 Acute Myeloid Leukemia with Pseudo-Chediak-Higashi Anomaly Exhibits a Specific Immunophenotype with CD2 Expression

H Chang, QL Yi. University Health Network, University of Toronto, Toronto, ON, Canada.

Background: Large pink or purple cytoplasmic granules, defined as peudo-Chediak Higashi (PCH) anomaly, have been described in rare cases of acute myeloid leukemia (AML). AML with PCH anomaly express common myeloid associated antigens such as CD13. CD33, but no specific marker(s) have been identified.

Design: We systematically characterized the morphologic, immunophenotypic and cytogenetic features of 5 de novo adult AML cases with PCH anomaly. The immunophenotype was evaluated by multi-parameter flow cytometric analysis.

Results: Of the 5 cases of AML with PCH, two were FAB-M2, 2 M1, and 1 M6. The bone marrow blast percentage ranged from 40-90%. The frequency of blasts containing PCH granules ranged from 1-8%. Auer rods were found only in one case with FAB-M2 morphology. In two cases, the blasts contained multiple PCH granules, whereas in the rest cases the blasts harbored only single cytoplasmic PCH. By enzyme cytochemistry, the PCH granules were myeloperoxidase (MPO) positive and non-specific esterase negative. On cytogenetic analysis, one case had t(8;21) (M2), one had complex chromosomal abnormalities, and the other 3 had normal karyotypes. In all 5 cases, flow cytometry revealed that the blasts expressed the progenitor cell marker CD34, the non-

lineage specific marker HLA-DR, and myeloid associated marker CD13, CD33, CD117 and MPO. Of particular interest, all 5 cases expressed the pan T-cell associated antigen CD2 (range, 42-99%). To compare the frequency of CD2 expression in AML without PCH anomaly, we retrospectively reviewed the immunophenotype of 140 cases of de-novo AML, and found 25 (17%) cases expressed CD2. On statistical analysis, CD2 expression was strongly correlated with AML with PCH anomaly ($p<0.01$).

Conclusions: Acute myeloid leukemia (AML) with peudo-Chediak-Higashi (PCH) anomaly represents a rare distinct morphologic entity. Our study suggests a link between a specific immunophenotypic marker, CD2 and AML with PCH anomaly.

1012 *Chlamydia psittaci* Is Variably Associated with Ocular MALT Lymphoma in Different Geographical Regions

E Chanudet, Y Zhou, C Bacon, A Wotherspoon, H Müller-Hermelink, P Adam, Y Li, R Wei, X Gong, Q Wu, R Ranaldi, G Goteri, S Pileri, H Ye, H Liu, J Radford, MQ Du. University of Cambridge, United Kingdom; Royal Marsden Hospital, United Kingdom; Würzburg University, Germany; Sun Yat-Sen Ophthalmologic Hospital, China; Changzheng Hospital, China; Hainan Province Hospital, China; Sun Yat-Sen Tumour Hospital, China; Pathology Institute, Italy; Unità Operativa di Emolinfopatologia, Italy; Christie Hospital, United Kingdom.

Background: Infectious agent plays an important role in the development of MALT lymphoma of various sites; this is best exemplified by the causative role of *Helicobacter pylori* infection in gastric MALT lymphoma. Recent studies from Italy showed evidence of *Chlamydia psittaci* infection in 80% of ocular adnexal lymphomas and eradication of *C. Psittaci* led to partial or complete regression of the disease in some cases. To further understand the aetiology of ocular MALT lymphoma, we screened ocular lymphomas and appropriate controls for 7 infectious agents commonly associated with chronic eye diseases.

Design: The presence of *Chlamydiae (C. trachomatis, C. pneumoniae, C. psittaci)*, herpes simplex virus type 1 and 2 (HSV1, HSV2), and adenovirus type 8 and 19 (ADV8, ADV19) was assessed separately by PCR of DNA from 92 archival cases of ocular MALT lymphoma, 10 ocular DLBCLs, 21 other non-Hodgkin lymphomas and 39 conjunctival biopsies without lymphoproliferative disorder, from 6 different geographical regions.

Results: *C. psittaci* was found at variable frequencies in ocular MALT lymphomas from different geographical regions: high in those from Germany (47%), Italy/Bologna (33%) and China/Canton (20%), but low in those from the UK (10%), Italy/Ancona (0%), and China/Hainan and Shanghai (0%). The frequencies in the latter group were similar to or lower than that those seen in conjunctival biopsies without lymphoproliferative disorder (10.2%) and non-Hodgkin lymphomas other than MALT and DLBCL (9.5%). Cases of DLBCL were only available from China/Canton; interestingly, they showed a high incidence (33.3%) of *C. psittaci* infection, as observed for MALT lymphomas from the same region. There was no difference in the incidence of *C. pneumoniae* and *C.trachomatisi,* HSV1, HSV2, ADV8 and ADV 19 infection between lymphoma and controls from different geographical regions.

Conclusions: *C. psittaci* was preferentially associated with ocular MALT lymphoma and such association varied according to the geographical origin.

1013 Secondary Cytogenetic Aberrations Do Not Affect Survival in Acute Myeloid Leukemia (AML) with t(8;21)(q22;q22)

L Chen, LJ Medeiros, W Chen, C Bueso-Ramos, LV Abruzzo, X Wang, P Lin. UT MD Anderson Cancer Center, Houston, TX.

Background: AML with t(8;21)(q22;q22) is a distinct type of AML generally associated with a favorable prognosis. However, some patients rapidly succumb to disease within months despite chemotherapy. It has been postulated that additional cytogenetic aberrations account for the diverse clinical behavior, but the data are controversial. In this study, we analyzed the impact of secondary cytogenetic aberrations on survival.

Design: We searched the files of the Cytogenetics Laboratory for patients with AML with t(8;21)(q22;q22) from 1995-2005. The t(8;21) was confirmed by conventional cytogenetics, RT-PCR, and/or FISH. We compared overall survival and event-free survival of patients with AML with isolated t(8;21) vs. those with AML with t(8;21) and other aberrations using the Kaplan-Meier method. A multivariate Cox proportional hazard model was used to analyze the impact of age, gender, CD19 expression, and therapeutic regimens on survival.

Results: 64 patients were diagnosed with AML with t(8;21); 1 patient who declined treatment was excluded. There were 29 males (46%) and 34 females (54%) with a median age of 39 years (range 9-76). 19 (30%) patients had isolated t(8;21) (group 1) and 44 (70%) patients had t(8;21) and other aberrations (group 2). Loss of a sex chromosome was found in 18 (29%) cases, followed by del(9) in 6 (1.0%) cases. The median survival for group 1 and group 2 was 2.2 years and 3.3 years, respectively. There was no survival difference between the two groups (p=0.69)(Table 1). Neither loss of a sex chromosome nor del(9) impacted on survival. All 63 patients received chemotherapy: 33 with fludarabine-based, and 30 with other regimens. The patients treated with fludarabine-based regimens had a significant lower risk of events (p=0.002), but overall survival probability was not significantly different.

Conclusions: Secondary cytogenetic aberrations are detectable in approximately 70% patients with AML with t(8;21). Neither age, gender, nor CD19 expression correlated with event-free or overall survival. Patients treated with fludarabine-based chemotherapy had better event-free survival, but not overall survival.

Table 1 (N=63)

variable	isolated t(8;21)	t(8;21) with secondary aberrations	p value
Age	47 (19-70)	38.5 (9-76)	0.30
CD19+	15/16	30/36	0.47
OS (years)	2.2 (1.5-NA)	3.3 (2.3- NA)	0.69
EFS (years)	1.0 (0.8-NA)	1.4 (1.0-NA)	0.38

OS-overall survival; EFS-event-free survival

1014 Clinical Implications of Quantitative and Qualitative Post-Treatment Detection of AML1-ETO in AML

L Chen, J Guo, D Jones, R Luthra, P Lin. The University of Texas MD Anderson Cancer Center, Houston, TX.

Background: t(8;21)(q22;q22) is associated with a distinct subtype of AML. Methods to detect the t(8;21) or its fusion product *AML1-ETO* include conventional cytogenetics (CG), fluorescent in-situ hybridization (FISH) and reverse transcription polymerase chain reaction (RT-PCR). In this study, we compared methods for detection of residual disease in post-therapy samples, including aspirate blast counts, FISH, CG and both qualitative and quantitative RT-PCR assays. The goal was to determine which method might predict overt clinical relapse.

Design: Bone marrow samples from 8 patients with t(8;21) positive AML were studied including four who remained in remission (group 1) and four who eventually relapsed (group 2). CG, FISH and RT-PCR were performed during clinical remission at 1-6 months intervals. Levels of *AML1-ETO* transcript were analyzed in parallel by quantitative TaqMan RT-PCR using a kit from Ipsogen (Marseille, France) and a qualitative PCR assay that used post-PCR hybridization with an AML1 probe. For molecular monitoring, two samples, one obtained during early remission and the other prior to relapse were analyzed.

Results: The post-treatment follow-up period was 13 to 62 months (median 17 months). All 8 patients had persistent AML1-ETO transcripts in both the qualitative and quantitative RT-PCR assays despite having negative CG and/or FISH results prior to relapse. In group 1, the percentage of AML1-ETO transcript normalized to ABL transcripts ranged from 0.003% to 0.26% with 1 case showing an increase from the first to second time-points (4 folds). In group 2, the pre-relapse AML1-ETO/ABL levels varied from 0.005% to 66% in 3 patients with 23, 75, and 626 fold increases from the first to second pre-relapse samples. There was insufficient material in the fourth case for analysis. Blast counts prior to overt relapse were 0-7 % (median 1 %).

Conclusions: RT-PCR was the most sensitive method in detecting residual disease in t(8;21)-bearing AML, but was persistently positive even in patients who did not relapse. With the quantitative RT-PCR assay, there were variable levels of AML1-ETO transcript in individual patients post-treatment suggesting that absolute transcript levels may not be a strong predictor of relapse. However, rising levels of AML-ETO in the post-treatment period was typical of those patients who relapsed and preceded clinical relapse by 1-2 months. These results support the use of quantitative AML1-ETO monitoring in the post-treatment interval.

1015 A Biologically Distinctive Subgroup of Acute Myelogenous Leukemia (AML) with Characteristic Nuclear Morphology, High Frequency of *FLT3* and *NPM* Gene Mutations

W Chen, G Rassidakis, J Li, M Roubort, D Jones, LJ Medeiros, C Bueso-Ramos. UT MD Anderson Cancer Center, Houston, TX.

Background: AML is a heterogeneous group of diseases. Mutation of the nucleophosmin (*NPM*) gene and the fms-like tyrosine kinase-3 (*FLT3*) gene of internal tandem duplication (ITD) are frequent genetic events in AML. In this study, we describe a morphologically distinctive subgroup of AML cases with *NPM* and *FLT3* gene mutations.

Design: We observed rare cases of AML, M1, in which blasts exhibit prominent nuclear invagination with frequent fishmouth-like nuclear indentation and often carried *FLT3* ITD. We thus searched for AML, M1 cases with such nuclear feature (study series, SS) from 1998 to mid-2005 at our institution. This yielded 24 patients, representing about 20% of AML, M1 and 1% of all AML. The age-matched control series (CS) of 20 AML, M1, without these nuclear features was established to compare the frequency of *NPM*, *FLT3* gene mutations, conventional cytogenetics (CG), immunophenotype by flow cytometry. *NPM* mutations were detected by PCR amplification of genomic DNA followed by direct sequencing.

Results:

	Characteristics of AML cases						
	age (y)	sex M : F	NPM mutations	FLT3 ITD	normal CG	lack of CD34	lack of HLA-DR
SS (n=24)	18-81, 62	6 : 18	5/9 (56%)	7/8 (88%)	16/23 (70%)	17/24 (71%)	11/24 (46%)
CS (n=20)	20-80, 65	13 : 7	2/15 (13%)	7/14 (50%)	9/20 (45%)	2/20 (10%)	2/20 (10%)
p value	p> 0.05	0.014	0.06	0.16	0.10	0.0002	0.0009

As illustrated in the table, AML cases with fishmouth-like nuclear morphology (SS) were significantly associated with female gender, high frequency of *NPM* and *FLT3* mutations, and lack of expression of CD34 and HLA-DR. There was statistical trend toward high frequency of normal CG in the SS. The median white blood cell count was 48 in the SS (1.4-335 $x10^9$/L), and 35 in the CS (0.7-550 $x10^9$/L). There was no significant difference in bone marrow blast percentage (median 90% in SS vs. 85% in CS) nor did expression of CD13, CD33 and CD117. Electron microscopic examination demonstrated condensed collections of mitochondriae within the indented/invaginated nuclear pockets. Complete remission rate was higher in AML cases in the SS (73% vs. 40%, p=0.032). However, there was no difference in the overall survival rate.

Conclusions: AML cases with characteristic fishmouth-like nuclear morphology may represent a rare but distinctive AML subgroup with specific clinical, immunophenotypic and genetic features.

1016 FOXP1 Expression in AIDS-Associated Diffuse Large B-Cell Lymphoma (DLBCL): Correlation with Prognostic Parameters in Patients from AIDS Malignancies Consortium Trial 010

X Chen, E Cesarman, E Hyjek, LD Kaplan, DT Scadden, A Chadburn. Weill Cornell Medical College, New York, NY; University of California-San Francisco, San Francisco, CA; Massachusetts General Hospital-Harvard, Boston, MA.

Background: Microarray gene expression patterns of germinal center (GC) and non-germinal center (non-GC) correlate with survival in immunocompetent DLBCL patients. CD10, BCL6, MUM1 and CD138 phenotypic patterns are surrogates for genetic studies with comparable survival data; BCL6/CD10 expression alone defines the GC while MUM1/CD138 expression identifies the poorer prognosis non-GC phenotype which is also associated with poorer outcome in non-uniformly treated HIV+ DLBCL patients. FOXP1, a transcription factor differentially expressed in resting and activted B cells, is an independent adverse prognostic marker expressed by non-GC DLBCLs in immunocompetent patients. We examined AIDS-associated DLBCLs from uniformly treated (CHOP or CHOP-rituxan) HIV+ patients in AMC 010, to determine if FOXP1 is a prognostic marker in this group.

Design: Slides of 32 AIDS-associated DLBCLs from AMC010 patients were available for FOXP1, CD10, BCL6, MUM1, BCL2, Ki-67 immunohistochemistry (IHC) and ISH for EBV (EBER). Expression of an antigen by >20% tumor cells was considered positive. FOXP1 and/or MUM1 expression defined the non-GC phenotype. FOXP1 expression was correlated with survival; BCL2, Ki-67 expression; EBV status. Overall survival (OS) based on GC vs non-GC phenotype was examined.

Results: IHC identified 15/32 FOXP1, 12/23 MUM1, 11/19 BCL6, 4/7 CD10, 16/27 BCL2, 11/29 EBER positive cases. There was no OS difference between GC and non-GC groups or FOXP1+ (85 wks) and FOXP1- (61 wks) cases (t-test). However, patient survival at study end was significantly greater in the FOXP1+ (232 wks) vs FOXP1- cases (156 wks; p=0.005) suggesting a better prognosis for those surviving initial therapy. FOXP1 expression did not correlate with EBV positivity, BCL2 or Ki67 expression (chi-square).

Conclusions: FOXP1 expression in AIDS-associated DLBCLs, in contrast to immunocompetent DLBCLs, is not associated with significantly poorer prognosis based on OS. In contrast, HIV+ patients with FOXP1+ DLBCLs, if they survive the initial therapy, may have significantly superior survival. However, correlation with clinical data, currently in progress, is needed to determine if this superior survival is related to tumor biology or HIV status.

1017 Comparative Analysis of Zap-70 Flow Cytometry Assessment Techniques with Regard to IgVH Mutational Status in Chronic Lymphocytic Leukemia (CLL)

Y Chen, LC Peterson, R Gupta, VT Shankey, A Khoong, A Evens, CL Goolsby. Feinberg School of Medicine, IL; John H Stroger, Jr Hospital, IL; Beckman-Coulter, FL.

Background: Recent studies report strong correlation between Zap70 expression determined by flow cytometry (FC) and mutational status of immunoglobulin heavy-chain variable-region (IgVH) genes in patients with CLL. This correlation has been questioned since other reported concordance rates vary. This may be due to differences in methods and reference cell populations used to evaluate Zap 70. In this study, multiple FC methods to assess Zap70 expression were compared to IgV_H mutational status to determine the best correlation with mutational status.

Design: FC was performed on 155 samples from 125 patients with CLL. One subjective and 5 objective methods were tested. Subjectively, Zap70 status was assessed based on Zap70 staining as compared to T/NK cells. The objective methods included using fixed integration regions (FIR) based on T/NK cells (Crespo et al) and normal B cells to define positive staining. The other objective methods analyzed the ratio of staining intensity (MESF) within the abnormal B cells to that of normal B, T or NK cells. Mutational status was determined using RT-PCR gene sequencing standard database techniques. Sequences were aligned with IgV_H genebank and homology ≥ 98% was considered germline.

Results: The subjective T/NK cells method yielded 63% sensitivity (SN) and 65% specificity (SP) for detecting germline status. Of the FIR methods, Zap70 expression determined by comparison with the T/NK cells produced better SN (75%) but at greatly reduced SP (35%); meanwhile, comparison with normal B cells gave a SN of 44% and SP of 75%. Of the objective MESF methods, the ratio of abnormal B cell Zap70 staining intensity compared to normal T cells (SN 66% and SP 57%) and normal B cells (SN 59% and SP 59%) was superior to normal NK cells (SN 61% and SP 44%).

Conclusions: All of the methods correlated but none of the methods demonstrated high sensitivity and specificity for mutational status in CLL. Of the objective methods, the semi-quantitative analysis of Zap70 staining as a ratio of abnormal B Zap70 MESF staining to that in normal T cells or B cells provides the best combination of sensitivity and specificity. The subjective T/NK cell method gave a similar result, but may be limited by its inherent subjectivity and possible lack of reproducibility between laboratories. The fixed integration region method using T/NK cells was sensitive to germline mutations but had low specificity.

1018 The TNF Family Memebers BAFF and APRIL Play an Important Role in Hodgkin Lymphoma

A Chiu, X Qiao, B He, E Hyjek, J Lee, E Cesarman, A Chadburn, DM Knowles, A Cerutti. Weill Medical College of Cornell University, New York, NY.

Background: B-cell activating factor (BAFF), and its homologue a proliferation-inducing ligand (APRIL), are TNF family members which promote B cell proliferation and survival. BAFF and APRIL also induce class switch DNA recombination (CSR), a process that requires activation-induced-cytidine deaminase (AID). BAFF and APRIL are normally produced by dendritic cells, macrophages, and granulocytes, but not by B cells. Their receptors [transmembrane activator and calcium modulator and cyclophylin ligand interactor (TACI), B cell maturation antigen (BCMA), BAFF receptor (BAFF-R)] are preferentially expressed by normal B cells. Previously we showed that, unlike normal B cells, the neoplastic B cells in non-Hodgkin's lymphoma (NHL) express BAFF and APRIL In this study we aim to elucidate BAFF, APRIL, TACI, BCMA, and BAFF-R expression in Hodgkin lymphoma (HL).

Design: IHC for BAFF, APRIL, TACI, BCMA, and BAFF-R was performed on frozen tissue sections of 6 cases of HL. In addition, the Reed-Sternberg (RS) cells from these cases were microdissected and analyzed for the expression of AID and CSR byproducts by RT-PCR. The expression of BAFF, APRIL, TACI, BCMA, BAFF-R, AID, and CSR byproducts was also analyzed in 5 HL cell lines cultured in the presence or absence of BAFF, APRIL, and cytokines.

Results: In all 6 HL cases, both the RS cells and the reactive infiltrate express BAFF and APRIL. Unlike NHL B cells, which express BAFF-R, the RS cells of the 6 HL cases and HL cell lines lack BAFF-R expression, but express TACI and BCMA. In the presence of BAFF or APRIL, HL cell lines are rescued from spontaneous or induced apoptosis through activation of the NF-κB classical pathway. The increased RS cell survival is associated with up-regulation of BCL-2 and BCL-XL, and down-regulation of BAX. Finally, in the presence of IL-4 and BAFF or APRIL, RS cell lines up-regulate AID expression and increase their spontaneous CSR activity.

Conclusions: BAFF and APRIL promote RS cell survival through both autocrine and paracrine pathways. By engaging TACI and BCMA, BAFF and APRIL attenuate RS cell apoptosis through up-regulation of NF-kB and other pro-survival proteins, including BCL family members. By up-regulating AID, TACI and BCMA might introduce genomic instability in RS cells. Finally, given that TACI, BCMA, and AID are B cell-specific molecules and that CSR is a B-cell confine process, our findings consolidate the notion that RS cells are derived from a B cell precursor.

1019 2006 Chronic Myelomonocytic Leukemia (CMML): The Role of Bone Marrow Biopsy Immunohistology

R Chiu, DP O'Malley, S Allen, M Czader, A Orazi. Indiana University, Indianapolis, IN.

Background: The WHO criteria for diagnosing CMML require a persistent peripheral blood monocytosis of > 1 x 10^9/L, with monocytes greater than 10%, *BCR/ABL* negativity, <20% blasts in BM or PB, and dysplasia. The BM of CMML is usually hypercellular and may demonstrate granulocytic and monocytic hyperplasia, although the latter may be minimal and difficult to demonstrate in the absence of cytochemistry. Besides the evaluation of BM cellularity and fibrosis, additional diagnostic roles for the BM biopsy (BMB) in this condition have not been explored. In this study, we examined whether BMB immunohistochemistry may be helpful in distinguishing CMML from cases of CMML in acute transformation (CMML-AT) and/or CMML from Ph' pos. chronic myeloid leukemia (CML).

Design: We stained BMB of 24 cases of CMML using CD34 (QBEnd10), CD68 (PG-M1), CD68 (KP1), and compared the results with those observed in 7 cases of CMML-AT and in 6 cases of CML. The presence of plasmacytoid monocytes nodules (PM Nods) was investigated by CD123 (IL-3R-alpha). CD42b (MM2/174), was used to highlight dysplastic megakaryocytes.

Results:

Results:	CD34* (±SD)	CD68/KP1* (±SD)	CD68/PG-M1* (±SD)	CD123 PM Nods
CMML	4.76(3.9)	33.05(27.4)	3.96(3.8)	6 (19%)
CMML-AT	39.00(20.1)	23.14(29.4)	2.83(0.8)	1 (14%)
CML	3.83(1.9)	90.83(8.7)	2.80(0.8)	0

* The results are expressed as mean (±SD) percentage of positive nucleated marrow cells. KP1, CMML vs. CML: p<0.001; CD34 CMML vs. CMML-AT: p<0.001; CML vs. CMML-AT: p<0.01

Conclusions: Our study of BMB demonstrates differences between the groups. CD34 immunohistochemistry is a useful marker to separate CMML from cases of the disease in acute transformation. The presence of PM Nods reactive with CD123, is restricted to CMML and is not present in CML. Overlap between CMML and CML is observed with CD68 immunostaining, a result similar to that observed by others. PG-M1 is more restricted to BM macrophages and monocytes than KP1, but the differences between CMML and CML are still non significant. Additionally, KP1, an antigen which is localized to lysosomes and neutrophil granules, seems more strongly expressed in the myeloid cells of CML than in CMML. This may be due to the presence of lesser/abnormal granularity in the myeloid cells and neutrophils in CMML in comparison with CML. Although CD42b facilitates detection of dwarf megakaryocytes often present in CMML, the distinction between those and the small forms seen in CML is still problematic.

1020 Aberrant Promoter Methylation of Multiple Genes in Malignant Lymphoma of B-Cell Type

YD Choi, KH Lee, JH Nam, CS Park, C Choi. National Institute of Scientific Investigation, Chang-Sung, Republic of Korea; Chonnam National University Medical School, Gwang-ju, Republic of Korea.

Background: Diffuse large B cell lymphoma (DLBCL) usually arise *de novo* but can represent transformation of a low grade B cell malignant lymphoma (LGBML), such as chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL), follicular lymphoma (FL), mantle cell lymphoma (ML), and marginal zone B-cell lymphoma (MZL). Of the genes regulated by methylation, we tried to search the gene, which is significantly different between LGBML and DLBCL.

Design: We examined 34 cases of DLBCL, 6 of CLL/SLL, 20 of MZL, 3 of ML, and 5 of FL by performing methylation specific PCR (MSP) of MGMT (O^6-methylguanine-DNA methyltransferase), DAP-*k* (death-associated protein kinase), hMLH1, E-cadherin, P57, HIC-1, and SHP1. In selected cases, immunohistochermistry was performed to see whether the MSP results were matched to protein expression.

Results: The methylation frequency was 30.5% (47.1% in LGBML and 18.8% in DLBCL) in MGMT, 70.7%(61.7% in LGBML and 77.1% in DLBCL) in DAP-*k*, 1.2% (2.9% in LGBML and 0.0% in DLBCL) in hMLH1, 61.0% (61.8% in LGBML and 60.4% in DLBCL) in E-cadherin, 48.8% (50.0% in LGBML and 47.9% in DLBCL) in P57, 56.1% (55.9% in LGBML and 56.3% in DLBCL) in HIC-1, and 79.3% (64.8% in

LGBML and 89.6% in DLBCL) in SHP1, respectively. The methylation frequency of MGMT was significantly higher in LGMT than DLBCL (p=0.006) and the frequency of SHP1 was higher in DLBCL than LGMT (p=0.007). Aberrant methylation of MGMT and SHP1 genes were well correlated with no expression of proteins of them in immunohistochemistry.

Conclusions: In malignant lymphoma of B cell type, DAP-*k* and SHP1 genes are more methylated than other genes. It is possible that progression of malignant lymphoma was related with aberrant methylation of MGMT and SHP1. Further large studies are needed to clarify this problem.

1021 Determination of B-Cell Clonality in Hodgkin Lymphoma Using the InVivoScribe IgH Assay

DJ Chute, JB Cousar, MS Mahadevan, KA Siegrist, LM Silverman, MH Stoler. University of Virginia, Charlottesville, VA.

Background: Molecular analysis of IgH gene rearrangement is commonly used to help establish a diagnosis of B-cell lymphoma. Previous microdissection studies of Hodgkin Lymphoma (HL) have shown Reed-Sternberg (RS) cells contain clonal IgH gene rearrangements. However, without microdissection, demonstration of clonality is rare. The InVivoScribe IGH assay is a FDA-approved kit, which is reported to have increased sensitivity for IgH gene rearrangements. If true, increased detection of clonal IgH rearrangements in HL could blur the criteria used to distinguish non-Hodgkin lymphoma (NHL) from HL.

Design: 35 recent cases of classical HL were selected along with 7 diffuse large B-cell lymphoma (DLBCL) cases and 7 follicular hyperplasia (FH) cases as controls. The HL cases were reviewed for morphologic subtype. The density of RS cells/10HPF on H&E sections was classified as low (<10), intermediate (10-24) and high (>24). In 17 cases with CD30 available, the density of CD30+ cells/10HPF was classified as low (<25), intermediate (25-49) and high (>49). DNA from 2-4 10 micron formalin fixed, paraffin embedded sections was extracted and purified using Qiagen columns. Polymerase chain reaction amplification of the IgH gene was performed using the InVivoScribe IGH Gene Clonality Assay for ABI detection according to the manufacturer's procedure. Dominant peaks were considered clonal if >3 times the height of the polyclonal background, and borderline if between 2-3.

Results: Overall, 10/35 (29%) HL cases were clonal, and 6/35 (17%) were borderline (BL). Breakdown by morphologic subtype showed 30 cases of nodular sclerosing HL and 5 cases of mixed cellularity HL, of which 7/30 were clonal (5/30 BL) and 3/5 were clonal (1/5 BL) respectively. Using H&E RS density, 0/8 low cases were clonal (1/8 BL), 5/15 intermediate were clonal (2/15 BL), and 5/12 high were clonal (3/12 BL). By CD30+ cell density, 0/7 low cases were clonal (2/7 BL), 0/5 intermediate were clonal (1/5 BL), and 4/5 high were clonal (1/5 BL). 6/7 DLBCL were clonal (1/7 BL). 0/7 FH were clonal (1/7 BL).

Conclusions: In difficult cases, IgH gene clonality may be misleading, as 29-46% of HL cases are clonal using the InVivoScribe IGH assay, depending upon the stringency of the criteria used. The presence of clonality is directly correlated with the density of RS cells, and CD30+ cell density is more accurate at predicting cases showing clonality than H&E. The RS density analysis and clonality data blurs the distinction between HL and NHL.

1022 hs.TRAIL/Apo2L Induces Apoptosis in Part of Chemotherapy Refractory Nodal Diffuse Large B-Cell Lymphomas

SG Cillessen, CJ Meijer, GJ Ossenkoppele, K Castricum, E Hooijberg, JJ Oudejans. VU University Medical Center, Amsterdam, Netherlands.

Background: Inhibition of the stress-induced, caspase 9 mediated apoptosis pathway is a probable cause for chemotherapy resistance in nodal diffuse large B-cell lymphomas (DLBCL). TRAIL/Apo2L might be an alternative form of therapy for chemotherapy DLBCL lymphoma patients because it induces apoptosis via the alternative caspase 8 mediated pathway.

Design: In isolated lymphoma cells of diffuse large B-cell lymphomas we investigated whether hsTRAIL/Apo2L induces apoptosis in lymphomas that are resistant to chemotherapy induced cell death and whether expression of Bcl-2 and/or XIAP interferes with sensitivity to hsTRAIL/Apo2L induced apoptosis.

Results: hsTRAIL/Apo2L strongly induced apoptosis in part of DLBCL samples including chemotherapy refractory lymphomas. hsTRAIL/Apo2L induced apoptosis was preferentially observed in DLBCL and B-cell lines that were relatively or completely resistant to Etoposide-induced apoptosis. Furthermore, hsTRAIL/Apo2L also induced apoptosis in DLBCL and B-cell lines showing high expression levels of Bcl-2 and/or XIAP. In hsTRAIL/Apo2L sensitive cells expression of the TRAIL receptor R1 and R2 and absence of R3 and R4 was observed.

Conclusions: We conclude that hsTRAIL/Apo2L induces apoptosis in part of chemotherapy refractory nodal DLBCL and that disruption of the caspase 9 mediated pathway and expression of Bcl-2 and XIAP does not confer resistance to hsTRAIL/Apo2L induced apoptosis in DLBCL and B-cell lines. Thus, based on our results hsTRAIL/Apo2L appears to be a valuable alternative treatment for patients with chemotherapy refractory DLBCL

1023 Aplastic Anemia May Acquire Monosomy 7 Associated with Dysthrombopoiesis

AM Cioc, TP Singleton, JP Neglia, MM Dolan. University of Minnesota Medical School, Minneapolis, MN.

Background: Aplastic anemia (AA) is marrow failure due to an inadequate number of hematopoietic cells in the bone marrow. Outcome depends on the etiology and treatment, which may include antithymocyte globulin (ATG), cyclosporine (CSA), other immunosuppressive regimens, and bone marrow transplant. A subset of patients may develop myelodysplastic syndromes (MDS) or acute leukemias. Prior reports have described a more aggressive clinical course in aplastic anemia with monosomy 7 (Mo 7), a cytogenetic abnormality associated with MDS. We describe patients with AA and normal cytogenetics followed by acquisition of Mo 7.

Design: We reviewed our pathology database from 2004 to 2005 for pediatric patients with AA and normal cytogenetics followed by acquisition of Mo 7. Patients with Fanconi anemia were excluded. The peripheral blood smears and bone marrow biopsies were evaluated for myelodysplastic features before and after development of Mo 7. The medical records were reviewed for treatment and outcome.

Results: Three pediatric patients had AA with normal cytogenetics followed by acquisition of Mo 7. These patients received similar therapeutic regimens, including ATG, steroids and CSA. Two boys, ages 9 and 12, were previously healthy; a 17-year-old female had a history of chronic active hepatitis. For each patient, three to five bone marrow morphologic and cytogenetic analyses were performed over 2-3 years before the development of Mo 7. Bone marrow biopsies were initially diagnostic of AA and later developed morphologic dysplasia characterized by an increased number of small hypolobulated megakaryocytes, highlighted with immunohistochemical stains for CD61. The marrow blast percentage was not increased, except for one patient with 4.6% blasts. In two patients, the megakaryocytic dysplasia became apparent at the time that Mo 7 was first detected; in one patient, Mo 7 preceded morphologic dysplasia by one month. Within weeks to months of the development of Mo 7, the three patients underwent bone marrow transplant, and each has remained disease free for 7-18 months after transplant.

Conclusions: Pediatric patients with AA and normal cytogenetics may develop Mo 7 and MDS associated with characteristic megakaryocytic dysplasia. Patients with AA and Mo 7 should be evaluated for these morphologic abnormalities.

1024 Myeloid/Natural Killer Cell Precursor Immunophenotype Is Present in Heterogeneous Acute Leukemias

AM Cioc, MM Dolan, TP Singleton. University of Minnesota, Minneapolis, MN.

Background: Myeloid/natural killer cell precursor acute leukemia (MNKAL) has been proposed as a distinct clinicopathologic entity characterized by extramedullary disease; immature morphology; expression of CD7, CD56, and myeloid antigens; and lack of myeloperoxidase and other B- or T-cell associated antigens.

Design: The flow cytometry and hematopathology database at the University of Minnesota was searched for acute myeloid and biphenotypic leukemias expressing CD56 on more than 20% of blasts. The glass slides, flow cytometry, and cytogenetics were reviewed to classify the CD56+ acute leukemias according to WHO criteria and to find cases of MNKAL. The patient charts were reviewed for blood counts, extramedullary disease, and prior myeloid disorders.

Results: Between March 2001 and April 2005, 240 cases of de novo and relapsed acute myeloid and biphenotypic leukemia were analyzed in the flow cytometry laboratory for CD56: 182 biopsies were CD56-, and 58 biopsies (52 patients) were CD56+. Using the WHO criteria, the CD56+ biopsies (42 biopsies, 36 patients) were classified as follows: acute myeloid leukemia (AML) with t(8;21) (3 cases), acute promyelocytic leukemia with t(15;17) (2 cases), AML M0/M1 (2 cases), AML M2 (3 cases), AML M4/M5 (2 cases), AML M7 (1 case), AML with multilineage dysplasia (1 case), AML evolving from myelodysplastic syndrome (MDS, 2 cases), AML evolving from MDS/myeloproliferative disorder (MPD, 4 cases), AML therapy-related (1 case), blast crisis of chronic myelogenous leukemia (CML, 2 cases), relapsed AML (11 cases), and relapsed acute biphenotypic leukemia (1 case). Seven cases (5 patients) fulfilled criteria for MNKAL. Using the WHO criteria, these biopsies were classified as follows: AML M0 (2 cases), AML evolving from MDS (1 case), AML evolving from MPD unspecified (1 case, 3 biopsies), and blast crisis of CML (1 case). The five index patients were between 46 and 77 years of age. Blood counts varied: white blood cells 1.6 to 25.2 x10 ^{9}/L, hemoglobin 8.4 to 12 g/dL, and platelets 31 to 282 x10 ^{9}/L. Extramedullary disease was present in 2 patients. Cytogenetic findings (4 of 5 patients) showed normal karyotype in AML M0 (1 case), loss of chromosome Y in AML evolving from MDS, complex cytogenetic abnormalities including losses of 5q and 7q in AML evolving from MPD, and t(9;22) in blast crisis of CML.

Conclusions: The myeloid/NK cell precursor immunophenotype is present in acute leukemias evolving from MDS/MPD. De novo cases are rare, accounting for 1% of acute myeloid and biphenotypic leukemias.

1025 CD30 Expression in Non-Transformed Mycosis Fungoides: Correlation with Proliferative Index and Clinical Features

BZ Clark, L Geskin, L Cassidy, SH Swerdlow. University of Pittsburgh, Pittsburgh, PA.

Background: Mycosis fungoides (MF) is a cutaneous T-cell lymphoma (CTCL) with a usually indolent course. Clinical and histologic transformation, however, may occur and can be associated with acquisition of CD30 expression. Little is known, however, about the expression of CD30 in non-transformed MF and its possible clinical and other pathologic associations. Therefore, CD30 and Ki-67 expression were studied in 15 cases of histologically non-transformed MF and correlated with clinical data concerning disease severity and treatment.

Design: After histologic/immunohistologic review, 15 cases of non-transformed MF with a consistent clinical history were selected for study. The number of CD30+ and Ki-67+ cells were counted and the proportion of each in the epidermis and dermis were calculated. The results were then correlated with the following clinical features (when available): stage at diagnosis (11 patients), maximum stage (11) and number of therapies (13).

Results: The mean age of the patients was 69.3 years (10 females, 5 males) and the average clinical follow-up from onset of rash was 6.8 years, (range: 11 months to 13 years). CD30+ cells were present in the dermis (mean 6.3 ± 6.4%) with 12 cases having 2-30% CD30+ cells in the epidermis and 3 cases having 66-78%, sometimes most prominent at the dermal-epidermal junction. Dermal but not epidermal CD30 positivity

correlated with the proportion of Ki-67+ cells (p<.01). Dermal CD30 expression did not correlate with epidermal CD30 positivity. A higher percentage of epidermal CD30+ cells was associated with a lower maximal clinical stage (ANOVA, p = 0.001) but it did not correlate with initial stage or number of therapies. There was a trend toward a higher number of therapies used for disease control with increasing dermal CD30 positivity (Pearson's correlation, p = 0.09) but no other correlations could be documented. No clinical correlations with Ki-67 positivity could be documented.

Conclusions: CD30 positivity in MF is not restricted to cases that have transformed and can be very prominent in the epidermis. These latter cases may be clinically distinctive; however, larger studies will be required. In contrast, cases with more numerous dermal CD30+ cells may reflect the cases with more numerous proliferating cells and possibly cases that require more treatment.

1026 Loss of Heterozygosity Identifies Genetic Differences between Megakaryocytes and Surrounding Hematopoietic Tissues in Chronic Myeloproliferative Disorders

B Coleman, L Cheng, A Orazi, M Wang, DP O'Malley. Indiana University, Indianapolis, IN.

Background: Chronic myeloproliferative disorders (CMPD) have a variety of underlying molecular and cytogenetic defects. Loss of heterozygosity (LOH) is a technique used to detect chromosomal imbalances in myeloid disorders, with several genetic loci being identified as commonly involved. We evaluated bone marrow core biopsies for the presence LOH at several loci.

Design: We evaluated 10 bone marrow core biopsies (essential thrombocythemia (ET) = 5; polycythemia vera (PV) = 2; chronic idiopathic myelofibrosis (CIMF) = 3). We examined LOH loci at 7q (D7S2554), 8q (D8S263), 9p (D9S157) which are common sites of genetic abnormality in CMPD. The megakaryocytes were microdissected, with LOH results compared to surrounding marrow elements, as well as control tissues. Histologic samples of benign tissues from the same patients were used as negative controls for LOH.

Results: Seven of the ten case evaluated had at least one abnormal LOH locus (4/5 ET; 1/2 PV; 2/3 CIMF). 4/7 cases (57%) with abnormal loci had differences between the megakaryocytic abnormalities and those of surrounding hematopoietic cells. No LOH was seen in the negative control tissues.

Conclusions: Our preliminary results suggest that LOH studies are frequently abnormal in marrow core biopsies in CMPD. Further, surprisingly frequent differences are seen between genetic abnormalities in megakaryocytes and surrounding hematopoietic tissues. Additional cases of CMPD and additional LOH loci (D9S161, D13S319, D20S108, TP53) will be tested.

1027 Expression of the Id2 Helix-Loop-Helix Protein in Hodgkin and Non-Hodgkin Lymphomas

CV Cotta, V Leventaki, LV Abruzzo, LJ Medeiros, GZ Rassidakis. UT MD Anderson Cancer Center, Houston, TX.

Background: Id2 belongs to the inhibitor domain (Id) family of helix-loop-helix proteins. The Id proteins are important in development, mainly due to their interaction with the E family of transcription factors. Animal models show Id2 to be an important regulator of hematopoiesis. Id2 is also a target for Rb, suggesting that it may play a role in tumorigenesis. The expression pattern of Id2 in lymphoid malignancies is unknown.

Design: Id2 expression status was investigated in 14 lymphoma cell lines by Western blot analysis using two specific polyclonal antibodies. By immunohistochemical methods, Id2 expression was assessed in 3 reactive lymph nodes, 159 non-Hodgkin lymphomas and 32 Hodgkin lymphomas.

Results: Id2 was expressed in all cell lines tested at variable levels, the highest in cells from higher grade tumors (DLBCL, ALCL) and the lowest in cells from mantle cell lymphoma. Immunohistochemical studies performed on 3 reactive lymph nodes showed Id2 localized to germinal centers, mainly in centroblasts, with a nuclear and nucleolar staining pattern. The mantle and marginal zones were negative. The results of Id2 stains in various types of lymphoma are shown in Table 1. Most of the high grade B-cell (DLBCL) or T-cell (ALCL) lymphomas were Id2 positive, with the majority (>90%) of cells expressing Id2 in a nuclear and nucleolar pattern. In follicular lymphomas the larger cells and many centrocytes were positive. Mantle cell lymphomas had mixed nuclear and nucleolar reactivity in most cells. Expression levels of Id2 were lower in low grade B-cell non-Hodgkin lymphomas such as CLL/SLL or MALT lymphoma. However, paraimmunoblasts in CLL/SLL cases were commonly positive for Id2. In Hodgkin lymphomas neoplastic Hodgkin and Reed-Sternberg cells were strongly positive for Id2.

Conclusions: In normal lymph nodes Id2 is detected in a subset of germinal center cells with a nuclear and nucleolar distribution. Most high grade lymphomas are strongly positive for Id2, while lower grade lesions show a weaker stain or are negative. These findings suggest that Id2 may be involved in lymphomagenesis or lymphoma progression.

Table 1

	Number of Cases	Id2 Positive
CLL/SLL	26	7 (26.9%)
MALT Lymphoma	12	6 (50%)
Mantle Cell Lymphoma	24	20 (83.3%)
Follicular Lymphoma	26	25 (96.2%)
DLBCL	38	37 (97.4%)
ALCL, ALK+	8	8 (100%)
ALCL, ALK-	25	23 (92%)
Hodgkin Lymphoma	32	30 (93.8%)

1028 pSTAT3 Expression in EBV-Positive and EBV-Negative Diffuse Large B Cell Lymphoma and Post-Transplantation Lymphoproliferative Disorders

DR Czuchlewski, GJ Solomon, E Hyjek, A Young, DM Knowles, W Tam, A Chadburn. Weill Medical College of Cornell University, New York, NY.

Background: STAT3, a transcription factor of the Signal Transducer and Activator of Transcription family which modulates expression of multiple genes involved in cell cycle progression and apoptosis, is constitutively active in several lymphoid malignancies and in Epstein-Barr virus (EBV)-positive lymphoblastoid cell lines. The EBV oncogenic viral protein LMP-1 activates JAK3, which phosphorylates STAT3 to pSTAT3, suggesting a role for STAT3 in LMP-1-mediated lymphomagenesis. We have found that monomorphic post-transplantation lymphoproliferative disorders (M-PTLDs), although EBV-positive, lack expression of LMP-1, while the polymorphic (P-PTLD) lesions are LMP-1 positive. The role of LMP-1-associated pSTAT3 expression in PTLDs and in other EBV-positive lymphomas has not been evaluated.

Design: Immunohistochemistry (IHC) for LMP-1 (DAKO) and pSTAT3 (Cell Signaling) and in-situ hybridization (ISH) for EBV (EBER; Novacastra) were performed in 20 cases of diffuse large B cell lymphoma (DLBCL; 7 EBV+, 13 EBV-) and 17 cases of PTLD (4 M-PTLD, 3 EBV+, 1 EBV-; 13 P-PTLD, all EBV+). A case was considered pSTAT3 positive if at least 20% of malignant cells exhibited nuclear expression.

Results: Fifteen of 37 cases (41%) were pSTAT3 positive, including 4/17 PTLD (24%; 0 M-PTLD; 4 P-PTLD), 6/7 EBV+ DLBCLs (86%) and 5/13 EBV- DLBCLs (38%). Overall, there was no statistically significant difference in pSTAT3 expression between EBV+ and EBV- cases. However, EBV+ DLBCLs were more likely to be pSTAT3+ (6/7) than were EBV- DLBCLs (5/13;p<0.05) or M-PTLDs (0/4;p<0.01). Overall, and within each category, LMP-1 positivity did not correlate with pSTAT3 expression. Among PTLD cases, pSTAT3 expression was identified only in P-PTLDs (4/13) and not in M-PTLDs (0/4).

Conclusions: Among DLBCLs, EBV+ cases are more likely to show pSTAT3 expression than EBV- cases, regardless of LMP-1 status. In contrast with EBV+ DLBCLs, M-PTLDs lack pSTAT3, suggesting a pathogenetic difference. STAT3 and LMP-1 expression did not correlate within either PTLD or DLBCL categories, casting doubt on a central role for pSTAT3 in LMP-1-mediated pathogenesis of these lesions.

1029 Alk-1 Expression in Extranodal Anaplastic Large Cell Lymphoma (ALCL) – Experience at a Tertiary Cancer Center in India

BK Dave, AM Mahajan, DM Maru. Tata Memorial Hospital, Mumbai, Maharashtra, India.

Background: Alk-1 expression is a frequent finding in systemic ALCL and infrequent finding in cutaneous ALCL. Majority of systemic ALCL include predominantly nodal disease with or without extranodal involvement. Immunoprofile of ALCL with primary extranodal involvement is unclear. We studied extranodal ALCL at a tertiary cancer center in India with focus on pathologic characteristics and immunohistochemistry findings.

Design: A 3 year retrospective analysis of extranodal ALCL diagnosed at Tata Memorial Hospital was performed. Cases with primary extranodal presentation with or without regional lymph nodes were included. The bulk of the disease was in extranodal sites in all the cases. All cases were histologically subtyped by two pathologists. Immunohistochemical stains for CD30 (clone Ber H2, M0751, Dako), Alk-1(clone ALK 1, M7195, Dako), LCA (clones PD7/26 and 2b11, M701,Dako), CD3(polyclonal, N1580, Dako)/CD43(clone DF-T.1,M0786,Dako) were reviewed. Alk-1 staining pattern was subclassified into cytoplasmic, nuclear and membranous or combination of any of these three patterns.

Results: Thirty cases studied included 20 men and 10 women. Average age was 35 years (range 4-82 years). Eleven cases were primary cutaneous ALCL (36.6%) and 19 noncutaneous (63.4%). The sites of involvement for the latter in order of frequency were soft tissue (n=12), stomach (n=2), bone (n=2), lung (n=1), buccal mucosa (n=1) and tonsil (n=1). Among the histological subtypes, classic variant (n=18) was the commonest followed by small cell (n=7) and neutrophil rich (n=4) variants. One case was unclassified. CD30 was positive in all cases. LCA was negative or focal positive in 9 cases. Alk-1 was positive in 14 cases (46.6%) and was negative in 16 cases (53.4%). The cytoplasmic pattern of staining for Alk-1 was the most frequent (n=9) followed by combined nuclear and cytoplasmic pattern (n=4) and combined cytoplasmic and membranous pattern (n=1). Eleven out of 19 non-cutaneous ALCL (58%) and 3 out of 11 cutaneous ALCL (27.7%) were Alk-1 positive. The noncutaneous sites of involvement with Alk-1 expression were soft tissue (n=9), bone (n=1) and lung (n=1).

Conclusions: This study shows frequent Alk-1 expression in ALCL with a primary extranodal presentation and in a small percentage of cutaneous ALCL. This group of localized extranodal ALCL may represent a novel subset of ALCL.

1030 Histologic Findings in Early Cutaneous T-Cell Lymphoma Correlate with T-Cell Clonality

CE Day, J Du, H Qian, X Xue, H Kamino, H Ratech. Montefiore Med Ctr, Bronx, NY; Albert Einstein Coll Med, Bronx, NY; New York Univ Med Ctr, New York, NY.

Background: The diagnosis of early (patch or plaque stage) cutaneous T-cell lymphoma (CTCL; mycosis fungoides) can be challenging because the pathognomonic features of late stage disease are often absent, the histologic patterns overlap with inflammatory skin conditions, and T-cell clonality is not always provable. In an attempt to enhance the accuracy of diagnosing early CTCL, we asked: which histologic features most closely correlate with T-cell clonality?

Design: We retrospectively reviewed hematoxylin and eosin stained skin biopsy specimens from 299 patients clinically suspected of early CTCL. The paraffin-embedded samples were studied for T-cell receptor (TCR) γ gene rearrangement using polymerase chain reaction-denaturing gradient gel electrophoresis and classified as 168 monoclonal (1 or 2 sharp bands), 81 oligoclonal (3 or more bands), and 50 polyclonal (smear). The non-parametric Kruskall-Wallis test was used to asses differences between the groups.

Results: In order to correlate skin biopsy findings with TCR-γ clonality, we semiquantitatively scored the degree of 24 histologic criteria: 0, absent; 1, mild; 2, moderate; 3, severe. In addition, we classified the distribution of epidermotropism: 0, none; 1, basal; 2, moderate; 3, diffuse; 4, microabscesses. These values were normalized and binned according to mono-, oligo-, or polyclonal TCR-γ gene rearrangements. The means of the 6 most discriminating features are listed in the table.

Conclusions: Monoclonal TCR-γ gene rearrangement is strongly associated with scores greater than 1.0 for dermal lymphocytic infiltration, distribution of epidermotropism, lymphocyte atypia, and degree of epidermotropism. Polyclonal TCR-γ gene rearrangement is strongly associated with scores greater than 0.9 for spongiosis and histiocytes. Oligoclonal TCR gene rearrangement is associated with intermediate scores for these 6 criteria. We conclude that the accuracy of diagnosing early CTCL might be improved by combining histologic analysis and TCR clonality testing into a single algorithm.

Histologic Features	MONOCLONAL	OLIGOCLONAL	POLYCLONAL	P-Value
Dermal lymphocytic infiltrate	1.81	1.30	1.06	<.0001
Epidermotropism, distribution	1.46	1.05	0.64	0.0001
Lymphocyte atypia	1.42	0.88	0.74	<.0001
Epidermotropism, degree	1.05	0.79	0.56	0.0004
Spongiosis	0.76	0.88	0.96	0.2385
Histiocytes	0.66	0.63	0.92	0.1603

1031 Chronic Lymphocytic Leukemia: Prognostic Indicators for Fludarabine, Cyclophosphamide, and Rituxan (FCR) Therapy

J De, SM O'Brien, L Abruzzo, CE Bueso-Ramos. UT - Houston Medical School; UT MD Anderson Cancer Center, Houston, TX.

Background: Chronic lymphocytic leukemia (CLL) patients experience long survival, but10-20% of patients experience progression. Possible markers of adverse outcome are vascular endothelial growth factor (VEGF) levels, expression of CD38 and Zap-70, and cytogenetic abnormalities (+12 and deletions at 11q22, 17p13, and 13q14). New treatment regimens with nucleoside analogs (fludarabine) and anti-CD20 monoclonal antibody (rituximab) have shown improved remission rate. Here we assessed prognostic indicators, B-cell caspase 3 activity, and infiltration patterns in patients treated with fludarabine, cyclophosphamide and rituximab (FCR).

Design: Fifty patients with CLL on FCR were included. Plasma VEGF (by ELISA) and B-cell caspase 3 activity (by fluorometric assay) at baseline were compared with those of 14 normal donors. Percent lymphocytes, tumor infiltration, and expression of CD38 (by flow cytometry) and Zap70 (by IHC) and cytogenetics/FISH were compared in patients with complete and partial response determined by repeat bone marrow exam and molecular IgG heavy chain gene rearrangement.

Results: Thirty two patients (64%) had complete response by flow and molecular studies. The rest had partial response, with 11 (61%) having minimal residual disease <1% lymphocytes or molecular evidence of lymphoma. Diffuse infiltration was more common in partial than complete responders (50% vs. 37%). CD38 was more commonly positive in partial responders (8 of 18 [44%] vs 7 of 32 [22%]). Zap-70 was more likely to be positive in complete responders (13 of 21 [62%] vs 2 of 10 [20%]). 17p deletion was detected in one patient who was a complete responder. 13q14.3 gene deletion with other imbalances was more common in partial responders (7 of 14 [50%] vs 5 of 30 [16%]). CLL patients had higher VEGF than normal controls (mean 93 vs 39 pg/ml, *p = 0.001*). Higher VEGF correlated with non-diffuse infiltration in complete responders (mean 128 pg/ml, *p=0.028*). All four patients with very high VEGF (>200 pg/ml) had non-diffuse infiltration and complete response. B-cell caspase-3 activity was lower in CLL cells than in normal cells (2.4 vs 13.1) but did not correlate with treatment outcome.

Conclusions: Plasma VEGF levels in excess of 200 pg/ml correlated with non-diffuse CLL and complete response to FCR. B-cell caspase 3 activity did not predict response to FCR therapy. Diffuse infiltration, CD38 expression and 13q14.3 gene deletion were indicators of incomplete response to FCR; however, Zap-70 expression was not.

1032 Celiac Disease Resistant to Gluten-Free Diet Is a Monoclonal Intra-Epithelial T-Cell Disease. A Study of 20 Cases

A de Mascarel, G Belleannee, C Merlio, P Dubus, JP Merlio. University Hospital, Bordeaux, France.

Background: Celiac disease (CD) is characterized by villous atrophy and an increase of intraepithelial lymphocytes (IEL) of more than 40/100 epithelial cells. The IEL usually exhibit a suppressor/cytotoxic phenotype (cd3+, cd4-, cd8+) and display a polyclonal profile for T-cell receptor rearrangement as opposed to the monoclonality of refractory CD with cd8- IEL.

Design: 20 CD were reviewed in our department. 16 CD were resistant to gluten- free diet and 4 were diagnosed at the same time than intestinal T-cell lymphoma. Formalin-fixed biopsies were studied by hematein-eosin stain and immunohistochemistry (cd3, cd8, cd56). PCR for the detection of TCR gamma chain gene rearrangement was performed on DNA extracted from histologically-selected formalin-fixed fragments.

Results: There were 9 men and 11 women with a mean age of 52 years (24-73). The mean follow up was 46 months. All had sub-total or total villous atrophy and failed to respond to gluten- free diet except the 4 with CD diagnosed at the same time than intestinal T-cell lymphoma. The mean increase in IEL was 75/100 epithelial cells (50-100). In one case with a cd8+ phenotype, the DNA was not available. In the 19 other cases, a monoclonal rearrangement of TCR gene was detected in 17 (89%) and a polyclonal profile in 2 (11%). The phenotype of IEL was cd3+, cd56- and either cd8- (n=7) or cd8+ (n=13). All the 7 cd8- CD exhibited a monoclonal TCR rearrangement . Three of them were associated with a T-cell lymphoma bearing the same monoclonal rearrangement (2 at the same time than CD and one after 43 months of follow-up). Interestingly, 10/12 cases (83%) of CD with a cd8+ phenotype were also found monoclonal and 2 of them were associated with a T-cell lymphoma diagnosed in the same time than CD and also exibiting the same rearrangement.

Conclusions: According to previous reports, all 7 cases of cd8- CD exhibited monoclonality and 3 of them were associated with a T-cell lymphoma displaying the same monoclonal rearrangement. However, we report for the first time that refractory CD with cd8+ phenotype of IEL were also found monoclonal (83%) and can be associated with a T-cell lymphoma (22%). Whatever the cd8+ or cd8- phenotype of IEL, CD resistant to gluten-free diet is a T-cell monoclonal disease and this phenotype does not seem to predict its association with a malignant T-cell lymphoma.

1033 Necrosis in Lymph Nodes Involved by CLL/SLL: A Rare Finding Virtually Always Associated with Herpes Simplex Virus Infection

RD DeHaan, PJ Kurtin. Mayo Clinic, Rochester, MN.

Background: Necrosis is a rare finding in lymph nodes involved by chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) and large cell transformation and infection are the most important differential diagnoses for necrosis in this context. Localized or systemic infection with herpes simplex virus (HSV) is one infection that commonly causes necrosis in lymph nodes and is known to be a rare complication of CLL/SLL. We hypothesized that HSV infection accounts for the majority of cases of necrosis in lymph nodes involved by CLL/SLL.

Design: Seven previously identified lymph node biopsies with CLL/SLL and geographic necrosis were selected from the files of our lymph node consultation practice. Additionally, one hundred cases of CLL/SLL were randomly selected from our files and reviewed for the presence of geographic necrosis. All cases with CLL/SLL and necrosis were analyzed by ISH for HSV DNA. The histologic features and results of HSV ISH for each case with necrosis were recorded.

Results: The diagnosis of CLL/SLL was confirmed in each case on the basis of morphology and immunophenotype. Of the one hundred randomly selected cases of CLL/SLL, only one (1%) demonstrated geographic necrosis. Cases with necrosis contained circumscribed necrotic areas composed of karyorrhectic debris surrounded by focal proliferations of immunoblasts and histiocytes. No sheets or tumefactive clusters of neoplastic large cells suggestive of large cell or Hodgkin transformation were identified. In all eight cases of CLL/SLL with necrosis, necrotic areas contained numerous cells positive for HSV DNA by ISH.

Conclusions: Necrosis is rare in lymph nodes involved by CLL/SLL. When present in this context, necrosis virtually always signifies HSV infection and not large cell transformation.

1034 The Accuracy of Interpretive Criteria for B-Cell Clonality Using PCR with Capillary Gel Electrophoresis for Immunoglobulin Heavy Chain (IgH) Rearrangements

A Djalilvand, CE Hill, KP Mann. Emory University, Atlanta, GA.

Background: Detection of clonal IgH gene rearrangements indicates clonality in hematolymphoid malignancies, highly suggestive of B-cell lymphomas/leukemias. The aim of this retrospective study is to determine precise criteria for accurate interpretation of true monoclonality using peak heights, peak area, and background heights and background area. Although no criteria have been published for IgH, several can be found for TCR including Lee, JMD, 2000 (ratio of peak height above background to background height); and Sprouse, AJCP, 2000 (ratio of peak height to the third highest peak height). In this study we compared a novel method (ratio of peak area to background area) with those published in the literature for TCR.

Design: Analysis was performed on archived patient samples which had been previously evaluated for IgH gene rearrangements as part of the patient's clinical evaluation in the Emory Molecular Diagnostics Laboratory. Normal and T/B-cell lymphoma samples were selected from 121 patients, including 37 controls, 30 with B-cell lymphoma, 5 with T-cell lymphoma, 2 with other hematopoietic malignancy (Hodgkin lymphoma and myelodysplastic syndrome), and 47 suspicious for hematopoietic malignancy. Diagnosis of B/T-cell lymphomas was accomplished with a combination of clinical history, histologic examination, flow cytometric analysis, and immunohistochemistry. Specimens were obtained from blood, bone marrow, fresh solid tissues, and formalin-fixed paraffin-embedded tissues. Samples were tested using multiplex PCR with fluorescently labeled primers (Invivoscribe Technologies, San Diego, CA) followed by capillary electrophoresis.

Results:

	IgH gene rearrangement					
	Emory: h1 area/bkgd area> 0.35		Lee: (h1-h0)/h0 > 3.0		Sprouse: h1/h0 > 2.0	
	Clone	No Clone	Clone	No Clone	Clone	No Clone
B cell Lymphoma	25	5	22	8	24	6
T cell Lymphoma	0	5	0	5	0	5
Other hematopoietic malignancy	0	2	0	2	0	2
Benign	4	33	3	34	6	31
Unknown/suspicious	20	27	18	29	21	26

Sensitivity and Specificity			
	Emory	Lee	Sprouse
Sensitivity	83%	73%	80%
Specifity	91%	93%	86%

Conclusions: Our novel method shows the highest sensitivity and similar specificity to the Lee method. All 3 methods; however, demonstrate similar results. In addition we have established that previously published criteria for determination of T-cell clonality can be applied equally well to B-cell clonality testing.

1035 HHV8-Negative Plasma Cell Variant of Castleman's Disease: An Unusual Plasma Cell Proliferative Disorder Characterized by Monotypic but Polyclonal Plasma Cells

A Dogan, ED Remstein, RF McClure, TC Diss, PJ Kurtin. Mayo Clinic, Rochester, MN; UCL Hospitals, London, United Kingdom.

Background: Two distinct subsets of the plasma cell variant of Castleman's disease (PVCD) are recognized. The first one is associated with HHV8 infection, occurs mostly in the context of immunosuppression and is characterized by an unusual proliferation of HHV8 positive, immunoglobulin (Ig) M/lambda restricted plasmablasts that are genetically polyclonal. The second group is poorly defined but is characterized by an interfollicular HHV8-negative plasma cell infiltrate and may be associated with systemic plasma cell disorders. In this study we investigated the pathological features of HHV8-negative PVCD.

Design: Clinical notes and paraffin sections from six lymph node biopsies involved by PVCD were reviewed and 20 plasmacytomas presenting in the context of systemic myeloma were used as controls. Immunohistochemistry for HHV8-LNA, Ig heavy and light chains was performed and clonal Ig gene rearrangements were analysed by PCR using BIOMED-2 primers for Ig genes on all specimens.

Results: All control plasmacytomas showed light chain restriction and either IgG or IgA heavy chain expression and 19 of 20 had clonal Ig gene rearrangements by PCR. The results for six PVCD cases are summarized in Table 1. All cases showed the presence of an infiltrate of interfollicular plasma cells that were monotypic for light chains but were polyclonal by PCR for Ig genes. Interestingly, two cases had plasmacytomas at the time of presentation and these were monotypic by immunohistochemistry and monoclonal by PCR for Ig genes.

Table 1: Pathological features of PVCD

Case	HHV8-LNA	Ig-IHC	Ig-PCR	Bone marrow	Clinical Syndrome	Other pathology
1	-	IgA/L	Polyclonal	N/A	MCD	
2	-	IgG/L	Polyclonal	N/A	N/A	
3	-	IgA/L	Polyclonal	-	MCD	Plasmacytoma
4	-	IgA/L	Polyclonal	-	MCD	
5	-	IgA/L	Polyclonal	+	POEMS	Plasmacytoma
6	-	IgA/L	Polyclonal	-	POEMS	

Conclusions: Our results suggest that at least a subset of HHV8-negative PVCD represent an unusual plasma cell disorder characterized by proliferation of monotypic but polyclonal plasma cells. It appears that occasionally monotypic and monoclonal plasma cell lesions such as plasmacytomas may arise from this background. These features are analogous to HHV8-positive PVCD where Ig lambda light chain restricted but polyclonal plasmablastic proliferation may give rise to monotypic and monoclonal plasmablastic lymphomas. Molecular pathogenesis of HHV8-negative PVCD is of great interest in understanding of stepwise development of systemic plasma cell proliferations.

1036 Usefulness of Routine Cytogenetic Analysis in Tissues Submitted for "Lymphoma Work-Up"

CH Dunphy, W Tang. UNC, Chapel Hill, NC.

Background: Non-Hodgkin's lymphomas (NHLs) are often associated with characteristic cytogenetic abnormalities (abns) (i.e., mantle cell lymphoma (MCL), follicular lymphoma (FL), Burkitt's lymphoma (BL), anaplastic large cell lymphoma (ALCL), etc). However, the utility of routine cytogentic studies (RCs) in tissues submitted for "lymphoma work-up (L-WU)" has not been well analyzed.

Design: We reviewed RC data of 261 tissues submitted for "L-WU" over 3 years, including cases initially appearing definitively benign. 4 non-hematopoietic malignancies were excluded. The 257 hematolymphoid processes were WHO-classified, but 64 yielded no cytogenetic results (NRs). The remaining 193 included 78 lymphoid hyperplasia/lymphadenitis (LH/L), 4 extramedullary hematopoiesis (EMH), 1 normal spleen & 1 with idiopathic thrombocytopenic purpura (ITP), 72 B-cell lymphomas (BCL) (6 SLL/CLL & 1 with Hodgkin lymphoma-HL transformation; 2 Richter's; 1 classic & 4 blastic MCL; 21 FL: 3 gr 1, 7 gr 2, 11 gr 3; 3 nodal & 3 extranodal marginal zone lymphoma-MZL; 5 diffuse large BCL-DLBCL; 18 DLBCL, follicle center cell (FCC) origin; 2 1° mediastinal BCL-PMBCL; 1 DLBCL with immunoblastic, high-grade features; 1 CD5+, CD23- de-novo DLBCL; 4 BL); 19 HL, 9 TCLs (1, PTCL; 1 angioimmunoblastic TCL-AITL; 6 ALCL; 1 mycosis fungoides), 4 post-transplant lymphoproliferative disorders (PTLD), 5 leukemias (1 pre-B ALL; 1 monocytic (MS) & 1 erythroblastic sarcoma (ES); 1-adult & 1 juvenile chronic myelomonocytic leukemia); and 1 systemic mastocytosis.

Results: Of 64 with NRs, FISH of 5 showed a t(14;18) (3 DLBLC, FCC origin; 1 FL; 1 BL also with a t(8;14)). Of the 77 LH/Ls, 4 showed abn RCs (1, multiple abns: follow-up(f/u) revealed diagnosis of FL in 1 month; 1, 1/20 cells-extra X; 2, 1 or 2 cells-del 2). 1 EMH showed del 20 (history of myelodysplasia (MDS)). 1 SLL showed a t(14;18). Increasing grades of FL showed increased lack of t(14;18) & increased del 6q abn. Nodal, but not extranodal, MZLs showed tri 3. 1/4 BL showed t(8;14) & t(14;18). 1/4 PTLDs showed abns, confirming clonality (DNA studies were negative.) The MS showed a t(9;11q23); the ES, numerous abns, establishing the diagnosis.

Conclusions: RCs in "L-WUs" seem indicated due to: a. Detection of rare abns indicating closer f/u for development of NHL. b. Detection of abns in FL (lack of t(14;18) & detection of del 6q) correlating with increasing grade. c. Detection of co-existent t(8;14) & t(14;18) in BL, known to predict an extremely poor prognosis. d. Ability to establish clonality in PTLD. e. Aid in diagnosis of MDS & leukemic infiltrates presenting as tissue masses.

1037 Primary Mediastinal B-Cell Lymphoma: Detection of Bcl-2 Gene Rearrangments by Polymerase Chain Reaction (PCR) Analysis

CH Dunphy, DP O'Malley, L Cheng, TY Fodrie, SL Perkins. University of North Carolina, Chapel Hill, NC; Indiana University, Indianapolis, IN; University of Utah, Salt Lake City, UT.

Background: Primary mediastinal large B-cell lymphoma (PMBCL) has a characteristic clinical presentation, morphology, and immunophenotype and represents a clinically favorable subgroup of diffuse large B-cell lymphoma (DLBCL). By gene expression profiling (GEP), PMBCL shares features with classical Hodgkin lymphoma (HL). Of further interest, bcl-6 gene mutations and bcl-6 and/or MUM1 expression in a number of PMBCLs have supported an activated/post-germinal center (A-PGC) cell origin. Several studies, including GEP, have failed to detect bcl-2 gene rearrangments (GRs) in PMBCL. An index case of t(14;18)+ PMBCL prompted our study of the incidence of bcl-2 GRs in PMBCL by PCR analysis and its possible clinical impact.

Design: 24 retrospectively identified, well-defined and -characterized PMBCLs (4 with cytogenetic results) from 3 institutions were analyzed for a bcl-2 GR with a PCR kit (*InVivo*Scribe Technologies). The formalin-fixed, paraffin embedded tissue blocks of 23 available cases were also analyzed by bcl-2 (Dako, LSAB2 kit) immunohistochemistry (IHC).

Results: Of the 4 with cytogenetic results, 1 had a t(14:18)(q32;q31). Of the 24 analyzed by PCR, 2 had no amplifiable DNA (aDNA), including the t(14;18)+ case. Of the 22 with aDNA, a bcl-2 GR was detected in 2. Bcl-2 protein expression by IHC analysis was variably detected in 20 (strongly,uniformly + in 5, including the 3 with a t(14;18) or a bcl-2 gene rearrangement ; moderately to weakly, subset + in 15) and negative in 3. The 2 cases with a bcl-2 gene rearrangement by PCR had good responses to conventional chemotherapy(CTX) +/- radiotherapy (RTX) and were in complete remission at last follow-up. However, the t(14;18)+ case experienced 2 recurrences, despite CHOP/Rituxan CTX and consolidative RTX.

Conclusions: 1. A subset (13%) of PMBCL in our series revealed a t(14;18) or a bcl-2 gene rearrangement by PCR, implying a GC cell origin. 2.Clinical follow-up of this subset showed a similar course to other PMBCLs in 2/3 cases, but a more aggressive course in the other. A larger study is necessary to determine a significant clinical impact in this subset. 4. GEP of this subset would be interesting to compare to PMBCLs of (A-PGC) cell origin.

1038 Histologic and Functional Analysis of Heat Shock Protein-90 (HSP90) in Multiple Myeloma

J Duus, G Venkataraman, HI Bahar, KF Izban, H Al-Masri, T Maududi, A Toor, S Alkan. Loyola University Medical Center, Maywood, IL; Elmhurst Memorial Hospital, Elmhurst, IL.

Background: All cells respond to stress such as elevated temperatures by making a distinct set of proteins known as the stress-related or heat-shock proteins. These proteins may play some protective role in cells exposed to stress. HSP90 is specifically required for structural folding and maintenance of conformational integrity of various proteins, including several associated with cellular signaling. Recent studies utilizing 17-allylamino-17-demethoxygeldanamycin (17-AAG), an inhibitor of HSP90, demonstrated anti-tumor effect in solid tumors.

Design: In order to test whether HSP90 could be targeted in multiple myeloma (MM) patients, we first investigated expression of HSP90 by immunofluorescence and flow cytometric analysis in a myeloma cell line (U266) and primary myeloma cells. Cytotoxicity assays were performed after treating U266 cells with 17-AAG and bortezomib (an inhibitor of the proteasome machinery licensed for use in multiple myeloma). Assessment of changes in levels of the BCL-2 family of proteins (bcl-2, bcl-xL and mcl-1) was performed using flow cytometry. In addition, western blotting was performed to analyze changes in HSP90 protein levels with treatment. Following demonstration of HSP90 expression in myeloma cells, archival samples of 32 MM patients were analyzed for HSP90 expression by immunoperoxidase staining.

Results: Myeloma cells in all patients showed strong cytoplasmic expression of HSP90 in all samples and 55 % of the cases also demonstrated concurrent nuclear immunopositivity. Treatment of U266 and primary MM cells with 17AAG resulted in significantly increased apoptosis compared to untreated control cells. The IC50 of 17-AAG in U266 was determined to be approximately 400nM as seen in the cytotoxicity assay. Analysis of anti-apoptotic BCL2 family proteins in MM cells incubated with 17-AAG revealed down-regulation of BCL-2, BCL-X_L and MCL-1 but there was no quantitative change in the levels of HSP90. Furthermore, while low concentration of bortezomib had no cell death, combination of 17AAG and bortezomib treatment revealed a synergistic apoptotic effect on the myeloma cell line.

Conclusions: Our data indicates that targeted inhibition of HSP90 may prove to be a valid and innovative strategy for the development of future therapeutic options for MM patients.

1039 *MYC* Translocations Involving Immunoglobulin Light Chain Loci in B-Cell Lineage Neoplasms Are Widely Dispersed

RR Einerson, ME Law, HE Blair, PJ Kurtin, RF McClure, RP Ketterling, HC Flynn, JE Allen, A Dogan, ED Remstein. Mayo Clinic, Rochester, MN.

Background: The variant *MYC* translocations t(2;8)(p11.2;q24.1)-*IGK/MYC* and t(8;22)(q24.1;q11.2)-*IGL/MYC* are recurrent anomalies in B-cell lineage malignancies. Detection of translocations involving *MYC* is important for diagnostic and prognostic purposes, but it is hampered by the widely dispersed breakpoints of the MYC gene and the lack of commercially available FISH probes for kappa (*IGK*) and lambda (*IGL*) immunoglobulin genes.

Design: Four FISH probes were developed and validated: 2 break apart (BAP) probes to detect *IGK* and *IGL* translocations, and 2 dual color dual-fusion (D-FISH) probes to detect the reciprocal *MYC* rearrangements *IGK/MYC* and *IGL/MYC*. Four Burkitt lymphoma (BL) cell lines chosen as gold standards and 15 archival B-cell lineage

malignancy specimens (paraffin-embedded tissue or fresh-fixed metaphase pellets) that were known to possess a translocation involving either *MYC* or 8q24 were evaluated for *IGK/MYC* and *IGL/MYC* rearrangements.
Results: All four BL cell lines were correctly identified as positive for *IGK/MYC* (2) or *IGL/MYC* (2). A *MYC* rearrangement was identified in all 15 archival specimens. Of the 4 cases that lacked karyotypic data, 3 had an *IGL/MYC* rearrangement and 1 had an *IGK/MYC* rearrangement. Of the 11 cases with a previous karyotype, 8 of 8 t(8;22)(q24.1;q11.2)-positive specimens had an *IGL/MYC* rearrangement and 2 of 3 t(2;8)(p11.2;q24.1)-positive cases had an *IGK/MYC* rearrangement. A translocation involving *MYC* and an unknown partner gene on 2p12 far telomeric of the IGK locus was identified by metaphase FISH performed on the third specimen. Separate experiments utilizing a novel homebrew break apart FISH strategy demonstrated that 33% (1of 3) of *IGK/MYC* cases and 45% (5 of 11) of *IGL/MYC* cases have breakpoints greater than 350 kb 3' of *MYC*.
Conclusions: These probe sets reliably detect variant *IGK/MYC* and *IGL/MYC* translocations in B-cell lineage malignancies. In addition, a novel breakpoint cluster region associated with the t(2;8)(p11.2;q24.1)-*IGK/MYC* and t(8;22)(q24.1;q11.2)-*IGL/MYC* translocations was identified that is further downstream from the MYC oncogene than previously described. Our results suggest the frequency of *MYC* translocations may have been underestimated by conventional FISH strategies.

1040 Identification of Chromosomal Translocation Partners Encoding Oncogenic Chimeric ALK Fusion Proteins by Tandem Mass Spectrometry

KS Elenitoba-Johnson, DK Crockett, JA Schumacher, CM Coffin, MS Lim. University of Utah, Salt Lake City, UT; ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT.
Background: Anaplastic lymphoma kinase (ALK) is a receptor tyrosine kinase that is involved in normal cellular development and in oncogenesis. In humans, full-length ALK protein expression is normally restricted to neural cells, pericytes and endothelial cells in the brain, suggesting an important role for ALK in normal neural development. The *ALK* gene at 2p23, is frequently involved in chromosomal translocations with an increasing list of partners with formation of oncogenic fusion proteins. Chromosomal translocations involving *ALK* are characteristic of two neoplasms; anaplastic large cell lymphoma and inflammatory myofibroblastic tumor (IMT). Identification of translocation partners is important for understanding the molecular pathogenesis, and for the diagnosis of various tumors. The methodologies that facilitate the identification of chromosomal translocation partners are important for the elucidation of the molecular pathogenesis of various tumors. We describe a mass spectrometry-based approach for the identification of *ALK*-fusion partners.
Design: The proteomic strategy involves anti-ALK antibody immunoprecipitation and one-dimensional gel electrophoresis followed by isolation of aberrant protein bands, parallel proteolytic digestions with multiple enzymes and analysis by tandem mass spectrometry for the identification of fusion partners of ALK.
Results: This approach accurately identified the reported NPM-ALK fusion protein in an anaplastic large cell lymphoma (ALCL)-derived cell line carrying the t(2;5)(p23;q35), and the *TPM3-ALK* in a clinical biopsy of IMT carrying the t(1;2)(q21;p23). In both samples, multiple overlapping peptides were generated providing greater than 80% protein coverage from both participating proteins providing definitive evidence for the juxtaposition of *ALK* to its fusion partners.
Conclusions: This study shows for the first time, the ability of mass spectrometry to identify oncogenic chimeric proteins resulting from chromosomal rearrangements. Similar strategies can be used for the identification of known and unknown translocation partners of chimeric fusion proteins involved in oncogenesis.

1041 Cdk6 and Cyclin D2 Control G1 Cell Cycle Progression in Multiple Myeloma and Are Absent in Prognostically Favorable t(11;14)+ Cases

S Ely, M DiLiberto, R Niesvizky, E Hatada, H Cho, DM Knowles, J Lane, S Chen-Kiang. Weill Medical College of Cornell University, New York, NY.
Background: Multiple myeloma (MM) is a cancer of bone marrow (BM) plasma cells (PC). Recurrent translocations (RT) are associated with outcomes. However, whereas RT-associated gene dysregulations have been shown to have physiologic consequences in other types of cancer, such data is limited in MM. In fact, t(11;14), the most common RT in MM, which leads to overexpression of cyclin D1, a positive cell cycle regulator, is paradoxically associated with a favorable prognosis. We studied the expression of cell cycle regulators and Rb phosphorylation, which controls mid G1 cell cycle progression, in MM to better understand cell cycle control and the physiologic significance of t(11;14).
Design: We studied 430 patients (80 normal, 58 MGUS, and 292 MM). Double immunohistochemistry (IHC) was performed on iliac cores for all patients and on lytic lesions (n=19) for coexpression of CD138, a PC antigen, along with cyclin D1, cyclin D3 and Cdk6, in comparison to phosphorylation of Rb. Expression of cyclin D2 was assessed by Western blotting (WB) of CD138+ bead-selected PCs from corresponding aspirates. FISH also was performed on the aspirates.
Results: Expression of cyclin D1, D2, or D3 was mutually exclusive in each patient. Expression of D2 was coordinated with Cdk6. While expression of D1 and D3 rarely resulted in Rb phosphorylation, coordinate up-regulation of D2 and Cdk6 did result in Rb phosphorylation and G1 cell cycle progression. Analysis of multiple biopsies (5 - 26) taken throughout the clinical course from 21 patients followed for an average of 6 years (31 - 166 months) showed that expression of D1 and D3 did not change throughout the clinical course in an individual patient. The pattern of D1 and D3 expression was uniform throughout each infiltrate, either seen in scattered cells or expressed by the entire MM population. However, Cdk6 was expressed in large focal aggregates in iliac cores, sometimes early in the course of disease. Also, Cdk6 expression was commonly seen in lytic lesions.
Conclusions: Our data show that in MM, G1 cell cycle progression is achieved primarily by the coordinate expression of Cdk6 with cyclin D2. Our finding that Cdk6 is commonly expressed in lytic lesions bolsters this idea. Because the expression of each D cyclin is mutually exclusive, the biologic reason for the association between D1 overexpression / t(11;14) and a favorable prognosis may be the concomitant lack of D2 and Cdk6 in such cases.

1042 The Chemokine CXCL13 (BCA-1) Is Expressed by Follicular Dendritic Cell Sarcoma and Attracts Intratumoral Lymphocytes Expressing CXCR5

F Facchetti, F Gentili, M Ungari, F Zorzi, S Pileri, M Uguccioni, G Arrigoni, C Doglioni, M Ponzoni. University of Brescia, Brescia, Italy; Poliambulanza, Brescia, Italy; University of Bologna, Bologna, Italy; University of Bern, Bern, Switzerland; San Raffaele Hospital, Milan, Italy.
Background: The homeostatic chemokine CXCL13 (also called B-cell-attracting chemokine 1, BCA1) is constitutively expressed in secondary lymphoid tissues and initiates lymphoid neogenesis, by attracting follicular B and T cells expressing its receptor CXCR5. In normal tissues follicular dendritic cells (FDCs) are considered to be the major source of CXCL13. FDC sarcoma is a rare tumor, with extremely variable morphology; the presence of intratumoral lymphocytes is an intrinsic feature of this neoplasm.
Design: We analyzed by immunohistochemistry the expression of CXCL13 and CXCR5 proteins in seven cases of FDC sarcoma as well as of CXCL13 mRNA in one case by in situ hybridization. Immunohistochemistry for CXCL13 and CXCR5 was performed on paraffin sections, respectively applying a goat polyclonal and a mouse monoclonal antibody (both from R&D Systems, USA). In situ hybridization for CXCL13 was performed as reported (J Clin Invest 1999; 104:R49).
Results: The tumors (4 from lymph nodes, 1 from skin, retroperitoneum, and mediastinum each) showed a classical fascicular spindle cell pattern (4 cases) or epithelioid cell growth (3 cases); intratumoral lymphocytes were abundant in 6 cases; in one they formed dense lymphoid nodules containing FDCs. The tissue relative to recurrence was available in two cases. All cases showed variable expression of FDC antigens CD21, CD23, CD35 and CNA.42. All tumors were positive for CXCL13; staining was intracytoplasmic (diffuse or Golgi-like). Notably, CXCL13 was the only retained marker retained by FDC in the biopsy of the recurrent case. Intratumoral CXCR5+ lymphocytes were recognizable in all cases and were particularly abundant in two; except for residual follicles, where normal CXCR5+ cells were also found, CXCR5+ lymphocytes were strictly associated with the neoplastic areas. CXCL13 mRNA was detected by in situ hybridization in tumor cells.
Conclusions: These data show that FDC sarcoma cells retain, as their normal counterpart, the expression of CXCL13 mRNA and protein. This feature suggests that this molecule is likely to be functional, as indicated by the recruitment of intratumoral lymphocytes expressing CXCR5. CXCL13 might represent a novel marker for FDC sarcoma diagnosis.

1043 The Architectural Pattern of FOXP3$^+$ T Cells Is an Independent Predictor of Survival in Patients with Follicular Lymphoma (FL)

P Farinha, E Campo, A Banham, H Masoudi, B Skinnider, JM Connors, K Shumansky, J Spinelli, RD Gascoyne. BC Cancer Agency, Vancouver, BC, Canada; Hospital Clinic, Barcelona, Spain; John Radcliffe Hospital, Oxford, United Kingdom; BCCA, Canada.
Background: The Leukemia/Lymphoma Molecular Profiling Project demonstrated that non-neoplastic cells in FL biopsy samples were important in FL outcome. We recently showed using tissue microarrays (TMA) that Lymphoma-Associated Macrophage (LAM) content is an independent adverse prognostic factor in advanced-stage FL patients. Tumor infiltrating macrophages have been described to cooperate with regulatory T-cells (Tregs) in an immunosuppressive microenvironment advantageous to the tumor. Tregs express CD4, CD25 and FOXP3. FOXP3 is crucial for their differentiation/activity and is thus far considered the best marker of Tregs. Our study aimed to test their prognostic relevance in FL.
Design: Between 1987 and 1993, 126 patients were enrolled on a phase II study of BP-VACOP chemotherapy with radiotherapy. All patients were treatment naïve, < 61 y and had advanced-stage FL. Paraffin blocks were available in 105 patients. The TMAs consisted of duplicate 1.0mm cores of diagnostic biopsies and were immunostained with CD4, CD8, CD25 and FOXP3 antibodies. Immuno-architectural patterns were determined and correlated with overall (OS) and progression-free survival (PFS). A Cox multivariate model was constructed.
Results: The median OS was 14.8 y and the IPI was predictive of OS ($p = 0.003$). Histologic grade was 76 grade 1, 20 grade 2 and 6 grade 3A. The immuno-architectural distribution of CD4 vs CD8, CD25 and FOXP3 cells within the tumor was significant. Some cases had a predominant intrafollicular or perifollicular localization of positive cells (follicular pattern) and others had not (diffuse pattern). 38 cases had a follicular pattern (FOXP3). A Cox multivariate model showed both IPI and FOXP3$^+$ patterns were independent variables ($p<0.0001$). 11/12 of the cases with high LAM had a follicular FOXP3 pattern.
Conclusions: Treg distribution within the tumor is an important independent prognostic factor in advanced-stage FL patients treated uniformly with an aggressive treatment. The FOXP3 follicular pattern may be a good surrogate for functionally active Treg cells within the tumor microenvironment.

Immuno-architectural patterns	CD4	CD4/CD8	CD25	FOXP3
OS	NS	NS	NS	0.0002
PFS	NS	0.046	0.004	0.0002

NS-not significant

1044 Tissue Proteomics, the Bcl-2/Bax Switch, and the Role of the Pathologist

AL Feldman, V Espina, C Gulmann, M Winters, LA Liotta, ES Jaffe. National Cancer Institute, Bethesda, MD; George Mason University, Manassas, VA.

Background: Selecting patients to receive novel anticancer agents targeting apoptotic pathways will rely on quantifying apoptotic protein levels in the neoplastic cells (Cory S, Adams JM. Killing cancer cells by flipping the Bcl-2/Bax switch. Cancer Cell. 2005;8:5-6). We recently demonstrated prognostic significance of the Bcl-2/Bax ratio in follicular lymphoma (FL) using laser-capture microdissection (LCM) and protein microarrays. Since most gene expression profiling of lymphoma has used whole tissue lysis (WTL) without LCM, we tested whether pathologist oversight and LCM were necessary for accurate proteomic characterization of apoptotic pathways in FL.

Design: We used reverse phase protein microarrays to analyze 18 cases of FL and 8 cases of follicular hyperplasia (FH) after LCM of neoplastic or reactive follicles, or after WTL. Mean levels of apoptotic proteins were compared in FL and FH, and in prognostic subgroups of FL (*good:* >10 yr survival; *poor:* death by 10 yr). Overall survival (OS) stratified by Bcl-2/Bax ratio was assessed using the Kaplan-Meier method.

Results: The Bcl-2/Bax ratio was elevated in FL, and this ratio segregated FL and FH more discretely when LCM was used (p<.0001, Mann-Whitney) than when WTL was used (p=.02). Use of WTL failed to identify a significant difference in Bcl-2/Bax ratios between FL patients with good vs poor outcomes (p=.67), whereas the ratio was significantly higher in patients with poor outcomes when LCM was used (p=.015). WTL also failed to identify a difference in OS between patients with high vs low Bcl-2/Bax ratios (median suvival, 11.1 yr vs 11.2 yr; p=.85, Mantel-Cox). Patients with high ratios showed a trend toward earlier death when LCM was used (median survival 7.6 yr vs 11.4 yr, p=.05).

Conclusions: Proteomic analysis demonstrated an association between the Bcl-2/Bax ratio and survival in FL patients only when LCM was used. This is likely due to the normal expression of apoptotic proteins in non-neoplastic immune cells (e.g. T cells) found in FL. While use of WTL has yielded molecular profiles that shed light on tumor-host interactions, pathologist involvement and tools such as LCM may be critical for accurate assessment of apoptotic pathways within neoplastic cells. These findings impact the strategy for treatment with novel targeted therapies, in which protein profiles will guide appropriate drug selection for cancer patients.

1045 Lymphoma of the Ocular Adnexa: A Study of 353 Cases

JA Ferry, C Fung, L Zukerberg, R Hasserjian, F Preffer, NL Harris. Massachusetts General Hospital, Boston, MA.

Background: Lymphomas are the most frequent malignancies involving the ocular adnexa. The distribution of WHO subtypes compared with other extranodal sites has not been fully established.

Design: We studied 353 cases of lymphomas involving the ocular adnexa diagnosed at Massachusetts General Hospital between 1974 and 2005 using morphology and immunophenotype to determine the types of lymphomas that occur in this site and to correlate their clinical and pathological features.

Results: There were 153 M and 200 F, aged 7-95 years (mean, 64). 280 patients had no history of lymphoma. They had marginal zone lymphoma (169 cases), follicular lymphoma (56), mantle cell lymphoma (MCL, 7), chronic lymphocytic leukemia (CLL, 4), low grade B-cell, not subclassified (16), precursor B lymphoblastic lymphoma (3), diffuse large B-cell lymphoma (DLBCL, 23), Burkitt lymphoma (1), and extranodal NK/T-cell lymphoma (1). Although they had no history of lymphoma, nearly all of the MCL and CLL patients and the Burkitt lymphoma patient had widespread disease on staging. 73 patients had a history of lymphoma. They had marginal zone lymphoma (13 cases, usually with an extranodal primary), follicular lymphoma (24, usually with a nodal primary), MCL (12), CLL (7), lymphoplasmacytic lymphoma (4), splenic marginal zone lymphoma (2), low-grade, not subclassified (5 cases), DLBCL (4), peripheral T-cell lymphoma (1) and nodular sclerosis Hodgkin lymphoma (1). Morphology and immunophenotype were similar to that seen in other sites. Some DLBCL were associated with large destructive masses involving adjacent paranasal sinuses or bone, suggesting an origin from an adjacent structure with ocular adnexal involvement occurring by direct extension. Cases of low-grade B-cell lymphoma, not subclassified, highlight the difficulty that may arise in distinguishing types of low-grade lymphoma, particularly when biopsies are small and artifactually distorted.

Conclusions: This is the largest series of ocular adnexal lymphomas reported. Ocular adnexal lymphomas occur predominantly in older adults, with a slight female preponderance. Most are low-grade B-cell lymphomas, with marginal zone lymphoma being by far the most common type. Marginal zone lymphoma typically involves the ocular adnexa primarily, while other low-grade B-cell lymphomas more often involve the ocular adnexa secondarily. High grade B-cell lymphoma only occasionally involves the ocular adnexa. T-cell lymphoma, NK-cell lymphoma and Hodgkin lymphoma are rare in this site.

1046 Identification of Recurrent Genomic Alterations in Burkitt and Burkitt-Like Lymphoma Using Array-Based Comparative Genomic Hybridization (CGH)

K Fu, WG Sanger, DD Weisenburger, DL Pickering, B Dave, TC Greiner, WC Chan. University of Nebraska Medical Center, Omaha, NE.

Background: Burkitt lymphoma (BL) is an aggressive B-cell lymphoma that is characterized by deregulation of the c-myc oncogene on chromosome 8q24. Although other genetic alterations beside c-myc translocation have been reported, little information regarding global genetic abnormalities in this rare entity is available.

Design: Twenty-one cases of BL or Burkitt-like lymphoma (BLL) with clinical information were selected from the Nebraska Lymphoma Study Group registry. Array-based CGH was performed using an array with 2580 BAC clones (SpectralChip 2600). FISH studies for c-myc and bcl-2 translocations were also performed. The findings were then correlated with patient age and overall survival.

Results: There were seven pediatric patients (Age range, 4-16 yrs; median, 8 yrs) and 14 adult patients (Age range, 32-85 yrs; median, 71 yrs). All pediatric cases and six of the 14 adult cases had a c-myc translocation. Two adult cases with c-myc rearrangement also had a bcl-2 translocation. Genomic imbalances were identified in 20 of 21 cases with 16 cases having more than one genomic alteration. A total of 170 DNA copy number changes (60 gains and 110 losses) were detected with an average of eight abnormalities per case (range, 0-46). The most frequent recurrent chromosomal segmental losses involved 17p13 (33%) and 6q21 (24%). The most frequent gains involved 1q21-25 (38%), 13q31-32 (29%), and 18q21 (24%). Fewer genomic imbalances were detected in cases with c-myc translocation (average, 3/case) than those without c-myc translocation (average,16/case). Among the cases with a c-myc translocation, fewer abnormalities were detected in pediatric patients (average, 2.5/case) than in adults (average, 4/case). Both cases with c-myc and bcl-2 translocations exhibited a genetic alteration involving the 13q13-21.1 region, which was not detected in any other case. Within the pediatric group, 3 patients had died and 4 were alive at the time of last contact. In contrast, all adult patients had died of disease within a median of 5.5 months after the initial diagnosis.

Conclusions: Genomic alterations in addition to c-myc translocation are common in BL/BLL cases. The most frequent recurrent genomic alterations involve chromosome regions 1q, 6q, 13q, and 17p. Fewer genomic alterations are detected in cases with c-myc translocation than those without the translocation,suggesting difference in pathogenesis.

1047 All Cases of Precursor T Acute Lymphoblastic Leukemia/Lymphoma (T-ALL) Exhibit Multiple Immunophenotypic Aberrancies

F Fuda, R McKenna, Y Xu, N Karandikar. The University of Texas Southwestern, Dallas, TX.

Background: Normal T-cell precursors (thymocytes) and lymphoblasts of T-ALL have similar immunophenotypic features. The ability to distinguish between the two cell types may be important in differentiating neoplastic processes from reactive proliferations of thymocytes. This study was designed to evaluate diagnostically useful aberrant antigen expression patterns in a diverse cohort of T-ALL.

Design: Consecutive diagnostic T-ALL specimens from 25 patients were analyzed by 4-color FC with panels of antibodies to >25 lymphoid and myeloid antigens. Cluster analysis was performed on ungated data using BD Paint-a-Gate software. Antigenic aberrancies/leukemia-associated immunophenotypes (LAIPs) were defined as an abnormal absence, over-expression or under-expression of antigens, compared to previously defined patterns observed in thymocytic differentiation and normal/reactive peripheral T lymphocytes.

Results: The cohort population ranged in age from 1 to 47 years; 15 were children (<18 years) and 13 were male. Sites of involvement included 7 lymph nodes, 5 pleural and 2 pericardial fluids, 4 mediastinal masses, 3 bone marrows, 2 neck masses, 1 kidney and 1 lung biopsy. All cases showed multiple immunophenotypic aberrancies, ranging from 5 to 15 (median 8). The most common aberrancy involved the pattern of expression of 4 antigens (CD3, CD4, CD8, CD1a), which was distinctly deviant in every case, in which all 4 markers were tested (24/24). A deviation from the normal expression pattern of these antigens could successfully distinguish leukemic blasts from maturing thymocytes or peripheral T-cells. In addition to the aberrant pattern of these markers, the most common aberrancies were under-expression of CD45 (18/24, 75% cases) and CD45RO (12/22, 55%) and overexpression of CD10 (15/25, 60%). Multiple other recurrent aberrancies were present at lower frequencies and included expression of at least one myeloid marker (9/25, 36%), expression of at least one B-cell marker (4/25, 16%), over- or under-expression of T-lineage markers CD7 (8/25, 32%), CD5 (12/25, 48%), or CD2 (7/21, 33%) and under-expression of TdT (7/20, 35%).

Conclusions: FC analysis identifies multiple immunophenotypic aberrancies in all cases of T-ALL. In particular, every T-ALL exhibits an aberrant pattern of CD3/CD4/CD8/CD1a expression. Thus, the evaluation of these 4 markers (preferably as a 4-color combination) provides a basis for distinction of neoplastic T-lymphoblasts from thymocytes and a marker for minimal residual disease assessment.

1048 Simultaneous *JAK2* V617F and *KIT* D816V Mutations in Systemic Mastocytosis

CD Galderisi, CL Corless, J Wolford, T Harrell, MC Heinrich, RD Press. Oregon Health and Science University, Portland, OR.

Background: The World Health Organization classifies systemic mastocytosis (SM) as a heterogeneous group of hematopoietic disorders characterized by abnormal growth and accumulation of mast cells in one or more organs. Activating mutations of *KIT* (particulary D816V) have been strongly implicated in the pathogenesis of these disorders. A well-conserved kinase auto-activating *JAK2* V617F mutation has recently been found in the majority of cases of Philadelphia chromosome negative myeloproliferative disorders (MPD) including polycythemia vera, essential

thrombocythemia, and chronic idiopathic myelofibrosis. The role of the V617F *JAK2* mutation in so-called atypical myeloproliferative diseases, including SM, has yet to be clarified. We assessed the presence and prevalence of *JAK2* V617F and *KIT* D816V in a group of SM cases.

Design: In cases with a clinical diagnosis of SM, DNA was extracted from paraffin-embedded bone marrow biopsy, blood, or fresh marrow aspirate. The samples were screened for *KIT* exon 11 and 17 mutations by PCR and denaturing HPLC; the *KIT* D816V mutation commonly reported in SM was also examined using a highly sensitive allele-specific PCR (AS-PCR). *JAK2* was amplified with flanking primers and the amplicons were digested with the restriction enzyme BsaXI, which specifically cleaves the *JAK2* wild type allele and allows precise allele discrimination after agarose gel electrophoresis.

Results: Three of four SM cases were positive for *KIT* D816V, consistent with published literature. In one of these cases the mutation was detectable only by AS-PCR (<20% mutant allele). Two of the *KIT* D816V-positive cases also harbored a heterozygous *JAK2* V617F mutation, including the case in which the D816V was in low abundance. The fourth SM case was negative for *KIT* D816V and positive for *JAK2* V617F. Screening of additional SM cases is underway.

Conclusions: Our results suggest that *JAK2* V617F can be found in patients with a clinical diagnosis of SM. Moreover, *JAK2* V617F and *KIT* D816V mutations may coexist in the same samples from these patients. While preliminary, this finding raises the intriguing possibility that within the context of a myeloproliferative disorder with excess mast cells, the dominant oncogenic kinase may be JAK2 instead of KIT. Whether *JAK2* and *KIT* mutations occur within the same or separate subpopulations of cells arising from MPD remains to be determined.

1049 The Extent and Distribution of Amyloid in 33 Autopsies on Patients with Primary (AL) Amyloidosis

J Gan, LH Connors, M Skinner, CJ O'Hara. Boston Medical Center, Boston, MA.

Background: More than 20 different proteins have been noted to form amyloid. Of the more common types, Primary (AL) amyloidosis, associated with abnormal immunoglobulin light chain folding and fibril formation, is the most aggressive and lethal form of the disease. While much has been written about AL amyloidosis, there has been no systematic assessment of amyloid deposition in the different organs of the body. In this study, we examined the extent and organ distribution of amyloid in 33 patients who died from AL amyloidosis in an effort to delineate possible organ trophism and pattern of deposition.

Design: 33 autopsy cases with confirmed AL Amyloidosis were retrospectively analyzed in our institution (mean age 60 years; M/F 22/11). Twenty-nine had monoclonal lambda light chain gammopathy; four had monoclonal kappa gammopathy. The presence, extent and distribution of amyloid were assessed using conventional stains (H&E, PAS, Congo red and Sulfated-Alcian Blue). The amyloid deposition was scored as followed: 0(no amyloid), 1+ (<25% of tissue), 2+ (25% - 50%), 3 + (50%-75 %) and 4+ (>75%) and the anatomical distribution noted (vascular, interstitial, glomeruli, pleural etc.)

Results:

Table 1. Distribution of Amyloid in Various Organs

Organs (33)	Heart	Spleen	Kidney	Lung	Adrenal cortex	Liver	Pancreas
0	4	12	10	3	15	21	25
1+	1	1	5	8	4	0	2
2+	7	1	4	9	4	3	1
3+	10	6	10	10	6	7	3
4+	11	13	4	3	4	2	2
% 3+4	64	57	42	39	30	27	15

Table 2. Amyloid Distribution in Different Organs

Organ	MAJOR INVOLVEMENT	MINOR INVOLVEMENT	SPARED
Heart	Myocardium, transmural	Pericardium	Coronary arteries
Spleen	Red pulp, blood vessels	White pulp only	—-
Kidney	Glomeruli, blood vessels	—	Peritubal, interstitium
Lung	Blood vessels, predominant alveoli, visceral pleura, variable	Parietal pleura	Bronchi, Bronchioles
Adrenal cortex	Cortex and small vessels	—	Medulla
Liver	Sinusoids, portal small vessels	—	—-
Pancreas	Blood vessels	Exocrine Parenchyma	Islets of Langerhans

Conclusions: 1. There is a difference in the extent of organ involvement in AL amyloidosis; the heart and spleen exhibited marked amyloid deposition, the kidney, lung and adrenal cortex exhibited moderate deposition and the liver and pancreas exhibited limited involvement. 2. Within each involved organ, there is variable deposition: small blood vessels are uniformly involved in all organs; the adrenal medulla and islets of pancreas are conspicuously spared. 3. This study supports the presence of particular organ/tissue trophism for amyloid deposition in AL amyloidosis.

1050 New Key Factors in the Outcome of Hodgkin's Lymphoma Cases: Tumor Microenvironment and Mitotic Checkpoint

JF Garcia, A Sanchez-Aguilera, C Montalban, P Dominguez, L Sanchez-Verde, MM Morente, M Garcia-Cosio, C Bellas, V Romagosa, J Menarguez, MJ Mestre, MF Fresno, MA Piris. MD Anderson International Spain, Madrid, Spain; Hospital Ramon y Cajal, Madrid, Spain; Fundacion Hospital Alcorcon, Madrid, Spain; Clinica Puerta de Hierro, Madrid, Spain; Ciudad Sanitaria y Universitaria de Bellvitge, Barcelona, Spain; Hospital Gregorio Marañon, Madrid, Spain; Hospital de Mostoles, Madrid, Spain; Hospital Central de Asturias, Oviedo, Spain.

Background: The factors that influence the outcome of Hodgkin's Lymphoma (HL) cases have not yet been fully elucidated, underscoring the demand for the recognition of biological risk factors and new potential therapeutic targets.

Design: We profiled samples from 29 patients with advanced HL treated with standard therapy, and compared the differentially expressed genes between patients with favorable and unfavorable clinical outcome, using supervised methods. Simultaneous analysis of normal lymphoid tissues, HL cell lines, and normal germinal center B-cells allowed the identification of differential gene signatures. The results were validated in an independent series of 235 HL patients by automated scanning and quantification of protein levels of 8 representative genes using tissue-microarrays (TMAs).

Results: 145 genes that were associated with outcome are grouped into a four signature system, representing two immune response signatures and two cell cycle signatures, mainly consisting of genes implicated in the regulation of mitosis and signaling/apoptosis. Moreover, many of these genes encode enzymes previously described as factors implicated in drug resistance. Analysis of protein expression of representative markers in the validation group of patients confirmed the existence of clear differences in survival between patients with high versus low protein expression levels. Analysis of the spindle checkpoint in cell lines confirmed the existence of disrupted transition through mitosis in HL cells.

Conclusions: Genes related with tumor microenvironment, signaling/apoptosis, and regulation of the G2/M transition and mitosis are associated with treatment response and outcome of HL patients. The results identify: 1) general processes implicated in treatment response 2) multiple new prognostic markers; and 3) potential therapeutic targets for HL patients.

1051 Identification of Genes Involved in Somatic Hypermutation in Small B-Cell Lymphomas

M Garcia-Cosio, L Tracey, M Aggarwal, P Algara, JF Garcia, A Rodriguez, N Martinez, FI Camacho, G Roncador, A Sanchez-Aguilera, E Ruiz-Ballesteros, M Mollejo, JA Garcia-Marco, MA Piris. Hospital Ramon y Cajal, Madrid, Spain; CNIO, Madrid, Spain; Hospital Virgen de la Salud, Toledo, Spain.

Background: Immunoglobulin (Ig) gene somatic hypermutation (SHM) is a biological and clinical relevant factor in different types of small B-cell lymphomas, such as chronic lymphocytic leukemia (CLL), splenic marginal zone lymphoma (SMZL) and mantle cell lymphoma (MCL). Nevertheless, both the mechanisms and markers of SHM are poorly characterized, with the partial exception of the role of the genes ZAP70 and AID

Design: With the purpose of identifying SHM surrogate markers in small B-cell lymphomas, we analyzed IgV_H mutational status and expression profiles of 93 small B-cell lymphoma patient samples including SMZL (24 cases), MCL (33 cases) and CLL (36 cases).

Results: Patients were classified into two groups: high SHM (>5% mutations) and low SHM (<5% mutations). T-test analysis with 100,000 permutations was performed and 39 genes were identified whose expression is significantly different ($p<0.005$, FDR<0.05) between high and low SHM groups. The results were combined with those of an experimental model of SHM induction. Upregulated genes are implicated in transcription, DNA repair and replication and chromosome maintenance, correlating well with previous hypotheses indicating that active transcription is necessary for SHM. Based on these observations, a group of 13 key genes, implicated in DNA repair, replication and transcription, were selected and protein expression was analyzed in a set of Tissue Micro Arrays containing 150 paraffin embedded small B-cell lymphoma cases, for which clinical data is available and SHM status is known

Conclusions: These proteins may provide a surrogate marker for prognosis and/or analysis of IgV_H SHM in patient samples using paraffin-embedded tissue samples.

1052 CD163: A Specific Immunohistochemical Marker of Acute Myeloid Leukemia with Monocytic Differentiation

DL Gardner, KK Reichard. University of New Mexico School of Medicine, Albuquerque, NM.

Background: Acute myeloid leukemia with monocytic differentiation (AMLMD) is diagnosed using a variety of tools: morphology, special stains, flow cytometry, and cytogenetics. CD68 and lysozyme immunostains may be of use in distinguishing AMLMD; however, staining in non-AMLMD may be seen. CD163, a hemoglobin scavenger receptor, is highly specific for monocyte/macrophage lineage. Because detection of AMLMD often has diagnostic and prognostic significance, we evaluated the utility of CD163 immunostaining in the diagnosis of AMLMD.

Design: 54 cases of AML were identified from the pathology files (46 had cytogenetic data). From the bone marrow core biopsy paraffin-embedded tissue blocks, 2mm tissue microarrays were constructed. An H&E stain confirmed the presence of leukemic cells in all cases, which were subclassified according to the 2001 WHO. Immunohistochemical stains for CD163 (clone 10D6, Novocastra), CD68 (clone KP1), lysozyme, CD34 and myeloperoxidase were performed. Cases were positive if ≥20% of tumor cells showed staining. CD163 staining was cytoplasmic in all cases except one (membrane).

Results: 26 of 54 total cases were identified as AMLMD (AML with inv(16), 11q23 and M4 or M5 by French-American-British (FAB) criteria). CD163 positivity was entirely restricted to AMLMD (100% specific).

Table 1. CD163 and CD68 immunostaining in AML.

AML Subtype	# Cases	No. (%) CD163+	No. (%) CD68+
AML w/ recurring gen. abn.			
t(8;21)	1	0 (0)	0 (0)
t(15;17)	4	0 (0)	0 (0)
inv16	4	1 (25)	4 (100)
11q23 abn.	3	1 (33)	3 (100)
Therapy-related AML (with 11q23 abn.)	2	1 (50)	2 (100)
AML with multilineage dysplasia	4	0 (0)	1 (25)
AML not otherwise categorized			
M4	11	6 (55)	9 (82)
M5a	3	3 (100)	2 (67)
M5b	3	2 (67)	2 (67)
All others	19	0 (0)	3 (16)

However, it showed suboptimal sensitivity (54%), compared to CD68 (85%).

Table 2. Sensitivity and specificity of CD163 and CD68 in AMLMD.

Parameter	CD163	CD68
Sensitivity	54	85
Specificity	100	92

CD163 showed improved detection of AML not otherwise categorized, FAB M5 compared to other AMLMD.

Conclusions: CD163 is a highly specific marker for the detection of AMLMD (100%). However, its sensitivity is suboptimal (54%), requiring correlation with results from other methodologies (e.g. NSE cytochemistry, CD68, cytogenetics). These findings demonstrate that CD163 is helpful in the evaluation of AML and may indicate a possible underlying cytogenetic abnormality with prognostic and diagnostic relevance.

1053 Lack of Expression of Platelet-Derived Growth Factor Receptor Beta (PDGFRβ) in Langerhans Cell Tumors: An In Situ Hybridization Study

TI George, RB West, S Blais, KD Montgomery, J Gotlib, M van de Rijn, DA Arber. Stanford University School of Medicine, Stanford, CA.

Background: Treatment with imatinib mesylate has recently been reported to improve Langerhans cell histiocytosis (LCH) in a few patients. Imatinib mesylate selectively inhibits the ABL, KIT, and PDGF kinases. Recent work has demonstrated that Langerhans cells in a single case of cerebral LCH were strongly positive for platelet-derived growth factor receptor (PDGFRβ) using immunohistochemistry, but were negative for KIT (*NEnglJMed* 2004;351:1034). We characterized PDGFRβ expression in 27 cases of Langerhans cell tumors using in situ hybridization in formalin-fixed, paraffin-embedded tissue.

Design: The archives of the Department of Pathology of Stanford University were searched for Langerhans cell tumors with available tissue. All hematoxylin and eosin-stained and available immunohistochemical stained slides were reviewed. 27 cases were identified (ages 6 months to 77 years with mean/median of 20/12 years, M/F=13/14) with sites of disease including bone (14), skin/scalp (7), soft tissue (6), palate (2), lymph node (2) and lung (1). 3 cases involved bone and soft tissue, with 1 of these cases also involving skin. In situ hybridization was performed using a probe directed at the 3' untranslated region of the PDGFRβ gene in a modification of the protocol described by West et al. (*AmJPathol* 2004;165:107). Positive and negative controls included tissue from a dermatofibrosarcoma protuberans and reactive tonsil, respectively. Staining was graded on a 0 to 3+ scale with strong punctuate nuclear and cytoplasmic staining greater than 50% of cells = 3+, 20-50% = 2+, less than 20% = 1+, and less than 5% = 0. Overexpression of PDGFRβ was defined as 2/3+ staining with negative staining defined as 0/1+.

Results: Overexpression of PDGFRβ was detected in 1 of 27 cases, from a solitary scalp lesion of a 64 year old female with a diagnosis of Langerhans cell sarcoma. The remaining 26 cases showed negative expression of PDGFRβ in Langerhans tumor cells and included diagnoses of Langerhans cell histiocytosis (25) and Langerhans cell histiocytosis with atypical features involving the labia of a 38 year old female.

Conclusions: The vast majority of Langerhans cell tumors lack overexpression of PDGFRβ mRNA. Overexpression of the PDGF-receptor system does not appear to play a role in most cases of LCH. This suggests that drugs which selectively inhibit the PDGF kinases, such as imatinib mesylate, would be effective in only a minority of LCH cases.

1054 A Sensitivity Analysis of Aberrant Myeloid Phenotypes in Normal Bone Marrows and Acute Myeloid Leukemia

MF Georgy, EG Weir, MJ Borowitz. Johns Hopkins University, Baltimore, MD.

Background: The recognition of aberrant phenotypes in acute myeloid leukemia (AML) is important for the detection of minimal residual disease (MRD). Aberrant phenotypes are characterized by the unusual coexpression of antigens, asynchronous expression of antigens and/or absence of normal antigens. MRD studies in AML often rely on the design of custom flow cytometry panels to detect aberrant phenotypes seen at diagnosis. However, few studies have assessed aberrant phenotypes in normal marrow specimens and their utility in routine phenotyping. In this study, we examine normal marrows to establish a frequency range for the expression of aberrant phenotypes, and correlate these findings with phenotypic data from cases of AML and myelodysplastic syndrome (MDS) with increased blasts.

Design: Fifteen normal marrow specimens from patients undergoing staging evaluations for non-Hodgkin lymphoma were analyzed by 4-color flow cytometry, in which > 200,000 events were collected per specimen. Analysis focused on the detection of aberrant antigen combinations in the blast population, which was identified on the CD45 versus right angle light scatter display. The frequency of blasts with an aberrant phenotype was expressed as a fraction of total events. The upper limit of normal was defined as the mean + 2 SD. The phenotypes with the greatest analytical sensitivity were then compared with the expression patterns from 403 cases of AML or MDS with increased blasts.

Results: Coexpression of CD34 and CD56 had the highest sensitivity, with a frequency of < 0.01% of events in normal marrows; this phenotype was observed in 22% of AML cases. Four additional phenotypes occurred with a frequency of < 0.1% of events: CD117+CD15+(seen in 45% of AML cases); CD2+CD13+(12%); CD10+CD13+(9%); and CD34+CD38-(4.5%). Expression of one or more of these phenotypes was observed in 69% of AML cases. The phenotype CD7+CD13+, while often considered aberrant, had a frequency of > 0.2% in normal marrow.

Conclusions: Except for CD34+CD56+, many aberrant phenotypes in AML occur in normal marrows at frequencies that limit sensitivity of MRD detection in routine analysis to ~0.1%. The incorporation of additional parameters such as brightness of antigen expression, or more complex antigen combinations in higher order flow cytometry, has the potential to further enhance sensitivity but at the cost of limiting the number of AML cases to which the observations apply.

1055 AKT – Mediated Serine 166 Phosphorylation of MDM2 in Anaplastic Large Cell Lymphoma

K Giaslakiotis, E Drakos, L Tian, F-X Claret, LJ Medeiros, GZ Rassidakis. The University of Texas MD Anderson Cancer Center, Houston, TX.

Background: MDM2 is an oncoprotein that negatively regulates p53 and, in addition, has a number of p53 independent activities that may contribute to tumorigenesis. Previously it has been shown that AKT is capable of phosphorylating MDM2 at Serine 166 and 186 residues, causing MDM2 stabilization and nuclear accumulation. Recently, we have demonstrated that total MDM2 is over-expressed in the majority of ALK+ and ALK- anaplastic large cell lymphoma (ALCL) tumors, which frequently express wild-type p53 at a variable level. ALCL tumors also commonly express activated (^{Ser473}phosphorylated) AKT. We hypothesized that MDM2 is stabilized in ALCL through phosphorylaton at serine 166 by AKT and contributes to cell cycle progression.

Design: Two ALK+ (Karpas 299, SUDHL-1) and one ALK- (Mac2a) ALCL cell lines were used. The cell lines were infected with an HA-tagged Myr-Akt adenovirus expressing constitutively active AKT. AKT was inhibited with treatment of cells with increasing concentrations of LY294002 or transient transfection with siRNA specific for *akt1* gene product. Cell lysates were prepared 48 hours after treatment. Western blot analysis was performed using standard methods and antibodies specific for ^{Ser166}pMDM2, total MDM2, AKT, ^{Ser473}pAKT, and p53. BrdU incorporation assay was used to assess changes in the S fraction of cell cycle. ^{ser166}pMDM2 expression was assessed in 49 ALCL tumors (27 ALK+, 22 ALK-) using immunohistochemistry and tissue microarrays.

Results: Forced expression of constitutively active AKT using an AKT-Myr adenovirus in Karpas 299, SUDHL-1 and Mac2a cells resulted in increased ^{ser166}pMDM2 levels. Inversely, inhibition of PI3K/AKT by LY294002 or selective inhibition of *akt1* gene expression using specific siRNA resulted in a concentration-dependent decrease of ^{ser166}pMDM2, which correlated with cell cycle arrest at G1-S phase. Cell cycle changes may be mediated in part by p53 as forced MDM2 phosphorylation resulted in increased p53 proteolysis in Mac2a cells. Using a 10% cutoff, ^{ser166}pMDM2 was strongly expressed in 23/27 (85%) ALK+ and 19/22 (86%) of ALK- ALCL tumors with a nuclear staining pattern.

Conclusions: These findings suggest that AKT activation is linked to MDM2 phosphorylation and stabilization in ALCL that may contribute to its oncogenesis via p53-dependent or independent pathways. Modulation of MDM2 phosphorylation or function may have beneficial theurapeutic implications in patients with ALCL.

1056 PAX5 Expression in Acute Myeloid Leukemia with and without t(8;21) Is Associated with CD19 Expression

SE Gibson, ED Hsi, HY Dong. Cleveland Clinic Foundation, Cleveland, OH; Genzyme Genetics, New York, NY.

Background: The expression of the B-cell transcription factor PAX5 has been described in a fraction of acute myeloid leukemia (AML) with t(8;21). CD19 and CD79a are transcriptional targets of PAX5. This may account for the common expression of CD19 in t(8;21)-AML. We hypothesize that PAX5 expression may not be limited to t(8;21)-AML and may also be associated with B-cell antigen expression in cases lacking t(8;21).

Design: Twenty-one cases of AML with t(8;21) and 52 cases of AML without t(8;21) were analyzed. Expression of CD19 and CD20 was identified by flow cytometry. CD22, CD79a, and PAX5 expression was studied by immunohistochemical staining of B5-fixed core biopsy or formalin-fixed clot sections. The pattern of staining for CD22, CD79a, and PAX5 was classified as strong (staining in 20% or more blasts), weak (staining in <20% blasts), or negative. The expression of PAX5 was characterized by nuclear staining.

Results: Seventeen cases were PAX5+ (16 strong and 1 weak) and 58 cases were negative. Of the 17 PAX5+ cases 12 were identified as AML with t(8;21) ($p<0.0001$, Fisher exact test). All 12 cases were associated with CD19 expression. The remaining 5 of the 17 PAX5+ cases lacked t(8;21). Expression of CD19 was detected in 4 of these cases. The 5th case had t(11;19)(q23p13.1) and CD20 expression without CD19 expression. There was a significant association between PAX5 expression and CD19 expression in the entire cohort of cases ($p<0.0001$, Fisher exact test). Even when excluding cases of t(8;21)-AML, there was still a significant association between PAX5 and CD19 ($p=0.0003$, Fisher exact test). No association between PAX5 and CD22 or CD79a expression was identified.

Conclusions: We confirm the common expression of PAX5 in AML with t(8;21). However, PAX5 is also exhibited in CD19+ AML lacking t(8;21). The close association of PAX5 with CD19 expression in AML with or without t(8;21) suggests that PAX5 is responsible at least in part for the aberrant expression of CD19 in AML. The mechanism underlying PAX5 expression in AML requires further investigation.

1057 Expression of B-Cell Transcription Factors in Acute Myeloid Leukemia

SE Gibson, ED Hsi. Cleveland Clinic Foundation, Cleveland, OH.

Background: The aberrant expression of the B-cell transcription factor PAX5 has previously been described in a subset of acute myeloid leukemia with t(8;21) in association with B-cell antigen expression. However, the expression of other transcription factors, particularly OCT-2 and its B-cell specific coactivator BOB.1, has not been described in AML. We hypothesize that the transcription factors OCT-2 and BOB.1 may also be expressed in AML and may be associated with B-cell antigen expression.

Design: Sixteen cases of AML with t(8;21) and 52 cases of AML without t(8;21) were studied. CD19 and CD20 expression was analyzed by flow cytometry. CD22, CD79a, PAX5, OCT-2 and BOB.1 expression was studied by immunohistochemical staining of B5-fixed core biopsy specimens or formalin-fixed clot sections. The pattern of staining for CD22, CD79a, PAX5, OCT-2 and BOB.1 was classified as positive (staining in 20% or more blasts) or negative. The expression of PAX5, OCT-2 and BOB.1 was characterized by nuclear staining.

Results: Expression of PAX5, OCT-2 and BOB.1 was seen in 9 (13%), 23 (34%) and 36 (53%) AML cases, respectively. Seven of the 9 PAX5+ cases were identified in t(8;21)-AML (P=0.0003, Fisher exact test), while OCT-2 and BOB.1 were more widely expressed. Neither OCT-2 nor BOB.1 expression correlated with PAX5 expression. Although PAX5 expression was strongly associated with CD19 or CD20 expression (P<0.0001, Fisher exact test), neither OCT-2 nor BOB.1 expression was associated with B-cell antigen expression (CD19, CD20, CD22, or CD79a). OCT-2 and BOB.1 co-expression was seen more frequently in non-t(8;21) AML cases (P=0.007, Fisher exact test), particularly in cases with myelomonocytic/monocytic differentiation (P=0.03, Fisher exact test).

Conclusions: OCT-2 and the B-cell transcription factors PAX5 and BOB.1 are expressed in AML. The expression of PAX5 is highly correlated with t(8;21) and aberrant B-cell antigen expression. However, OCT-2 and BOB.1 expression are not associated with PAX5, t(8;21), or B-cell antigen expression. This suggests that the mechanism underlying the expression of OCT-2 and its B-cell specific coactivator BOB.1 is independent of PAX5 expression. The significance of the expression of OCT-2/BOB.1 in AML is unknown but may be related to monocytic differentiation.

1058 Methylation Analysis in Mantle Cell Lymphoma

TC Greiner, DL Klinkebiel, L Tang, DD Weisenburger, WC Chan, JK Christman. University of Nebraska Medical Center, Omaha, NE.

Background: Mantle cell lymphoma is characterized by a proliferation signature, composed of genes identified by mRNA expression profiling, that correlates with overall survival. Methylation of cell cycle and tumor suppressor genes has been described in some cases of mantle cell lymphoma, but has not been systematically studied. We hypothesized that the methylation status of key genes would be associated with a high proliferation signature and poor survival.

Design: DNA was extracted from 24 cases simultaneously with RNA extraction. The cases had been previously analyzed by mRNA gene expression profiling, and a proliferation signature value (PSV, range -2.04 to +1.76, for good to poor survival) was generated from 20 genes. Methylation-specific PCR was performed on the promoter regions of 12 genes (ATM, CDH1, DAPK, MGMT, p14, p16, p27, p53, p57, p73, RARB, TIMP-3) after bisulfite pretreatment of DNA.

Results: The number of cases seen with the specified number of methylated genes was as follows: 1 gene-5 cases; 2 genes-5 cases; 3 genes-7 cases; 4 genes-6 cases; 5 genes-1 case. The most frequently methylated genes were CDH1 and DAPK in 71% of cases, TIMP-3 in 66% of cases, and RARB in 54% of cases. Rare cases (4%) had methylated p16 or p27 genes. No methylation was seen in ATM, MGMT, p14, p53, p73 or p57. A direct correlation was seen between the number of methylated genes and the mean PSV: 1-2 methylated genes, PSV = -0.44; 3 methylated genes, PSV = -0.13; and 4-5 methylated genes, PSV = 0.70. Whereas a PSV of -0.44 falls within the quartile of cases with a long median survival (6.7 years), a PSV of 0.70 falls within the quartile with very poor median survival (0.83 years).

Conclusions: Cases of mantle cell lymphoma with a high number of methylated genes also have a high proliferation signature which predicts for poor survival.

1059 Immunoglobulin Heavy Chain Expression in B-Cell Lymphomas and Reactive Lymph Nodes Analyzed by Flow Cytometry

D Grier, S Al-Quran, Y Li, R Braylan. University of Florida, Gainesville, FL.

Background: Discriminating between reactive and malignant lymphoid processes based on histology and immunohistochemistry can be problematic. The diagnosis of B-cell lymphomas is often dependent on the detection of clonal immunoglobulin (Ig) light chain expression. However, some B-cell lymphomas lack this expression, which may preclude a reliable interpretation of the data. We analyzed the expression of Ig heavy chains (HC) using flow cytometry (FCM) to determine if there are differences in HC expression that can help distinguish benign reactive lymph nodes from B-cell lymphoma, and also differentiate various subtypes of B-cell lymphoma.

Design: Previously collected FCM data from 92 lymph nodes were re-analyzed with attention given to the HC expression. B-cells were selected by their surface expression of CD20 and CD19. The mean fluorescence intensity (MFI) of IgA, IgG, IgM and IgD expression, measured exclusively on B-cells, was determined using non-specific reagents as controls. The MFI for reactive lymph nodes and various lymphoma subtypes were compared.

Results: HC expression on B-cells varied among different types of hyperplasias. In dominant follicular hyperplasia, discrete populations of mantle and germinal center cells were recognized by FCM mainly based on size and CD20 expression.Normal mantle cells had a high expression of IgM and IgD while IgA and IgG expression were barely detected. IgG expression was weak in germinal center cells while the other HC were poorly or not expressed. In mixed hyperplasia, there was a blurring of B cell compartments and IgD and IgM expression was high. In lymphomas there was variable or no expression of a dominant HC. Compared to reactive nodes, lymphomas generally had a more variable expression of IgM. Of note, IgD expression, while variable, was significantly higher in reactive lymph nodes than in B-cell lymphomas. Small lymphocytic lymphomas had a lower expression of IgM than mantle cell lymphomas. B cells in a lymphoma that lacked Ig light chain expression demonstrated a single dominant HC expression.

Conclusions: FCM is a useful tool in the analysis of normal B-cell compartments in follicular hyperplasia. IgM can be used in distinguishing mantle cell lymphomas from small lymphocytic lymphomas. Expression of IgD should be useful in differentiating reactive follicular hyperplasia from B-cell lymphoma. Heavy chain analysis is also useful in cases when B-cell lymphoma cells lack Ig light chain expression.

1060 Expression of CXCL13, a Chemokine Highly Upregulated in Germinal Center T Helper Cells, Distinguishes Angioimmunoblastic T-Cell Lymphoma from Peripheral T-Cell Lymphoma, Unspecified

KL Grogg, AD Attygalle, WR Macon, ED Remstein, PJ Kurtin, A Dogan. Mayo Clinic, Rochester, MN; University College, London, United Kingdom.

Background: The germinal center T helper cell (GC-Th) is the putative cell of origin for angioimmunoblastic T-cell lymphoma (AITL), based on tumor expression of CD3, CD4 and frequently CD10, similar to normal GC-Th. We recently reported diffuse expression of CXCL13, a chemokine critical for germinal center formation and one of the most highly upregulated genes in the GC-Th subset, in the majority (86%) of AITL cases (Grogg et al, *Blood* 2005; 106:1501-2). We extended this work by evaluating CXCL13 expression in peripheral T-cell lymphomas of unspecified type (PTCL-u), to determine the specifity of this marker for AITL.

Design: Archived paraffin-embedded tissues from 28 patients with an established diagnosis of PTCL were studied. The majority (26) were nodal-based lymphomas; others involved the skin (1), and lung (1). At a minimum, paraffin sections in each case were stained with antibodies to CD3, CD4, CD8, CD10, CD20, CD21 and CXCL13.

Results: By WHO classification criteria, 22 cases were considered PTCL-u, and 6 were reclassified as AITL based on disorganized proliferation of CD21-positive follicular dendritic cell (FDC) meshworks and perivascular clear cells. CXCL13 expression by the neoplastic cells was observed in 2 of 22 (9%) PTCL-u cases; none expressed CD10. The 2 PTCL-u cases positive for CXCL13 showed a Lennert's lymphoma-like histology, with an extensive histiocytic infiltrate. In one case the neoplastic cells were CD4-positive; the other case displayed a phenotype consistent with gamma/delta T cells. Five of 6 cases reclassified as AITL showed diffuse CXCL13 staining, with 2 of these showing partial CD10-positivity.

Conclusions: CXCL13 expression is a distinctive feature of AITL when compared to PTCL-u. The combined results of our studies indicate diffuse CXCL13 expression in 86% of AITL cases and only 9% of PTCL-u cases. This finding provides further support for the GC-Th as the cell of origin for AITL, and given its specificity may be diagnostically useful. The role this cytokine plays in organization of the lymphoid follicle may explain the dysregulated proliferation of FDCs as well as B cells that is characteristic of AITL. Immunomodulatory strategies targeting the downstream effects of CXCL13 expression may prove helpful in AITL treatment.

1061 Comparison of FISH and PCR for Detection of t(14;18) in Follicular Lymphomas

K Gu, R Hawley, M Cankovic, L Whiteley, J Sanchez, A Adeyinka. Henry Ford Hospital, Detroit, MI.

Background: Follicular lymphomas (FL) comprise approximately 22% of non-Hodgkin lymphomas worldwide. The reported incidence of t(14;18) in FL ranges from 70% to 95%. Detection of t(14;18) can be of assistance in the diagnosis of FL and utilized to monitor the level of residual disease. Detection techniques include conventional cytogenetic analysis, Southern blot analysis, FISH, and PCR-based assays. Only FISH and PCR are applicable to fixed tissue. The aim of this study was to compare the relative sensitivities of FISH and multiplex PCR in the detection of t(14;18) in FL in formalin-fixed paraffin-embedded tissue.

Design: 50 cases of FL and 10 cases of reactive lymphoid hyperplasia were studied; excisional lymph node biopsies were utilized in all cases. The FL were histologically and, in most cases, immunophenotypically defined; all FL included in the study had a predominantly follicular pattern of infiltration. For FISH, 3-4μm formalin-fixed paraffin-embedded tissue sections were examined by fluorescence microscopy following pretreatment and hybridization with LSI IgH/BCL2 dual color/dual fusion probe (Vysis). For PCR, DNA was isolated from formalin-fixed paraffin-embedded tissue sections (Qiagen QIAamp DNA mini kit) and analyzed for MBR, mcr, and JH breakpoints by multiplex PCR and capillary electrophoresis (In Vivo Scribe bcl2/JH translocation assay and Applied Biosystems ABI 3100).

Results: We were able to detect the t(14;18) in 36 of 50 FL cases (72%) with FISH and 32 of 50 cases (64%) with PCR. Ten out of the 36 samples that were positive for t(14;18) by FISH were negative by PCR, whereas 6 (including 3 cases of probe failure) of the 32 samples that were positive by PCR were negative by FISH. With PCR, rearrangements involving MBR were detected in 26 of 50 cases (52%), and rearrangements involving mcr were identified in 6 additional cases (12%). Rearrangements were not detected in any of the 10 reactive lymphoid hyperplasia cases with FISH. A rearrangement involving the MBR was found at low level in 1 of the 10 reactive cases by PCR.

Conclusions: Our results indicate that both FISH and PCR can be effective means of detection of t(14;18) in formalin-fixed paraffin-embedded tissue in the majority of cases of FL. FISH proved to be the more sensitive of the two techniques with a detection rate of 72% vs. 64%.

1062 Percentage Hematogones by Flow Cytometric Analysis Is Lower in Patients with Myelodysplastic Syndrome Than in Cytopenic Patients without Myelodysplastic Syndrome

R Gupta, RP Hasserjian, WN Rezuke, JA DiGiuseppe, JP Hunt. Baystate Medical Center, Springfield, MA; Massachusetts General Hospital, Boston, MA; Hartford Hospital, Hartford, CT.

Background: Myelodysplastic syndromes (MDS) comprise a group of clonal disorders characterized by ineffective hematopoiesis and traditionally defined by peripheral blood cytopenias and dyspoiesis involving one or more myeloid cell lines. Diagnostic morphologic abnormalities are somewhat subjective, and may also be caused by medications, toxins or infections. Recently, increased apoptosis among B-cell precursors (hematogones (HG)) has been reported in the bone marrow (BM) of MDS patients. We sought to determine if this increased HG apoptosis is manifested by a difference in HG percentage between MDS patients and patients with cytopenias due to other causes.

Design: Pathology databases from two institutions were searched for adult patients in whom MDS was a clinical consideration and whose BM samples were evaluated by flow cytometry. The HG percentage (as a fraction of nucleated BM cells) was determined using 4-color flow cytometry and was compared between groups of patients with and without diagnostic features of MDS (as determined on the basis of clinical information, follow-up, morphology, and, when available, cytogenetics). Patients with indeterminate or equivocal findings were excluded. Because of differences in preparation and analysis, patient cohorts from each institution were analyzed separately.

Results: The bone marrow HG percentage was significantly lower in patients with MDS compared with those without MDS for both cohorts of patients. See table.

Hematogone Percentage

	Institution 1				Institution 2		
	Mean	Median	Range		Mean	Median	Range
MDS, n=49	0.38	0.07	0-2.8	MDS, n=38	0.09	0.04	0-0.63
no MDS, n=88	1.32	0.93	0-6.34	no MDS, n=28	0.5	0.26	0-3.28
		p<0.00001				p=0.0001	

Conclusions: 1. In the patient cohorts at both institutions, the bone marrow HG percentage in patients with MDS was lower than that in patients with cytopenias but without diagnostic features of MDS. 2. Because of overlap in the percentages of HG between these two groups of patients, this parameter alone cannot be used to establish a diagnosis of MDS. 3. However, in conjunction with morphologic, clinical and cytogenetic features, the HG percentage may be a useful ancillary parameter in distinguishing patients with MDS from those with other causes of cytopenias.

1063 Myelodysplastic Syndrome with Isolated Del(20q): A Distinct Clinicopathologic Entity?

R Gupta, V Johari, RP Hasserjian. Baystate Medical Center, Springfield, MA; Massachusetts General Hospital, Boston, MA.

Background: The cytogenetic abnormality del(20q) occurs myeloid neoplasms and is a favorable prognostic feature in myelodysplastic syndrome (MDS). In our practice, we have noted cases of thrombocytopenic patients who were initially felt to have immune thrombocytopenia (ITP), but were subsequently found to have an isolated del(20q) on bone marrow cytogenetics.

Design: We retrieved 11 bone marrow samples with isolated del(20q)(excluding MPD and AML cases) obtained from 9 patients at two institutions over a 5-year period. For comparison, we also retrieved a control group of 17 bone marrow samples obtained from adult patients with thrombocytopenia not related to MDS (12 ITP, 1 splenic sequestration, and 4 transient thrombocytopenias). Three observers reviewed all 28 cases (biopsies, aspirates, CBC results, and peripheral smears) blinded to the diagnosis and cytogenetics results. The observers scored morphologic parameters and evaluated the likelihood of MDS for each case.

Results: The 9 patients with del(20q) included 8 males and 1 female with a median age of 73 years (range 58-83). The indication for bone marrow biopsy was thrombocytopenia in 7 patients and lymphoma staging in 2 patients. 6 patients had normocytic anemia (median hematocrit 36%), 7 were thrombocytopenic (median platelet count 83 x 10^9/L), and 1 was neutropenic. On review, a diagnosis of MDS (refractory cytopenia with multilineage dysplasia) was rendered in only 1 case; all three observers favored a reactive process in 6 cases and one or more observers raised the possibility of MDS in the remaining 4 cases. Hematologic parameters were similar in the del(20q) and control groups, with the exception of a lower absolute neutrophil count in the del(20q) cases (median 2.5 x 10^9/L, p=0.019). There were no significant differences between the groups in bone marrow cellularity, megakaryocyte number, or dysplasia assessment in any lineage. Among the 4 del(20q) patients with at least 1 year of clinical follow-up, 3 have stable peripheral blood counts (at 1, 2, and 5 years) and 1 has progressive pancytopenia.

Conclusions: MDS cases with del(20q) commonly manifest with thrombocytopenia, mild normocytic anemia, and a relative lack of morphologic dysplasia. Most of these cases fall into the WHO category of MDS, unclassifiable, but their clinicopathologic features suggest classification as a distinct MDS subgroup. Cytogenetic studies in elderly patients with thrombocytopenia may be helpful in avoiding misdiagnosis of MDS with del(20q) as ITP.

1064 Immunophenotypic Study of Basophils by Multiparameter Flow Cytomtry

XH Han, JL Jorgensen, Y Shi, S Awagu, E Schlette, W Chen. UT MD Anderson Cencer Center, Houston, TX.

Background: Basophils are the lowest frequency constituents of blood cells and basophilic leukemia is very rare. The immunophenotypic profile (IP) of basophils is thus not yet fully established. As the patterns of CD45 expression and light scatter properties in basophils are similar to those of myeloblasts, this could potentially lead to inaccurate evaluation of myeloblasts. Therefore, the knowledge of the IP on basophils is imperative.

Design: IP of basophils in peripheral blood (n=13) or marrow aspirates (n=7) in 5 lymphoma and 15 chronic myelogenous leukemia (CML) cases were studied by 4-colour flow cytometry (FC). The panel included: CD3, CD4, CD9, CD11b, CD13, CD19, CD22, CD25, CD33, CD34, CD36, CD38, CD45, CD64, CD117, CD123 and HLA-DR.

Results: IP of basophils in negative lymphoma staging cases was consistent, and served as a normal control. This group included 3 males (M), 2 females (F), aged 39 to 71 years (median 61). Basophils in all cases were positive for CD9, CD13, CD22, CD25, CD33, CD36, CD38 (bright), CD45 (dimmer than lymphocytes and brighter than myeloblasts) and CD123 (bright), and were negative for CD3, CD4, CD19, CD34, CD64, CD117 and HLA-DR. In a subset of cases, basophils were positive for CD11b (2/5). The comparison group of CML patients included 9 M, 6 F, aged 19 to 73 years (median 61); 7 patients in chronic, 5 in accelerated and 3 in blast phase. Compared to control cases, basophils in CML cases aberrantly expressed CD64 (8/15) and HLA-DR (3/15), lost expression of CD25 (3/14), CD33 (1/15) and CD22 (2/15), showed decreased intensity of CD38 (13/15), CD123(5/15), and showed variable intensity of CD13 (bright 1/15, partial 1/15) andCD9 (bright 1/15, dim 2/15). The patterns of expression of CD11b, CD36 and CD45 were similar. In cases with expression of CD34 (1/15) and CD117 (7/15), 4 showed morphologically immature basophilic precursors. In all CML cases basophils possessed 1-5 immunophenotypic aberrancies. Myeloblasts showed a distinct IP, as they typically expressed CD34, CD117 and HLA-DR, dimly expressed (compared to basophils) CD38, CD45 and CD123, and lacked expression of CD22.

Conclusions: Basophils expressed myeloid markers and characteristically expressed CD9, CD22, CD25, CD38 (bright), CD45, CD123 (bright), and lacked expression of CD34, CD117 and HLA-DR. This IP distinguished them from myeloblasts, which were positive for CD34, CD117 and HLA-DR, and typically had moderate expression of CD38, CD123 and CD45. Basophils in all CML cases exhibited one or more immunophenotypic aberrancies.

1065 Ki67/CD23 Expression Pattern Distinguishes Progressive Transformation of Germinal Centers (PTGC) from Nodular Lymphocyte Predominant Hodgkin Lymphoma (NLPHL)

S Hao, J Yang, P Lin, K Dresser, B Woda. University of Massachusetts Medical School and UMass memorial Medical Center, Worcester, MA; Fallon Clinic, Worcester, MA; UT MD Anderson Cancer Center, Houston, TX.

Background: Immunohistochemical (IHC) characterization of neoplastic cells and background reactive lymphocytes is often used to distinguish NLPHL from PTGC. We have previously demonstrated that follicular immunoarchitecture, defined by Ki67+ follicle center cells (FCC), CD23+ follicular dendritic cells (FDC) and CD23+ mantle zone lymphocytes (MC), discriminates reactive from neoplastic follicles. Here, we further examined the utility of the Ki67/CD23 expression pattern in differentiating NLPHL from PTGC.

Design: Ki67 (Dako) and CD23 (Vector) IHC were performed on paraffin-sections of 27 archived lymph node specimens of PTGC and NLPHL. Of these, 7 were known cases of PTGC (4) or NLPHL (3). The remaining 20 "unknown" cases were examined by 2 pathologists, blinded to the original histopathology and immunophenotype, and classified exclusively according to pattern of Ki67/CD23 expression.

Results: In PTGC, clusters of intermingling Ki67+ FCC and CD23+ FDC were distributed near the center of the nodules and were surrounded by prominent CD23+ MC. The IHC double staining highlighted the close complementary relationship of Ki67+ FCC and CD23+ FDC in PTGC. Admixed with PTGC were often follicles showing reactive follicular hyperplasia (RFH) with polarized Ki67+ FCC and concentric CD23+ FDC in the light zones. In contrast, Ki67+ FCC and CD23+ FDC in NLPHL were randomly distributed throughout the follicles and devoid of the prominent CD23+ MC. In some NLPHL CD23+ FDC may be absent or distributed peripherally. Distinct Ki67/CD23 expression in PTGC allowed correct identification of 10 of 10 NLPHL and 9 of 10 PTGC (sensitivity = 100%, specificity = 90%) in the unknown series. The single false positive diagnosis we encountered in a PTGC was later reclassified as RFH with extensive follicle lysis, on review of the routine histology.

Conclusions: Ki67/CD23 highlights the different follicular immunoarchitecture of reactive and neoplastic follicles and is a highly valuable diagnostic adjunct in differentiating PTGC from NLPHL. The complementary relationship of Ki67+ FCC and CD23+ FDC and the prominent CD23+ MC are consistent features of PTGC and are not seen in NLPHL. Follicle lysis can sometimes mimic NLPHL on Ki67/CD23 criteria alone and histologic correlation is required.

1066 Detection of the Activating JAK2 V617F Mutation in Paraffin-Embedded Bone Marrow Biopsies of Patients with Chronic Myeloproliferative Disorders

T Horn, M Kremer, T Dechow, WM Pfeifer, B Geist, J Duyster, L Quintanilla-Martinez, F Fend. Technical University Munich, Munich, Germany; Beth Israel Deaconess Medical Center and Harvard Medical School, Boston, MA; GSF Research Center for Environment and Health, Oberschleissheim, Germany.

Background: The recent identification of the activating V617F mutation in the JAK2 tyrosine kinase in a large proportion of patients with Ph- chronic myeloproliferative

disorders (CMPD) represents a major breakthrough for the understanding of CMPD. The aim of our study was to determine the suitability of trephine bone marrow (BM) biopsies for detection of the V617F JAK2 mutation and to assess its frequency in patients with CMPD.

Design: A total of 152 formalin-fixed, paraffin-embedded BM biopsies with CMPD and related disorders classified according to WHO criteria, as well as normal controls, were studied for the presence of the V617F JAK2 mutation, using both an allele-specific PCR, as well as a nested PCR with subsequent restriction enzyme digestion with BsaXI. Sequencing was performed in selected cases.

Results: Only 6 (4%) of the 152 trephine BM biopsies were not evaluable due to poor DNA quality. The V617F JAK2 mutation was detected in 27/28 cases (96%) of polycythemia vera, 17/23 (74%) cases of essential thrombocythemia (ET), 28/45 (62%) cases of chronic idiopathic myelofibrosis, including prefibrotic stages, 6/8 (75%) cases of CMPD unclassified and 2/4 (50%) cases of MDS/MPS. Ph+ chronic myelogenous leukemia (4 cases), reactive (secondary) erythrocytosis or thrombocytosis (15) and normal controls (19) were all negative for the V617F mutation. Based on the ratio of digested versus undigested PCR product in the BsaXI digestion and sequencing results, 20/54 (37%) evaluable cases were considered homozygous for the V617F mutation. None of the ET cases showed evidence of homozygosity.

Conclusions: Detection of the V617F JAK2 mutation is feasible in paraffin-embedded BM biopsies and represents a major advance for the diagnostic evaluation of CMPD. The high incidence of the mutation in ET in this series - in contrast to previous studies on peripheral blood - warrants further investigation.

1067 t(14;19) in B-Cell Lymphoid Neoplasms: Clinicopathologic, Immunophenotypic and Cytogenetic Study of 8 Cases

YO Huh, GZ Rassidakis, L Abruzzo, LJ Medeiros, MJ Keating, D Catovsky. UTMD Anderson Cancer Center, Houston, TX; Royal Marsden Hospital, London, United Kingdom.

Background: The t(14;19) is a rare recurrent cytogenetic abnormality in patients with B-cell neoplasms, most frequently reported in chronic lymphocytic leukemia (CLL). This translocation juxtaposes the *BCL3* gene at chromosome 19q13 with the immunoglobulin heavy chain gene locus at 14q32.

Design: We searched the files of the Cytogenetics Laboratory at M. D. Anderson Cancer Center and the Royal Marsden Hospital in London for lymphoid neoplasms with t(14;19). Eight cases were identified. The medical records and all relevant laboratory reports were reviewed. The morphologic features of peripheral blood and bone marrow (BM) aspirate smears, clot section and core biopsy and lymph node were examined. The imunophenotype was scored according to a system proposed by Moreau et al (1997). *BCL3* immunohistochemical stain was performed on BM trephine biopsy, clot or lymph node sections.

Results: The clinical, morphologic, immunophenotypic and cytogenetic data are summarized in the table. Lymphadenopathy was present in 6 of 8 patients and splenomegaly was found in 2 patients. By immunophenotype, no one had a typical CLL score of 4-5. The majority of patients had score of 1 or 2 and 2 patients had score of 3. In all patients, BM biopsy showed interstitial growth pattern and *BCL3* was positive. Cytogenetically, in addition to t(14;19), trisomy 12 was found in 6 patients and other additional changes were present in 5 patients.

Conclusions: The t(14;19) is associated with an atypical immunophenotype for CLL with low CLL scores, an interstitial growth pattern in BM biopsy and overexpression of *BCL3*. Trisomy 12 is frequent and additional cytogenetic changes are common in this subgroup of patients.

Summary of 8 Patients with t(14;19)

Age/ Gender	LN/ Spleen*	CLL Scores**	BM Histology	BCL3	Cytogenetics	Treatment	Outcome
55 M	Yes/ Yes	3	Interstitial	Pos	+12, t(14;19)	FCR, allo stem cell	Alive, CR
44 F	Yes/ No	1	Interstitial	Pos	+12, t(14;19), complex	BMT from sibling	Alive, CR
33 M	Yes/ No	1	Interstitial	Pos	+12, t(14;19), complex	FCR	Alive, CR
68 M	Yes/ No	3	Interstitial	Pos	+12, t(14;19)	FCR	Lost to follow-up
40 M	Yes/ No	2	Interstitial	Pos	t(14;19), t(2;10)	FCR	Alive, CR
66 M	Yes/ Yes	1	Interstitial	Pos	t(14;19), 6q21-	FCM	Alive
50 M	No/ No	1	Interstitial	Pos	+12, t(14;19), 6q21-	CHOP	Alive
76 F	Yes/ No	1	Interstitial	Pos	+12, t(14;19)	CBL, FCR	Died of sepsis

* Enlarged lymph node/Splenomegaly, ** CLL Scores by Immunophenotype

1068 Apoptotic Gene Expression Profiling in Low-Grade B-Cell Lymphomas using Oligonucleotide Arrays

D Hui, N Satkunam, R Lai. U of Alberta, Edmonton, AB, Canada; U of Alberta and Cross Cancer Institute, Edmonton, AB, Canada.

Background: The pathogenesis of low-grade B-cell lymphoma is incompletely understood, but multiple defects in the apoptotic pathway have been identified in these tumors. By prolonging cell survival, these defects promote tumorigenesis, and also may be responsible for resistance to chemotherapy. Only a relatively small number of studies have been performed examining the gene expression profile of these tumors, but no consistent patterns in the apoptotic gene expression have been reported.

Design: Using oligonucleotide arrays including 112 apoptotic genes, we aimed to examine the apoptotic gene expression profile in two of the most common low-grade B-cell lymphomas - small lymphocytic lymphoma/chronic lymphocytic leukemia (SLL/CLL) and follicular lymphoma (FL). One of the focuses of the study was to compare results before and after chemotherapy in order to identify chemotherapy-induced changes in the expression of apoptotic genes. 6 SLL/CLL patients and 7 FL patients were included in this study. RNA extracted from multiple frozen tumors from each patient collected at different time was used.

Results: The overall pattern of apoptotic gene expression was remarkably similar among samples from previously untreated SLL/CLL (n=3) and FL (n=6). Gene expression in untreated SLL/CLL also highly correlated with that in untreated FL(Pearson correlation coefficient, 0.94). A small number of genes were consistently highly expressed, regardless of the diagnosis (SLL/CLL versus FL) or exposure to chemotherapy; and these genes included MCL1, TNFRSF1B and TNFRSF7, all of which are associated with anti-apoptotic functions. When comparison was made between untreated SLL/CLL (n=3) and previously treated SLL/CLL (n=4), we identified 6 relatively highly expressed genes that had changes >2 fold, and these genes included survivin, TP53, TNFRSF1A and LTBR (upregulated) and BCLAF1 and BCL2A1 (downregulated). In one SLL/CLL patient in whom results from pre-treated and post-treated samples were available, we found >2 fold changes in all of these 6 genes except LTBR.

Conclusions: The apoptotic gene expression profile is strikingly similar between SLL/CLL and FL, suggesting a common fundamental defect in the apoptotic pathway in these two diseases. We have also identified upregulation of potentially important anti-apoptotic genes, such as survivin, in post-chemotherapy SLL/CLL cases, suggesting that these molecules may be responsible for resistance to chemotherapy.

1069 Novel Anti-Hemoglobin F Antibody in Bone Marrow Can Predict a Response to Antithymocyte Globulin and Cyclosporine in Patients with Aplastic Anemia

R Ichihashi, M Ito, I Sugiura, N Mori, S Kojima. Nagoya University Graduate School of Medicin, Nagoya, Aichi, Japan; Nagoya First Red Cross Hospital, Nagoya, Aichi, Japan; Toyohashi Municipal Hospital, Toyohashi, Aichi, Japan; Nagoya University School of Medicine, Nagoya, Aichi, Japan.

Background: The distinction between hypoplastic myelodysplastic syndrome (MDS) from Aplastic anemia (AA) by morphology alone is sometimes difficult. It is required to invent a new tool to differentiate two diseases. We developed a novel polyclonal antibody by immunizing a rabbit with synthetic peptide of human HbF, which can detect of HbF-containing erythroblasts in paraffin-embedded hematpoietic tissues. HbF is a useful marker to distinguish MDS from AA. Although 42 of 51 MDS patients with cytogenetic abnormalities were positive with HbF, only 2 of 12 patients with typical AA were positive in previous study. (Ichihashi R, et al : Blood 102: 428a, 2003). On the basis of previous findings, we hypothesized that HbF expression on bone marrow erythroblast might be applied to predict to IST in AA patients.

Design: We investigated bone marrow samples from 46 patients with acquired AA who received IST with antithymocyte globulin (ATG) and cyclospoline (CSA) between 1992 and 2005. The diagnosis of AA was established by bone marrow findings and peripheral blood counts. 34 Patients had severe AA and 12 had moderate AA. Patient's age ranged from 2 to 80 years (median : 35.4years). Formalin fixed, paraffin embedded bone marrow tissues were immunostained.We defined that HbF was positive when more than 3 erythroblasts were stained in a cluster.

Results: Overall response rate was a 60.9percentage of patients with CR and PR at 6 months after IST. Higher probability of response to IST was observed in patients with HbF-negative AA. Although 10 of 11 patient (90.9%) with HbF-negative AA responed to ISF, 18 of 35 patient (51.4%) with HbF-positive AA responded. Of 18 patients who did not respond to IST, HbF positive erythreblasts were detected in 17 patients (94.4%).($P<0.05$)

Conclusions: These results suggest that staining of HbF positive erythroblast in pretreatment bone marrow samples may be helpful to predict a good response to IST.

HbF and CyA/ATG response

	HbF+	HbF-	Total
CR/PR	18	10	28
NR	17	1	18
Total	35	11	46

1070 Telomerase Component hTERT Is Overexpressed by Flow Cytometry in Myelodysplastic Syndrome

KA Iczkowski, D Coco, JA Iturraspe, CG Pantazis, R Braylan. U of FL, Gainesville, FL; VA Med Center, Gainesville, FL; Hematopathology Assoc, Gainesville, FL; Munroe Reg Med Center, Ocala, FL.

Background: The myelodysplastic syndromes (MDS) are diagnosed by cytopenias, dysplasia, quantification of myeloblasts, and cytogenetics,. Telomerase is a ribonucleoprotein complex comprising an RNA component and a catalytic reverse transcriptase component (hTERT). hTERT is expressed in the vast majority of human neoplasms, but has not been studied in MDS. Flow cytometric CD16 expression, among other markers, has been cited as altered in MDS (Blood 2002;100:2349-56).

Design: Myeloid marrow cells from 19 patients (7 MDS, 3 myeloproliferative disorder (MPD), 5 AML, and 4 CML) were studied by multidimensional flow cytometry. Exclusion criteria were infection or growth factor treatment. Data were compared with findings in 58 patients with non-neoplastic, reactive changes. A monoclonal antibody to hTERT was conjugated with FITC, and used to measure granulocytes. In all cases, telomerase fluorescence was normalized to that from an IgG isotype control.CD16 % reactivity in granulocytes measured by flow cytometry was interpreted by visual and calculated means. hTERT granulocyte reactivity was compared with that of CD16. The promyelocytic leukemia cell line HL60/MX2 was used as a positive control with each run. hTERT expression was statistically compared between dysplastic/neoplastic and non-neoplastic, 'reactive' conditions.

Results: hTERT expression was elevated in dysplastic/neoplastic groups ($p<0.0001$) compared to reactive specimens. By pairwise comparison, maximal differences were found between AML and reactive ($p=0.0003$), and between MDS and reactive ($p<0.0001$). A negative correlation between hTERT expression and visual (-0.42050, $p=0.0014$) and calculated (-0.45827, $p=0.0095$) CD16 expression was found, but only

in the reactive group. No significant correlation of hTERT with CD16 was identified in the MDS, AML, or CML groups. CD16 expression differed significantly across all 5 groups (visual p=0.015, calculated p=0.024) but, unlike hTERT, not between the reactive and neoplastic groups. A discriminant analysis of neoplastic and reactive groups using only hTERT and/or CD16 expression was done. hTERT alone had the lowest error rate (18%) and calculated CD16 expression had a higher error rate (40%).
Conclusions: hTERT expression is significantly altered in granulocytes of patients with myeloid abnormalities, possessing 82% accuracy in discriminating MDS from reactive, non-neoplastic bone marrow.

1071 Expression of CD44s and CD44v6 in High and Low Grade Lymphomas

KA Janatpour, J Chen, A Afify. University of California, Davis Medical Center, Sacramento, CA.
Background: Adhesion molecule receptors contribute to the aggressiveness of lymphomas and appear to determine specific tissue-dissemination patterns associated with certain lymphoma subtypes. CD44, an 85-90 kDa integral transmembrane protein, belongs to a distinct family of adhesion molecule receptors which have a postulated role in matrix adhesion, lymphocyte activation and homing. CD44 is expressed in a standard form (CD44s), as well as a myriad of CD44 variants (CD44v). The aim of this study was to investigate expression of CD44s and one of its variants, CD44v6, in both low and high-grade lymphomas and to determine their prognostic significance in these diseases.
Design: Archived paraffin-embedded tissue sections from 12 normal lymph nodes, 19 follicular lymphomas (representing low grade lymphomas) and 24 diffuse large B-cell lymphomas (representing high grade lymphomas) were retrieved from our hematopathology files. Corresponding tissue sections, immunohistochemistry, and flow cytometry results were reexamined in all cases to confirm the diagnosis. Tissue sections from each case were stained for CD44s (1:1000, Bender MedSystems, CA) and CD44v6 (1:1500, Bender MedSystems, CA). Positive staining was defined as uniform, strong, membranous staining in at least 10% of cells. Sections from tonsil and squamous cell carcinoma served as positive controls for CD44s and CD44v6, respectively.
Results: Staining was consistent throughout in control tissues. In normal lymph nodes, CD44s was expressed predominately in the interfollicular zone (90% of lymphocytes), and to a lesser extent, in the mantle zone (50% of lymphocytes), but was not expressed in the germinal center lymphocytes. In contrast, CD44v6 was not expressed in any region of normal lymph nodes. CD44s was expressed in 58% (11/19) of follicular lymphomas and 79% (19/24) of diffuse large-B cell lymphomas. CD44v6 was expressed in none (0/20) of the follicular lymphomas, and in 21% (5/24) of diffuse large-B cell lymphomas.
Conclusions: Normal lymph nodes express CD44s mainly in interfollicular lymphocytes and do not express CD44v6. Follicular lymphomas demonstrate upregulation of CD44s compared to its expression in normal germinal center cells from which the lymphoma originates. The exclusive expression of CD44v6 in diffuse large-B cell lymphomas indicates that CD44v6 expression is of poor prognostic significance.

1072 Non-Hodgkin Lymphoma Showing Abnormalities of c-myc Including Dual Translocations Involving c-myc and Bcl2: A Clinicopathologic Study

R Jastania, V Kukreti, K Chun, M Al Shraim, D Bailey, B Patterson, M Crump, S Boerner, W Geddie. University Health Network, Toronto, ON, Canada.
Background: Non-Hodgkin lymphomas (NHL) with translocations or amplification of c-myc are known to have a poor prognosis, especially when t(14:18) is also present. Many are classified from surgical or cytology specimens as diffuse large B-cell lymphomas (DLBCL) or follicular lymphoma in transformation. A retrospective review of clinical and laboratory findings in a series of these patients was undertaken to determine features that would predict the need for testing for these oncogenes at presentation.
Design: A cytogenetic database (2002-2005) was reviewed to find cases assessed by a Vysis® dual colour break-apart probe for c-myc (8q24.1). 27 patients were identified that did not fulfill criteria for diagnosis of Burkitt or atypical Burkitt lymphoma but had cytogenetic abnormalites of c-myc on FISH . Microscopy was reviewed by two pathologists and correlated with immunophenotypic, clinical, and FISH data.
Results: The age range was 26-83 years. 67% had Stage 3-4 disease, 38% had B symptoms and 30% had CNS involvment. 24 cases were diagnosed as DLBCL and 3 as FL-Grade3/3. 15 cases showed rearrangement of c-myc (Group 1) and 12 cases showed only extra c-myc copies (Group 2). 8 patients showed combined t(14;18) and c-myc translocation. The majority of DLBCL's were centroblastic. Most cases showed moderate to severe pleomorphism with some bizarre forms but two were monomorphic centroblastic. All cases with dual translocations showed bright sIg, moderate CD19 and CD20 and bright CD10. No other immunophenotypic markers showed a consistent pattern. MIB1 positive cells ranged from 60-90%. All patients were treated with anthraclycline based chemotherapy. 3 from Group 1 and 5 from Group 2 are currently in complete remission with follow-up less than one year. Median survival of the remaining patients in Group 1 was 9 months (range 1-13) and in Group 2 was 5 months (range1-21).
Conclusions: Both c-myc gene rearrangement and extra copies of c-myc appear to confer poor prognosis in NHL. Not all patients with extra copies or translocation of c-myc satisfy criteria of Burkitt or atypical Burkitt lymphoma and they showed no consistent morphologic findings to distinguish them from other DLBCL's. Testing for c-myc abnormalities by FISH should be initiated in NHL patients with unusually aggressive clinical behaviour and patients with follicular lymphoma in transformation.

1073 Expression of the Novel Cytotoxic Protein Granzyme M Detected by Immunohistochemistry in T and NK-Cell LGL Leukemia

D Jevremovic, WG Morice, CA Hanson, PJ Kurtin. Mayo Clinic, Rochester, MN.
Background: Large granular lymphocytic leukemia (LGLL) is an indolent lymphoproliferative disorder of cytotoxic T and NK cells. Detecting an immunophenotypically distinct cell population is fundamental in establishing a diagnosis of LGLL. In bone marrow (BM) biopsies this can be accomplished by paraffin immunohistochemistry using antibodies to CD8 and the cytotoxic granule proteins TIA-1 and granzyme B (GrB). These immunostains reveal the presence of histologically subtle, disease specific interstitial and intravascular LGLL infiltrates, however they do not distinguish the T and NK cell types.]Granzyme M (GrM) is a recently described novel cytotoxic protein expressed by NK cells. Normal αβ T cells only express GrM after prolonged in vitro stimulation. In this study GrM expression was studied in a group of T and NK cell LGLL to determine if either GrM was aberrantly expressed in T-LGLL or if GrM staining may help in distinguishing these LGLL subtypes.
Design: Paraffin-embedded BM biopsies from 19 T-LGLL and 10 NK-LGLL collected from 1993 to 2005 were studied. Immunohistochemistry was performed using antibodies CD3, CD20, CD8, CD56, CD57, TIA-1, GrB and GrM. Both interstitial clusters (≥8 adjacent cells) and intravascular staining of cells positive for CD8, TIA-1 and GrB in BM biopsies has been reported as a disease specific, clonality-associated finding T and NK LGLL. Each study case was assessed for the presence of these findings for all of the antibodies tested.
Results: Increased numbers of BM cytotoxic lymphocytes were present in all of the T-LGLL and NK-LGLL cases. As summarized in the Table, LGLL associated staining patterns were seen in most of the T and NK cases with antibodies to TIA-1 and GrB, in keeping with previous reports. Furthermore, these staining patterns were present with antibodies to GrM in the majority of the T and NK LGLL. The number of cells positive for GrM closely mirrored that seen with GrB.
Conclusions: GrM immunostaining reveals that this antigen is expressed by T and NK LGLL, with the disease specific patterns previously described for TIA-1 and GrB usually present. The GrM positivity of T LGLL is aberrant as this antigen is not expressed by normal cytotoxic T-cells, providing further evidence to support that this is a disorder of T-cells exposed to prolonged stimulatory signals.

Disease Specific Staining for Cytotoxic Granule Proteins in LGLL

	TIA-1	GrB	GrM
T-LGLL (n=19)	89%	84%	89%
NK-LGLL (n=10)	70%	90%	70%

Interstitial Clusters (≥8 cells) and/or Intravascular Staining

1074 Value of Blood Cell Chimerism Evaluation in Long Term Post-Transplant Survivors among AML and ALL

XY Jinag, GD Sinclair, M Wellings, P Zhao, S Low, M Engel, Z Matotek, A Mansoor. Calgary Laboratory Services, Calgary, AB, Canada; University of Calgary, Calgary, AB, Canada.
Background: Currently bone marrow (BM) or peripheral blood stem cell (PBSC) transplantation for acute leukemia is a standard therapy. During post transplant period detection of residual recipient blood cells is important for determining success of transplantation, extent of blood cell chimerism, and detecting molecular relapse, especially in cases where disease specific markers are not available. Value of peripheral blood cell chimerism among long term (≥ 5yrs) post-transplant survivors in acute leukemia patients is not well reported.
Design: We assessed variable number tandem repeat (VNTR) at 8 specific loci on 6 different chromosomes (TPO, D4S243, D7S460, D16S310, D21S11, VWF1756, VWF1880 and VWF2266) using polymerase chain reaction (PCR) technique among serial samples from long term (≥5 yrs) transplantation survivors of acute leukemia. Peripheral blood or BM specimens at 3 months, 6 months and 1 year after transplant, then yearly for 5 or more years were analysed. The samples were initially screened with 3 VWF locus primers. Non-informative samples were subjected to subsequent amplification at additional VNTR loci. Amplified products radiolabelled with 32^{P}-dCTP during the PCR reaction were analysed by mini-polyacrylamide gel electrophoresis.
Results: The 33 patients (22 AML and 11 ALL) ranged in ages of 18-59 yrs (20 M; 13F, M: F 2: 1) were included in analysis. The VNTR based PCR analysis detected <1% of recipient cells at informative loci. Approximately 70% of AML and ALL donor recipient pairs were informative using the 3 VWF VNTR loci only. No significant differences were found in age, sex or time to complete chimerism between AML and ALL long-term survivors. 19 of 22 (83%) patients showed complete chimerism after 6 months, with no signs of clinical relapse after 5 years. Among >80% patients, BM and peripheral blood specimens showed concordance at 3 months post transplant, but all had converted to complete blood cell chimerism by 6 months.
Conclusions: Peripheral blood is adequate for long term monitoring of blood cell chimerism after BM/PBSC transplantation in AML/ALL patients. Complete chimerism by 6 months is a good indicator for long-term remission. A set of 3 VWF (VWF1756, VWF 1880, and VWF2266) primers can be used as a screening panel for efficient laboratory practice.

1075 Novel Anti-ZAP-70 Antibody for Flow Cytometry in Chronic Lymphocytic Leukemia: Comparison with ZAP-70 Immunohistochemistry and IgV_H Mutation Status

JL Jorgensen, D Jones, RG Luthra, DG Cooper, A Brahmandam. UT MD Anderson Cancer Center, Houston, TX.
Background: Expression of ZAP-70 is associated with unmutated immunoglobulin heavy chain variable region genes (IgV_H-U) and poor prognosis in B-cell chronic lymphocytic leukemia (CLL). Reliable clinical flow cytometry (FC) assays have been difficult to establish, as many anti-ZAP-70 antibodies show a suboptimal signal-to-noise ratio, and an imperfect correlation with IgV_H mutation status.
Design: We studied fresh peripheral blood or bone marrow specimens from thirty-five patients with CLL. Samples were stained for intracytoplasmic ZAP-70 using the novel monoclonal antibody 1164 (kindly provided by BD Biosciences, San Diego, CA). Threshholds were set using isotype-matched negative control antibodies. Mutational

status of the IgV_H genes was assessed by RT-PCR, followed by standard Sanger DNA sequencing. Divergence from germline IgV_H segments (IMGT database) was calculated using VBASE, with 2% or less changes over codons 1-94 of IgV_H regarded as unmutated. Bone marrow core biopsies or clot sections from a subset of these cases were stained for ZAP-70 by immunohistochemistry (IHC) using 2F3.2 (Upstate Cell Signaling Systems, Lake Placid, NY).

Results: With 1164 staining by FC, most specimens were strongly positive, with 30/35 (86%) cases showing >60% of cells above the isotype threshhold. The remaining 5 cases (14%) showed <40% of cells positive. All IgV_H-U cases showed the expected positivity by FC (Table 1), which is a significantly higher rate than seen in most FC studies. However, only about half of cases with mutated IgV_H genes (IgV_H-M) were negative by FC. By IHC, 18/27 cases (67%) were positive for ZAP-70. 17/18 IgV_H-U cases were positive by IHC (Table 1), while 1/9 IgV_H-M cases was positive. Nearly all cases (17/18) which were positive by both FC and IHC were IgV_H-U (Table 2), and all three cases which were negative by both assays were IgV_H-M. Most discordant cases (positive by FC but negative by IHC) were also IgV_H-M.

Conclusions: FC with the 1164 anti-ZAP-70 antibody provides strongly positive staining in all IgV_H-U cases, and all IgV_H-M cases were negative. However, the FC-positive cases also include some with mutated IgV_H genes. IHC staining for ZAP-70 in this series showed a strong correlation with the IgV_H mutation status.

ZAP-70 staining vs. IgVH status

	Unmutated	Mutated
FC positive	24	6
FC negative	0	5
IHC positive	17	1
IHC negative	1	8

Number of IgVH mutated cases, vs. IHC and FC

	FC positive	FC negative
IHC positive	1/18	0/0
IHC negative	5/6	3/3

1076 Growth Factor Therapy May Unmask Hypoplastic Myelodysplastic Syndrome Mimicking Aplastic Anemia

A Kanugo, S Konoplev, LJ Medeiros, P Lin. UT Houston Medical School; UT MD Anderson Cancer Center, Houston, TX.

Background: The distinction between hypocellular myelodysplastic syndrome (MDS) and aplastic anemia (AA) can be difficult as the paucity of neoplastic cells in MDS prevents detection of dysplastic changes and/or cytogenetic abnormalities. Growth factor therapy is a potent stimulant of normal and neoplastic hematopoiesis. In this study we hypothesized that growth factor therapy may unmask some cases of hypoplastic MDS that initially mimic AA.

Design: The files of our institution were searched for cases that presented with pancytopenia, hypocellular bone marrow, and were clinically considered to be AA at initial presentation (1998-2005). The criteria for inclusion were cases that had no morphological evidence of dysplasia or cytogenetic abnormalities at time of presentation and than subsequently were found to have dysplasia and/or abnormal cytogenetics upon growth factor therapy. Bone marrow specimens at initial diagnosis of AA and at the time of diagnosis of MDS were reviewed.

Results: Nineteen cases were identified. There were 7 men and 12 women with a median age of 54 years (range, 24 - 79). All patients had hypocellular bone marrow with a median cellularity of 5% (range, 1-15%) at initial evaluation. In addition to growth factors, 4 received ATG, 2 received ATG and prednisone, 6 received ATG and cyclosporin, and 2 received allogeneic BMT. The median time to MDS diagnosis was 11 months (range, 2-63) when the median bone marrow cellularity was 30% (range, 5-90%). Cellularity was significantly higher than that at presentation in 15 of 19 patients, $p<0.001$. Dysplastic changes were observed in 18 patients: 12 erythroid only, 2 erythroid and granulocytic, 2 erythroid and megakaryocytic, and 2 all 3 lineages. Ring sideroblasts were identified in 8 patients. 11 patients had cytogenetic abnormalities detected including 1 patient without dysplastic changes: monosomy 7 (n=4), complex abnormalities (n=2), trisomy 8 (n=2), monosomy 7 and 8q+ (n=1), inversion 5 (n=1), trisomy 15 (n=1). Six patients died and 13 patients are alive with a median follow-up of 36 months (range, 2-132 months). Overall 5-year survival for the group was 62%.

Conclusions: Some MDS cases initially can present in hypoplastic phase mimicking AA. Growth factor therapy appears to unmask the MDS. Growth factor stimulation may simply allow better detection of dysplastic changes by increasing cell number or foster cell culture growth for detection cytogenetic abnormalities.

1077 The Utility of CD26 in Flow Cytometric Evaluation of Peripheral Blood in Sezary Syndrome

K Kelemen, C Goolsby, JL McLaughlin, R Gupta, T Kuzel, J Guitart, LC Peterson. Northwestern University Feinberg School of Medicine, Chicago, IL.

Background: A highly specific flow cytometric marker of the circulating neoplastic cells in Sezary syndrome is yet to be identified. Recently, the loss of the CD26 antigen (dipeptidyl-aminopeptidase IV) has been proposed as a marker of circulating Sezary cells. The aim of our study was to correlate the CD26 status with the presence of monoclonal TCR gene rearrangement and with levels of circulating convoluted lymphocytes in the peripheral blood of patients with clinical features of Sezary syndrome.

Design: The study included 44 flow cytometric analyses from 20 patients with clinical suspicion of Sezary syndrome. CD2, CD3, CD5, CD7, CD4, CD8 and CD26 measurements were performed with high resolution flow cytometry. TCR gene rearrangement was studied by PCR in the peripheral blood concurrently to the flow analysis. Circulating Sezary cells were quantitated by microscopic examination of the peripheral blood and expressed as % of lymphocytes as well as absolute number/mm^3 blood.

Results: Of the 44 analyses, 38 showed CD7 deletion, 4 had deletion of CD2 and 2 cases had both CD7 and CD2 deletion. Loss of the CD26 antigen of the CD4+ T cells was observed in 22 of the 44 total analyses (50%). Monoclonal TCR gene rearrangement was demonstrated by PCR in 21 of the 44 specimens. Within this group,CD26 loss was observed in 11 analyses (52%). When using morphological criteria, 28 had more than 5% convoluted lymphocytes in the peripheral blood and 22 had more than 1000 Sezary cells/mm^3 blood at the same time when the flow cytometry was done. In these groups CD26 deletion was observed in 60% and 68% of analyses respectively. Sixteen cases had concomitant monoclonal TCR gene rearrangement and morphological evidence of more than 1000/mm^3 circulating Sezary cells. The CD26 deletion was present in 11 analyses in this group (68%).

Conclusions: The loss of CD26 expression on CD4+ T cells failed to identify 48% of Sezary syndrome patients when monoclonal T cell rearrangement was used as criteria to define Sezary syndrome and similarly missed 32 % of patients when morphological criteria were used. We conclude that the sensitivity of CD26 marker is lower than suggested by the literature and that this marker cannot replace traditional diagnostic modalities, such as morphology, detection of major T cell antigen aberrancies by flow cytometry or molecular analysis.

1078 Intrachromosomal Translocations of BCL6: An under Recognized Mechanism of BCL6 Deregulation in B-Cell Non-Hodgkin Lymphomas

CE Keller, S Nandula, E Vakiani, B Alobeid, VS Murty, G Bhagat. Columbia University, New York, NY.

Background: Balanced reciprocal translocations involving chromosome (chr) 3q27 that juxtapose BCL6 with the promoter of a variety of partner genes are a well-established mechanism of BCL6 deregulation in a subset of follicular lymphomas (FL) and diffuse large B-cell lymphomas (DLBCL). We have observed inversions, deletions, and additions involving chr 3q27 in both FL and DLBCL. The aim of our study was to investigate the frequency of these structural chromosomal alterations and determine whether they represented intrachromosomal translocations involving BCL6.

Design: G-banded karyotypes of all cases of FL and DLBCL submitted for cytogenetic analysis at our institute were examined for structural alterations involving chr 3q27. Cases were separated into 2 groups, those with balanced reciprocal translocations involving chr 3q27 and those with other types of chr 3q27 alterations. FISH for BCL6 was performed using dual color break-apart probes. Morphologic evaluation and grading of B-NHL was performed on H&E stained tissue sections.

Results: Eight of 92 (8.7%) cases with informative karyotypes (M:F=2:6, age 33-79, mean 58.4) that included 0/12 FL1, 2/19 (10.5%) FL2, 1/9 (11.1%) FL3a, and 5/52 (9.6%) DLBCL had balanced reciprocal translocations involving BCL6 and a variety of partner loci while 7/92 (7.6%) cases (M:F=2:5, age 51-88, mean 68), including 0/12 FL1, 2/19 (10.5%) FL2, 2/9 (22.2%) FL3a, and 3/52 (5.8%) DLBCL had intrachromosomal BCL6 translocations confirmed by FISH. The latter types of translocations included ***additions*** {3(q27) [n=1]}, ***inversions*** {3(q26.1;q27), 3(p24;q27), and 3(q24;q27), 1 case each}, and ***deletions*** {3(q21q37) [n=1] and 3(q25q27) [n=3]}.

Conclusions: We observed intrachromosomal translocations between BCL6 and known as well as uncharacterized loci on chr 3 that appear to constitute a novel mechanism of BCL6 deregulation in both FL and DLBCL. These types of translocations appear to occur at a similar frequency as balanced reciprocal translocations involving BCL6 but have not been previously characterized most likely due to the difficulty in detecting terminal alterations of chr 3q by G-band karyotyping.

1079 The Spectrum of B-Cell Non-Hodgkin Lymphomas with Combined IgH-BCL2 and BCL6 Translocations: Evidence for Early Acquisition of BCL6 Translocations

CE Keller, S Nandula, E Vakiani, VS Murty, B Alobeid, G Bhagat. Columbia University, New York, NY.

Background: Distinct and independent pathogenetic pathways have been proposed for B-cell non-Hodgkin lymphomas (B-NHL) associated with IgH/BCL2 and BCL6 translocations. Both translocations are occasionally observed in diffuse large B-cell lymphomas (DLBCL) and acquisition of BCL6 translocations by IgH-BCL2+ follicular lymphomas (FL) has been associated with an increased risk for transformation to aggressive DLBCL. We undertook this study to discern the morphologic spectrum of B-NHL with combined IgH/BCL2 and BCL6 translocations detected by conventional karyotypic analysis and their impact on the clinical course of B-NHL.

Design: We searched our departmental cytogenetic database to identify all cases of B-NHL in which combined IgH/BCL2 and BCL6 translocations were detected by G-band karyotype analysis. FISH was performed to confirm both translocations. H&E stained sections were used for morphologic analysis and grading. Immunohistochemical (IHC) staining for a variety of B-cell antigens, including CD10, Bcl6, and Bcl2 was performed. Student's t-test was used for statitistical analysis.

Results: Dual IgH/BCL2 and BCL6 translocations were detected in 6/92 B-NHL diagnosed at our institute. These cases included 4/40 (10%) FL (M:F=1:3, age 33-79 yrs, mean 64 yrs), representing 3/19 (16%) FL2 and 1/9 (11%) FL3a, and 2/52 (4%) DLBCL (M:F=1:1, age 52 and 88 yrs). Partner genes/loci involved by the BCL6 translocation could not be identified in both DLBCL and 2 FL but chromosomal loci 10(q11.2) and 12(q24.1) were involved in 1 case each of FL. Mean number of chromosomal abnormalities was 8.0 in FL compared to 17.0 in DLBCL ($p<0.05$). IHC profile of all cases with dual translocations was CD10+ Bcl6+ Bcl2+. No patient with FL had evidence of transformation 1 month-6 yrs (mean 3.9 yrs) post diagnosis and only 1 (FL2) of 3 patients (1 FL2, 1 FL3 and 1 DLBCL) who underwent bone marrow biopsy at our institute had evidence of marrow involvement.

Conclusions: Our findings suggest that BCL6 abnormalities are acquired early in the progression of a subset of IgH-BCL2+ FL. Moreover, accrual of additional cytogenetic

abnormalities, rather than acquisition of BCL6 translocations per se, might dictate the clinical course and play a more important role in progression of FL to aggressive DLBCL.

1080 Prognostic Significance of Cyclin D1, Cyclin D3, and FGFR3 Immunohistochemistry in Plasma Cell Myeloma

TW Kelley, ED Hsi, MA Hussein, JR Cook. Cleveland Clinic Foundation, Cleveland, OH.

Background: Gene expression profiling has identified several subsets of plasma cell myeloma including those overexpressing FGFR3 or D type Cyclins. Prior studies have suggested some subsets may be associated with prognostic differences. In this report, we describe the results of immunohistochemistry for Cyclin D1, Cyclin D3, and FGFR3 in a series of 94 plasma cell myeloma patients treated with chemotherapy.

Design: All patients were treated with chemotherapy, including at least one thalidomide-based regimen. Immunohistochemical staining for CD138, Cyclin D1, Cyclin D3, and FGFR3 was performed on B5-fixed bone marrow core biopsies or formalin fixed clot preparations. Cases were scored as Cyclin D1 positive (D1+) if nuclear staining in was present in ≥5% of plasma cells, and Cyclin D3 positive (D3+) if ≥20% of plasma cell nuclei were positive. Cases were classified as FGFR3 positive (FGFR3+) when ≥20% of plasma cells showed cytoplasmic or membrane staining. Overall survival was measured from the time of initial diagnosis to death or last followup.

Results: Of 94 cases tested, 35 (37%) were D1+FGFR3-, 5 (5%) were D1-FGFR3+, 52 (55%) were D1-FGFR3-, and 2 (2%) were D1+FGFR3+. 2of 92 cases (2%) were D3+, both of which were negative for FGFR3 and Cyclin D1. Overall survival was compared in four subsets defined by patterns of FGFR3 and Cyclin D1 expression. The median overall survival in D1-FGFR3+, D1-FGFR3-, D1+FGFR3-, and D1+FGFR3+ subsets was 28 months, 61 months, 89 months, and undefined, respectively (p=0.093, log-rank test).

Conclusions: Cases with a FGFR3+CyclinD1- phenotype represented the subset with shortest median survival, although the number of FGFR3+ cases identified remains small and overall survival differences did not reach statistical significance. Cyclin D1, Cyclin D3, and FGFR3 positivity by immunohistochemistry is most often seen in mutually exclusive subsets, but occasional cases may be positive for more than one of these markers. Aberrant expression of Cyclin D3 or FGFR3 protein does not appear to contribute to the pathogenesis of most cases of plasma cell myeloma.

1081 An Immunohistochemical Evaluation of MUM1-Positive Follicular Lymphoma: Correlation with CD10, BCL6 and Marginal Zone Differentiation

TW Kelley, RC Beck, ED Hsi. Cleveland Clinic Foundation, Cleveland, OH.

Background: Follicular lymphoma (FL) typically expresses the germinal center B-cell markers CD10 and BCL6. When comparing intrafollicular (IF) and extrafollicular (EF) compartments, these markers are often heterogeneously expressed. MUM1 is a transcription factor involved in B-cell maturation. In a previous study, we found evidence to suggest that MUM1 was expressed in a subset of FL with a poor outcome. Therefore, we evaluated the expression patterns of MUM1, CD10, BCL6 and BCL2 in a series of MUM1+ FLs to determine if there was any relationship between MUM1 and these markers.

Design: 24 previously identified MUM1+ (at least 1+ on scale below) cases of FL were studied using immunohistochemistry (IHC) for MUM1, CD10, BCL6 and BCL2. Features evaluated included marginal zone (MZ) differentiation and marker expression in IF and EF compartments (in conjunction with CD138, CD20 and CD3). Frequency of MUM1 staining was scored as follows: 0 (negative; <1%), 1+ (1-5%), 2+(6-20%), 3+(21-50%), 4+(>50%). Intensity of MUM1 staining was scored as 0 (negative), 1(weak), 2(strong).

Results: IF MUM1 expression was 4+ in 2 cases (8.3%), 3+ in 8 cases (33.3%), 2+ in 7 cases (29.2%), 1+ in 6 cases (25%) and 0 in 1 case (4.2%). EF MUM1 staining was 4+ in 1 case (4.2%), 3+ in 3 cases (12.5%), 2+ in 8 cases (33.3%), 1+ in 5 cases (20.8%), 0 in 3 cases (12.5%) and not evaluable in 4 cases due to paucity of the EF component. Considering cases with evaluable EF and IF components, MUM1 positivity was greater in IF areas in 10 of 20 cases (50%), equal in EF and IF components in 5 cases (25%) and greater in the EF component in 5 cases (25%). For the CD10+ cases (23 of 24), expression patterns were: EF not evaluable 3 cases, EF>IF 2/20 cases (10%), EF=IF 4/20 cases (20%), EF<IF 14/20 cases (70%). For the BCL6+ cases (22 of 24), expression patterns were: EF not evaluable 4 cases, EF<IF 18/18 cases (100%). MZ differentiation was seen in 7 of 24 cases. Interestingly, these MZ cells expressed MUM1 thereby imparting a unique and readily identifiable MZ pattern to the MUM1 staining.

Conclusions: MUM1 is expressed in a subset of cases of FL in both the IF and EF compartments and, along with CD10 and BCL6, tended to be decreased in the EF compartment. In cases with MZ differentiation, the MZ cells expressed MUM1, suggesting post-germinal center maturation. This characteristic pattern of MUM1 staining may be an adjunct to morphology in identifiying FLs with MZ differentiation, a finding which has previously been associated with a poorer outcome.

1082 Comparison of Marrow Plasma Cell Count by Aspirate Morphologic Examination, Flow Cytometric Analysis and Biopsy CD138 Immunohistochemical Staining in Patients with Monoclonal Gammopathy

HS Khalidi, P Kotylo. Clinical Pathology Associates, Louisville, KY.

Background: Marrow plasma cell count (PCC) is essential for accurate diagnosis and classification of plasma cell neoplasms. There is a wide discrepancy between PCC estimates by marrow aspirate morphologic examination (ME) and flow cytometric analysis (FC). An accurate marrow PCC is often not possible with aparticulate hemodiluted marrow aspirates. Immunohistochemical staining (IHC) of marrow biopsy for CD138 (syndecan-1) is an alternative method for estimation of PCC. The aim of this study is to compare these methods and determine which of them provides the most accurate PCC.

Design: This is a retrospective study of patients diagnosed with plasma cell dyscrasia, including MGUS, indolent/smoldering myeloma and plasma cell myeloma. All bone marrow specimens diagnosed in 2004-2005 in four hospitals were retreived. Specimens included in this study had aspirate smears, aspirate flow cytometric analysis and marrow clot and/or core biopsy. ME was performed by a 300-cell count of the most cellular aspirate smears. FC of aspirate specimens was performed using a 22 antibody panel, including CD38, CD138 and cytoplasmic kappa and lambda. IHC of marrow biopsy paraffin sections for CD138 was performed.

Results: 54 cases of plasma cell neoplasms were studied. All patients had a documented monoclonal gammopathy. The average and range of PCCs in these cases, and the number of cases with PCCs of <10%, 10-30% and >30% performed by ME, FC and CD138 IHC are shown in the following table:

Comparison of plasma cell counts in 54 cases by 3 methods

Number of cases=54	ME	FC	CD138 IHC
Plasma cell% mean	15.8	5.0	28.9
Plasma cell% range	3 - 80	0.1 - 52	4 - 95
Number of cases with <10% plasma cells	29	45	8
Number of cases with 10-30% plasma cells	17	7	29
Number of cases with >30% plasma cells	8	2	17

PCCs of all cases by FC were significantly less than those estimated by aspirate ME (P=0.0005) and biopsy CD138 IHC (P=0.0005). CD138 IHC resulted in higher PCCs than those obtained by ME (P=0.004) and demonstrated the extent and focality of marrow plasma cell distribution. In hemodiluted specimens, PCC by ME and FC were extremely low as compared to those obtained by CD138 IHC.

Conclusions: Marrow plasma cell estimate by CD138 IHC of biopsy is a more sensitive and accurate method than ME and FC of aspirate specimen. It generates higher PCCs, which may result in upgrading the category of plasma cell neoplasm. CD138 IHC is the only reliable method for PCC when aspirate specimens are hemodilute.

1083 Mixed Lineage Kinase-3 (MLK3) Expression Is a Poor Prognostic Factor in Diffuse Large B-Cell Lymphoma, Associated with an Activated B-Cell Subtype

F Khanani, MS Almiski, O Basturk, J Frank, MO Kurrer, E Levi. Harper Hospital, Wayne State University, Detroit, MI; University Hospital, Zurich, Switzerland; John D Dingell VAMC, Detroit, MI.

Background: Mixed lineage Kinase-3 (MLK3) is a protein kinase that is part of the MAPK signaling system which plays a role in the activation of the JNK and ERK pathways. Recently, MLK3 gene was shown to be overexpressed in follicular lymphomas in a gene array study. In this study we were interested to see the expression of MLK3 in diffuse large B-cell lymphomas and to analyze any relationship with germinal center phenotype.

Design: A tissue array containing 94 cases of diffuse large B-cell lymphoma was utilized. The followup data on these cases were available. Immunohistochemical staining was performed utilizing Santa Cruz antibody (MLK3, C20, sc-536). In addition, CD10, bcl2, bcl6, MUM1 and PAK1 stains were performed and correlated with MLK3 expression. Cases staining with more than 50% staining of tumor cells were considered positive.

Results: MLK3 expression on normal tonsil was minimal to negligible. In the diffuse large B-cell lymphomas 42 of 94 (%44) cases were stained. MLK3 expression correlated strongly with pAkt, PAK1, and MUM1. There was no association with bcl6 or CD10. In univariate and multivariate analyses MLK3 expression was a poor prognostic factor (p=0.015).

Conclusions: MLK3 expression is a bad prognostic factor in diffuse large B-cell lymphomas. Contrary to a previous report we found MLK3 expression to be associated with an activated B-cell phenotype. Strong association with PAK1 suggests that both PAK1 and MLK3 are potential targets for intervention in diffuse large B-cell lymphomas.

1084 ZAP-70 Expression by Immunohistochemical and Quantitative RT-PCR Methods Correlate with Somatic Hypermutation of Immunoglobulin Heavy Chain Genes in CLL/SLL

RJ Knoblock, LV Abruzzo, KR Coombes, EJ Schlette, LJ Medeiros, MJ Keating, D Jones, JH Admirand. The University of Texas MD Anderson Cancer Center, Houston, TX.

Background: Zeta-associated protein-70 (ZAP-70) expression has been proposed as a surrogate marker of unmutated somatic hypermutation (SHM) status of the immunoglobulin heavy-chain variable region (IgV_H) genes in a subset of chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) patients with poor prognosis. In this study, we assessed the correlation of ZAP-70 expression, measured by immunohistochemistry (IHC) and semi-quantitative real-time reverse transcription-polymerase chain reaction (QRT-PCR) assay, with SHM status in 63 patients with CLL/SLL.

Design: The study group included 63 previously untreated CLL/SLL patients (44 men, 19 women) with a median age of 58 years (range 27 to 76 years). ZAP-70 expression was evaluated by IHC on bone marrow biopsy and/or clot sections, and scored as 0 (negative), 1+ (weakly positive), and 2+ (strongly positive). QRT-PCR for ZAP-70 mRNA was performed on RNA extracted from purified peripheral blood (PB) B-cells (B-CLL) in 60 patients and categorized as positive (≤25 cycles) or negative (>25 cycles). The SHM status of the IgV_H gene was performed on RNA extracted from B-CLL and cases were categorized as unmutated (≥98% homology to the germline sequence) or mutated (<98% homology to the germline sequence).

Results: There were 25 mutated and 38 unmutated cases. 28 patients showed no expression (0), 26 showed 1+ expression, and 9 showed 2+ expression of ZAP-70 by IHC. For statistical analysis, cases with 1+ and 2+ expression were combined. ZAP-70 by QRT-PCR was negative in 21 cases and positive in 39 cases. ZAP-70 detected by

IHC strongly correlated with ZAP-70 mRNA expression detected by QRT-PCR ($p=3.707x10^{-9}$, Fisher's Exact Test) (Table 1). ZAP-70 expression by IHC and by QRT-PCR also significantly correlated (p=0.004 and p=0.0002, respectively, Fisher's Exact Test) with SHM status (Table 2).

Conclusions: There is a strong correlation between ZAP-70 expression measured by IHC and QRT-PCR methods. ZAP-70 expression measured by IHC and QRT-PCR significantly correlates with SHM status, although a small number of discordant cases are present by both methods.

Table 1

ZAP70 IHC	ZAP70 QRTPCR-	ZAP70 QRTPCR+
0	20	7
1+ and 2+	1	32

Table 2

SHM STATUS	ZAP70 QRTPCR-	ZAP70 QRTPCR+	ZAP70 IHC-	ZAP70 IHC+ (1&2+)
MUTATED	15	8	17	8
UNMUTATED	6	31	11	27

1085 Expression of mTOR Signaling Proteins in Mantle Cell Lymphoma

RJ Knoblock, E Drakos, G Reyes, V Leventaki, LJ Medeiros, GZ Rassidakis. The University of Texas MD Anderson Cancer Center, Houston, TX.

Background: Mantle cell lymphoma (MCL) is a distinctive type of B-cell lymphoma associated with aggressive clinical course, the t(11;14) chromosomal translocation and overexpression of cyclin D1. Results from previous transgenic mouse studies have shown that the t(11;14) is not sufficient for tumorigenesis. Additional molecular events may contribute to MCL oncogenesis through deregulation of the cell cycle and apoptosis. In this study, we hypothesized that proteins downstream from the mammalian target of rapamycin (mTOR), including p70S6K, rpS6, eIF-4E and 4E-BP1, are activated and may contribute to tumor cell proliferation and survival in MCL.

Design: Three MCL cell lines (Mino, Z-138 and JeKo-1) were used to demonstrate mTOR suppression. Transient transfection of Jeko and Z-138 cells with specific mTOR siRNA was performed and cell lysates were prepared 48 hours following transfection. Apoptosis and cell cycle analysis were performed using Annexin V staining (by flow cytometry) and BrdU incorporation assays. Immunohistochemistry (IHC) using a tissue microarray of MCL neoplasms was performed for p-p70S6K, p-rpS6, eIF-4E and 4E-BP1. Each case was graded as 0 (negative), 1+ (weakly positive) or 2+ (strongly positive). 1+ and 2+ cases were combined using a 10% positive cutoff for statistical analysis.

Results: Silencing of mTOR expression using mTOR-specific siRNA decreased phosphorylation of p70SK, rpS6 and 4E-BP1 and induced cell cycle arrest and apoptosis in MCL cell lines. These changes were associated with decreased expression of cyclin D1 and downregulation of the anti-apoptotic proteins cFLIP, BCL-XL and Mcl-1, thus outlining the importance of the mTOR pathway in tumor cell survival in MCL. Expression of mTOR pathway proteins in MCL neoplasms is summarized in Table 1. There was a statistical trend between the percentage of p70SK-positive cells and blastoid morphology. eIF-4E and 4E-BP1 are part of the same transcriptional unit and showed statistically significant co-expression (p=.009, Fischer's Exact Test).

Conclusions: These findings suggest that activation of the mTOR signaling pathway may contribute to cell cycle progression and cell survival in MCL. mTOR signaling phosphoproteins represent potential therapeutic targets for the treatment of patients with MCL.

Table 1. Frequency of mTOR Proteins in MCL

mTOR Protein	p70	pS6	eIF-4E	4E-BP1
Poitive/Total	12/22	5/20	20/35	18/31

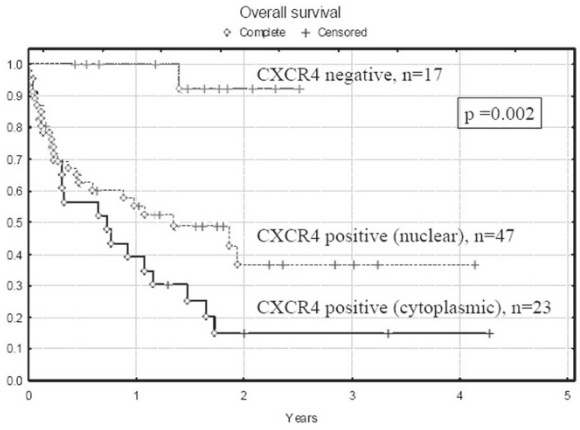
1086 Overexpression of CXCR4 in AML Predicts Adverse Overall and Progression-Free Survival Independently of *Flt3* Gene Mutation

S Konoplev, G Rassidakis, E Estey, H Kantarjian, M Konopleva, M Andreeff, G Kubiak, LJ Medeiros. UT MD Anderson Cancer Center, Houston, TX.

Background: CXC chemokine receptor 4 (CXCR4) is involved in trafficking of normal and neoplastic hematopoietic cells. CXCR4 expression is reported to correlate with *flt3* mutation and predict poorer prognosis in AML (Blood 2004;104:550-7). In this study, we analyze the prognostic significance of CXCR4 expression in a group of AML patients with diploid karyotype and without *flt3* mutation.

Design: The study was 87 AML patients with diploid karyotype and no evidence of *flt3* mutation treated at our institution, 1997-2003. Bone marrow biopsy specimens obtained at diagnosis were stained with anti-CXCR4 antibody (1:500, clone 44716, R+D Systems, Minneapolis, MN). Response to therapy and overall and progression-free survival data were obtained with most recent follow-up in June 2005.

Results: There were 44 men and 43 women with a median age of 63 years (range, 18-82). Median follow-up was 53 wks (range, 1-223). All patients received intensive chemotherapy according to institutional protocols. Fifty-six patients achieved complete remission. Twenty-four patients relapsed. Forty-four patients died, including 10 patients with no evidence of disease. CXCR4 was negative in 17 and positive in 60: predominantly nuclear in 47, and predominantly cytoplasmic in 23. The complete remission rate did not correlate with CXCR4 staining (55% predominantly nuclear, 65% predominantly cytoplasmic, and 82% negative). Expression of CXCR4 (cytoplasmic or nuclear) correlated with poorer overall survival as shown in figure 1 (p=0.002, log rank). Predominantly cytoplasmic (n=17) versus predominantly nuclear (n=26) CXCR4 expression correlated with poorer progression-free survival (p=0.03, log rank).



Conclusions: CXCR4 expression and localization correlated with overall and progression-free survival in this group of AML patients. These data suggest that CXCR4 expression predicts prognosis independently of flt3 gene mutation status. Novel therapeutic approaches targeting CXCR4 may benefit patients with AML.

1087 Activated STAT3 but Not MAP Kinase p38 Is Expressed in Acute Promyelocytic Leukema (APL)

S Konoplev, R Luthra, G Rassidakis, LJ Medeiros, P Lin. UT MD Anderson Cancer Center, Houston, TX.

Background: APL is typically characterized by the t(15;17)(q22;q22) resulting in *PML/RARa* fusion. Studies of animal models have shown that *PML-RARa* alone is insufficient for leukemogenesis and requires an additional "hit". *FLT3* gene mutations are the most common secondary genetic aberration in APL and are more common in the microgranular variant (M3v) than the hypergranular variant (M3). *FLT3* mutations are known to collaborate with *RML-RARa* to induce APL, however, the mechanisms involved are unknown. Since signal transducer and activator of transcription 3 (STAT3) and mitogen-activated protein kinase (MAPK) are targets of *FLT3* signaling, (Nat Rev Cancer 2003;3:650-65) we assessed expression of activated/phosphorylated STAT3 (pSTAT3) and MAPK in APL.

Design: We included cases of APL in which the t(15;17)(q22;q22) and *PML/RARa* fusion were detected in the bone marrow aspirate samples by conventional cytogenetics, fluorescence *in-situ* hybridization (FISH) and/or reverse transcription-polymerase chain reaction (RT-PCR). *FLT3* gene mutations of either internal tandem duplication (ITD) or D835 point mutations (PM) type were detected by fluorescent-based PCR. The corresponding bone marrow biopsy specimens were stained with monoclonal antibodies specific for pSTAT3 (Santa Cruz Biotechnology, Santa Cruz, CA) and MAP kinase p38 (Cell Signaling Technology, Beverly, MA).

Results: The study group included 11 men and 16 women with a median age of 38 years (range 20-74). The median follow-up was 25 months (range 1-86). Seven patients died, 20 were alive, and the 5-year overall survival was 74%. *FLT3* gene mutations (6 ITD and 2 PM) were identified in 8 of 20 (40%) cases assessed. pSTAT3 was expressed in 15 of 27 (56%) cases. MAPK p38 was not expressed in any cases studied. pSTAT3 expression did not correlate with *FLT3* gene mutations (p=0.373), short or long forms of *PML/RARa* transcript (p=0.682), M3v morphology (p=0.658), or overall survival (p=0.426).

Variant	Total	MAPK p38	pSTAT3	FLT3 mutation	PML/RARa
M3	22	0/22 (0%)	12/22 (55%)	6/16 (38%)	13 long/ 7 short
M3v	5	0/5 (0%)	3/5 (60%)	2/4 (50%)	1 long/ 3 short

Conclusions: Activated STAT3 is expressed in approximately half of APL cases. pSTAT3 expression did not correlate with *FLT3* gene mutations in this study, suggesting that pSTAT3 expression is mediated by mechanisms other than *FLT3* gene mutations. MAPK p38 is unlikely to be involved in APL pathogenesis.

1088 Overexpression of bFGF Is Frequent in Megakaryocytes of Chronic Myeloproliferative Disorders and MDS/AML with Fibrosis, but Is Not Correlated with the Presence of the JAK2 VAL617Phe Mutation

M Kremer, T Horn, A Tzankov, T Dechow, L Quintanilla-Martinez, F Fend. Technical University, Munich, Germany; University, Innsbruck, Austria; GSF Research Center, Neuherberg, Germany.

Background: Megakaryocyte-derived basic fibroblastic growth factor (bFGF) has been implicated in the pathogenesis of bone marrow (BM) fibrosis in chronic myeloproliferative disorders (CMPD). This prompted us to investigate the potential role of bFGF in myelodysplastic syndromes/acute myeloid leukaemia (MDS/AML) with fibrosis in comparison to CMPD. Since JAK2 is downstream of bFGF, we additionally investigated the correlation between bFGF expression and the recently described VAL617Phe JAK2 mutation, which is frequent in Ph- CMPD.

Design: Immunohistochemical analysis of bFGF in paraffin-embedded trephine biopsies was performed in 25 MDS/AML with fibrosis (4 MDS unclass., 4/11 RAEB1/2, 6 AML), 19 MDS/AML without fibrosis, and 29 CMPD. 19 idiopathic thrombocytopenic purpura and 4 normal BM trephines served as negative controls. The percentage of megakaryocytes showing nuclear bFGF staining was scored. Presence of the VAL617Phe Jak2 mutation was detected by mutation-specific primers using DNA extracted from the BM trephines.

Results: Based on the findings in normal BM, nuclear expression of bFGF in >10% of megakaryocytes was considered as positive. BFGF was detected in 72% of MDS/AML with fibrosis compared to 21% in MDS/AML without fibrosis (p<0.005). Notably, RAEB-1/2 showed almost identical bFGF positivity as AML with fibrosis, with 80% and 83% respectively. BFGF expression was frequent in CMPD, with 100% in essential thrombocythemia (ET), 88% in polycythemia vera (PV) and 90% in osteomyelofibrosis (OMF), with CML being negative. The Val617Phe JAK2 mutation was detected in 50% of ET, 87% of PV, 81% of OMF, and in one Ph- CML, respectively, but was negative in MDS/AML. JAK2 mutations were mostly associated with bFGF overexpression, except for five cases showing either bFGF without associated JAK2 mutation (4 cases) or vice versa (1 case).

Conclusions: Nuclear overexpression of bFGF in MDS/AML is strongly associated with the presence of fibrosis. Besides the induction of fibrosis, its nuclear accumulation is possibly also involved in the increased proliferation of megakaryocytes, since CMPD without fibrosis also revealed overexpression of bFGF. Our preliminary data also suggest that bFGF expression and JAK2 mutations are independent factors in the pathogenesis of CMPD.

1089 Usefulness of Core Biopsy in the Diagnosis and Follow up of Malignant Lymphomas. A Multidisciplinary Approach in 100 Patients

JI López, JL del Cura, R Zabala, A Fdez-Larrinoa, MV García-Menoyo, E Fuertes, FJ Bilbao. Basurto Hospital, Basque Country Univ (UPV/EHU), Bilbao, Spain.

Background: The role of ultrasound-guided core biopsy (USCB) in the diagnosis and management of malignant lymphomas (ML) is a controversial issue that has not been accepted generally. The purpose of this study is to evaluate its usefulness and limitations in the diagnosis of MLs and in the identification of their recurrences.

Design: Over a 5-year period (2000-2004), 100 MLs were biopsied under ultrasound control with 18G BioPince® needles. If needed, molecular studies [IgH, TCR, t(14;18) MBR region] were done. Four diagnostic categories were considered [large B-cell lymphomas (LBCL), small B-cell lymphomas (SBCL), Hodgkin's disease (HD), and miscellaneous]. Conventional lymph node biopsy was done in 47 cases for diagnostic accuracy. Clinical data and haematological studies were the diagnostic reference in the remaining cases.

Results: Overall, USCB diagnosis of lymphoma was accurate in 88 cases. Among them, LBCL (36 cases), SBCL (34 cases), HD (15 cases), and miscellaneous (15 cases) [including T-cell (5), T-cell-rich B cell (2), NK cell (1), Burkitt (1), MALT (1) lymphomas and ML, NOS (5)] were diagnosed. Three HDs, 1 NK cell lymphoma, 1 follicular lymphoma, and 1 LBCL were not correctly diagnosed by the pathologists. In addition, the radiologists did not reach the target in 6 cases. Post-punction relevant secondary complications were not observed in any case.

Conclusions: USCB is a safe, efficient and reliable method in the diagnosis of MLs. The diagnostic success greatly depends on tight clinico-pathological communication. This procedure should be especially considered as the first diagnostic step in complicated clinical situations in which a conventional lymph node biopsy may be technically difficult or risky for the patient.

1090 Somatic Hypermutation of the Immunoglobulin Heavy Chain (*IgH*) Gene in Mantle Cell Lymphoma

R Lai, SV Lefresne, BC Franko, X Shi, D Hui, I Mirza, A Mansoor, HM Amin, Y Ma. U of Alberta, Edmonton, AB, Canada; U of Calgary and Calgary Laboratory Services, Calgary, AB, Canada; U of Texas MD Anderson Cancer Center, Houston, TX; Nevada Cancer Institute, Las Vegas, NV.

Background: Mantle cell lymphoma (MCL) is a distinct subtype of B-cell neoplasm characterized by the t(11;14)(q13; q32) abnormality and cyclin D1 overexpression. A few previous studies have analyzed the somatic hypermutational status of *IgH* in MCL, and results from the two larger studies suggest that the vast majority of MCL tumors have unmutated *IgH*, which correlates with the 'antigen-inexperienced' phenotype. However, the PCR primers employed in these two studies may only detect a relatively restricted repertoire of *IgH* rearrangements.

Design: To fully assess the rearranged *IgH* repertoire in MCL, we employed two primer sets, FR256/J_H an FR1c/J_H, both of which have been previously shown to be highly sensitive in amplifying rearranged *IgH*. We employed formalin-fixed, paraffin-embedded tissues from 40 cases of MCL, all of which were diagnosed according to the World Health Organization Classification Scheme. Amplifiable PCR products were subcloned, and 5 subclones from each case were sequenced. Interpretation of the somatic hypermutation status was determined according to published methods.

Results: Monoclonal *IgH* bands were identified in all 40 cases. An average of 4.1 interpretable DNA sequences were obtained. At the time of writing this abstract, analysis of the data was completed in 21 cases. 13 of 21 (62%) cases had at least one clone that was hypermutated and were classified as the 'memory B-cell' phenotype. Five of these 12 cases had hypermutated clones as the predominant species (i.e. ≥50% of the clones). Furthermore, we identified evidence of clonal evolution in 10 of 21 (48%) cases and 8 of these cases had the 'post-germinal center B-cell' phenotype. Clinically, the average age of these 21 patients was 68 years, with 18 men and 3 women. With this relatively small sample size (i.e. 21), cases with the 'memory B-cell' phenotype showed a trend for better clinical outcome (p=0.24, Kaplan Meier).

Conclusions: In contrast with the previously published data, we found that a significant proportion of MCL has hypermutated *IgH* and clonal evolution. Our data suggest that the somatic hypermutational status of MCL may be predictive of the clinical outcome.

1091 Immunophenotyping and Clonality Analysis in Diagnosis of Refractory Coeliac Disease

H Liu, R Brais, K Payne, Y Huang, H Ye, CM Bacon, J Woodward, M-Q Du. Addenbrooke's NHS Trust, Cambridge, United Kingdom; West Suffolk Hospital, Bury St Edmunds, United Kingdom; University of Cambridge, United Kingdom.

Background: A small proportion of coeliac disease (CD) patients who fail to improve after a gluten-free diet may develop into refractory CD (RCD) but identifying these patients is often difficult on clinico-pathological grounds. Although abnormal immunophenotype (cytCD3$^+$CD8$^-$) and monoclonality of intraepithelial T-cells (IEL) are associated with RCD, their specificity and sensitivity in diagnosis of RCD remain to be evaluated.

Design: Consecutive duodenal biopsies were retrieved from 11 CD, 6 "suspected RCD" (with clinical symptoms or abnormality in serology or histology but not fulfilling the criteria for RCD), 11 RCD and 2 enteropathy-type T-cell lymphoma (ETL) cases. The histology was reviewed and IEL phenotype examined by CD3/CD8 double immunostaining. T cell clonality was determined by PCR of the rearranged TCR genes with BIOMED-2 protocols using DNA samples from whole tissue sections or microdissected IEL or lamina propria lymphocytes. In one ETL, clone specific PCR was performed to trace the tumour cells in intestinal biopsies before ETL diagnosis.

Results: All biopsies from 11 CD cases showed normal IEL phenotype and polyclonal TCR gene rearrangement. Of 6 cases of "suspected RCD", 5 showed abnormal immunophenotype (cytCD3$^+$CD8$^-$), of which 2 displayed persistent identical monoclonality in follow-up biopsies, 1 displayed transient monoclonality and the other 2 were polyclonal. The remaining "suspected RCD" case showed normal immunophenotype and polyclonal T cells. Among 11 RCD cases, 4 showed an abnormal immunophenotype and 2 of them displayed persistent identical monoclonality. The remaining 7 RCD cases showed normal immunophenotype, including 2 with persistent identical monoclonality and 1 with transient monoclonality. In one ETL, tumour-related clonal T-cells were detected in histologically and immunophenotypically tumour-free duodenal biopsies 4 years before the lymphoma diagnosis. Interestingly, the clonal T-cells were found in both IEL and lamina propria in 4 RCD and 1 ETL cases.

Conclusions: Abnormal immunophenotype (cytCD3$^+$CD8$^-$) and monoclonality are not a feature of classic CD, but are only associated with 36% and 45% of RCD respectively, with 64% of cases showing either of the abnormalities. Combined immunophenotypic and clonality analysis would maximise their potential utility in RCD diagnosis and in identification of CD cases at risk of progression.

1092 Cyclin D1- and t(11;14)-Positive B-Cell Neoplasms Resembling Marginal Zone B-Cell Lymphoma – A Morphologic Variant of Mantle Cell Lymphoma

A Mansoor, M Akbari, I Auer, R Lai. U of Calgary and Calgary Laboratory Service, Calgary, AB, Canada; U of Alberta, Edmonton, AB, Canada.

Background: Mantle cell lymphoma (MCL) is a distinct subtype of aggressive B-cell neoplasm characterized by positivity for CD5 and/or CD43, cyclin D1 overexpression and the t(11;14) abnormality. Most cases of MCL, labeled as the classical variant, are composed of a monotonous population of lymphoid cells of small-to-medium cell size, arranged in a nodular, diffuse or mantle zone pattern. A 'marginal-zone B-cell' or 'monocytoid B-cell' variants have been briefly mentioned in the World Health Organization Classification of Hematopoietic Neoplasm, but details of these variants have not been fully described in the literature.

Design: We described the morphologic, immunophenotypic and molecular features of 3 cases of marginal-zone lymphoma (MZL)-like variant of MCL. Imunohistochemistry (IHC) was performed using formalin-fixed, paraffin-embedded tissues, standard antigen-retrieval and IHC techniques. PCR and FISH detecting the t(11;14) were done using previously published methods.

Results: The pathologic features of these 3 cases are summarized in the Table. All 3 tumors were composed of a monotonous cell population of monocytoid B-cells, characterized by a medium-cell size, abundant clear cytoplasm, and irregular nuclear contours. Large cells were conspicuously absent in all cases. Plasmacytoid features were recognized in case 1. Two cases were examined for Ki-67 labeling by IHC; which showed 60% and 30% positive cells for case 1 and 3, respectively. Aggressive clinical behavior was observed in all 3 cases, with one patient (case 3) presented in the leukemic phase.

Conclusions: We described 3 cases of MZL-like B-cell neoplasm which are best classified as MCL, based on the cyclin D1 expression, t(11;14), and their aggressive clinical behavior. The absence of CD5/CD43 positivity does not exclude the diagnosis of MCL, and the conspicuous absence of large lymphoma cells in a MZL-like tumor should prompt the inclusion of MCL as a differential diagnosis.

PATHOLOGIC FEATURES OF 3 CASES OF MZL-LIKE MCL

CASE #	AGE	SEX	BIOPSY SITE	INFILTRATION PATTERN	IHC	t(11;14) BY PCR	t(11;14) by FISH
1	73	M	LEFT AXILLARY LYMPH NODE	nodular, with many residual germinal centers	POSITIVE: CD20, cyclin D1; NEGATIVE: CD5, CD43, CD23	YES	ND
2	59	M	CERVICAL LYMPH NODE	diffuse	POSITIVE: CD20, cyclin D1; NEGATIVE: CD5, CD43, CD23	YES	YES
3	75	M	SALIVARY GLAND	diffuse	POSITIVE: CD20, cyclin D1, CD5, CD43; NEGATIVE: CD23	NO	YES

ND = not determined

1093 Utility of Combined Fine-Needle Aspiration / Core Biopsy and Flow Cytometry in Diagnosing and Subclassifying Lymphoma

I Mansoor, B Nelson, C Goolsby. Northwestern Memorial Hospital, Chicago, IL.

Background: The primary diagnosis of non-Hodgkin's lymphoma (NHL) based on fine-needle aspiration/core biopsy (FNABX) is controversial. We reviewed our experience with FNABX and flow cytometry (FC) to determine their usefulness and limitations in diagnosis and classification of NHL according to World Health Organization scheme and to differentiate them from reactive conditions.

Design: A computer search of our pathology archives was performed for FNABX of all lymph node (LN) and extranodal tissue that had FC, performed during the period 01/04 to 08/05 to evaluate for lymphoma. Clinical findings and any subsequesnt follow-up biopsy were analyzed.

Results: 210 consecutive FNABX with flow cytometery were done to evaluate for NHL. 69 lymphomas (36 Primary DX, 33 recurrent DX), 5 multiple myeloma, 104 benign / reactive, 6 LN with metastatic carcinoma and 26 inconclusive studies. FC yielded meaningful results in 178 (85%) cases. Anatomic sites were neck LN 127, abdomen / reteroperitonium 37, axilla 4, thorax / mediastinum 22, groin LN 5, thyroid 6, Spine / paraspinal 8 and orbit 1. 26/184 (14%) diagnostic cases had follow-up biopsy. The biopsy findings concurred with the initial FNABX/FC diagnosis in 17 NHL and 9 reactive cases. 17/26 (65%) cases with insufficient material for FC had follow-up biopsy and 7 showed metastatic carcinoma, 10 reactive conditions. Lymphoma was subclassified in 57/69 (83%) cases (follicular 23, CLL 11, MZL 4, DLBCL 17, MCL 2) and 12/69 cases with monoclonal B cells were classified as B cell lymphoma, NOS. Material collected through FNABX was adequate to run a mean number of 10 markers per case (range 4-26, SD 6.5) and a mean number of 3 (range 1-6, SD 1.7) for FC studies.

Conclusions: FNA combined with FC is useful for diagnosing primary and recurrent NHL. NHL can be accurately diagnosed using FNABX and FC combined and up to 83% can be subclassified according to the WHO scheme. Small sample size precluded grading of all Follicular lymphoma and FC in minority of cases.

1094 Frequency of *BCL2* Breakpoints in Follicular Lymphoma and Correlation with Histologic Grade

MR Mariappan, C Shum, DA Arber. Stanford University Medical Center, Stanford, CA.

Background: Follicular lymphoma is characterized by the presence of the t(14;18), in which *BCL2* is juxtaposed to *IGH*. The most common translocations studied involve the major breakpoint region (*MBR*), and minor cluster region (*mcr*), but three other breakpoints also occur with some frequency (*ICR*, *3'BCL2*, *5'mcr*). This study attempts to correlate histologic grade in follicular lymphoma with the five different breakpoints.

Design: 221 cases of follicular lymphoma diagnosed between 1983 and 1997 at Stanford University were used for the study. DNA was extracted from frozen material and real-time PCR was performed with primers targeting *MBR*, *mcr*, *3'BCL2*, *5'mcr* and *ICR* breakpoints in *BCL2* and *JH* primers. Fluorescence in situ hybridization (FISH) using a breakapart probe targeting *BCL2* (Vysis, Downers Grove, IL) was carried out in cases that were PCR negative. Available archived slides were reviewed to confirm the histologic grade, using WHO criteria.

Results: Correlation between histologic grade and breakpoints, as evaluated by PCR, is shown in Table 1. PCR evidence of t(14;18) was detected in 170 cases (76.9%). *MBR* (112) followed by *ICR* (31) were the most common breakpoints. Of the 51 cases that were negative for the studied breakpoints by real time PCR analysis, 37 cases were positive by FISH analysis using the *BCL2* breakapart probe, suggesting the presence of alternative *BCL2* breakpoints. The combined FISH and PCR results identified 207 cases (93.7%) with evidence of t(14;18). Correlation between histologic grade and PCR negative cases are shown in Table 2. Non-parametric testing (two-way ANOVA) for significant differences between histologic grade and the 5 PCR breakpoints was not significant. However, the PCR negative group was significantly associated with grades 2 and 3 (p=0.006). .

Conclusions: Evidence of the t(14;18) was detected in 93.7% of follicular lymphomas with the combined PCR and FISH method. Follicular lymphomas that are negative for the five most commonly described *BCL2* breakpoints by PCR tend to have a higher histologic grade (grade 2 or 3). Most of these cases have FISH evidence of a *BCL2* translocation, suggesting the presence of alternative *BCL2* breakpoints.

Correlation of breakpoints with histologic grade

	MBR	ICR	3'BCL2	mcr	5'mcr	Total
Grade 1	43	17	4	5	2	71
Grade 2	47	6	5	3	1	62
Grade 3	22	8	4	3	0	37
Total	112	31	13	11	3	170

BCL2 PCR negative cases

	BCL2 FISH positive	BCL2 FISH negative
Grade 1	13	7
Grade 2	13	1
Grade 3	11	6

1095 Correlation of KIT D816V Mutation Analysis and Immunophenotypic Analysis of Bone Marrow Aspirates in Patients Evaluated for Systemic Mastocytosis

I Maric, A Siddon, W Fu, J Stoddard, J Robyn, D Metcalfe, P Noel. CC/NIH, Bethesda, MD; NIAD/NIH, Bethesda, MD.

Background: Systemic mastocytosis is a rare disorder characterized by abnormal accumulation of mast cells in bone marrow and other organs. Recent data indicate that it is a clonal disorder of mast cells, and the presence of point mutation in c-kit receptor was demonstrated. Some studies have shown that neoplastic marrow mast cells also display aberrant immunophenotypic characteristics, most notably CD2 and CD25. In our study, we have examined expression patterns of CD2, CD25 and multiple other surface antigens in marrow mast cells obtained from patients evaluated for KIT D816V point mutation. In addition, we have analyzed maturation patterns of other hematopoietic cell lineages in these patients and compared them with normal bone marrow maturation patterns.

Design: Patients undergoing evaluation in our institution for mast cell disease were prospectively studied. Their bone marrow biopsies were histologically evaluated for WHO major diagnostic criteria for systemic mastocytosis (presence of multifocal collections of 15 or more mast cells). The presence of KIT D816V point mutation was investigated in marrow aspirates and peripheral blood samples by RT-PCR RFLP. Immunophenotypic analysis of marrow aspirates was performed by multiparameter flow cytometry.

Results: Total of 24 patients was included in this study. 17 patients were positive for KIT D816V mutation and 7 patients were negative. 15 of 17 patients positive for KIT D816V mutation met major WHO histological criteria for systemic mastocytosis. Immunophenotyping showed that in all patients with detectable mutation, marrow mast cells were positive for CD2, CD25, CD11c and CD35. In addition, high levels of expression of CD59, CD63 and CD69 were noted. All 7 patients negative for KIT D816V mutation did not show aberrant expression of the above antigens and did not meet histological criteria for systemic mastocytosis. The marrow aspirates from patients positive for mutation also showed increase in number of eosinophils and immature B-cells compared to normal marrows and negative patients.

Conclusions: In our patient population, we have found complete correlation between the presence of KIT D816V mutation in marrow aspirates and the expression of CD2, CD25, CD11c and CD35 antigens on marrow mast cells. In addition, high levels of CD59, CD63 and CD69 antigen expression were noted in these patients.

1096 Dlk1 and MNDA Protein Expression in Myeloid Progenitors: Utility of Dlk1 as an Immunohistochemical Marker of the Megakaryocytic Lineage

SA McClintock-Treep, MH Jagasia, SA Goodman, RC Briggs, DR Head. Vanderbilt University Medical Center, Nashville, TN; Veterans Administration Hospital, Nashville, TN.

Background: Delta-like protein (Dlk1) is a member of the epidermal growth factor-like family. Studies have shown that Dlk1 message was more than double in more than half of MDS patients versus healthy controls. Only a minority of AML patients had elevated Dlk1 expression; interestingly, these cases demonstrated at least bilineage dysplasia and were presumably MDS-related AML. Thus, upregulation of Dlk1 may distinguish MDS from other disorders. Other studies have shown increased Dlk1 message in the megakaryocyte lineage. Myeloid Nuclear Differentiation Antigen (MNDA), a nuclear protein expressed in myeloid precursors, regulates Dlk1 in a manipulated cell line. MNDA message is reportedly decreased in MDS patients. Therefore, MNDA and Dlk1 are implicated in MDS pathogenesis, but their inter-relationship is uncertain. We examined expression of Dlk1 and MNDA protein in MDS and AML.

Design: Marrow samples of patients with MDS (10), acute megakaryocytic leukemia (3), and controls (10) were analyzed. Clinical information and morphology were reviewed blinded to study results. Anti-human Dlk1 polyclonal antibodies (C-19 goat and H118 rabbit; Santa-Cruz Biotechnology, Santa Cruz, CA, USA) and anti-human MNDA monoclonal antibody (rat antibody; Calbiochem, San Diego, CA, USA) were incubated for 1½ hours with deparaffinized sections. Reactivity was detected with avidin-biotin immunoperoxidase (Vector Laboratories, Inc., Burlingame, CA) and reviewed for expression of MNDA and Dlk1.

Results: Controls revealed uniform high MNDA expression in G-M cells. Mononuclear cells were negative for Dlk1, but megakaryocytes revealed strong cytoplasmic staining. Dlk1 staining highlighted megakaryocytes in MDS samples, but was negative in other cells. MNDA staining of mononuclear cells in MDS was lower than controls. Finally, acute megakaryocytic leukemia marrows revealed intense cytoplasmic reactivity with Dlk1, with only rare MNDA reactivity.

Conclusions: Dlk1 and MNDA proteins are not co-expressed in marrow cells. Dlk1 protein is expressed in megakaryocytes and megakaryoblasts but not in other marrow cells. It is not a marker of MDS; however, it may be useful as a megakaryocytic lineage marker. MNDA protein expression is decreased in myeloid progenitors in MDS and may be a useful marker of MDS.

1097 Comparison of Lichen Planus Versus Mycosis Fungoides: An Immunohistochemical Study

SA McClintock-Treep, KS Hamilton, SG Bregman, SJ Olson, KS Parman, MM Zutter. Vanderbilt University Medical Center, Nashville, TN; St Thomas Hospital, Nashville, TN.

Background: Mycosis fungoides (MF) is largely indolent; however, it has been reported that 8-23% of patients undergo transformation to a large cell lymphoma which is a more aggressive form of the disease. Studies have looked at expression profiles of MF as a group versus other non-Hodgkin lymphomas; however, few have studied cell cycle regulator expression in contrast to transformed MF and benign cutaneous lymphoid disorders. This study aims to analyze expression of bcl-2, junB, myc (p62), STAT3 and p53 in cases of MF, transformed MF, and lichen planus with the intention of discovering immunophenotypic trends between the three groups.

Design: The initial study set consisted of 16 MF patients (6 with transformation, 10 without transformation) and 12 skin biopsies from non-neoplastic controls (lichen planus). Immunohistochemical studies for expression of bcl-2, junB, STAT3, p53 and myc were performed. After the sections were blindly graded, the respective diagnoses were revealed to determine possible relationships.

Results: Expression of myc was negative in all 12 cases of lichen planus, 8/10 MF cases and 5/6 cases of MF with transformation. JunB was strongly expressed in all lichen planus cases, 8/10 MF cases and 5/6 transformed MF cases. Bcl-2 was expressed in all but one case of lichen planus. The non-transformed cases were largely negative (8/10),

while 4/6 of transformed cases were bcl-2 positive. All cases of lichen planus and 6/10 MF cases were negative for p53, while 5/6 cases of transformed MF showed strong p53 positivity. All cases, with the exception of one transformed MF, were strongly STAT3 positive.

Conclusions: The initial aim of this study was to collect immunohistochemical data in order to establish a profile which is predictive of transformation in MF; alternatively, it was found that "neoplastic markers" were also expressed in benign cutaneous lymphoid conditions. Expression of the selected cell cycle markers were not found to delineate differences between non-transformed and transformed MF.

	bcl-2	p53	junB	myc	STAT3
Normal Controls (+)	92%	0%	100%	0%	100%
Normal Controls (-)	8%	100%	0%	100%	0%
MF (+)	20%	40%	80%	20%	100%
MF (-)	80%	60%	20%	80%	0%
Transformed MF (+)	67%	83%	83%	17%	83%
Transformed MF (-)	33%	17%	17%	83%	17%

1098 *JAK2*V617 Mutational Analysis for the Diagnostic Work-Up of Chronic Myeloproliferative Disorders

C McMahon, JA Kant, FE Craig, SH Swerdlow, SA Monaghan. University of Pittsburgh School of Medicine, Pittsburgh, PA.

Background: Analysis for *BCR/ABL* greatly facilitates the diagnosis of chronic myeloid leukemia (CML). Establishing the diagnosis of other chronic myeloproliferative disorders (CMPDs) is often more difficult. Recently, a mutation in a cytoplasmic tyrosine kinase, *JAK2*, has been reported in a significant number of well-established non-CML CMPDs . To determine the diagnostic utility of *JAK2* mutational analysis, we compared morphology, cytogenetics and mutational status in *BCR/ABL* negative bone marrow examinations performed for diagnostic evaluation of suspected CMPD.

Design: Archival DNA from bone marrow samples submitted for *BCR/ABL* testing were analyzed for *JAK2* V617F mutation by allele-specific PCR. Cases that had been positive for *BCR/ABL* were excluded. Based on blind review of blood and bone marrow slides and basic clinical data, the morphologic and clinical features were tabulated and the cases were classified based on the strength of evidence for diagnosis of a purely myeloproliferative disorder: MPD 1 = features do not support a diagnosis of primary myeloid disorder, or strongly suggest another entity such as myelodysplastic/ myeloproliferative disease (MDS/MPD); MPD2 = characteristic for CMPD, but non-specific; MPD3 = findings strongly support CMPD without evidence of MDS/MPD.

Results: The *JAK2* mutation was detected in 8 of 22 samples (36.4%) for which bone marrow examinations were available for review. Most of the cases with strong morphologic evidence for a CMPD had *JAK2* mutations (4 of 5). Positive *JAK2* mutation clarified the diagnosis in 2 of 4 cases where the morphology was considered compatible, but not specific for CMPD (MPD2). Of the cases that had little morphologic evidence of CMPD (MPD1), *JAK2* mutation was found in 2 of 13 cases (15%). One such case was interpreted as likely reactive, but was found to have trisomy 8 and *JAK2* mutation. Another case with a del 20q12 and *JAK2* mutation was interpreted as acute myeloid leukemia likely evolving from a MDS/MPD.

Morphologic CMPD scores and JAK2 V617F status

MPD score:	1	2	3	total
JAK2 V617F Positive	2	2	4	8
JAK2 V617F Negative	11	2	1	14

Conclusions: *JAK2* mutational analysis is an important addition to the workup of possible CMPDs. In many cases that are positive for the *JAK2* mutation, morphologic evaluation of the bone marrow strongly supports a diagnosis of CMPD. However, a minority of patients that did not have morphologic features to suggest a CMPD were nonetheless positive for the *JAK2* V617F mutation.

1099 Expression Levels of Active Caspase 3 Predict Response to Treatment in Diffuse Large B-Cell Lymphomas

M Melachrinou, SF Assimakopoulos, P Matsouka, A Symeonidis, DS Bonikos. Medical School, University of Patras, Patras, Greece.

Background: Diffuse large B-cell lymphomas (DLBCLs), the most common types of lymphomas, represent a biologically and clinically heterogeneous group. Although these neoplasms can respond to chemotherapy, a large number of patients are still resistant to treatment. In vitro studies suggest that resistance to chemotherapy may be caused by inhibition of the apoptotic machinery. The aim of this study was to investigate whether the expression of apoptosis-regulating proteins in DLBCLs is related with the rate of apoptosis and may predict tumor chemosensitivity.

Design: Formalin-fixed, paraffin-embedded tissue from 39 cases of DLBCLs were immunostained with antibodies for bcl-2, mcl-1, bax, caspase 3 (CPP32), active caspase 3 and PARP-1 using Envision detection kit. The rate of apoptosis as measured by DNA fragmentation was evaluated in each case applying TUNEL assay. The percentage of TUNEL-positive cells to the total number of the tumor cells measured in four high power fields was reported as apoptotic index (AI). Clinical data were available for 29 cases (19 chemosensitive, 10 chemoresistant).

Results: Bcl-2, mcl-1, bax, CPP32 and PARP-1 immunostaining, in >10% of tumor cells, was detected in 71.8%, 92.3%, 89.7%, 61.5% and 92.3% of cases, respectively. Positive immunostaining for active caspase 3 was detected in all cases. The percentage of active caspase 3-positive cells ranged from 0.62% to 16.71% (median, 5.11%). A significant higher immunoexpression of active caspase 3 was identified in chemosensitive cases (10.52%, mean) compared to chemoresistant (2.75%) ($p = 0.009$). The AI ranged from 0.02% to 24.08% (median, 6.85%). A significantly higher AI was shown in cases with complete response to treatment (10.04%, mean) than in resistant cases (5.04%) ($p = 0.006$). A positive correlation was found between the immunoexpression of active caspase 3 and the AI ($r = 0.675$, $p = 0.032$).

Conclusions: Among the studied apoptosis-related proteins only active caspase 3 exhibited a significant positive correlation with AI. Cases with complete response to treatment showed significantly higher apoptotic levels compared to these with resistant disease, as detected either by TUNEL assay or expression of active caspase 3. High numbers of active caspase 3-positive tumor cells predict a favorable response to chemotherapy in DLBCL patients.

1100 Myelodysplastic Syndromes Post-Solid Organ Transplantation: Therapy Related Myelodysplasia or A Consequence of Immunosuppression?

M Menes, E Vakiani, C Keller, V Murty, B Alobeid, G Bhagat. Columbia University, New York, NY.

Background: Hematopoietic disorders post-solid organ transplantation are well recognized and have a multifactorial etiology. There is, however, a growing awareness of an increased risk for myelodysplastic syndromes (MDS) or acute leukemias (AL) post transplantation, especially in patients treated with azathioprine. The aim of our study is to describe the morphologic spectrum of marrow findings, cytogenetic abnormalities, and clinical course of patients who developed MDS post-solid organ transplantation.

Design: We encountered 5 patients, over a 10-yr period (1994-2004), who had undergone solid organ transplantation and fulfilled the clinical and pathologic criteria for MDS. Morphology was assessed on H&E stained sections of BM biopsies and Wright-Giemsa stained aspirate smears. G-band karyotype analysis was performed in 4/5 patients.

Results: The patients, 3 males and 2 females (age 48-64 yrs, mean 58 yrs), had received heart (n=2), lung (n=2), and kidney (n=1) transplants 1.8-21 yrs (mean 7.4 yrs) prior to diagnosis of MDS. Immunosuppressive regimens consisted of cyclosporine, FK506, and prednisone (n=4) and azathioprine and prednisone (n=1). Three of 5 patients presented with peripheral cytopenias and 1 with neutrophilia. Marrow cellularity ranged from hypocellular (n=2) to hypercellular (n=3). According to the WHO criteria the MDS were classified as refractory anemia (n=1), refractory cytopenia with multilineage dysplasia (n=2), RAEB-1 (n=1), and MDS/MPD unclassifiable (n=1). Cytogenetic abnormalities included del(20)(q11) (n=2), complex karyotype including del(20)(11) and –5 (n=1), and del(5q)(q12q22) and del(13)(q14) (n=1). None received any treatment for MDS and none developed AL in the follow up period. Three patients are alive (2, 3.6, and 8 yrs post diagnosis), 1 patient was lost to follow-up, and 1 died due to transplant related complications 6 months post diagnosis.

Conclusions: We observed a spectrum of MDS in post transplant patients that were associated with both good and poor risk cytogenetic abnormalities. Previously reported chromosomal abnormalities due to prolonged azathioprine use were not observed. Our findings raise the question whether MDS post transplantation are the result of drug toxicity, representing "therapy related" disorders, or the consequence of defective immune surveillance due to prolonged immunosuppression.

1101 Splenic Marginal Zone Lymphoma. Searching for Diagnostic and Prognostic Markers

M Mollejo, FI Camacho, L Sanchez-Verde, E Ruiz-Ballesteros, P Algara, J Menarguez, MA Cruz, MA Piris. Hospital Virgen de la Salud, Toledo, Spain; Centro Nacional de Investigaciones Oncologicas, Madrid, Spain; Hospital Gregorio Marañon, Madrid, Spain.

Background: Splenic Marginal Zone Lymphoma lacks diagnostic and prognostic markers that could facilitate its consistent diagnosis and risk-adapted clinical management. Thus, the diagnosis of SMZL is often hindered by the lack of specific molecular markers. Once diagnosed, most cases run an indolent clinical course; however there is a subset of cases who have a more aggressive clinical behaviour

Design: A series of 108 SMZL cases was included in this study. Immunostaining for Cyclin D3, MUM1, SYK, TCL1, CD38, MIB1, P53, ZAP70, CD138, IGD, Cyclin D1, BCL6 and other B-cell markers was performed. The results were related to IgVH mutational status and outcome. A series of non-SMZL 89 cases small B-cell lymphomas involving the spleen was included for comparison purposes

Results: The study revealed that SMZL expresses some markers absent in normal marginal zone B-cells in the spleen, such as TCL1 (62% cases), SYK (61%), MUM1 (27%), ZAP70 (47% cases) CD38 (55%), CyclinD3 (14%) and p53 (6%). All the SMZL here analyzed were negative for Bcl6 and Cyclin D1. The expression of CD38, ZAP70 and the absence of IgVH mutations (51% cases) was associated with shorter overall survival.

Conclusions: 1) SMZL cases display a degree of heterogeneity that mimics the observations in B-CLL. 2) CyclinD3 is only an infrequent finding in these cases. 3) TCL1 overexpression as a clue for the analysis of the pathogenesis of SMZL. 4) Multiple immunohistochemical or molecular markers can be used for prognosis.

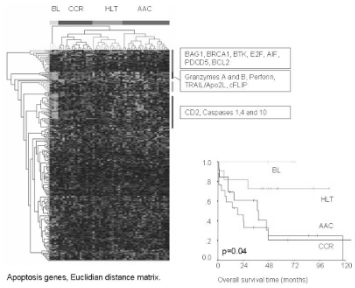
1102 Chemotherapy Refractory Primary Nodal Diffuse Large B-Cell Lymphomas Are Characterized by High Expression Levels of Apoptosis Inducing Genes

JJ Muris, B Ylstra, CJ Meijer, SA Cillessen, GA Meijer, W de Boer, P de Bruin, JJ Oudejans. VU University Medical Center, Amsterdam, Netherlands; Antonius Hospital, Nieuwegein, Netherlands.

Background: Clinical outcome in patients with diffuse large B cell lymphomas (DLBCL) is highly variable and difficult to predict. Resistance to apoptosis of lymphoma cells is a probable mechanism causing intrinsic resistance to apoptosis and eventual fatal clinical outcome.

Design: In this study we investigated whether patients with primary nodal diffuse large B-cell lymphomas can be stratified in groups with different clinical outcome by genome wide expression profiling and by profiling of only apoptosis inducing, regulating and executing genes. For this in duplicate spotted 15 K oligonucleotide micro arrays were used to investigate genome wide expression levels of 48 DLBCL

samples. Profiling of apoptosis genes was done by hierarchical clustering of the selected apoptosis related genes.
Results: Hierarchical clustering of the 10% genes with strongest variance resulted in accurate identification of GCB and ABC like DLBCL and a third group characterized by an intense host response. Unsupervised clustering of only the set of genes involved in apoptosis (N=247) improved clinical stratification with the identification of 1 group with highly favorable clinical outcome resembling hyperplastic lymphoid tissue (HLT) and 2 groups with very poor outcome, one characterized by high expression levels of pro- and anti apoptotic genes involved in stress-induced apoptosis (AAC) and one group characterized by an intense cellular cytotoxicity immune response (CCR). The results were validated using the previously published LLMPP database.
Conclusions: We conclude that expression profiling of apoptosis regulating genes DLBCL results in 3 groups of DLBCL with differences in clinical outcome. The results indicate that chemotherapy refractory DLBCL are characterized by an intense cellular cytotoxic immune response or by constitutive activation of the apoptosis cascade. Moreover, 6 apoptosis inhibiting genes were identified in chemotherapy refractory DLBCL that may be targets for future therapy with specific small antagonistic molecules.



1103 Identification of JAK2 Mutation V617F in Myeloproliferative Disorders Using FRET Probes and Melt Curve Analysis

G Murugesan, MA Verbic, H Szpurka, S Aboudola, ED Hsi, J Maciejewski, K Kottke-Marchant, RR Tubbs. The Cleveland Clinic Foundation, Cleveland, OH.
Background: A single point mutation (V617F) in the cytoplasmic tyrosine kinase JAK2 is an activating clonal mutation implicated in non-CML myeloproliferative disorders (MPD) including polycythemia vera (PV), essential thrombocythemia (ET) and myeloid metaplasia with myelofibrosis (MF). This finding has significant impact on the diagnosis and therapeutic approaches to these diseases. A simple, rapid and reliable molecular test can greatly enhance the diagnosis of MPD. We have developed and validated such a test employing fluorescent probes and melt curve analysis.
Design: A total of 162 DNAs isolated from peripheral blood granulocytes (46) and formalin-fixed bone marrow clot sections (116) in patients with MPD (56) and non-MPD (106) previously genotyped for JAK2 mutation V617F (nucleotide change G>T) were available for the study. Of 162 DNAs, 111 had been genotyped by allele-specific PCR (AS-PCR) and the remaining 51 by direct sequencing. These 162 DNAs were genotyped by melt-curve analysis using the primers and probes designed in-house with the software provided with the LightTyper. As the LightTyper is a high-throughput platform, genotyping either in a 96- or 384-well format, we have also adapted the assay on the LightCycler for a low-throughput and routine analysis.
Results: Genotyping of 111 DNAs by AS-PCR resulted in 68 wild types (GG) and 43 mutants, which included both heterozygotes (GT) and homozygous mutants (TT). Of 51 DNAs sequenced, 46 were wild type and 5 were heterozygotes. In combination, these two methods yielded a total of 114 wild types and 48 mutants. In comparison, the number of wild types (GG), heterozygotes (GT) and homozygote mutants (TT) identified using the LightTyper were: 114, 42 and 6, respectively, a concordance of 100% between FRET probe melt curve analysis and reference genotypes. A sub-set of 30 DNAs, earlier genotyped by sequencing, allele-specific PCR and the LightTyper, were cross-validated using the LightCycler with 100% accuracy and reproducibility. Forty-two (75%) of the MPD (PV, ET and MF) patients had mutation compared to six (6%) non-MPD patients confirming the general trend of this mutation among MPD patients.
Conclusions: We have demonstrated that the JAK2 mutation V617F can be genotyped using FRET probes and melt curve analysis. It is a rapid, reliable and reproducible assay, and can be an effective molecular test in the diagnosis of MPD.

1104 Non-Anaplastic Peripheral T-Cell Lymphomas in Children

T Muzzafar, RE Hutchison, M Link, M Chang, J Laver. SUNY Upstate Medical University, Syracuse, NY; Stanford University Medical Center, Palo Alto, CA; University of Florida, Gainesville, FL; Virginia Commonwealth Univ Health System-MCV, Richmond, VA.
Background: Non-Hodgkin lymphomas (NHL) in childhood and adolescence consist predominantly of Burkitt, lymphoblastic, diffuse large B-cell and anaplastic large cell lymphomas. Non-anaplastic peripheral T-cell lymphomas (PTCL) are rare in young patients and are not well characterized. A high proportion of adult patients with PTCL have poor risk disease with frequent relapse and unfavorable outcome, and it is generally assumed that pediatric PTCL is similar. There is, however, scant data on the spectrum of histologic types or treatment response of PTCL in young patients.
Design: We identified 20 pediatric patients diagnosed with PTCL and negative for CD30 and/or ALK between 1992 and 2000 on one of two treatment protocols of the Pediatric Oncology Group (now of the Children's Oncology Group) for localized NHL (#9219) or advanced stage large cell lymphoma (#9315). All cases were centrally reviewed. The cases were initially categorized as per the Working Formulation and REAL classification. Diagnoses were translated into the WHO classification based on the histology, available immunohistochemistry and clinical findings.
Results: Of the 20 patients, 9 were male and 11 female. The median age was 12.6 years (range 0.7 to 16.9 years). The primary sites of involvement at onset included lymph nodes (5), skin (3), nasal sinuses (3), small intestine (2), soft tissue (2), bone marrow (2), mediastinum (1), breast (1) and liver (1). Histological subtypes included PTCL, unspecified (12), extra-nodal NK/T-cell lymphoma of nasal type (4), subcutaneous panniculitis-like T cell lymphoma (1) and enteropathy-type T- cell lymphoma (1). The 12 cases of PTCL, unspecified, were heterogeneous regarding cell size and composed of diffuse large cells (5), diffuse mixed small and large cells (6) or diffuse small cells (1). Additionally, 2 cases were re-classified as histiocytic sarcoma. Out of 10 patients with localized disease, only 2 relapsed and 9 (90%) continue to survive. Out of 10 patients with advanced disease, 6 relapsed and 5 (50%) survive.
Conclusions: These results suggest that histologic subtypes of non-anaplastic PTCL in children and adolescents parallel those in adults but are frequently cured with modern therapy, particularly when localized.

1105 Immunohistochemical Detection of Cyclin D1 and CD20: Prognostic Significance in Patients with Multiple Myeloma

M Narbaitz, P Barreiro, M de Dios Soler, C Martin, I Slavutsky, A Marti, A Cotliar, M Leguiza, C Corrado. Academia Nacional de Medicina (ANM); ANM, Buenos Aires, Argentina; Hospital San Roque, Gonnet, Buenos Aires, Argentina.
Background: Overexpression of cyclin D1 in Multiple Myeloma (MM) has been the subject of different studies and its prognostic significance remains controversial, while the clinical impact of CD20 is currently under study. The goal of our study was to investigate the expression of Cyclin D1 and CD20 in patients with MM at diagnosis, and correlate these findings with overall survival.
Design: Bone marrow biopsies taken at diagnosis in 49 patients with MM were retrieved from our files. Clinical data and additional cytogenetic information were taken from clinical records. The samples were fixed in Bouin's fixative and embedded in paraffin. H&E and Giemsa stained sections were reviewed for plasma cell morphology. Immunostaining for Cyclin D1 protein (clone RBT14, Cell Marque, Sta. Barbara, CA, USA) and CD20 expression (L26, Dako, Sta. Barbara, CA, USA) was made by means of the avidin-biotin peroxidase complex (ABC) method (Vectastain, Vector, Burlingame, CA, USA). The cut off value for Cyclin D1 positivity was 10%. Positive and negative controls were run in parallel.
Results: Forty nine patients were included: 23 females and 26 males. Median age was 59 years. Most patients were Durie and Salmon clinical stage 3 (82%). Thirty one patients had well-differentiated morphology (63%). Expression of nuclear Cyclin D1 was detected in 26 patients (54%) 30 of which (67%) showed well differentiated plasma cells. CD20 reactivity was detected in eight cases (16%), all of them composed of mature tumor cells. Overall median survival for the whole group was 40 months and was not affected neither by Cyclin D1 (p=0.494) nor by CD20 expression (p=0.957) (log rank test). Further analysis of the cases according to the percentage of Cyclin D1 positive cells revealed 13 patients with more than 50% and 13 patients with less than 50% positive cells. No significant differences (p=0.153) in survival were found between these patients.
Conclusions: Our results failed to show any differences in survival between CD20+ and Cyclin D1+ cases and the group of negative ones. Of note was the finding of Cyclin D1 + without the corresponding t(11;14) by conventional cytogenetics, a fact described by others in a small number of patients, in whom a FISH analysis was additionally performed, and which is probably related to gene deregulating mechanisms other than t(11;14).

1106 RNA-Binding Protein VICKZ Is Expressed in a Germinal Center Associated Pattern among Lymphoma Subtypes

Y Natkunam, G Vainer, S Zhao, G Amir, E Pikarsky, AS Hammer, S Hamilton-Dutoit, R Levy, J Yisraeli, IS Lossos. Stanford University School of Medicine, Stanford, CA; Hebrew University-Hadessah Medical School, Jerusalem, Israel; Aarhus University Hospital, Aarhus, Denmark; Sylvester Comprehensive Cancer Center & University of Miami, Miami, FL.
Background: Recent effort in the molecular characterization of diffuse large B-cell lymphoma (DLBCL) has led to the recognition that patients with DLBCL of germinal center origin exhibit a better overall survival. Thus, identification and characterization of markers of germinal center derivation are of importance in dissecting prognostic subclasses of DLBCL. The VICKZ (Vg1 RBP/Vera, IMP1, 2, 3, CRD-BP, KOC, ZBP-1) family members are RNA-binding proteins that recognize specific RNA targets and have been implicated in diverse cellular functions including cell polarity, migration, proliferation and tumorigenesis/metastasis.
Design: We generated a novel antibody that recognizes all three isoforms of VICKZ protein and characterized its expression in normal lymphoid tissue and in 868 non-Hodgkin and Hodgkin lymphoma by immunohistochemistry on tissue microarrays.
Results: In normal tonsils, VICKZ protein showed a germinal center-specific pattern of expression with staining localized to the cytoplasm. Staining for VICKZ protein was present in 76% (126/165) of follicular lymphoma, 78% (155/200) of DLBCL, 90% (9/10) of mediastinal large B-cell lymphoma, and 100% (2/2) of Burkitt lymphoma. A subset of mantle cell lymphoma (11%, 2/19), extranodal (8%, 2/25), and nodal (20%, 1/5) marginal zone lymphoma and lymphoblastic lymphoma (25%, 4/13), showed VICKZ staining. The majority of lymphocyte predominant Hodgkin (92%, 12/13) and classical Hodgkin (94%, 101/108) lymphoma were found to be positive. Among T cell lymphoma, anaplastic large cell lymphoma were positive (75%, 6/9).
Conclusions: The differential expression pattern of VICKZ protein in lymphoma subtypes suggests a potential utility for VICKZ in the identification of subgroups of DLBCL associated with different prognoses. Additional work is in progress to correlate

VICKZ protein expression with other germinal center markers such as HGAL, BCL6 and CD10 as well as with prognostic subclasses of DLBCL.

1107 Expression of the Human Germinal Center Associated Lymphoma (HGAL) Protein Identifies a Subset of Classical Hodgkin Lymphoma of Germinal Center Derivation and Improved Outcome

Y Natkunam, ED Hsi, S Zhao, P Elson, B Pohlman, R Levy, IS Lossos. Stanford University School of Medicine, Stanford, CA; Cleveland Clinic Foundation, Cleveland, OH; University of Miami, Miami, FL.

Background: The Human Germinal center-Associated Lymphoma (HGAL) gene was identified as a candidate molecule that predicted improved outcome in diffuse large B-cell lymphoma. Previously, we showed that HGAL mRNA and protein are expressed in germinal centers (GC), GC-derived lymphomas and a substantial proportion of classical Hodgkin lymphoma (CHL). Here, we investigate whether HGAL protein expression could serve to distinguish biologically distinct subgroups of CHL.

Design: Ninety cases of CHL on a tissue microarray of 1.0 mm cores of paraffin tissue were used. Percent of HGAL-stained Hodgkin cells was scored on a 3-tiered scale as negative (0%), weak (<30%), and strong (>30%), as previously described (Natkunam et al. Blood 2005, 105:3979-86). These cutoffs were chosen before results were correlated with clinical endpoints.

Results: Twenty-five cases (28%) showed no staining for HGAL and 25 (28%) had weak staining; HGAL was strongly expressed in 40 cases (44%). Thirteen of the 90 patients had died and 19 had failed (i.e. relapsed or died). The overall 5-year survival (OS) and failure-free survival (FFS) were 87% + 4% and 79% + 4%, respectively. HGAL expression was associated with improved OS in univariate analysis (p=0.04, no vs weak/strong staining), as were age < 45 years (p<0.001), stage I or II (p=0.02), and possibly histology (nodular sclerosing vs other, p=0.09). However, in multivariate analysis with age, stage and histology, HGAL expression no longer had an impact (p=0.93). HGAL did not impact FFS in either univariate (p=0.13) or multivariate analysis (p=0.87).

Conclusions: The expression of HGAL in a subset of CHL suggests that a substantial proportion of CHL retain characteristics of lymphomas of GC derivation. The association with improved OS in univariate but not multivariate analysis suggests that HGAL may be a biologic marker related to known clinical parameters of improved OS and FFS. Comparative studies with well-characterized GC markers, BCL6 and CD10, and with BCL2, MUM1 and Blimp-1 are underway to further define biologically distinct subsets of CHL.

1108 The T-Cell Oncoprotein LMO2 Is Expressed in Normal Germinal Center B-Cells and in Germinal Center-Derived B-Cell Lymphoma

Y Natkunam, R Levy, S Zhao, B Taidi, IS Lossos. Stanford University, Stanford, CA; University of Miami, Miami, FL.

Background: We recently developed a multivariate model based on the expression of six genes - *LMO2, BCL6, FN1, CCND2, SCYA3 and BCL2* - that independently predicts survival in patients with diffuse large B-cell lymphoma (Lossos et al, NEJM 2004: 1828). Validation of the prognostic significance of the six cognate proteins by immunohistochemistry (IHC) is highly desirable for routine clinical application. LMO2 encodes a transcription factor that regulates angiogenesis and erythropoiesis, and is the most frequent site of chromosomal translocation in childhood precursor T-acute lymphoblastic lymphoma (T-ALL). The significance of its association with prolonged survival in DLBCL or in B-cell biology is unknown.

Design: Using a GST-LMO2 fusion protein to immunize mice, we generated a monoclonal anti-LMO2 antibody and characterized LMO2 protein expression in normal lymphoid tissue and 915 lymphomas utilizing IHC and tissue microarrays.

Results: LMO2 protein is expressed in normal germinal center (GC) B-cells and is localized to the nucleus. Mantle and marginal zones as well as interfollicular and paracortical regions lack staining. The expression of LMO2 protein in lymphoma subtypes is tabulated below.

Conclusions: Our results indicate that the LMO2 protein is expressed in normal GC and in GC-derived lymphomas. It is rare in lymphomas of non-GC derivation, mature T and NK lymphomas and myelomas. A significant proportion of T-ALL express this protein, consistent with its role in t(11;14) (p13;q11) or t(7;11)(q35;p13). In addition, a subset of B-ALL also expresses LMO2. Its restricted expression pattern suggests that LMO2 may have a potential role in the diagnosis and prognosis of specific lymphoma subtypes. Studies are underway to correlate LMO2 protein expression with other GC markers including HGAL, BCL6 and CD10, and to address its potential utility in predicting outcome in patients with DLBCL.

LMO2 Protein Expression in Lymphoma Subtypes

	Total Positive	Percent Positive
Follicular lymphoma	80/159	50%
Diffuse large B-cell lymphoma	92/197	47%
Mediastinal large B-cell lymphoma	8/13	62%
Burkitt lymphoma	1/4	25%
Marginal zone lymphoma	1/37	2%
Mantle cell lymphoma	0/18	0%
Small lymphocytic lymphoma/CLL	0/36	0%
Precursor B-lymphoblastic lymphoma	5/12	42%
Precursor T-lymphoblastic lymphoma	8/14	57%
Peripheral T & NK cell lymphoma	5/116	4%
Plasma cell neoplasms	3/174	2%
Lymphocyte predominance Hodgkin lymphoma	9/15	60%
Classical Hodgkin lymphoma	0/107	0%

1109 Prognostic Significance of Bcl-2 and Bcl-6 Expression in Patients with Nodular Sclerosis Hodgkin Lymphoma (NSHL)

H Naushad, P Aoun, LM Smith, Z Pan, P Bierman, TC Greiner, WC Chan, DD Weisenburger. University of Nebraska Medical Center, Omaha, NE.

Background: Classical Hodgkin lymphoma (HL) is curable by modern radiation and chemotherapy in a majority of patients. However, those who relapse are often therapy-resistant and have a poor prognosis. Bcl-2 expression in HL has been associated with a poor outcome, whereas the prognostic significance of CD20 and EBERs expression is controversial. Therefore, we examined the immunophenotypic profile of Reed-Sternberg cells in NSHL using a panel of newer antibodies and improved methods, and correlated the findings with survival.

Design: We evaluated 92 patients with NSHL who were uniformly staged and treated with standard therapy by the Lymphoma Study Group between 1983 and 1999. The patients were divided into two groups based on the age at diagnosis (<45 yrs and ≥ 45 yrs). Clinical features including age, sex, stage, B-symptoms, bulky disease >10 cm, mediastinal involvement, and LDH levels were evaluated. CD20, CD79a, bcl-2, bcl-6, and PAX-5 expression by immunohistochemistry; and kappa, lambda and EBERs by *in situ* hybridization, were evaluated using routine and tissue microarray sections to determine the predictive effect on overall survival (OS) and event-free survival (EFS).

Results: The median age of the patients was 30 yrs (range 14-79 yrs) and the majority (77%) were < 45 yrs old. None of the usual clinical factors was predictive of outcome in either age group. Overall, 26% of the cases were CD20+, 13% were CD79a+, 59% were bcl-2+, 27% were bcl-6+, 51% were PAX-5+, and 11% were EBERs+. None of the cases was positive for kappa or lambda by *in situ* hybridization. Bcl-2 and bcl-6 expression was predictive of survival in the entire group. However, bcl-2 was expressed in 70% of the older age group and was the only marker predictive of poor OS (p=0.0034) and EFS (p=0.04) in this group. In contrast, bcl-6 was expressed in 27% of the younger age group and was the only marker predictive of better EFS (p=0.023), but not OS, in this group. None of the other markers predicted OS or EFS in either age group.

Conclusions: Both bcl-2 and bcl-6 expression predict outcome in NSHL. Bcl-2 expression is a significant adverse prognostic indicator in older patients, whereas bcl-6 expression is a favorable indicator in younger patients. Analysis of biological markers by age group appears to be important in NSHL.

1110 Co-Expression of CD15 and CD30 in Anaplastic Large Cell Lymphoma

V Neppalli, K Fu, WC Chan, TC Greiner, DD Weisenburger, P Aoun. University of Nebraska Medical Center, Omaha, NE.

Background: The distinction between ALCL and Hodgkin lymphoma (HL) is sometimes difficult due to overlapping morphological features. Co-expression of CD15 and CD30 is typical of HL; however, cases of ALCL expressing both of these markers have been reported. Therefore, we examined the pathologic features of ALCL cases with CD15 and CD30 co-expression.

Design: Thirty cases of ALCL stained for CD15 and CD30 during 1995-2005 were identified in the files of the Lymphoma Study Group. Five cases with CD15 and CD30 co-expression were identified. The diagnosis of ALCL was confirmed by review of the morphologic features and immunostains for CD2, CD3, CD4, CD5, CD7, CD8, CD15, CD20, CD30, CD43, CD45, CD45RO, CD79a, ALK, clusterin, EMA, TIA-1, granzyme B, and EBERs. T-cell receptor gamma chain gene rearrangement studies were performed by PCR from paraffin-embedded tissue in 3/5 cases.

Results: There were 23 men and 7 women with a median age of 39 years (range, 12-80 years), and all patients presented with nodal disease. Five cases showed co-expression of CD15 and CD30 in a golgi and membranous pattern in >30% of the tumor cells, and 4/5 cases demonstrated Reed-Sternberg-like cells and variants in a variable inflammatory background. The background lymphocytes showed a normal immunophenotype for T-cells and B-cells. Immunostains for CD20, CD79a, EMA, and EBERs were negative in all 5 cases. CD3 was negative in 3/5 cases and CD45 was variably positive in 2/5 cases. All 5 cases were negative for ALK; however, hallmark cells were identified in 4/5 cases. Other features of ALCL including partial or subtotal effacement of the nodal architecture and sinusoidal infiltration were present. Additional immunostains showed a cytotoxic phenotype typical of ALCL (TIA-1+ 4/4 cases, and granzyme B+ 4/4 cases) with CD4 expression in 4/5 and CD8 expression in 1/5 cases. Clonal T-cell receptor gamma chain gene rearrangements were identified in all 3 cases studied to date.

Conclusions: Co-expression of CD15 and CD30 by the tumor cells in ALCL, along with the presence of Reed-Sternberg-like cells and variants, poses a diagnostic pitfall in favor of HL. A high index of suspicion coupled with extensive immunophenotypic and molecular analysis are essential for an accurate diagnosis in such cases.

1111 Using Flow Cytometric Analysis To Detect Early Stem Cell Diseases

TC Netzel, Y Li. University of Florida, Gainesville, FL.

Background: Hematopoietic stem cell disorders (myelodysplastic syndrome, MDS and/or myeloproliferative disorders, MPD) are malignant disorders of hematopoietic progenitors in which the bone marrow is composed of abnormal clonal hematopoietic cells. However, the neoplastic blasts are few in number in the early stages of these diseases, making clinical diagnosis a challenging process.

Design: Flow cytometric data for 267 bone marrow aspirates examined in the University of Florida Department of Pathology in 2004 were reviewed and re-analyzed using the WoodList and CellQuest flow cytometry data analysis software packages. Cases of acute myeloid leukemia, and acute lymphoblastic leukemia were not included. Parameters studied included forward and side light scatter patterns; differential cell counts; aberrant expression of antigens including CD64, CD16, CD56, and CD7; and levels of expression of CD34, CD38, and CD13. Flow cytometric data for the control group of 216 cases (normals, aplastic anemia, multiple myeloma, non-Hodgkin lymphoma, and other non-neoplastic disorders) were analyzed to determine the mean and standard deviation for each parameter analyzed. A reference range was determined for each parameter. Results for the remaining 51 cases were compared to these ranges in order to

find patterns which may be of use in the diagnosis of early stem cell diseases. Final diagnoses were made using morphologic, clinical, and cytogenetic data. A scoring system was developed in which parameters which fell outside the reference range were given a points value. The points for each case were totaled and a cutoff score was chosen.
Results: The sensitivity and specificity of the scoring system for detecting MDS/MPD were 64% and 92%, respectively. The positive predictive value and negative predictive value were 31% and 98%, respectively. The scoring system was then applied to 54 additional cases. Analysis and scoring was performed without prior knowledge of the diagnosis in these cases. The sensitivity and specificity for detecting MDS/MPD in these cases were 100% and 96%, respectively. The positive predictive value and negative predictive value were 71% and 100%, respectively.
Conclusions: While the sensitivity of this scoring system is fairly modest (74%), the negative predictive value is quite high (98%). Our data show that a careful, but relatively simple analysis of flow cytometric data, with particular attention to statistical analysis of differential cell counts, forward and side light scatter properties, and levels of antigen expression, should be an integral part of the diagnostic workup of MDS.

1112 Identification of Novel RUNX1 (*AML1*) Translocation Partner Genes in Acute Myeloid Leukemia

TT Nguyen, LN Ma, ML Slovak, CD Bangs, AM Cherry, DA Arber. Stanford Medical Center, Stanford, CA; City of Hope National Medical Center, Duarte, CA.
Background: The *RUNX1* (formerly known as *AML1*) gene is the most frequent target for chromosomal translocation in leukemia. Previous studies have reported at least 14 *RUNX1* translocation partners [*Semin Hematol* **36**(suppl 7):59,1999]. However, only a few translocations have been well characterized in acute myeloid leukemia (AML).
Design: Frozen sample from six patients from Stanford University or the City of Hope National Medical Center with balanced translocations involving 21q22, the *RUNX1* locus, were studied. Complete chromosome karyotype analysis was performed on the peripheral blood or bone marrow from these patients. A *RUNX1* split was confirmed in all cases by FISH analysis using a commercially available probe (Vysis, Downers Grove IL). A cDNA Panhandle (*PNAS* **97**:9597, 2000) or 3'RACE (Invitrogen, Carlsbad CA) PCR approach was utilized to identify unknown *RUNX1* translocation partner genes. The cDNA PCR products were subcloned (Invitrogen, TOPO TA cloning) and subsequently sequenced.
Results: The six patients had reciprocal translocations involving 21q22/*RUNX1* and chromosomes 1, 3, 4, 8, and 12. Translocation partner genes were detected in five of the six cases, with the negative case being one case with a t(8;21) failing to demonstrate a partner gene. The method detected the *ETO/AML1* (*RUNX1/RUNX1T1*) fusion transcript in one other patient with a t(8;21) and *MDS1/RUNX1* in a patient with t(3;21). Three additional novel *RUNX1* translocation partners on 4q31.3 (*SH3D19* [NCBI Entrez]), 1p35 (*YTHDF2*) and 1q21.2 (*ZNF687*) were identified in three other patients. The translocation events occurred between exon 3 and exon 7 of the *RUNX1* gene. Although *RUNX1* is also frequently mutated in AML, the hybrid *RUNX1* -translocation partner transcripts contained infrequent *RUNX1* mutations. *SH3D19* encodes a cytoplasmic protein EBP whose suppression of Ras-induced cellular transformation can be inhibited by nuclear recruitment (*Blood* **103:**1445, 2004). The t(4;21) in our patient creates a hybrid EBP-RUNX1 protein retaining RUNX1's DNA binding domain which may result in nuclear localization of the chimeric protein and inhibition of EBP's Ras-suppressive functions.
Conclusions: We identify three previously unreported *RUNX1* translocation gene partners. These hybrid *RUNX1* -translocation partner transcripts infrequently contain mutated *RUNX1* suggesting that these translocations are critical events in leukemogenesis. Future studies would be useful to further characterize these novel fusion protein products.

1113 Expression of Angiogenic Cytokines in Idiopathic Myelofibrosis

H Ni, N Nanaji, S Wu, G Barosi, R Hoffman. University of Illinois, Chicago, IL; IRCCS, Policlinico San Matteo, Pavia, Italy.
Background: Idiopathic myelofibrosis (IM) is a chronic Philadelphia chromosome negative myeloproliferative disorder. IM is a stem cell disorder in which a multipotent hematopoietic stem cell acquires a clonal proliferative advantage and its progeny inappropriately releases fibrogenic and angiogenic factors into the bone marrow microenviroment. We and others have previously demonstrated increased bone marrow angiogenesis in IM (Ni H. et al. *Mod Pathol* 18:243A, 2005). In this study, we evaluated bone marrow expression of angiogenic cytokines in IM patients.
Design: Paraffin-embedded tissues from 20 IM patients at the time of initial diagnosis and 10 controls were analyzed. Expression of vascular endothelial growth factor (VEGF) and basic fibroblastic growth factor (bFGF) was evaluated by immunohistochemical staining using antibodies against VEGF and bFGF
Results: In controls, weak expression of bFGF was observed in megakaryocytes, endothelial cells and histiocytes. IM patients showed a significantly higher degree of expression of bFGF in megakaryocytes and endothelial cells. Weak expression of VEGF was seen in the megakaryocytes of controls. Six patients with the prefibrotic form of IM exhibited strong expression of VEGF in megakaryocytes. The distribution and intensity of staining for VEGF in the remaining 14 IM patients were similar to that of the controls.
Conclusions: The results suggest that the higher degree of angiogenesis in the bone marrow of patients with IM is associated with increased expression of b-FGF and VEGF in megakaryocytes and endothelial cells in the bone marrow. These studies suggest that bFGF and VEGF likely lead to the increased marrow microvessel density in IM. VEGF probably plays a role in angiogenesis in the early stage of the disease.

1114 Bone Marrow Morphology of Myelodysplasia with Monosomy 7

K Nigro, F Racke, N Rosenthal, H Meyerson. University Hospitals of Cleveland, Cleveland, OH; The Johns Hopkins University, Baltimore, MD; The University of Iowa Hospitals, Iowa City, IA.
Background: Cytogenetic abnormalities of chromosome 7 are associated with a poor prognosis in patients with myelodysplastic syndrome (MDS). However, the morphologic features associated with abnormalities in chromosome 7 have not been established. Bone marrows from a subset of MDS patients with monosomy 7 as the sole cytogenetic abnormality were analyzed to determine the morphologic features associated with this karyotype.
Design: 12 MDS patients with monosomy 7 were identified from a search of the cytogenetic databases at University Hospitals of Cleveland and The University of Iowa Hospitals from 1995-2003. An additional patient was included in the study who had monosomy 7 and trisomy 21. Morphologic and clinical features of these cases were retrospectively reviewed. A bone marrow dysplasia score (DS) was assigned to each hematopoietic lineage as follows: no dysplasia = 0, 1-$\leq$10% of cells within one lineage exhibiting dysplasia = 1+ (mild dysplasia), 11-$\leq$50% = 2+ (moderate dysplasia), and 51-100% = 3+ (severe dysplasia).
Results: All patients had moderate to severe dysplasia of megakaryocytes with most patients (10/13) showing severe dysplasia. One patient had no identifiable megakaryocytes on the aspirate smear or core biopsy. In all cases, the megakaryocytic abnormalities consisted of either small hypolobate megakaryocytes or megakaryocytes with separate nuclear lobes. Dysplasia in the erythroid and granulocytic lineages was mild in most instances (erythroid DS of 0 or 1+ in 9/13 patients, granulocytic DS of 0 or 1+ in 12/13). Blast percentage was $\leq$5% in 9/13 patients. Megakaryocyte hyperplasia was identified in 8/13 patients. 8/13 patients had platelet counts less than 150,000. Cellularity was variable with 11/13 cases being normo- to hypercellular. 11/13 patients had an International Prognostic Scoring System score of $\leq$1.5.
Conclusions: Myelodysplasia with monosomy 7 is associated with severely dysplastic megakaryocytes and a low marrow blast percentage. Dysplasia in other lineages is mild. These morphologic findings may resemble that seen in 5q- syndrome.

1115 Development of a Real Time PCR Assay for the JAK2 V617F Mutation in Myeloproliferative Disorders

RJ Olsen, D Tang, DW Bernard, DH Farkas, JCC Chang. Baylor College of Medicine, Houston, TX; Cornell University, Ithica, NY; The Methodist Hospital, Houston, TX.
Background: Chronic myeloprolifertive disorders (MPDs) such as polycythemia vera (PV), essential thrombocythemia (ET) and idiopathic myelofibrosis (MF) are clonal stem cell dyscrasias characterized by abnormal myeloid proliferation. Although strict diagnostic criteria have been established, differentiation from reactive conditions remains difficult. A specific mutation (V617F) in the JAK2 gene has been recently recognized as having diagnostic value in MPDs. No assay is currently available for routine evaluation of the JAK2 mutation. We report the development of an appropriate assay.
Design: Specific primers were designed to amplify a 163-bp region of JAK2. Fluorescence resonance energy transfer (FRET) probes were designed such that the 5' probe overlaps the mutated codon and the 3' probe anneals immediately downstream. Patients with a biopsy proven diagnosis of a MPD or reactive condition were selected by retrospective review of pathology reports (The Methodist Hospital, Houston, TX). DNA was extracted from 20 peripheral blood smears, 20 unstained bone marrow (BM) aspirates, 20 stained BM aspirates and 20 BM clot sections. Human erythroleukemia (HEL) and multiple myeloma (RPMI8226) cell lines were used as positive and negative controls. PCR and melting curve analysis were performed on the LightCycler platform.
Results: The JAK2 region was successfully amplified in all patient specimens. Wild type amplicons demonstrated a melting temperature of 54^{o} C, whereas those containing the V617F mutation melted at 45 ^{o}C. Titration studies showed that the relative size of each curve was proportional to allele frequency. V617F was identified in patients previously diagnosed with PV, ET, MF and acute myeloid leukemia (AML) transformed from MPD. Patients with secondary conditions or *de novo* AML demonstrated a wild type genotype. One case of AML transformed from PV was initially positive for the mutation; however, it was not detectable following treatment. Results were verified by DNA sequencing.
Conclusions: The real time PCR assay described here may be used to identify the JAK2 V617F mutation in MPD patient specimens and can be easily integrated into the operations of a molecular diagnostics laboratory. Furthermore, this assay will semi-quantitatively detect the mutation in fresh or archived materials. This provides a new tool for studying the change in the proportion of mutated alleles present during disease progression.

1116 Differential Utility of Various Markers in the Flow Cytometric Detection of Paroxysmal Nocturnal Hemoglobinuria (PNH) in Blood and Marrow

H Olteanu, NJ Karandikar, RW McKenna, Y Xu. University of Texas Southwestern Medical Center, Dallas, TX.
Background: Flow cytometry (FC) is considered the "gold standard" for detecting glycosyl-phosphatidylinositol (GPI)-deficient blood cells, but there are no standardized criteria for its use in the diagnosis of PNH. In this study, we examined the utility of various markers on different cell populations in the diagnosis of PNH. We also evaluated bone marrow specimens, which are generally considered less suitable than blood due to variable expression of GPI-linked antigens during hematopoietic cell differentiation.
Design: Blood specimens from 15 PNH+ patients and 15 concurrently drawn healthy control subjects were analyzed for expression of CD14, CD16, CD24, CD55, and CD59 using 4-color FC. Different leukocyte lineages were discriminated by CD45/light scatter analysis. Marrow granulocytes and monocytes were analyzed using limited

panels, including CD14, CD16, and/or CD55 in 8/15 PNH+ patients and 10 normal marrows.

Results: All 15 patients revealed subpopulations of CD16/CD55-deficient granulocytes, CD14/CD55-deficient monocytes and CD55/CD59-deficient erythrocytes. Clone size varied from 11-99% (mean 64%) in 13 patients with a clinical diagnosis of PNH, and was 1.7% in a patient with aplastic anemia and 3.4% in a patient with hepatitis C. The size (mean±S.E.) for CD16/CD55-deficient granulocytes and CD14/CD55-deficient monocytes in the control group was 0.12±0.07% and 0.13±0.08%, respectively. CD59 showed very limited utility for detecting PNH+ monocytes. Normal monocytes exhibited significantly dimmer CD59 expression than normal granulocytes. PNH+ monocytes exhibited only slightly diminished levels of CD59. The change of mean fluorescence intensity of CD59 on blood PNH monocytes was significantly smaller than for PNH granulocytes (43±10 vs. 146±23; p=0.0005). FC analysis of 8 PNH+ marrows identified abnormal patterns of CD14 expression on monocytes and CD16 expression on granulocytes in 3 (38%) and 5 (63%) cases, respectively. A combination of CD16/CD55/CD45/CD14 detected distinct populations of CD16/CD55-deficient granulocytes and CD14/CD55-deficient monocytes in 2/2 PNH+ marrows and in none of 10 control marrows.

Conclusions: GPI-deficient monocytes express only marginally lower CD59, making it a less robust marker in detecting monocytic PNH clones as compared to CD14 and CD55. Furthermore, our study demonstrates that a limited CD16/CD55/CD45/CD14 panel is useful to screen GPI-deficient clones in marrows when submitted for FC analysis of cytopenias.

1117 Granzyme B Leakage-Induced Cell Death Is a Crucial Mechanism of Necrosis in Nasal-Type NK/T Cell Lymphoma

S Park, H Jin, D Lee, Y Ko. Samsung Medical Center, Sungkyunkwan University, Seoul, Korea.

Background: Extensive necrosis is common histologic finding of nasal-type NK/T cell lymphoma and has been speculated to be caused by vascular occlusion or release of EBV-associated inflammatory cytokine. Granzyme B is one of serine proteases and induces cytotoxic T lymphocyte-mediated target cell death. In the cytotoxic T or NK cells, granzyme B is localized within cytotoxic granules, thus does not attack oneself. When released into the cytosol, the serine proteinase inhibitor 9 (PI-9) inhibits granzyme B acitivity and protects T or NK cells from toxic effect of granzyme B. Because nasal-type NK/T cell lymphoma commonly expresses abundant granzyme B, this study is designed to explore whether the granzyme B plays a role in the necrosis of nasal-type NK/T cell lymphoma.

Design: Tissue microarray of 26 nasal-type NK/T cell lymphoma was studied with TUNEL assay, and immunohistochemical stains for active caspase 3, PARP-1/p89, and Bcl-2. HANK-1 cell line (NK/T cell lymphoma, granzyme B+) and NKL cell line (granzyme B-) were analyzed with western blot analysis for cytochrome C, active caspase 3, and PARP-1/p89. Immunoprecipitation/immunoblot was performed to identify binding of granzyme B and PI-9. To localize the granzyme B, immunogold labeling and immunofluorescence staining were performed and observed with transmission electron microscopy and confocal laser scanning microscopy respectively.

Results: 1) Expression level of granzyme B in tumor tissue was correlated with apoptosis rate(p=0.015), and the levels of active caspase 3(p=0.036) and PARP-1/p89 (p=0.040). 2) The level of granzyme B in tumor tissue was well correlated with degree of necrosis of tumor tissue (p=0.002) 3) The level of apoptosis and necrosis was correlated with prognosis of the patient (p=0.035) 4) Granzyme B-positive HANK-1 cell line showed increased spontaneous cell death comparing to granzyme B-negative NKL cell line. In un-stimulated state, HANK-1 cells released cytochrome C into the cytosol with cleavage of caspase 3 and PARP-1. Treatment with granzyme B inhibitor and caspase inhibitor decreased cleavage of PARP-1. 5) By immunogold labeling, granzyme B was identified within the cytolytic granules as well as in the cytosol. Confocal microscopy and immunoprecipitation assay confirmed colocalized PI-9 and granzyme B which formed SDS-resistant complex.

Conclusions: Granzyme B leakage induces cell death in NK/T cell lymphoma with caspase-dependent mechanism, which leads to extensive necrosis commonly seen in NK/T cell lymphoma.

1118 JH and TCRγ Gene Rearrangements in Acute Leukemia in Correlation with Phenotype and Cytogenetics

Y Piao, L Tsao, J Zhong, G Bhagat, B Alobeid. Columbia University, New York, NY.

Background: Clonal rearrangements of the JH (JH+) and TCRγ (TCR+) genes are commonly seen in B and T cell lymphomas, respectively, but can also be detected in acute lymphoblastic leukemia (ALL). Cross-lineage JH+ and/or TCR+, however, can occur in acute myeloid leukemia (AML) and ALL. We investigated the frequency of such rearrangements in acute leukemia (AL) and correlated with the phenotype and cytogenetics.

Design: We reviewed 42 AL: 23 AML, 9 B-ALL, and 10 T-ALL (age 6 days to 83 yrs; M/F 23/19). Samples with leukemic cells were analyzed by PCR, using primers for the variable region (FR3) and joining region of the JH gene, and J1/2, JP, JP1/JP2, V1, V9 and V10/11 regions of the TCR-γ gene. For statistical analysis, two- tailed Fisher's exact test was used.

Results:

	MLL+ AML	MLL- AML	AML	MLL+ B-ALL	MLL- B-ALL	B-ALL	T-ALL
JH+	4/5	3/18	7/23	4/4	4/5	8/9	3/10
TCR+	1/5	1/18	2/23	2/4	5/5	7/9	6/10
JH+ & TCR+	1/5	none	1/23	2/4	4/5	6/9	2/10

MLL rearrangements (*MLL+)* are found in 5 AML and 4 B-ALL. The remaining show normal karyotype or various aberrations. The sensitivity and specificity of JH+ for B-lineage in AL is 88.9% and 75.8%, respectively; and of TCR+ for T-lineage in AL is 60% & 71.9%, respectively. Cross-lineage clonal rearrangement is present in all subtypes of AL. In B-ALL, the majority (77.8%) is TCR+, similar in frequency to T-ALL (60%). Interestingly, JH+ (30.4%) and TCR+ (8.7%) are both detected in AML, but JH+ is more frequent, especially in MLL+ AML (80.0%) versus MLL- AML (16.6%) (p = 0.02). JH+ is found in 8/9 (88.9%) MLL+ AL and 10/33 (30.3%) MLL- AL. TCR+ is found in 3/9 (33.3%) MLL+ AL and 12/33 (36.3%) MLL- AL. Combined TCR+ and JH+ are detected in 42.1% ALL (66.7% B-ALL and 20% T-ALL), 4.3% AML, 3/9 (33.3%) MLL+ AL, and 6/33 (18.1%) of MLL- AL.

Conclusions: JH+ is most commonly seen in B-ALL, and is relatively more sensitive and specific compared to TCR+ for T-ALL. ALL is significantly more likely to show combined JH+ and TCR+ than AML (p = 0.03). Although JH+ is rather specific for B cell lineage, it can be seen in AML and T-ALL. In AML, JH+ is significantly more common in *MLL+* AML (p=0.02). JH+ and/or TCR+ do not correlate with any other recurrent cytogenetic aberrations. The latter findings suggest that *MLL+* AML are genotypically mixed lineage even when phenotypically appear committed to a single lineage.

1119 CD4+ CD56+ Hematodermic Neoplasms ("Blastic NK-Cell Lymphomas") Express the Immature Plasmacytoid Dendritic Cell Marker BDCA-2

ME Pilichowska, JL Pinkus, MD Fleming, GS Pinkus. Tufts-New England Medical Center, Boston, MA; Brigham and Women's Hospital, Boston, MA; Children's Hospital Medical Center, Boston, MA.

Background: Lineage-negative hematodermic neoplasm ("blastic natural killer [NK] cell lymphoma") is a rare entity of unknown origin with a CD3- CD4+ CD56+ CD43+ HLA-DR+ immunophenotype and frequent involvement of skin, peripheral blood and bone marrow. Recently, CD123 expression by tumor cells linked its origin to immature dendritic cells (DCs), a composite group of myeloid and lymphoid early-committed cells capable of differentiating into antigen presenting DCs. By flow cytometry, myeloid DCs represent the majority of human peripheral blood DCs and are positive for BDCA-1 (blood dendritic cell antigen-1), CD11c(high), CD123(low), CD13, and CD33. Plasmacytoid dendritic DCs are BDCA-2 (blood dendritic cell antigen-2) positive, CD13-, CD33-, CD123(high), and produce interferon (IFN)-α when triggered by antigens. Our goal was to evaluate a possible plasmacytoid DC derivation for these rare neoplasms.

Design: Three cases of CD4+CD56+ lineage-negative neoplasms were retrieved from the files of Brigham & Women's and Children's Hospitals based on availability of frozen tissue. Immunohistochemical studies were performed on cryostat and/or paraffin sections for BDCA-1, BDCA-2, CD123, MxA protein, fascin, CD13, CD33, lysozyme, B (CD19) and T cell antigens (CD3, CD4, CD8, CD7, TCRα/β, TCRγ/δ, CD45RO), BDCA-3, CD43, CD34, TdT, CD68, CD1a, TIA-1 and granzyme. Flow cytometric, cytogenetic and molecular studies for B-cell (IgH) and T cell receptor gene rearrangements also were performed.

Results: All patients (1 M:2F; 16-77 yrs) presented with cutaneous lesions. Two patients revealed subsequent bone marrow involvement and/or leukemic dissemination and have expired. One patient was recently diagnosed. Tumor cells comprising the cutaneous neoplasms were CD4+, CD56+, CD123+, BDCA-2+, MxA+, TdT+, CD68+, and CD43+ and negative for fascin, a mature dendritic cell marker. All other markers and in situ hybridization for EBV (EBER) were negative. Flow cytometric studies were concordant with immunohistochemical findings. Cytogenetic analysis revealed complex chromosomal abnormalities. Molecular genetic studies revealed no IgH or T cell receptor gene rearrangements.

Conclusions: This study is the first to demonstrate immunohistochemical expression of BDCA-2 and MxA protein for three CD123+CD4+ CD56+ hematodermic neoplasms and provides evidence for an immature plasmacytoid DC derivation.

1120 Polymorphisms Affecting the Expression of Certain Cytokines Associate with the Myelodysplastic Syndromes

MP Powers, A Raza, H Nishino, Y Lou, CC Chang. The Methodist Hospital and Weill Cornell Medical School, Houston, TX; University of Houston, Houston, TX; BCM, Houston, TX.

Background: Myelodysplastic syndromes (MDS) are characterized by ineffective hematopoesis and excessive apoptosis. Certain cytokines are significantly elevated or depressed in MDS, and may contribute to the ineffective hematopoesis. Numerous polymorphisms have been identified in and around the genes encoding the cytokines, and some of these polymorphisms associate with either increased or decreased expression of the corresponding cytokine. These polymorphisms associate with different autoimmune and/or neoplastic diseases and may contribute to their pathogenesis. It is unknown whether these cytokine polymorphisms are associated with, and may therefore be playing a role in the pathogenesis of, MDS.

Design: DNA was isolated from the peripheral blood or BM aspirate of 23 MDS patients diagnosed at the Methodist Hospital within the last 2 years. The genotypes for 11 polymorphisms (SNPs), associated with the altered expression of 7 different cytokine genes, were determined using the PEL-FREEZ cytokine genotyping kit (Dynal/Invitrogen). The allele frequencies and genotype frequencies were compared to similar populations in both the dSNP database and in the published literature using the chi-squared test.

Results: In our MDS population, when compared to the controls, the -308A/A genotype of the *TNFα* gene (p=0.06) and the *TGFβ1* allele +29T and genotype +29T/T(p=0.025, p=0.059 respectively), each associated with higher levels of expression, were overrepresented in our MDS population. Additionally, the +1902G polymorphism of the *IL4Rα* gene, associated with lower receptor activity, was overrepresented in our population (p=0.007); however, the results for the +1902 G/G genotype, and our analyses of *IL-6*'s polymorphisms, were inconclusive due to insufficient power. Polymorphisms in *IL-2* and *IL-10* did not show any significant trends, but this may have been limited by the number of subjects.

Conclusions: Polymorphisms associated with increased expression in the cytokines *TNFα* and *TGFβ1*, and a polymorphism associated with less *IL4Rα* activity, may be overrepresented in our MDS population suggesting that increased *TNFα* and *TGFβ1*, and decreased *IL4Rα*, activities may contribute to either the susceptibility and/or pathogenesis of MDS. Further studies, with more patients, are being performed to confirm the above findings and enhance the statistical analyses of the rare genotypes that may show an association with MDS.

1121 Malignant Lymphomas of the Orbit: Role of *Chlamydia Psittaci* Infection and *MALT1* Translocations

GW Procop, U Reischl, M Hartke, RR Tubbs, SH Swerdlow, JR Cook. Cleveland Clinic Foundation, Cleveland, OH; University of Regensberg, Regensberg, Germany; University of Pittsburgh School of Medicine, Pittsburgh, PA.

Background: A recent study suggested that many MALT lymphomas and diffuse large B-cell lymphomas (DLBCL) arising in the orbit are associated with *Chlamydia psittaci* infection. Translocations involving the *MALT1* gene, including *API2/MALT1* or *IGH/MALT1* translocations, have also been reported in some MALT lymphomas and DLBCL of the orbit. However, the incidence *C. psittaci* infection, and whether *C. psittaci* is found in cases with or without *MALT1* translocations, remains unclear. We therefore studied 35 orbital lymphomas, including 32 MALT lymphomas and 3 DLBCL, for evidence of *C. psittaci* infection and *MALT1* translocations.

Design: First round and nested PCR studies were performed using primer sets for the *omp1* and *omp2* genes in *C. psittaci*. β-globin specific primers were employed as a positive internal control. In 2 patients, serologic studies for antibodies to *Chlamydia* species were also performed. Paraffin section interphase FISH studies for *MALT1* translocations were performed using dual color, break-apart probes spanning the *MALT1* locus.

Results: Each of 34 cases tested were negative for *C. psittaci* DNA, with appropriate amplification of β-globin DNA in each case. Of 32 cases that could be analyzed for *MALT1* translocations, all were negative. Gains of the *MALT1* locus, consistent with polysomy of chromosome 18q, were identified in 2 of 3 DLBCL (67%) and 9 of 29 MALT lymphomas (31%).

Conclusions: These results suggest that polysomy for chromosome 18q is more important in the pathogenesis of orbital MALT lymphomas and DLBCL than *MALT1* translocations. In contrast to a recent report from a single European institution, this study shows no evidence of *C. psittaci* infection in malignant lymphomas of the orbit diagnosed at two institutions in the United States. These findings may reflect geographic variation in *C. psittaci* infection. At this time, antibiotic therapy directed against *C. psittaci* appears unwarranted for patients in the United States with DLBCL or MALT lymphomas of the orbit.

1122 CKS1B Gene Amplification Is a Frequent Event and Associated with Tumor Progression in Multiple Myeloma

C Qi, D Reece, JC Reader, Y Ning, H Chang. University Health Network - Princess Margaret Hospital, Toronto, ON, Canada; University of Maryland, Baltimore, MD.

Background: Structural abnormalities at Chromosomal 1q are frequently detected in MM but the specific target gene is still obscure. CKS1 (cyclin kinase subunit 1) is a crucial regulator of cell cycle progression mapped at 1q21. Overexpression of CKS1 has shown to be associated with aggressive phenotype and poor prognosis of several types of human carcinomas. The role of CKS1 in multiple myeloma (MM) has not been established.

Design: We evaluated the copy number of CKS1B gene by interphase fluorescence in situ hybridization (FISH) in 57 primary MM, 22 plasma cell leukemia (PCL) and 14 human myeloma cell lines (HMCL). In addition, Q-RT-PCR was performed to determine the CKS1B expression in 5 HMCLs.

Results: Interphase FISH analysis revealed 3-8 copies of CKS1B in 23 of 57 (40%) primary MM samples, indicating CKS1B amplifications. This amplification was detected in 10 of 32 (31%) MM at the time of diagnosis, and in 13 of 25 (52%) at refractory/relapsed stage disease. Of the 22 PCLs, 9 were primary, 13 secondary. CKS1B amplification was detected in 12 (55%) of the PCL cases, including 5 (55%) primary and 7 (54%) secondary cases. In serial bone marrow specimens from 8 patients with MM who relapsed after high-dose chemotherapy with autologous stem cell support, 3 had CKS1B amplification at relapse but were not seen at diagnosis. While one had CKS1B amplification at both stages, there was a significant increase of percentage and copy numbers of CKS1B in the relapsed cases (44% with mainly 3 signals at diagnosis; 75% with 3-8 signals at relapse). The other 3 cases were negative for CKS1B amplification at either stage. Furthermore, multiple copies of CKS1B gene were detected in 13 (93%) of 14 HMCLs. By Q-RT-PCR, we found that the CKS1B expression levels from 5 HMCLs with multiple CKS1B signals by FISH ranged from 1.5 to 3.46 times higher than that from the OCI-MY5 cell line with 2 copies of CKS1B gene, indicating that gains of CKS1B copy number in MM cell lines resulted in an increased CKS1B expression.

Conclusions: Our results indicate that CKS1B is frequently amplified in MM, especially in relapsed/refractory patients and in patients with PCLs. CKS1B gene may be the target of chromosome 1q abnormalities in MM and the amplification of CKS1B may represent a secondary change associated with therapeutic resistance and MM progression.

1123 The Transcription Factor CCAAT/Enhancer Binding Protein (C/EBP)β Is Constitutively Expressed in ALK-Positive Anaplastic Large Cell Lymphomas (ALCL), and Is Dependent upon NPM-ALK Kinase Activity

L Quintanilla-Martinez, S Pittaluga, T Davies-Hill, C Miething, N Anastosov, M Rudelius, J Duyster, ES Jaffe, F Fend, M Raffeld. GSF-Research Center for Health and Environment, Neuherberg, Germany; National Cancer Institute, NIH, Bethesda, MD; Technical University of Munich, Munich, Germany.

Background: C/EBPβ is one of a six member family of CCAAT/enhancer binding proteins (C/EBP). These transcription factors are involved in the regulation of various aspects of cellular growth and differentiation, and have been implicated in oncogenesis. Although C/EBPβ has important functions in both B and T-cell differentiation, its expression has not been well studied in lymphoid tissues. The aim of this study was to investigate whether C/EBPβ expression might contribute to lymphomagenesis.

Design: The expression of CEBPβ was analyzed by immunohistochemistry and Western blot in normal lymphoid tissues and in 229 well-characterized lymphomas (107 T-cell NHL, including 45 ALK-postive ALCL, 93 B-cell NHL, 9 NLPHD and 20 HL), and in 19 lymphoma cell lines. The influence of NPM-ALK on C/EBPβ was investigated by studying the effects of enforced expression of NPM-ALK and a kinase inhibitable modified NPM/ALK in two different cell line models (Ba/F3 and 32D).

Results: Normal lymphoid tissues and the vast majority of B-, T-cell NHL and HL lacked detectable C/EBPβ. In striking contrast, most cases of ALK-positive ALCL (40/45; 88%) strongly expressed C/EBPβ. Very weak staining was observed in a smaller percentage of ALK-negative ALCL cases (8/29; 28%) and in HD cases (5/20; 25%). Western blot analysis confirmed the strong expression of C/EBPβ in the ALK-positive ALCL cases, and demonstrated elevated levels of the LIP isoform, which has been associated with increased proliferation and aggressiveness in carcinomas. Transfection of C/EBPβ negative cell lines with both NPM-ALK and a kinase inhibitable modified NPM-ALK construct resulted in the induction of C/EBPβ, and demonstrated dependence upon the NPM/ALK kinase activity.

Conclusions: In this study, we describe for the first time the constitutive expression of the transcription factor C/EBPβ in ALK-positive ALCL cases. In addition, we demonstrate that C/EBPβ expression is induced through the kinase activity of NPM-ALK. We suggest that C/EBPβ is likely to play an important role in the pathogenesis and unique phenotype of this lymphoma.

1124 Clinical Significance of Surface Immunoglobulin (sig) Expression in Diffuse Large B-Cell Lymphoma (DLBCL). Is IgM Expression Associated with Aggressive Disease?

MTS Rad, DA Stewart, I Auer, J Luider, R Lai, A Mansoor. University of Calgary / Calgary Laboratory Services, Calgary, AB, Canada; Tom Baker Cancer Center, Calgary, AB, Canada; University of Alberta, Edmonton, AB, Canada.

Background: The clinico-pathological heterogeneity among DLBCL is well established. International prognostic index (IPI) is currently used to predict outcome, although it does not account the biological diversity of DLBCL. Gene expression profile studies had addressed this issue and categorised significantly better overall survival in patients (pts) with germinal center B-like DLBCL vs. activated B-cell. Since anitgen stimulation plays a significant role in lymphomagenesis, we hypothesize that there is a difference between the biology of DLBCL expressing surface IgM vs IgG . We evaluated flow-cytometric (FCM) parameters (sIg, CD3, CD4/CD8) in relation with overall survival (OS), IPI, staging and relapse in DLBCL pts.

Design: DLBCL pts between 1997-2001 at our institution, in which FCM and survival data was available were included. FMC was performed using standard protocols. All pts were staged (Ann Arbor) & treated with CHOP +/- radiation. The cut-off percentages for CD3, CD4 , CD8 were determined by pilot studies and their correlation to OS, IPI, staging and relapse was analysed by two-tail t-test.

Results: 31 pts (15 M/16 W; age 32-78 yrs; mean 62 yrs) satisfied inclusion criteria. The sIg expression was IgG (10/31), IgM (12/31), null (8/31), IgG/IgM (1/31). CD3+ cells ranged between 5-78% in tissue samples. CD4 and CD8 were available in 20 pts. IPI was <=2 in 21/31 pts and 3-4 in10/31. There were 15/31 pts with stage I-II disease while 16/31 were in III-IV. The overall survival (OS) was 57+/- 26 months (range; 8-95 months). 17/31 pts had a history of relapse. Significant differences for OS noted between DLBCL expressing IgG (72 months) vs. IgM (35 months) ($p<0.01$) and IPI (IgG=1 vs IgM =3; $p<0.004$). IPI / OS was not statistically significant in null group. Higher % of CD3+ cells (>35%), had significant impact on OS / IPI ($p<0.02$ / <0.001). CD4 + cells >20% had similar effect on OS/IPI ($p<0.004/0.04$). Lower % of CD8+ cells (<15%) showed significant correlation with relapse ($p<0.002$).

Conclusions: FCM analysis in DLBCL pts may provide valubale prognostic information. Biological differences between IgG+ vs. IgM+ DLBCL and correlation of tumour infiltrating T lymphocytes and its subsets needs further exploration in DLBCL pts.

1125 Aberrant Expression of CD5 and CD10 in Hairy Cell Leukemia: Correlation with Clinical Presentation and Outcome

A Rahemtullah, S Rezk, SA Wang, RP Hasserjian. Massachusetts General Hospital, Boston, MA; UMass Memorial Medical Center, Worcester, MA.

Background: Hairy cell leukemia (HCL) is a B-cell neoplasm with clinicopathologic features and treatment distinct from other low-grade B-cell lymphomas. We report our experience with HCL aberrantly expressing CD5 and/or CD10, markers important in classifying low-grade B-cell neoplasms but rarely expressed in HCL.

Design: Pathology reports from 111 HCL cases identified in the surgical pathology files of 2 institutions over a 10-year period (1995-2005) were retrieved. All available materials from cases with coexpression of CD5 and/or CD10 were reviewed, including bone marrow biopsy and aspirate slides, peripheral blood smears, immunohistochemistry, and flow cytometry scattergrams. Clinical presentation, treatment information, and follow-up were obtained from these cases.

Results: Among the 111 cases, 4 expressed CD5 (4%), 10 expressed CD10 (9%), and 1 case expressed both CD5 and CD10. These 15 cases included 13 males and 2 females with a median age of 50 years (range: 30-75). Splenomegaly was present in 11 cases, absent in 3, and unknown in 1. Absolute monocytopenia at presentation was noted in 9 of 11 cases with available data. All cases exhibited bright CD20 and surface immunoglobulin expression. 13/15 cases demonstrated a CD103+CD11c+CD25+ immunophenotype typical of HCL; one CD5+ case lacked CD25 expression and one CD10+ case had not had HCL markers done, but did have findings on bone marrow pathology consistent with the diagnosis. All cases showed typical HCL morphology on smears and in the bone marrow biopsy sections with the exception of the CD25- case, which contained medium to large cells with irregular nuclei and pale cytoplasm with hairy projections. 13/14 of the patients with available follow-up information were initially treated with cladribine. 7/9 CD10+ cases achieved a complete response to cladribine, while 2 had residual disease 4 and 6 months post-therapy; 2 patients subsequently relapsed 4 and 5 years after initial presentation. Three CD5+ cases and the single CD5+CD10+ case achieved a complete, sustained response to cladribine. The CD5+CD25- case with unusual morphology was treated with interferon, but the patient subsequently died of disseminated disease 5 months after presentation.

Conclusions: This study highlights the importance of recognizing HCL in the differential diagnosis of CD5+ and CD10+ B-cell lymphomas. These cases exhibit clinical, morphologic and other immunophenotypic features similar to typical HCL and respond to cladribine therapy similarly.

1126 Gene Expression Profile of Primary Pulmonary Malt Lymphoma: Insights into Molecular Pathogenesis

ED Remstein, AL Oberg, AA Leontovich, BW Morlan, JL Myers, PJ Kurtin, A Dogan. Mayo Clinic, Rochester, MN.

Background: Primary pulmonary MALT lymphomas (P-MALT) are low-grade malignant lymphomas of B-cell origin. Approximately 50% of P-MALT harbor a balanced chromosomal translocation, most commonly t(11;18)(q21;q21)/*API2-MALT1* (30-35%) and t(14;18)(q32;q21)/*IGH-MALT1* (10-15%). The cases with and without the translocations are morphologically and immunophenotypically indistinguishable but may have a different pathogenesis and natural history. In this study gene expression profiling was used in an attempt to identify different molecular pathways involved in the pathogenesis of P-MALT.

Design: Thirty P-MALT with known translocation status (8 t(11;18)/*API2/MALT1*+ cases, 3 t(14;18)/*IGH-MALT1*+ cases, 19 cases without translocation) were studied. Gene expression profiling was performed on the Affymetrix U133 Plus 2.0 platform using RNA extracted from frozen tumor samples. Group comparisons were performed on normalized data via linear mixed effects models.

Results: Analysis of the differentially expressed genes in P-MALT with translocation versus without translocation showed that MALT1 was the top-ranked differentially overexpressed gene in translocation-positive cases. Other genes of interest that were differentially expressed included transcription factor, WT1; protein tyrosine phosphatase, PTPRK implicated in B-cell lymphoma development; TNF receptor-associated signaling molecule, TRAF4; GPI-anchored cell surface molecule, LY6G5C involved in immune activation and tyrosine kinase receptor; and EPHA4 (Ephrin) implicated in lymphomagenesis. In addition, expression of NF kappaB pathway-associated genes was separately analyzed. Again, MALT1 was the top-ranked differentially overexpressed gene in translocation-positive cases. Other genes of interest that were differentially expressed included hematopoietic growth promoting cytokines, CSF3(GCSF), IL6 and IL3; receptor for B-cell growth factors BAFF/APRIL, TNFRSF13B (TACI); adhesion molecule, CD44; inhibitor of apoptosis, BIRC6 (BRUCE); growth factor, IGF1; and non-receptor tyrosine kinase, BMX. Interestingly other genes implicated in the pathogenesis of MALT lymphoma such as BCL10, FOXP1, BIRC3 (API2), CD80 and CD86 were not differentially expressed between the two groups.

Conclusions: Our results indicate that MALT1 overexpression is the critical oncogenic event in translocation-positive P-MALT cases. The differences in overall and NF kappaB-associated gene expression patterns suggest that the molecular pathogenesis of the two groups is different.

1127 Expression of Inhibitor of Apoptosis Proteins in B-Cell Non-Hodgkin Lymphomas and Hodgkin Lymphomas

Y Ren, N Akyurek, GZ Rassidakis, LJ Medeiros. MD Anderson Cancer Center, Houston, TX.

Background: Increased resistance to apoptosis is involved in the pathogenesis of many hematological disorders. Members of the inhibitor of apoptosis protein (IAP) family have been shown to inhibit both the external and intrinsic apoptosis pathways. IAPs are suggested to play a role in resistance to chemotherapy and may be targets for therapy. Expression of IAPs has been found in a wide variety of cell lines and primary tumors, but little is known about IAP expression in lymphomas. We assessed expression of three IAP family members, cIAP1, cIAP2 and XIAP, in large number of B-cell lymphoma cell lines and primary tumors.

Design: The study group included 240 cases of B-non-Hodgkin lymphoma (NHL), 40 cases of Hodgkin lymphoma (HL), and 14 cell lines (10 B-NHL and 4 HL). Expression of cIAP1, cIAP2, and XIAP were determined by immunohistochemistry and Western Blot analysis. For immunostaining, tumors were analyzed using either tissue microarrays or full tissue sections. The following antibodies were used: cIAP1 (polyclonal, RD Systems, Minneapolis, MN); cIAP2 (polyclonal, Santa Cruz Biotechnology, Santa Cruz, CA), and XIAP (monoclonal, BD Transduction Labs, Lexington, KY).

Results: cIAP1 was positive in 13/14 cell lines (1 DLBCL-), cIAP2 was positive in 11/14 (2 MCL- and 1 DLBCL-), and XIAP was strongly positive in all cell lines. In 240 B-cell NHL cases, cIAP1 was expressed in 175 (73%), cIAP2 in 115 (48%), and XIAP in 37 (15%). In 40 HL cases, cIAP1 was positive in 30 (75%), cIAP2 in 25 (63%), and XIAP in 23 (58%). Specific types of NHL and HL are listed in the table.

Conclusions: IAPs are commonly expressed in many types of B-NHL and HL. Apoptotic mechanisms mediated by IAPs are likely to be involved in the pathogenesis of lymphomas. IAPs may be novel targets for the treatment of patients with B-cell lymphoma.

Expression of IAP Proteins in B-cell NHL and HL

	No.	cIAP1	cIAP2	XIAP
NHL				
Follicular	30	29 (97%)	17 (57%)	0
Mantle cell	38	18 (47%)	1 (3%)	0
CLL/SLL	9	5 (56%)	6 (67%)	0
LPL/WM	7	4 (57%)	5 (71%)	1 (14%)
N-MZL	7	7 (100%)	0	3 (43%)
E-MZL	10	5 (50%)	10 (100%)	0
SMZL	8	4 (50%)	1 (13%)	1 (13%)
DLBCL	38	34 (92%)	14 (37%)	10 (26%)
Burkitt	12	7 (58%)	9 (75%)	4 (33%)
Pre-LBL/ALL	28	28 (100%)	26 (93%)	16 (57%)
Plasma cell tumors	53	34 (64%)	26 (49%)	2 (4%)
HL				
N-LPHL	6	5 (83%)	5 (83%)	5 (83%)
Classical HL	34	25 (74%)	20 (59%)	18 (53%)

1128 Abnormal Cytoplasmic Localization of ARF in Acute Myeloid Leukemia (AML) with *NPM* Gene Mutations

Y Ren, V Leventaki, M Routbort, T McDonnell, LJ Medeiros, GZ Rassidakis, W Chen. MD Anderson Cancer Center, Houston, TX.

Background: Somatic mutations of the nucleophosmin (*NPM*) gene have been recently reported in AML. These mutations disrupt the nucleolar-localization signal, causing abnormal cytoplasmic accumulation of NPM (NPMc+). However, the underlying mechanism(s) by which *NPM* mutations contribute to leukemogenesis are unknown. NPM normally shuttles between the cytoplasm and nucleus, and associates with various proteins including the cyclin-dependent kinase inhibitor p14 or alternative reading frame (ARF). NPM has been suggested to interact with ARF, and exert its tumor-suppressor function via ARF-p53 pathway. We tested the hypothesis that *NPM* gene mutations in AML may result in aberrant subcellular localization of ARF, and subsequently affect p53.

Design: The study group included 9 cases of NPMc+ AML-M5b and 3 cases of NPMc- AML-M1. NPMc+, a surrogate marker for *NPM* gene mutation, was determined by immunofluorescence (IF) on bone marrow (BM) smears. ARF and p53 expression were detected using fixed, routinely processed BM core biopsy sections and immunohistochemical methods.

Results: By IF, AML-M5b blasts demonstrated NPM exclusively localized to the cytoplasm indicating presence of NPM gene mutation (table). By contrast, AML-M1 blasts showed nuclear localization consistent with absence of *NPM* gene mutations. The staining patterns of ARF and p53 were also different in these two groups. Moderate to strong cytoplasmic expression of ARF and strong nuclear expression of p53 were observed in AML-M5b with *NPM* gene mutations. The staining pattern of ARF and p53 in residual normal maturing hematopoietic precursors was similar to that of blasts in AML-M1 without *NPM* gene mutations.

Expression of ARF and p53 in AML

AML subtype	NPMc+	ARF	p53
AML-M5b	+	strong, N/moderate to strong, C	strong, N
AML-M1	-	strong, N/negative to weak, C	weak, N

N, nuclear; C, cytoplasmic stain

Conclusions: Abnormal cytoplasmic localization of ARF is present in NPMc+ AML. Strong nuclear p53 expression indicates its stabilization, possibly through a disrupted ARF-MDM2-p53 pathway, that may, at least in part, contribute to leukemogenesis.

1129 Ultra Sensitive Detection of Mixed Chimerism in Post-BMT Patients Using Real-Time PCR Analysis of Insertion Deletion Polymorphisms

MG Rhode, JK Howard, JA Thorson. University of Michigan, Ann Arbor, MI.

Background: Early detection and treatment of disease relapse is vital to the success of allogeneic bone marrow transplantation (BMT) for hematologic malignancies. Re-emergence of host derived cells, i.e., mixed chimerism (MC), increases prior to relapse, and laboratory assessment of hematopoietic chimerism is routine in the follow up of BMT patients. Current DNA-based chimerism assays utilize PCR amplification of short tandem repeat (STR) markers to distinguish donor from recipient derived cells and have a detection limit of approximately 1% under optimal conditions. Using these assays, early indications of relapse may go undetected, limiting the effectiveness of some treatment options. Our objective was to develop an assay with the ability to detect MC below the 1% level, allowing recognition of relapse at an earlier stage.

Design: A panel of 16 diallelic insertion/deletion (indel) polymorphisms as well as the beta-globin (*HBB*) gene were assayed by real time PCR. Pre-transplant genomic DNA from donor/recipient pairs representing 100 BMT cases (50 with related donors, 50 with unrelated donors) seen at the University of Michigan were assayed for the presence or absence of the 16 polymorphisms using a high throughput, micro-volume assay performed in 384 well plates. Markers were deemed informative when present in the pre-transplant recipient sample as well as absent from the donor sample. The degree of MC in 26 post-transplant recipient samples was assessed using relative quantification of informative markers by normalization to the *HBB* gene, with pre-transplant DNA as a 100% standard. Accuracy and detection limits of quantitative measurements were evaluated with known dilutions of recipient DNA and by comparison to a standard STR assay.

Results: Overall informativity of the 16 marker panel was 80% for 100 cases, and equal informativity was obtained for related and unrelated donor/recipient pairs. MC was detected to a level of 0.01% recipient DNA. Quantification correlated well with values

determined by STR assay. For 7/12 post-transplant samples judged to be donor only by STR assay, the indel PCR assay detected MC at levels of 0.01 to 6.0%.
Conclusions: Real time PCR analysis of indel polymorphisms is a rapid and accurate method for quantification of MC in post-BMT patients. The assay provides a detection limit 2 orders of magnitude lower than current state of the art assays, potentially allowing detection of impending relapse and initiation of appropriate therapy at earlier time points.

1130 MALT Lymphoma of the Dura: Analysis of Clinical, Histological and Genetic Features

KA Rizzo, B Streubel, A Chott, L Sorbara, S Kumar, S Pittaluga, M Raffeld, ES Jaffe. National Institutes of Health, National Cancer Institute, Bethesda, MD; Vienna General Hospital, Medical University of Vienna, Vienna, Austria; VA Medical Center, Washington, DC.
Background: Extranodal mucosa-associated lymphoid tissue (MALT) lymphomas present rarely as meningeal mass lesions, with an anatomic distribution similar to that of meningiomas. Recent studies have suggested that MALT lymphoma associated chromosomal alterations show a nonrandom anatomic distribution. We undertook a study to determine the clinicopathological features, immunophenotype and genetic alterations found in primary MALT lymphomas of the dura.
Design: 18 primary dural MALT lymphomas were retrieved from the consultation files of the Hematopathology Section, NCI. $H_{\&}E$ and immunostained slides were reviewed. Clonality was further investigated by PCR amplification of IgH genes. FISH was performed on interphase cells as previously described to detect t(14;18)(q32;q21) involving *IGH* and *MALT1,* rearrangements of *BCL10*, translocations of *FOXP1*, aberrations of *IGH* and *BCL6*, and trisomies of 3, 12 and 18. RT-PCR was used to detect the *API2-MALT1* fusion transcript.
Results: The male:female ratio was 1:3.5, and the age range was 37-62 years old (median 49). The majority of cases, 72%, demonstrated plasmacytic differentiation. 14 cases, 79% (11/14) exhibited light chain restriction and 64% (7/11) expressed kappa light chain. PCR for IgH rearrangement performed on 14 cases show monoclonality in 71% (10/14). FISH and RT-PCR analysis of common MALT associated chromosomal translocations and trisomies of 9 cases demonstrated genetic alterations in 3 cases. One case was positive for trisomy 18. One case was positive for trisomy 1 and trisomy 3. One case was positive for trisomy 18 and trisomy 3. However, none of the cases were positive for trisomy 12.
Conclusions: MALT lymphoma of the dura is a low grade B cell lymphoma, which presents radiographically as a localized mass lesion. There is a female predominance, as with other forms of MALT lymphoma. Monoclonality could be demonstrated in 16 cases (89%), either by light chain restriction or PCR of IgH genes. One-third of the cases investigated exhibited numerical aberrations previously reported in MALT lymphoma. However, translocations such as the t (11;18)(q21;q21) most commonly observed in gastric and pulmonary MALT lymphomas, were not seen in any case.

1131 Tumor Infiltrating T-Cells in Nodular Lymphocyte Predominant Hodgkin Lymphoma Express CXCL13: A Chemokine Critical in Germinal Center Formation

AC Roden, KL Grogg, A Dogan, WR Macon. Mayo Clinic, Rochester, MN.
Background: The germinal center (GC) microenvironment not only is an essential niche for generation of B-cell response, but also is considered to be critical in the development of most human lymphoid neoplasms. Recently it has been shown that GC T-helper (Th) cells express CXCL13, a chemokine essential in GC development and critical for B-cell entry into GC. Based on these observations, we hypothesized that CXCL13 may play a role in the pathogenesis of B-cell lymphomas of GC origin, such as nodular lymphocyte predominant Hodgkin lymphoma (NLPHL). In this study we report CXCL13 expression by tumor infiltrating T-lymphocytes (TITL) in NLPHL and compare the expression pattern to classical Hodgkin lymphoma (CHL) and T-cell/histiocyte-rich large B-cell lymphomas (TCRLBCL).
Design: Paraffin sections were prepared on 18 samples of CHL, 11 samples of NLPHL, and 11 samples of TCRLBCL. All cases were classified according to the current WHO classification. Using standard immunohistochemistry techniques, all samples were stained with antibodies against CXCL13 and CD57.
Results: The staining results are summarized in Table 1.

Table 1: % T-cells expressing CXCL13

Tumor Type	<5%	5-20%	20-40%	>40%
NLPHL (n=11)	-	-	7 [64%]	4 [36%]
CHL (n=18)	8 [44%]	9 [50%]	1 [6%]	-
TCRLBCL (n=11)	6 [55%]	3 [27%]	2 [18%]	-

There was a strikingly increase in cytoplasmic expression of CXCL13 by TITL in cases of NLPHL with over 20% of TITL expressing CXCL13 in all samples. CXCL13 expressing TITL were mostly within the nodules, often forming rosettes around the tumor cells. In contrast, in the majority of the cases with CHL and TCRLBCL, CXCL13 was expressed by less than 20% of TITL and they were randomly distributed. The staining pattern of CD57 was similar to the staining pattern of CXCL13 in most cases suggesting that these antigens were co-expressed by TITL.
Conclusions: Our data show that TITL in NLPHL are phenotypically identical to normal GC-Th cells expressing both CD57 and CXCL13. Given the biological role of CXCL13 and the intimate asssociation of CXCL13 positive TITL with the tumor cells, it is likely that this chemokine plays a role in the pathogenesis of NLPHL. Furthermore, our findings suggest that CXCL13 immunostaining may be a useful tool in the differential diagnosis of NLPHL from TCRLBCL.

1132 Evaluation of CD52 Expression in Hematopoietic Neoplasms by Standard Immunohistochemistry: Implications for the Expanded Use of Alemtuzumab (CAMPATH-1H) in the Treatment of Hematologic Malignancies

SJ Rodig, JS Abramson, GS Pinkus, MA Shipp, JL Kutok. Brigham & Women's Hospital, Boston, MA; Dana-Farber Cancer Institute, Boston, MA.
Background: CD52 is a GPI-linked glycoprotein expressed by B cells, T cells, monocytes and macrophages. The humanized monoclonal antibody alemtuzumab (CAMPATH-1H) is specific for CD52 and is FDA-approved for the treatment of relapsed or refractory chronic lymphocytic leukemia (CLL). The utility of CAMPATH in the treatment of other lymphoid and non-lymphoid malignancies has been explored; however, a comprehensive survey of CD52 expression among the various classes of hematopoietic neoplasms has not been completed. In addition, the detection of CD52 has generally relied on flow cytometry and cannot be performed in a retrospective manner.
Design: Using standard immunohistochemistry, 223 hematolymphoid neoplasms were analyzed using a CD52 specific rat monoclonal antibody (CAMPATH-1G; Serotec, UK). Rabbit anti-rat secondary followed by Envision plus anti-rabbit (DAKO) and DAB chromogen were used for detection. Tissue samples included formalin, B5, and Zenker's fixed, paraffin-embedded bone marrow and lymphoid tissue.
Results: All low-grade B cell lymphoproliferative disorders (CLL/SLL, follicular lymphoma, lymphoplasmacytic lymphoma, mantle cell lymphoma) express CD52. However, variable expression of the antigen was noted among select tumor types. For example, plasma cells are largely negative for CD52 in LPL, and proliferation centers show higher expression of CD52 in CLL/SLL. In contrast to low grade B cell lymphomas, a subset of diffuse large B cell lymphomas (22%) were negative for CD52. Unlike the B cell lymphomas, a minority of mature T cell neoplasms (32%) showed CD52 expression. Among acute leukemias, the vast majority of cases of precursor-B ALL express CD52, but only rare cases of precursor-T ALL were CD52 positive. Among AMLs, only AML-M4Eo subtype was CD52 positive. Finally, all cases of Hodgkin lymphoma and multiple myeloma tested were negative for CD52 expression.
Conclusions: We demonstrate robust detection of CD52 by standard immunohistochemistry on paraffin embedded tissues and provide an expanded analysis of CD52 expression in hematopoietic malignancies. In addition, the data reveal that CD52 is not universally expressed among T and B cell lymphoproliferative disorders and acute leukemias, and, thus, target validation among select tumor subtypes will result in more rational use of CAMPATH therapy.

1133 Expression of TRAF1 and Nuclear C-Rel Distinguishes Primary Mediastinal Large B Cell Lymphoma from Nodal Diffuse Large B Cell Lymphoma

SJ Rodig, KJ Savage, NL Harris, MA Shipp, ED Hsi, RD Gascoyne, JL Kutok. Brigham & Women's Hospital, Boston, MA; British Columbia Cancer Agency, Vancouver, BC, Canada; Massachusetts General Hospital, Boston, MA; Dana Farber Cancer Institute, Boston, MA; Cleveland Clinic Foundation, Cleveland, OH.
Background: Primary mediastinal large B cell lymphoma (PMLBCL) and diffuse large B cell lymphoma (DLBCL) are neoplasms with distinct clinical characteristics, but overlapping morphology and near identical immunophenotypic profiles. Recent studies reveal that unique gene expression patterns differentiate these two entities and reinforce the notion that these tumors are biologically distinct; yet, a reliable means to distinguish these tumors during routine surgical pathology practice has not been reported or validated against a large cohort of cases.
Design: TRAF1 is a component of the TNF signaling cascade that favors anti-apoptotic/NFkappa B mediated effects and has been shown to be differentially expressed between PMLBCL and DLBCL by gene expression profiling studies. We assessed the protein expression of TRAF1 and the NFkappa B component, c-Rel, by standard immunohistochemical techniques. Formalin fixed paraffin embedded cases from 251 large cell lymphomas (78 cases of PMLBCL and 173 cases of DLBCL by WHO criteria) obtained from four separate institutions were analyzed.
Results: Cytoplasmic TRAF1 expression was seen in 48 of 78 (62%) cases of PMLBCL, but only 20 of 173 (12%) cases of DLBCL. In addition, robust nuclear localization of c-Rel in neoplastic cells was present in 31 of 48 (65%) cases of PMLBCL and 27 of 125 (22%) cases of DLBCL . Finally, combined TRAF1 expression and nuclear localization of c-Rel was seen in 24 of 45 cases (53%) of PMLBCL, but only 3 of 156 cases (2%) of DLBCL. The sensitivity and specificity for this marker combination in distinguishing PMLBCL from DLBCL are provided below.
Conclusions: The combined expression of TRAF1 and the nuclear localization of c-Rel is highly specific in distinguishing PMLBCL from DLBCL and is applicable to routine surgical pathology practice. These findings also imply that in a subset of DLBCLs, activation of NFkappa B is independent of TRAF1.

Antigen (localization)	Sensitivity*	Specificity*
TRAF1+ alone (cytoplasmic)	62%	88%
c-Rel alone (nuclear)	65%	78%
TRAF1+ & c-Rel(nuclear)	53%	98%

* for PMLBCL

1134 Phenotypic Analysis of T Cell Acute Lymphoblastic Leukemias with Genetic Alteration of the *TAL1* Locus

ER Rudzinski, Q Wang, S Olsen, MA Thompson. Vanderbilt University Medical Center, Nashville, TN.
Background: Over-expression of the TAL1/SCL transcription factor occurs commonly in cases of T-cell acute lymphoblastic leukemia (T-ALL), either due to a t(1;14)(p34;q11) translocation involving the *TAL1* locus on chromosome 1 (3%) or due to deletions 5' of *TAL1* which bring the *TAL1* gene under the control of the *SIL* promoter (12-26%). The target genes regulated by TAL1 which cause leukemogenesis are as yet unknown. Two groups have reported microarray analyses demonstrating a unique gene expression signature of T-ALLs over-expressing TAL1(Blood *106*: 274, 2005; Cancer Cell *1*: 75,

2002). Among these up-regulated genes may be targets of TAL1 regulation or co-expressed oncogenes. This study was designed to determine whether immunohistochemical detection of LCK, AML1, STAT5a, and c-kit correlate with disruption of the *TAL1* locus. *LCK*, *AML1*, and *STAT5a* are shown by microarray analysis to be upregulated in T-ALLs overexpressing TAL1, and *c-kit* has been shown to be a potential target of TAL1 transcriptional regulation *in vitro*.

Design: Nuclei were isolated from paraffin blocks in 13 archival cases of T-ALL, and FISH using a SIL-TAL1 break-apart probe (DakoCytomation) was performed. Nuclei were scored for either separation of signals, indicating a translocation, or loss of red signal, indicating deletion of the region 5' of *TAL1*. Paraffin sections were examined by immunohistochemistry using antibodies to LCK, AML1, STAT5a, and c-kit (CD117). Positive cases were those with greater than 25% of blasts staining with at least 1+ intensity.

Results: Of the 13 cases examined by FISH, 6 showed alteration of the *TAL1* locus, including 2 translocations and 4 5' deletions. 7 cases showed an intact *SIL-TAL1* locus; however, one case showed 4 copies of *SIL-TAL1*. 4 of the 6 cases with an altered *TAL1* locus expressed AML1 and STAT5a protein by immuno-histochemistry and 2 of 6 expressed LCK, but none co-expressed c-kit. For the cases with an intact *TAL1* locus, 3 of 5 expressed AML1, 3 of 6 expressed LCK, 1 of 5 expressed c-kit, and 4 of 6 expressed STAT5a. The Fisher Exact Probablility test showed no significant correlation between alteration of the *TAL1* locus and AML1, STAT5a, LCK or c-kit expression.

Conclusions: Both AML1 and STAT5a were expressed in a majority of the cases showing alteration of the *TAL1* locus; however, these markers do not reliably predict *TAL1* dysregulation. Although *c-kit* is implicated as a target of TAL1 regulation *in vitro*, c-kit protein was not detected in cases showing alteration of the *TAL1* locus.

1135 Secondary Genetic Alterations in Mantle Cell Lymphoma Influence Gene Expression and Improve the Proliferation-Based Prognostic Model

I Salaverria, S Bea, A Zettl, J Valls, V Moreno, G Ott, HK Muller-Hermelink, LM Staudt, E Campo, A Rosenwald. Hospital Clinic, Barcelona, Spain; University of Wurzburg, Wurzburg, Germany; IDIBELL Catalan Institute of Oncology, Barcelona, Spain; NCI, Bethesda.

Background: Mantle cell lymphoma (MCL) is characterized by 11q13 translocations leading to the overexpression of the *CCND1* gene. Additional alterations in cell cycle mechanisms play an important role in the progression of the tumors and proliferation activity is the strongest prognostic parameter in these patients. Additional secondary genetic alterations have been associated with the progression of the disease. However, it is not know whether these chromosomal aberrations may add prognostic information to the proliferation based model.

Design: Comparative genomic hybridization (CGH) was performed in 71 untreated MCL patients previously examined by cDNA microarrays (Lymphochip). Cases were classified by a proliferation signature based on the average expression of 20 proliferation-related genes

Results: Common alterations were gains of 3q21-q29(32%), 8q21-q24(11%), 15q21-q26(11%) and 18q11-q23(11%) and losses of 1p21-p31(52%), 13q14-q34(40%), 11q21-q23(28%), 6q22-q27(22%), 6q15-q21(18%), 9p21-p24(18%), 8p21-p23(13%), 13q11-q13(17%) and 17p13(13%). No recurrent high-level DNA amplifications were detected. Classical (n=58) and blastoid (n=13) variants differed in losses of 9p21-p24 ($p<0.001$). Cases with more chromosomal imbalances (>2) had poorer overall survival than cases with 0-1 alterations ($p=0.0001$). Losses of 9p and 17p were significantly associated with losses of *INK/ARF* and *p53* genes by RQ-PCR and were associated with high number of chromosomal imbalances($p<0.05$). Losses of 9p21-p24 ($p<0.0001$), 9q21-q34 ($p=0.015$) and 8p21-p23 ($p=0.03$) and gains of 8q21-q24 ($p=0.03$) were associated with shorter survival. A multivariate analysis showed that gains of 3q27-q29, and losses of 9p21 and 9q22-q32 were significantly associated with inferior survival independently of gene expression -based proliferation signature.Correlation between CGH and gene expression identified potential target genes deregulated in more frequently altered regions.

Conclusions: These results indicate that MCL harbor a high number of secondary genetic alterations that predict outcome. Importantly, gains of 3q27-q29 and losses of 9p21 and 9q22-q32 provide prognostic information that is independent of the expression-based proliferation signature.

1136 Bcl-2 Expression Levels in Chronic Lymphocytic Leukemia: Correlation with Other Prognostic Indicators

RL Sargent, FE Craig, SH Swerdlow. University of Pittsburgh School of Medicine, Pittsburgh, PA.

Background: Chronic lymphocytic leukemia (CLL) was considered an accumulative disorder of abnormally long-lived cells; however, more recently it has been suggested that apoptotic abnormalities are not of significance, particularly in explaining why the CD38 positive, ZAP-70 positive, IGH unmutated subset is more aggressive than the subset lacking these prognostic features. Nevertheless, bcl-2 levels are variably elevated in CLL and thought by some to be of prognostic importance. For this reason, bcl-2 expression levels were assessed in CLL and compared to other well-documented prognostic indicators.

Design: Three color flow cytometric studies were performed on 51 CLL cases (30 peripheral blood, 16 bone marrows, 4 lymph nodes, 1 pleural fluid) to evaluate CD38 and ZAP-70 expression (positive=>20%) and bcl-2 mean fluorescence intensity (MFI) in CD19+/CD5+ B-cells. The ratio of bcl-2 MFI in B versus T-cells was also calculated (MFI-R).

Results: Absolute and relative B-cell bcl-2 levels varied greatly among the CLL cases (MFI 45-152; MFIR 0.92-2.85). Analysis of the B-cell MFI and the MFI-R did not demonstrate statistically significant differences between the CD38 positive vs CD38 negative cases (MFI 92±29 vs 106±27; MFIR 1.73±0.53 vs 1.73±0.36) nor between ZAP-70 positive vs ZAP-70 negative cases (MFI 102±25 vs 97±30; MFIR 1.85±0.42 vs 1.71±0.46). There was an inverse correlation between B-cell CD38% positivity and bcl-2 MFI ($r=-0.30$, $p<0.04$). CD38 positivity correlated with ZAP-70 positivity ($p<0.04$); however, 65% of the CD38+ cases were ZAP-70 negative. Analysis using a lower cutoff for ZAP-70 positivity did not alter the bcl-2 results but did show a stronger correlation with CD38 expression ($p<0.01$).

Conclusions: These findings demonstrate that greater numbers of CD38 positive cells are significantly associated with less intense staining for bcl-2; however, a statistically significant association could not be identified between the variable bcl-2 levels in CLL and either CD38 or ZAP-70 positivity greater than 20%. Although the findings suggest that increased bcl-2 expression may play a more critical role in the cases with fewer CD38 positive cells, levels of bcl-2 do not appear to be a major determinant of the difference between the indolent and more aggressive groups of CLL.

1137 c-Maf Expression in Angioimmunoblastic T-Cell Lymphoma

E Sasaki, N Mori, T Sakaguchi, Y Yamashita, S Takahashi. Nagoya University Graduate School of Medicine, Nagoya, Aichi, Japan; University of Tsukuba, Tsukuba, Ibaraki, Japan.

Background: The oncoprotein c-Maf was recently found to be overexpressed in about 50% of multiple myelomas (MM) and c-Maf plays an important role in survival and expansion of MM through promoting cyclin D2, CCR1 and integrin β expression. WHO defines that angioimmunoblastic T-cell lymphoma (AILT) is a peripheral T-cell lymphoma characterised by systemic disease, a polymorphous infiltrate involving lymph nodes, with a prominent proliferation of high endothelial venules and follicular dendritic cells. One of the authors found high levels of c-Maf in AILT by RT-PCR. In this study, we subsequently examined T-cell lymphomas with a particular focus on AILT for c-Maf and D-type cyclins (cyclins D1, D2 and D3) expression immunohistochemically.

Design: We selected twenty-two cases of AILT, nine cases of adult T-cell leukemia/lymphoma (ATL), nine cases of peripheral T-cell lymphoma, unspecified (PTCL-U), and eleven cases of mycosis fungoides (MF). AILT cases were diagnosed on the basis of standard morphologic criteria and immunohistochemical detection. AILT, ATL, PTCL-U and MF cases were immunostained with c-Maf and D-type cyclins.

Results: C-Maf expression was seen in 13/22 (59%) cases of AILT, 2/9 (22%) of ATL, 2/9 (22%) of PTCL-U and 0/11 (0%) of MF. Among the c-Maf positive AILT cases, double immunostaining revealed that c-Maf positive cells were also immunopositive for CD4, CD43 and CD45RO but negative for CD8 and B-cell markers. Cyclin D1 was immunopositive in 10/13 (77%) cases, cyclin D2 in 9/13 (69%) and cyclin D3 in 7/13 (54%) of the c-Maf positive AILT cases.

Conclusions: Double immunostaining showed that c-Maf positive cells are neoplastic cells because AILT is believed as a CD4 positive T-cell lymphoma and we concluded that c-Maf expression is relatively specific for AILT compared to other types of T-cell lymphoma. These findings may provide new insight into the pathogenesis of AILT and suggest that c-Maf may be a useful diagnostic marker for AILT. Though c-Maf expression affects the expression of cyclin D2 in MM, the role of c-Maf in cyclin D expression in AILT awaits future investigation.

1138 Over-Expression of CD7 in CD4(+) T Cells in Classic Hodgkin Lymphoma

AC Seegmiller, NJ Karandikar, SH Kroft, RW McKenna, Y Xu. University of Texas Southwestern Medical Center at Dallas, Dallas, TX.

Background: Hodgkin lymphoma (HL) is characterized by Hodgkin and Reed-Sternberg (HRS) cells in a background of reactive inflammatory cells. While HRS cells usually are not identified by flow cytometry (FC), a large number of T cells with a high CD4:CD8 ratio are frequently detected. We assessed the diagnostic utility of the immunophenotypic features of T cells in the distinction of HL from reactive lymphadenopathy.

Design: Using an institutional FC database, 54 consecutive excisional lymph node biopsies from HIV(-) patients diagnosed as HL and 140 as reactive lymphadenopathy were identified. The T-cell CD4:CD8 ratio and CD7 mean fluorescence intensity (MFI) in T cells were determined using 4-color FC. To assess the diagnostic utility of FC in HL, receiver operator characteristic (ROC) curves were constructed for CD4:CD8 and CD7 MFI; optimal cut-offs were applied to fine needle aspirate (FNA) specimens from classic HL and reactive lymph nodes.

Results: Immunophenotyping of T cells revealed two major findings. First, CD4:CD8 was significantly higher in nodular sclerosis HL (7.4±1.0), but not in lymphocyte-rich classic HL (6.3±1.2), mixed cellularity HL (3.9±0.7), or nodular lymphocyte predominant HL (6.6±1.2), compared to reactive lymph nodes (4.5±0.2) ($p=0.01$, 0.07, 0.52, and 0.13, respectively). Second, CD7 MFI was significantly higher in CD4(+) T cells in classic HL than in those from reactive lymph nodes (118±8.4 vs. 57±2.0; $p<0.001$). This difference was much more pronounced in patients <=40 years of age (141±9.9 vs. 56±3.1; $p<0.001$) than in patients >40 years of age (82±12 vs. 57±2.5; $p=0.06$). Furthermore, CD7 expression in CD4(+) T cells was higher in classic HL ($n=50$) than in NLPHL ($n=4$) (MFI=123±8.6 vs. 46±4.3, $p<0.001$). ROC curve analysis yielded an area under the curve of 0.62 for CD4:CD8 and 0.86 for CD7 MFI in the discrimination of classic HL from reactive nodes. Using a CD7 MFI cut-off point that yielded a sensitivity and specificity of 70% and 92% for the diagnosis of HL versus reactive lymphadenopathy, 8 of 10 HL and 10 of 12 reactive lymph node FNAs were correctly identified.

Conclusions: This study demonstrates that there are immunophenotypic differences between the reactive T cells in HL and reactive lymph nodes that may aid in differentiating these conditions, particularly in younger patients and when only scant material is available for diagnosis. The functional significance of over-expression of CD7 in CD4(+) T cells in HL is a potential area for future study.

1139 Immunophenotypic Differentiation between Neoplastic Plasma Cells in Non-Hodgkin Lymphoma and Plasma Cell Myeloma

AC Seegmiller, Y Xu, RW McKenna, NJ Karandikar. University of Texas Southwestern Medical Center at Dallas, Dallas, TX.

Background: Extranodal marginal zone lymphoma and lymphoplasmacytic lymphoma may show marked plasmacytic differentiation. Thus, it may be difficult to differentiate these lymphomas from plasmacytoma or myeloma, especially with limited diagnostic material. This study delineated the immunophenotypic differences between lymphoma- vs myeloma-associated plasma cells and evaluated their utility in the diagnoses of these disorders.

Design: Using an institutional flow cytometry (FC) database, we identified 41 consecutive B-lineage non-Hodgkin lymphomas showing plasmacytic differentiation, defined by a distinct subpopulation of cells expressing bright CD38. The immunophenotypic features of these plasma cells were compared to those of 41 consecutive bone marrow myelomas, using four-color FC. To evaluate the utility of the results, we also analyzed 7 isolated extramedullary plasmacytomas and 6 myeloma-associated extramedullary lesions.

Results: The neoplastic plasma cells from non-Hodgkin lymphomas showed a distinct immunophenotype compared to plasma cell myeloma. They were significantly more likely to express CD19 [39/41 (95%) vs 4/41 (9.8%), p<<0.001], CD45 [30/33 (91%) vs 17/41 (42%), p<<0.001] and surface immunoglobulin [31/41 (76%) vs 18/41 (44%), p=0.006], and less likely to express CD56 [6/18 (33%) vs 29/41 (71%), p=0.01]. Those expressing CD56 did so at a significantly lower level than plasma cell myelomas, with no overlap in mean fluorescence intensities [106±18 vs 1220±178, p<<0.001]. There was no significant difference in CD20 expression [13/41 (32%) vs 11/41 (20%), p=0.81]. As expected, all 6 myeloma-associated extramedullary lesions exhibited immunophenotypes similar to that of bone marrow myeloma, as did 5 of 7 isolated extramedullary plasmacytomas. Interestingly, the other 2 tumors, both of which involved the thyroid gland, showed lymphoma-like immunophenotypes, suggesting that they may indeed represent extranodal marginal zone lymphomas (MALT-type) with extensive plasmacytic differentiation.

Conclusions: Neoplastic plasma cells associated with non-Hodgkin lymphomas bear an immunophenotype that is distinct from that of plasma cell dyscrasias, in that they express CD19, CD45 and surface immunoglobulin and are negative or dim for CD56. These differences may be useful in distinguishing plasma cell processes from non-Hodgkin lymphomas with extensive plasmacytic differentiation, especially with limited biopsy material.

1140 CD30 Positive Cells in Nodular Lymphocyte Predominant Hodgkin Lymphoma (NLPHL): A Clinicopathologic Evaluation

RM Seliem, WL Wang, RP Hasserjian, JA Ferry, NL Harris, LR Zukerberg. Massachusetts General Hospital, Boston, MA.

Background: Nodular lymphocyte predominant Hodgkin lymphoma (NLPHL) is a distinct disorder with characteristic clinical, morphological and immunological features, including a nodular growth pattern with or without a diffuse component, expression of CD20, BCL6, CD79a and CD45 on tumor cells, lack of CD30 and CD15, and a good prognosis. Reed-Sternberg cells of classical Hodgkin lymphoma (CHL) are typically CD20 negative and CD15 and CD30 positive; however, CD20 may be expressed and CD15 may be absent in some cases. Thus CD30 is often the most decisive marker for distinguishing between CHL and NLPHL. We questioned whether CD30 expression occurs in NLPHL and whether CD30 positive cases have distinct morphological or clinical features.

Design: We retrospectively analyzed the morphologic and immunophenotypic patterns of 217 cases of NLPHL evaluated in our institution from 1998 to September 2005. The morphologic features and staining patterns with antibodies to CD30, CD15, CD20, CD3, and CD21 were evaluated. Clinical follow up including staging, therapy, and survival was obtained when possible.

Results: Neoplastic cells focally expressed CD30 in 18 cases. There were 15 males and 3 females aged 9 to 71 years (median 54 years). The patients generally presented with cervical, inguinal or soft tissue masses. In 17 cases, CD30 staining was fainter in tumor cells than in CD30 positive immunoblasts present in the background, while in one case staining was strong. The L&H cells in all cases were CD20 positive and CD15 negative. BCL6, Oct-2, BOB-1 and CD45 immunostains were positive in the L&H cells in all cases tested. 12 cases showed variable degrees of sclerosis and fibrous band formation. Follow-up was available for 10 patients, from 4 months to 18 years (median 2 years). Nine patients had no disease recurrence. One patient presented initially with diffuse large B-cell lymphoma, relapsed 8 years later with NLPHL, then had a second relapse of diffuse large B-cell lymphoma with residual NLPHL and died of acute respiratory distress 3 months after the final diagnosis.

Conclusions: CD30 is weakly expressed by neoplastic cells in approximately 8% of NLPHL. The clinical features at presentation and the prognosis are similar to those of CD30 negative NLPHL. The presence of CD30 staining of the neoplastic cells was associated with more frequent sclerosis. Faint CD30 expression by L&H cells in NLPHL can be seen and should not lead to diagnostic confusion.

1141 Appropriate Categorization of High Grade B-Cell Lymphomas with Classical Burkitt Morphology but Lacking MYC Translocation

DW Sevilla, JZ Gong, BK Goodman, PJ Buckley, AS Lagoo. Duke University Medical Center, Durham, NC.

Background: Burkitt lymphoma is a very aggressive mature B-cell neoplasm composed of monomorphic medium-sized cells in which translocation involving MYC is a constant genetic feature. The WHO classification defines "atypical Burkitt lymphoma" or "Burkitt-like lymphoma" as cases with more pleomorphic cells than Burkitt lymphoma which still have the required MYC translocation. Very high proliferation fraction determined by immunohistochemistry is acceptable as a surrogate marker for MYC translocation if genetic information is lacking. However no clear guidelines are given for classification of cases in which classical Burkitt morphology and high proliferation fraction are not accompanied by MYC translocation.

Design: 23 patients presenting with highly aggressive B-cell neoplasms with typical Burkitt cytomorphology (uniform, non-cleaved cells, small nucleoli, cytoplasmic vacuoles), histology ("starry sky" pattern) and immunophenotype (CD20+,CD10+,sIg+) were studied. In all cases with solid tumors, the proliferation fraction was assessed by immunohistochemical staining and MYC translocation was examined by fluorescence in situ hybridization or conventional cytogenetics.

Results: 19 of 23 patients were male. The patients ranged in age from 4 years to 61 years (median 35 years). A rearrangement of MYC was present in 10 cases but could not be demonstrated in 13. The patients in the latter group were somewhat younger (median age 16 years versus 43 years), but otherwise the clinical features were comparable. The Ki-67 staining in all cases without a demonstrable MYC arrangement was >95% and the morphology of the cells was that of classical Burkitt lymphoma. Only two patients (one with MYC translocation and one without) showed weak expression of TdT. All patients received similar chemotherapy and showed complete initial response.

Conclusions: The presence of very high proliferation fraction and classical Burkitt morphology do not always correlate with MYC translocation and cannot be used reliably to diagnose Burkitt lymphoma / leukemia as currently defined in WHO classification. In the absence of clear guidelines in the WHO classification, cases with classical Burkitt morphology, high proliferation fraction, but lacking MYC translocation are best categorized as high grade B-cell lymphoma with Burkitt-like features. Optimal therapy for these patients can be determined only if these cases are classified in a uniform fashion.

1142 Decrease of p38 MAPK Activation in Myeloproliferative Disorders

M Shahjahan, CH Dunphy, N Singhal, L Garza, Y Zu, C Chang. The Methodist Hospital, Houston, TX; University of North Carolina, Chapel Hill, NC.

Background: Activation (phosphorylation) of p38 mitogen-activated protein kinase (MAPK), a member of the MAPK family, by cytokines and growth factors has been shown to play a critical role in regulating hematopoiesis. In vitro studies have shown that p38 MAPK may participate in the differentiation and proliferation of AML and CML cell lines. The goal of the current study is to preliminarily evaluate the role of p38 MAPK in myeloproliferative disorders.

Design: Activation (phosphorylation) of p38 MAPK was analyzed by IHC using an antibody specific to phosphorylated p38 MAPK (Thr180/Tyr182, Cell Signaling Technology) in the formalin- fixed decalcified bone marrow core (BMC) biopsies from 32 MPD patients (14 CMLs; 4 PVs; 4 ETs; 5 MFs, 5 MPD-NOSs by WHO classification). Controls included 7 BMC biopsies free of MDS, MPD, leukemia or other hematologic malignancies. The degrees of activation of p38 MAPK in erythroid, myeloid, or megakayoctyic lineages were evaluated by scoring the % of cells in each lineage showing cytoplasmic/nuclear staining. The degree of p38 MAPK activation was defined as minimal (<5% of cells with cytoplasmic/nuclear p38 MAPK staining), weak (5-20% of cells), moderate (21-50% of cells) or strong (> 50% of cells) for each lineage.

Results: In erythroid lineage, all MPD cases show absent/minimal p38 MAPK activation; while p38 MAPK activation was moderate (6/7) to strong (1/7) in controls. In myeloid and megakaryocytic lineages, strong p38 MAPK activation was more commonly observed in controls than in MPDs (myeloid lineage: 7/7 vs. 4/32, p<0.0001; megakaryocytic lineage: 6/6 vs 6/32, p=0/0003, Fisher's Exact). The majority of MPDs showed weak (10/32 myeloid, 10/32 megakaryocytic) to moderate (18/32 myeloid, 10/32 megakaryocytic) p38 MAPK activation in myeloid and megakaryocytic lineages.

Conclusions: The vast majority of MPDs, independent of subtypes, show significant decrease of p38 MAPK activation in all 3 lineages, with the erythroid lineage being the most dramatic. This data suggests that dysregulation of p38 MAPK pathway may play a role in MPDs.

1143 Effects of Rituximab on the Immunophenotype of Benign B-Cell Precursors: Implications for Flow Cytometric Minimal Residual Disease Detection in Precursor B-Acute Lymphoblastic Leukemia

K Siami, S Awagu, DG Cooper, DA Thomas, Y Huh, D Jones, W Chen, JL Jorgensen. UTMDACC, Houston, TX.

Background: Anti-CD20 therapy using rituximab may be used for precursor B- acute lymphoblastic leukemia (B-ALL) with CD20+ blasts. Changes may occur in the immunophenotype (IP) of marrow B-cell precursors (hematogones) after rituximab therapy, and this may complicate assays for minimal residual disease (MRD) by flow cytometry (FC). We aimed to define rituximab-induced changes in the IP of hematogones, and to determine which FC markers can best distinguish them from B-ALL.

Design: Bone marrow aspirates from 22 B-ALL patients were evaluated for MRD by 4-color FC, using an extensive marker panel (CD9, CD10, CD13, CD15, CD19, CD20, CD22, CD33, CD34, CD38, CD45, CD45RA, CD52, CD66c, CD79a, BCL-2, κ, λ, and TdT). Patients treated with high-dose chemotherapy and rituximab (HDC-R, 18 patients with 101 specimens) were compared with HDC-only patients (4 with 30 specimens), with reference to the IP of the original B-ALL. The FC results were correlated with PCR data (for immunoglobulin heavy chain [IgH] rearrangements, and bcr-abl rearrangements in Ph+ cases), and clinical outcomes.

Results: Specimens from HDC-only patients showed the expected spectrum of B-cell maturation, with a subset of CD20+ cells. In contrast, most of the HDC-R patients (17/18) showed a distinct IP in at least one specimen: near-complete absence of CD20+ cells, and a proportional increase in immature CD34+TdT+ B-cells. This IP was often similar to the original B-ALL. However, in most cases the patterns of expression of CD38 (uniformly bright), CD58 (moderate) and/or CD9 (spectrum; most cells positive)

allowed discrimination from residual B-ALL. Rituximab effects were seen in 64/85 specimens from 17/18 HDC-R patients, and lasted in some cases for at least 6 months. All MRD-negative specimens by FC were also negative by PCR with one exception, a specimen from early in the course of therapy, with subsequent remission. 12 specimens from 6 patients were MRD+ by FC (4 patients during early therapy only, and 2 patients prior to clinical relapse).

Conclusions: Rituximab can cause an apparent block in marrow B-cell maturation, leading to a potential false-positive diagnosis of residual B-ALL. We recommend the use of an extensive marker panel, including at least CD9, CD38 and CD58, in order to distinguish hematogones from B-ALL.

1144 ZAP70 Expression in CLL – A Correlative Study Using Flow Cytometry, Immunocytochemistry and Western Blot Analysis

GW Slack, J Wizniak, L Dabbagh, X Shi, R Lai. University of Alberta, Edmonton, AB, Canada; Cross Cancer Institute, Edmonton, AB, Canada.

Background: Zeta-associated-protein-70 (ZAP70) is a T-cell receptor signaling protein that is normally expressed in T-cells but not mature B-cells. Previous studies have shown that ZAP70 expression is detectable in a subset of chronic lymphocytic leukemia (CLL) cases and ZAP70 expression correlates with worse clinical outcome in early-stage disease. Detection of ZAP70 in CLL is therefore useful and is commonly assayed using flow cytometry (FC). However, interpretation of the FC data can be difficult and no data has been published that establishes an optimal 'cut-off' value for positivity.

Design: The aim of this study was to establish the optimal 'cut-off' for ZAP70 by FC and validate the assay's utility. Peripheral blood samples from 45 consecutive, newly diagnosed CLL patients were assessed for ZAP70 expression by FC, immunocytochemistry (IC) (39/45) and Western blot analysis (WB) (7/45). IC was performed using ficoll-separated peripheral blood mononuclear cells, and WB was performed using CLL cells (CD5+CD19+) isolated by a flow cytometric sorter. Results from IC and WB, scored as positive or negative for each case, were correlated with the FC results, expressed as percentages of ZAP70+ cells in the CD19+ cell population.

Results: By FC, the percentage of ZAP70+ cells in most (>85%) cases were either <20% or >30%. We found that >25% ZAP70+ cells by FC correlated perfectly with ZAP70 positivity by IC and WB. We also found that the threshold of ZAP70 expression in B-cells was best set by the isotype negative control rather than ZAP70 expression levels within T-cells. As a group, using 25% as the cut-off, 19 of 45 (42%) cases were ZAP70+ by FC, which is comparable to that described in the previous studies. By correlating ZAP70 expression with clinical and pathologic parameters, we found that the ZAP70 positivity was significantly associated with atypical morphology (p=0.03, Fisher exact test).

Conclusions: We found that 25% is the optimal 'cut-off' for assessing ZAP70 expression in CLL by FC. Since the percentage of ZAP70+ CLL cells is either <20% or >30% in most cases, determination of ZAP70 positivity using the 25% 'cut-off' is usually straightforward. Overall, our data support the premise that FC is a rapid and reliable method for detecting ZAP70 expression in CLL. Our finding that ZAP70 expression is significantly associated with atypical morphology in CLL is in keeping with the concept that ZAP70 is a useful prognostic marker for this disease.

1145 Cyclin D1 Positive Multiple Myeloma: Loss of the 3'UTR Is Associated with High Cyclin D1 mRNA Levels, but Does Not Correlate with Proliferation Rate or Genomic Deletions

J Slotta-Huspenina, I Koch, M Richter, K Bink, M Kremer, K Specht, J Krugmann, L Quintanilla-Martinez, F Fend. Technical University Munich, Munich, Germany; GSF Research Center, Neuherberg, Germany; University of Innsbruck, Innsbruck, Austria.

Background: The cell cycle protein cyclin D1 (CyD1) is deregulated in a subset of B-cell neoplasms. Multiple myeloma (MM) shows upregulation of CyD1 in 50% of cases, due either to a t(11;14) translocation or associated with trisomy 11. Recently, the expression of a truncated, more stable form of the CyD1 mRNA lacking the 3'UTR (untranslated region) has been found to be associated with higher CyD1 mRNA levels, an increased proliferation rate and poor prognosis in t(11;14)+ mantle cell lymphoma (MCL). The aim of our study was to investigate the significance of the 3'UTR-deficient CyD1 mRNA variant in CyD1+MM.

Design: RNA and DNA were isolated from microdissected paraffin-embedded samples of 43 MM overexpressing CyD1 and CyD1+ MM (U266, KMS12) and MCL cell lines (Granta519, Jeko-1, Rec-1, NCEB, JVM2). CyD1 levels in relation to the housekeeping gene TBP and the ratio of short and long CyD1 mRNA transcripts were quantified by real-time RT-PCR. Deletions or mutations of the 3'UTR locus were analysed by genomic qPCR and sequencing, respectively. The presence of a t(11;14) or trisomy 11 was studied by interphase FISH.

Results: Among the 43 CyD1+ MM, 19 showed a t(11;14) (mean D1/TBP ratio=463) and 20 a trisomy 11 (mean D1/TBP ratio=23) by FISH. Both CyD1mRNA transcripts were expressed in 35/42 cases. The amount of the 3'UTR-deficient mRNA as percentage of total CyD1 mRNA ranged from 0 – 96.7% (mean=55.3%). Predominance of the 3'UTR-deficient mRNA was associated with higher CyD1 mRNA both in t(11;14)+ MM cases (p=0,009), as well as in CyD1+ cell lines. However, loss of 3'UTR was also found in MM samples with intermediate CyD1 levels. High CyD1 mRNA and loss of 3'UTR did not correlate with the proliferation rate assessed by Ki67 staining. Loss of the 3'UTR region on the DNA level was only found in the MCL cell line Jeko-1, but not in primary MM cases or the other cell lines. No mutations were detected in the proximal 3'UTR region.

Conclusions: Similar to MCL, loss of 3'UTR in t(11;14)+ MM is associated with high CyD1 mRNA levels. In contrast to MCL, there was no association between proliferation and loss of 3'UTR, nor genomic deletions or mutations of the 3'UTR region. This indicates that the abundance of the two mRNA variants in CyD1+ MM is regulated post-transcriptionally.

1146 Comparison of Plasma Cell Enumeration by Flow Cytometry to Morphologic Assessment

KJ Smock, SL Perkins, DW Bahler. University of Utah Health Sciences Center, Salt Lake City, UT.

Background: Accurate enumeration of bone marrow plasma cells (PCs) is an important component of the diagnosis and treatment of PC dyscrasias. Both flow cytometry analysis and morphology are commonly used for this purpose. However, it is unclear how accurate flow cytometry is compared to morphology because comparison studies have not been previously reported. In addition, if flow cytometry sometimes underestimates PC numbers, as has been commonly assumed, the frequency of underestimation is not known and factors that may affect the magnitude of underestimation have yet to be identified.

Design: PC percentages determined by 4-color flow cytometry and morphologic analysis were compared from 30 bone marrow specimens with increased PCs (mean 34% $\pm$ 20, range 12%-77%). PC percentages by flow were determined by quantitating cells with bright cytoplasmic light chain expression and/or cells with bright CD38 that had dim to negative expression of CD45. PC percentages by morphology were based on counts of 500 leukocytes from the diagnostic aspirate smears and 300 leukocytes from smears made with the flow cytometry specimens.

Results: PC percentages from morphologic analysis of the diagnostic aspirate smears were on average 78% higher ($\pm$ 20%) than those determined by flow cytometry. They were also 69% higher ($\pm$ 28%) than those determined by morphologic analysis of the flow cytometry specimens. Only 11 of 30 flow specimens had >10% PCs by morphologic analysis, with 6 cases having morphologic percentages markedly higher than the flow cytometry determined percentages (mean difference 48% $\pm$ 16%, range 36%-76%), and 5 cases having similar flow and morphologically determined PC percentages. Differences in PC percentages determined by morphology and flow cytometry from the flow cytometry specimens did not appear to correlate with differences in specimen viability or processing times.

Conclusions: PC percentages determined morphologically from diagnostic bone marrow aspirate smears are usually much higher than those determined by flow cytometry. Morphologic analysis of the flow cytometry smears suggests that much of this difference is related to poorer quality of the flow specimens. In addition to poorer specimen quality, the process of performing flow cytometry also appears to significantly reduce PC percentages in the majority of cases. These studies help define limitations of flow cytometry for determining PC percentages in marrow specimens which may be important when making diagnostic decisions and in assessing treatment responses.

1147 Plasma Cell Dyscrasias: Flow Cytometric vs Histologic Detection, How Do They Compare?

JH Smouse, DM Dorfman, J Vergilio. Brigham and Women's Hospital, Boston, MA.

Background: Flow cytometry (FCM) is an important tool in the detection of plasma cell dyscrasias (PCD), offering rapid discrimination of normal and neoplastic plasma cells. The classification of PCD relies on integration of serologic, radiologic and histologic data, of which marrow plasmacytosis is an important criterion in the diagnosis of multiple myeloma. Few studies correlate immunophenotypic detection of PCD with traditional histologic techniques. Here, we compare detection using a simple 3-color FCM panel performed on marrow aspirates with that using standard histology and immunohistochemistry (IHC) from corresponding biopsy (BMB) and aspirate smears (BMA).

Design: 151 aspirates from 125 patients were submitted to Brigham and Women's Hospital between January 2004 and July 2005 for assessment of a PCD using FCM. Neoplastic PC were identified by expression of CD38 and CD138 with CD56 (intermediate) and/or monotypic cytoplasmic immunoglobulin light chain (cIgL). These results were retrospectively compared to those reported in tandem BMB and BMA, the former evaluated with a combination of $H_{\&}$E/Giemsa stains and IHC [CD138, kappa, lambda] and the latter assessed with a 500 differential cell count on a Wright-Giemsa smear.

Results: Concordant results were obtained with FCM and BMB in 116 (77%) cases [95 PCD+, 21 PCD-] while discordant results were observed in 35 (23%) cases. Of those concordant PCD+, the median percentage of PC detected with BMB, BMA and FCM was 25% [range: 2-98%], 21% [range: 1-96%], and 2% [range: 0.04-60%], respectively. With FCM, 74% had monotypic cIgL, 79% were CD56+ and in 27 cases [18% overall], CD56 coexpression was the sole abnormality. Of the 35 discordant cases, 30 [20% overall] were FCM-/BMB+. 19/30 involved <10% intertrabecular space with a negative BMA [<5% PC] while 11 cases involved 5-65% (median, 30%) intertrabecular space with a positive BMA [2-87% PC (median, 15%)]. 5 discordant cases [3% overall] were FCM+/BMB- of which all contained CD138+, CD56+ PC [0.2-2%]. Of these, two also exhibited monotypic cIgL and two were BMA+ [8-16% PC].

Conclusions: A 3-color FCM panel including CD38, CD138, CD56 and cIgL offers high specificity (100%) and moderate sensitivity (74%) in the detection of PCD when compared to BMB (herein the "gold standard") and BMA (sensitivity 83%, specificity 79%). FCM results are qualitative, not quantitative. CD56 is a useful marker of PCD, detectable in the absence of monotypic cIgL. The discrepancies in FCM-, BMB+ cases may reflect specimen hemodilution, sampling artifact or PC loss during manipulation.

1148 Expression of the DNA Excision Repair Gene OGG1 in Chronic Myeloproliferative Disorders and Acute Myelogenous Leukemia

X Sun, E Wang, C Zuo, DM Jones, Y Gong, L Ai, AB Glassman, CF Ginsberg, HM Kantarjian, C Fan. University of Texas MD Anderson Cancer Center, Houston, TX; University of Arkansas for Medical Sciences, Little Rock, AR.

Background: Chromosome rearrangement or gene mutation caused by genomic instability is associated with many human malignancies including leukemia. It has been shown that overexpression of DNA repair glycosylase can cause imbalance in base excision repair, leading to paradoxically increased DNA breakage. Chronic

myeloproliferative disorder (CMPD) consists of Philadelphia chromosome (Ph) positive chronic myelogenous leukemia (CML or Ph+ CMPD) and Ph- CMPD. A Ph results from chromosomal translocation t(9;22). In this study, we compared the expression of 8-oxoguanine DNA N-glycosylase 1 (OGG1), a base excision DNA repair gene, in Ph+ CMPD and diploid Ph- CMPD. We also examined OGG1 promoter methylation in correlation with OGG1 mRNA expression in acute myeloid leukemia.
Design: Bone marrow samples of normal control (6 cases), Ph+ CMPD (10 cases), Ph- CMPD (9 cases), AML (21 cases) were obtained from our institutional tissue bank according to an IRB approved protocol. All the Ph+ CMPD and Ph- CMPD cases were at chronic phase. Total RNA was extracted, cDNA prepared, and duplicated real-time RT-PCR reactions performed for OGG1 mRNA expression. Real-time RT-PCR data were expressed as relative expression using the median of the control as 1 and analyzed by student t-test. In addition, genomic DNA was prepared from the AML samples, modified with sodium bisulfite, followed by analysis of OGG1 promoter methylation by methylation specific PCR on a methylation hotspot previously detected in head and neck cancer (-585 to -355 in relation to transcription start site).
Results: The OGG1 mRNA levels were significantly higher in Ph+ CMPD (median 2.34) than in Ph- CMPD (median 1.01) (p=0.049). The median of OGG1 mRNA level in AML was 0.05 and that of the methylation ratio was 0.24. There was no significant correlation between the mRNA expression and the promoter methylation.
Conclusions: Ph+ CMPD has higher OGG1 expression than does Ph- CMPD, suggesting that increased expression of OGG1 may contribute to the genomic instability in Ph+ CMPD. Promoter methylation may not contribute significantly to the OGG1 mRNA expression levels in AML.

1149 Lack of PRDM1α Expression in Hodgkin/Reed-Sternberg Cells Is Possibly Mediated by a MicroRNA

W Tam, P Landgraf, N Iovino, M Gomez, S Fan, YF Liu, LHC Tan, A Chadburn, KL Wright, R Sheridan, C Sander, T Tuschl, DM Knowles. Weill Medical College of Cornell University, New York, NY; The Rockefeller Univ, New York, NY; H Lee Moffitt Cancer Center and Research Institute, Univ of South Florida, Tampa, FL; Memorial Sloan Kettering Cancer Center, New York, NY.
Background: The PR (PRDI-BF1-RIZ) domain zinc finger protein 1 (PRDM1), a master regulator of plasma cell differentiation normally expressed at late stages of B-cell differentiation, has been implicated as a tumor suppressor in diffuse large B-cell lymphomas. We test whether inactivation of *PRDM1* may also play a role in the transformation of Hodgkin/Reed-Sternberg (H/RS) cells in Hodgkin lymphomas (HL).
Design: We analyzed the *PRDM1* gene sequence for mutation and quantified expression of PRDM1α, the functional isoform of PRDM1, at the RNA and protein levels in 4 HL cell lines by real-time quantitative RT-PCR and Western blotting, respectively. 14 classical HL cases were also analyzed by immunohistochemistry for PRDM1α expression. Results of PRDM1α expression analysis were correlated with microRNA profiling data.
Results: PRDM1α was undetectable in all HL cell lines tested except for the EBV(+) L591 line. H/RS cells in primary HL cases also lack PRDM1α, implying a desynchrony between IRF4 and PRDM1 expression. The absence of PRDM1α in H/RS cells is not due to impaired gene transcription, since similarly abundant *PRDM1α* transcripts are present in the HL cell lines as the myeloma line U266 which expresses high levels of *PRDM1α* protein. In addition, no mutations were detected in the coding region or the splice junctions of *PRDM1*. Two evolutionarily conserved binding sites for the microRNA miR-9 are predicted in the 3' untranslated region of *PRDM1* mRNA by computer algorithm. Moreover, miRNA profiling by cloning identified miR-9 as one of the most abundant miRNAs within the EBV(-) HD cell lines. miR-9 clones were detected at a significantly lower frequency in the PRDM1α(+) L591 and U266 cell lines, implying a negative correlation between levels of PRDM1α and miR-9. These results suggest that the lack of PRDM1α accumulation in H/RS cells may be translationally determined and regulated by microRNA(s).
Conclusions: Inactivation of *PRDM1* by microRNA-mediated translation repression may represent a novel molecular lesion involved in HL pathogenesis.

1150 Relapse Rates in Patients with AML in Relation to Type of Transplant and CD56 Positivity

K Taylor, D Jaye, M Graiser, K Easley, E Waller. Emory University, Atlanta, GA.
Background: CD56 antigen expression has been reported to be a poor prognostic factor in AML, particularly with AML with favorable cytogenetics (APL and t(8;21) sub-types) and CD56+ leukemia including a subset of cells with properties similar to Type 2 dendritic cells (DC2s), which are immunosuppressive in allogeneic transplantation. We investigated the prognostic significance of CD56 expression in AML blasts in patients undergoing high dose chemotherapy and stem cell transplantation. We hypothesized that CD56+ AML cells would inhibit the graft versus leukemia effect lending to higher relapse later in allogeneic transplantation versus CD56- AML.
Design: We analyzed post-transplant survival among 163 patients with AML who underwent chemotherapy and transplantation. The immunophenotype of the AML blasts at diagnosis and/or relapse were determined by multi-parameter flow cytometry and then correlated with patient outcomes recorded from the BMT database at Emory University. The median age was 42.3 years and 56.8% were male. 46 patients (28%) received autografts,68 patients (42%) underwent allogeneic transplantation, 14 (9%) patients had partially matched related donors, and 35 (21%) of patients received matched unrelated donors grafts.
Results: There was a trend of excess relapse of allograft patients who expressed CD56+. In patients with CD56 positivity, 21/28 (75%) patients receiving allogenic trasplantation relapsed compared to their autologous counterparts (6/11 or 55%). This is compared to the CD56 negative patients with allogenic transplantation, whose relapse rate was 58/75 (77%), identical in incidence to those receiving autologous transplants 27/35 (77%). Chi-square analysis showed p=0.2. Too few patients had blasts that co-expressed CD56 and CD11c or CD56 and CD4 to find a significant association with these phenotypes and outcome.
Conclusions: These results concur with our original hypothesis but were only suggestive and not statistically significant (p=0.2), possibly as a result of too few patients immunophenotyped with these markers in our database. A larger study with more AML patients typed for CD56 is needed to definitively prove or disprove the significance of this trend. Additional studies done by this group will be presented in the poster in which we were able to show that some of these CD56+ AML cases showed specific (BDCA-2) DC2 markers as well as a DC2 specific phage positivity.

1151 The Alternative NF-kB Pathway Is Active in the Majority of Diffuse Large B-Cell Lymphomas

B Timar, A Chadburn, DM Knowles, E Cesarman. Weill Medical College of Cornell University, New York, NY.
Background: The nuclear factor kappa B (NF-kB) is a transcription factor that plays an important role in many human neoplasms by inducing the expression of genes involved in cellular survival and proliferation. Two major signaling pathways lead to the translocation of NF-kB dimers from the cytoplasm to the nucleus: the classical pathway, where the p50/p65 heterodimers are translocated into the nucleus, and the alternative pathway where IKKalpha selectively phosphorylates p100 associated with RelB. This results in the degradation of p100 into p52 and in the nuclear translocation of this transcriptionally active p52:RelB dimer. Previous gene expression data have indirectly indicated that NF-kB is active in the non-germinal cell (non-GC) subset of diffuse large B cell lymphomas (DLBCL). However, direct analysis of actual NF-kB proteins in DLBCL specimens, and assessment of alternative pathway activation, has not been reported previously.
Design: We analyzed a panel of 8 DLBCL cell lines, as well as 19 patient primary DLBCL samples, to determine the presence of components of the alternative NF-kB pathway, and activation based on p100 cleavage and nuclear localization by immunoblot analysis using antibodies to RelB, p52, histone H1, tubulin and actin. Two color immunofluorescence analysis was performed with antibodies to p52, RelB, and BCL-6, and evaluated with fluorescence microscopy. Classification into GC and non-GC subgroups was performed with antibodies to Bcl-6, CD10, MUM-1 and FoxP1.
Results: We show that the NF-kB p100 precursor protein is cleaved and p52 is present in the nuclei of 5/8 DLBCL cell lines and 18/19 primary DLBCL samples. RelB was also identified in the nuclei of all DLBCL cell lines and 13/19 primary DLBCL samples. We found nuclear p52 and RelB in cases classified immunohistochemically as GC and post-GC. In primary DLBCL specimens showing nuclear p52 and RelB by immunoblot, p100/p52 was localized in the nucleus of many large tumor cells. p52 also colocalized with the BCL-6 protein, indicating that the tumor cells harboring GC features possess active components of the alternative NF-kB pathway.
Conclusions: We have shown that the alternative NF-kB pathway is active in the vast majority of DLBCLs in vivo regardless of their immunophenotypic subclassification into GC or post-GC type. Consequently, this pathway is likely involved in the survival of DLBCL cells and inhibitors of NF-kB that target the alternative pathway may be useful in the treatment of most patients with DLBCL.

1152 Hematological Parameters as Indication for Staging Bone Marrow Biopsies in Post-Transplant Lymphoproliferative Disorders

L Tsao, DG Savage, G Bhagat, B Alobeid. Columbia University Medical Center, New York, NY.
Background: Post-transplant lymphoproliferative disorders (PTLD) are complications of solid organ transplants. Of the spectrum of PTLD, monomorphic PTLD (m-PTLD) closely resembles diffuse large B-cell lymphoma (DLBCL). Bone marrow (BM) involvement in m-PTLD is an indicator of poor prognosis. Currently, staging BM biopsies are standard for patients with m-PTLD. Recently, clinical parameters have been used as indication for BM biopsy in early-stage DLBCL patients. However, m-PTLD usually present at higher stage and have higher incidence of BM involvement. We examine if similar clinical parameters are predictive of BM involvement in m-PTLD patients.
Design: A retrospective analysis of 32 patients who developed m-PTLD after various solid organ transplants (21 heart, 8 renal, 2 liver, 1 heart/lung). The patients' age ranged from 2 to 75 yrs (male:female 21:11). Clinical laboratory results for hemoglobin (Hgb), white blood count (WBC), and platelet count (PLT) from the time of initial diagnosis were reviewed. The criteria of Hgb<10 g/dL, WBC<$4x10^3$, or PLT<$100x10^3$ was set as indication for BM biopsy. Statistical analysis performed using two-tailed Fisher exact test.
Results: Of the 32 patients with m-PTLD, 8 patients had BM involvement at diagnosis. Seven (87.5%) of the 8 patients with BM involvement met at least one criterion indicating the need for staging BM biopsy. Fourteen (58.3%) of the 24 patients without BM involvement did not meet any of the three criteria indicating the need for staging BM biopsy.
Conclusions: Patients with m-PTLD with BM involvement are significantly more likely to fulfill at least one of the hematological criteria used as indication for staging BM biopsy (p=0.04). Only one patient with BM involvement did not meet the criteria for BM biopsy. However, this patient presented with a leukemic m-PTLD with WBC $30.1x10^3$. Because transplant patients frequently present with leukopenia, anemia, and thrombocytopenia due to immunosuppression or infection, the criteria used in this study are expected to over predict BM involvement in m-PTLD patients. However, over half the patients without BM involvement did not need biopsies, effectively reducing the number of required staging BM biopsies.

1153 Cytogenetic Abnormalities in B-Cell Post-Transplant Lymphoproliferative Disorders: Should Florid Follicular Hyperplasia Be Considered a Precursor?

E Vakiani, C Keller, V Murty, B Alobeid, G Bhagat. Columbia University, New York, NY.

Background: Alterations of a variety of oncogenes have been reported in B-cell post-transplant lymphoproliferative disorders (PTLD) but there is a paucity of data regarding cytogenetic abnormalities occurring in PTLD. We analyzed the nature and frequency of karyotypic abnormalities in a large set of B-cell PTLD and sought correlations with the EBV status and clinical outcome. Due to the controversial nature of florid follicular hyperplasia (FFH) as a precursor lesion, cases of post-transplant FFH were also analyzed.
Design: G-banded karyotype analysis was performed on 24 cases from 22 patients (M: F=15:7, age range 2-72 yrs) with B-cell PTLD, 14 monomorphic PTLD (M-PTLD) including 11 DLBCL and 2 Burkitt lymphomas (BL), 7 polymorphic PTLD (P-PTLD), and 1 Hodgkin lymphoma (HL) and on 13 cases of FFH (M: F=7:6, age range 2-9 yrs) involving the tonsils (n=12) and lymph node (n=1). Fluorescence in situ hybridization (FISH) for IgH and/or Bcl6 rearrangement was performed in a subset (6 PTLD, 2 FFH). In situ hybridization for EBER was performed on all cases. Fisher's exact test was used for statistical analysis.
Results: Clonal karyotypic abnormalities were noted in 9/22 (41%) PTLD (7/14 M-PTLD, 1/7 P-PTLD, 1/1 HL), 7/22 (32%) PTLD (4/14 M-PTLD, 3/7 P-PTLD) had a normal karyotype, and 6/22 (27%) PTLD (3/14 M-PTLD, 3/7 P-PTLD) had no analyzable metaphases. Three of 13 (23%) FFH had clonal and 1 FFH had non-clonal abnormalities and 8/13 (61%) FFH had normal karyotypes. Two of 22 (9%) PTLD (1 M-PTLD, 1 P-PTLD) and 4/13 (31%) FFH had simple karyotypic abnormalities while 7/22 (32%) PTLD (6 M-PTLD and 1 HL) had complex abnormalities. FISH for IgH and Bcl6 confirmed translocations in 2 cases (1 M-PTLD and 1 FFH) and 1 case (M-PTLD), respectively. Non-random cytogenetic abnormalities involved translocations of chromosome (chr) 1q21 (n=3, including 1 FFH) and 14q32 (n=2, including 1 FFH), gains of chr 7 (n=3), 2, and 12 (n=2 cases each), and loss of chr 22 (n=2, including 1 FFH). ISH for EBER was positive in 8/13 FFH, 5/7 P-PTLD, and 9/14 M-PTLD. Cytogenetic abnormalities did not correlate with EBV status, time since transplant, or clinical outcome.
Conclusions: Karyotypic abnormalities noted in PTLD are similar to those observed in B-NHL in immunocompetent patients but bcl6 translocations are infrequent. Detection of similar cytogenetic aberrations in FFH and PTLD support the inclusion of FFH as a precursor lesion and indicate a germinal center origin of at least a subset of chromosomal abnormalities.

1154 Proliferation of Crippled B-Cell Clones in Post-Transplant Lymphoproliferative Disorders: Rescue by EBV or Consequence of Immune Deregulation?

E Vakiani, L Pasqualucci, C Keller, B Alobeid, G Bhagat. Columbia University, New York, NY.

Background: The majority of B-cell post-transplant lymphoproliferative disorders (PTLD) are associated with Epstein-Barr virus (EBV) infection, however the role of EBV in the pathogenesis of PTLD is unclear. We analyzed the mutational status of immunoglobulin heavy chain (IgH) variable region genes for evidence of antigen selection in EBV+ and EBV- PTLD and to determine whether EBV plays a role in the survival of B-cell clones with crippling IgH mutations, as reported previously.
Design: PCR amplification of IgH genes was performed using V_H family-specific primers with a J_H primer mix on genomic DNA from 12 cases of PTLD (M:F=7:5, age 2-75 yrs, mean 35.5 yrs), 7 monomorphic (M-PTLD) and 5 polymorphic (P-PTLD). PCR products were subjected to direct sequencing and the results compared to the germline sequences in the IMGT database. The ratio of replacement (R) to silent (S) mutations in the complementary determining regions and framework regions (FR) was analyzed using a multinomial distribution model. In situ hybridization for EBER and immunohistochemical (IHC) stains for BCL6, MUM1, and CD138 were performed on all cases.
Results: Clonal IgH gene rearrangements were identified in 11/12 (92%) PTLD (4/5 P-PTLDs, 7/7 M-PTLDs). Three of 5 P-PTLD and 3/7 M-PTLD were EBV+. Two EBV- M-PTLD had a germinal center (GC) phenotype (BCL6+, MUM1-, CD138-) and the rest had a post-GC phenotype (BCL6-, MUM1+/-, CD138-). A functional rearrangement was identified in 9/11 (82%) PTLD and crippling mutations were found in 2/11 (18%) PTLD (deletion in an EBV+ P-PTLD and insertion of a stop codon in an EBV- GC derived M-PTLD). All monoclonal PTLDs had a signature of GC transit as evidenced by the presence of somatic hypermutation (range 3.8%-19.5%). There was no significant difference in the average mutation frequency between P-PTLDs and M-PTLDs and between EBV+ and EBV- PTLD. A lower than expected number of R mutations in FR indicating selection due to antigen pressure was found in 7/9 (78%) cases with functional rearrangement (2/3 P-PTLD, 5/6 M-PTLD) in both EBV – (n=3) and EBV + cases (n=4).
Conclusions: The majority of PTLDs in our study, both EBV+ and EBV-, had evidence of prior antigen selection. Since a crippling mutation was detected in 1 case each of EBV+ and EBV- PTLD, our results suggest that immune deregulation, in addition to rescue by EBV as previously suggested, might play an important role in the survival of B-cell clones with sterile IgH rearrangements in a minority of PTLD.

1155 Diffuse Large B-Cell Lymphoma and MALT-Lymphoma of Breast Uncommonly Have malt1 Gene Rearrangements

JR Valbuena, SS Talwalkar, LV Abruzzo, J Admirand, GZ Rassidakis, S Konoplev, CE Bueso-Ramos, LJ Medeiros. MD Anderson Cancer Center, Houston, TX; University of Louisville, Louisville, KY.

Background: Non-Hodgkin lymphomas can involve the breast, either localized and presumably arising in the breast, or as part of systemic disease. In the literature, diffuse large B-cell lymphoma (DLBCL) is most common. MALT-lymphoma can also involve the breast. The pathogenesis of these neoplasms and their relationship to each other is uncertain.
Design: 31 lymphomas localized to the breast were initially identified. There were 30 women and 1 man with a median age 60.5 years (range 27-85). After routine histology and immunohistochemical staining to determine B- or T-cell lineage, each neoplasm was classified using the World Health Organization system as: 19 DLBCL, 9 MALT-lymphoma, 1 Burkitt lymphoma, 1 precursor B-cell lymphoblastic lymphoma, and 1 peripheral T-cell lymphoma. Subsets of DLBCL (n=9) and MALT-lymphoma (n=4) were immunohistochemically assessed for expression of BCL-10 and the p65 subunit of NF-kB. BCL-10 expression was categorized as either cytoplasmic, cytoplasmic and nuclear, or negative and graded as weak, moderate, or strong. NF-kB expression was categorized as cytoplasmic (not activated) or nuclear +/- cytoplasmic staining (activated). In addition, 13 neoplasms were assessed by fluorescence in situ hybridization (FISH) for malt1 gene rearrangements using routinely processed tissue sections and a dual color breakapart probe (Vysis, Inc.).
Results: In 9 DLBCL cases, the pattern of BCL-10 expression was weak or moderate cytoplasmic (n=6), weak nuclear and cytoplasmic (n=1), or negative (n=2). NF-kB p65 expression was positive in a cytoplasmic pattern. In 4 MALT-lymphoma cases, BCL-10 pattern was weak or moderate cytoplasmic (n=3) and moderate nuclear and cytoplasmic (n=1). NF-kB p65 was positive in a cytoplasmic pattern. Using FISH, there was no evidence of malt1 gene rearrangements in all 9 DLBCL and 4 MALT-lymphoma assessed.
Conclusions: The inability to detect malt1 gene rearrangements in all DLBCLs and MALT-lymphomas tested indicates that the t(11;18) and t(14;18) are uncommon in breast lymphomas. The generally weak and cytoplasmic pattern of BCL-10 expression is consistent with the FISH results and suggests that the t(1;14) is uncommon. The absence of nuclear NF-kB p65 expression also indicates that NF-kB activation is uncommon. These results raise the possibility that MALT-lymphoma and DLBCLs arising from MALT-lymphoma in the breast have a distinct pathogenesis from MALT-lymphomas at other sites.

1156 Integrated CGH- and Expression-Array Profiling of Mantle Cell Lymphoma

JH van Krieken, M Schraders, P Jares, E Schoenmakers, E Campo. Radboud University Medical Center, Nijmegen, Netherlands; Hospital Clinic, University of Barcelona, Spain.

Background: Mantle cell lymphoma (MCL) is characterized by the translocation t(11;14)(q13;q32), which leads to the overexpression of cyclin D1. However, it is known that the overexpression of cyclin D1 alone is not sufficient for the development of MCL.We want to identify additional candidate genes that are involved in the development of MCL using an integrated profiling approach.
Design: To identify additional chromosomal changes in MCL, we previously analyzed 17 cases with array-based genome-wide comparative genomic hybridization using a 3.6k BAC array with a resolution of 800 kb. Further, we performed expression array analysis using the GeneChip® Human Genome U133 Plus 2.0 Array from Affymetrix, which provides comprehensive coverage of the transcribed human genome on a single array.
Results: Generating huge amounts of data with arrays is relatively straightforward. The challenge lies in developing methods to analyze this large amount of data and to link the array-CGH data to the expression array data. We focused on areas of chromosomal loss or gain. More specifically, on minimal common regions (mcr's), which are chromosomal aberrations present in at least 5 cases. Unsupervised clustering of the expression values of the genes within each mcr was performed. For several mcr's, e.g. 1p22.1-31.1, 6q23.2-27 and 11q22.3-23.3, the clustering tree showed two groups, one with the chromosomal aberration and one without. Subsequently, statistical analysis of gene expression levels within mcr's was performed to determine the genes that are differentially expressed. In the regions with genomic loss, only a subset of genes has down regulated expression levels, but even a smaller subset of genes has up regulated expression levels. Genes within a mcr of genomic loss that are transcriptionally down regulated might be regulated by gene dosage and are potential candidate genes for the development of MCL. Thus far, we have identified several expressed sequence tags and only a few interesting known genes as candidate genes.
Conclusions: This study shows that an integrated profiling approach is a good method to identify potential candidate genes. The relevance of several potential candidate genes is currently under investigation by using independent techniques.

1157 T(8;13) Positive "Bilineage Lymphomas" with T-Cell and Myeloid Components: Report of 4 Patients with the 8p11 Myeloproliferative Syndrome (EMS)

F Vega, LJ Medeiros, LV Abruzzo. UT MD Anderson Cancer Center, Houston, TX.

Background: EMS is a distinct chronic myeloproliferative disorder characterized by myeloid hyperplasia, eosinophilia and precursor lymphoblastic lymphoma (LBL) associated with balanced chromosomal translocations involving 8p11, most commonly t(8;13)(p11;q12). About 75% of EMS patients present with or develop precursor T-LBL. The disease is aggressive and rapidly transforms to acute leukemia, usually of myeloid phenotype. In this study, we describe the morphologic and immunophenotypic features of 4 "bilineage lymphomas" positive for t(8;13) with precursor T-LBL and a myeloid components.
Design: We reviewed lymph node biopsy specimens in 4 patients with EMS positive for t(8;13). Immunohistochemical studies were performed on the lymph node biopsy specimens using antibodies specific for lymphoid and myeloid markers including: CD1a, CD3, CD15, CD43, CD68, CD117, lysozyme, myeloperoxidase and TdT.
Results: The patients, 3 men and 1 woman, ranged in age from 9 to 18 years. All patients presented with generalized lymphadenopathy and had a high white blood cell count (range:14.0 to 60.0 $x10^9$/L). Morphologically, each lymph node was extensively

replaced by a blastic neoplasm with two cellular components: small to medium-sized cells with scant cytoplasm resembling lymphoblasts, and large immature cells with more abundant eosinophilic cytoplasm with a myeloid immunophenotype. In all cases, the myeloid component was located around residual lymphoid follicles and/or blood vessels. The precursor T-LBL component predominated in two cases and the myeloid component predominated in two cases. Numerous eosinophils and prominent high endothelial venules were present in all the cases. The immunophenotype of both cellular components is shown in the table. Interestingly, CD3 was positive in both cellular components.

Conclusions: EMS positive for t(8;13) should be suspected in lymph nodes involved by a hematological neoplasm in which precursor T-LBL and myeloid components are present. These neoplasms, for which we use the term "bilineage lymphoma" are analogous to bilineage acute leukemias. The presence of T-cell and myeloid components in these neoplasms supports origin from a transformed stem cell.

Immunophenotypic Findings

	Myeloid Component	Precursor T-LBL Component
CD1a	0/3	4/4
CD3	3/3	3/3
CD43	3/3, strong	2/3 weak
TdT	0/4	4/4
CD15	2/2, focal	0/2
CD68	3/3	0/3
CD117	2/4, focal	0/4
Lysozyme	4/4, focal	0/4
MPO	2/3, focal	0/3

1158 Immunohistochemical Study of NF-κB Expression in De Novo Diffuse Large B Cell Lymphoma

C Vejabhuti, SR Sanchez, SL Perkins, CC Chang. Baylor College of Medicine, Houston, TX; The Methodist Hospital, Houston, TX; University of Utah, Salt Lake City, UT.

Background: Nuclear factor κB (NF-κB) signaling pathway regulates the survival of normal and malignant B cells by controlling the expression of cell death regulatory genes. Recent gene expression profiling studies using microarray techniques have identified two main subgroups of diffuse large B cell lymphomas (DLBCLs), germinal center-like (GC) and activated B cell-like (ABC) lymphomas, correlating with prognosis. Furthermore, the NF-κB signature was found to be associated with the ABC DLBCL subgroup suggesting NF-κB role in tumorigenesis in this group. NF-κB inhibitors as a potential target treatment were recently developed and may become available for clinical usage soon. We hence investigated the NF-κB expression using immunohistochemical (IHC) methods in DLBCL and evaluated the association of the expression and clinical outcomes.

Design: Tissue microarray blocks were constructed using tissues from 91 archived cases of de novo DLBCL in immunocompetent patients. For each case, three representative 0.6 mm or 1.2 mm cores were included in the microarray blocks. IHC staining with antibodies to NF-κB p105/p50 was performed on the tissue microarrays.

Results: Twenty-seven (36%) of 74 cases with adequate tissue in the microarray slide showed nuclear NF-κB expression (> 20% of neoplastic large lymphocytes with moderate to strong nuclear staining). Forty (54%) cases showed cytoplasmic NF-κB expression (greater than 20% of neoplastic large lymphocytes with moderate cytoplasmic staining). The median follow-up was 44 months. Only IPI scores were shown to be independent prognostic indicators (Kaplan-Meier survival (KMS), $p < 0.002$, log rank test). Although cytoplasmic NF-κB expression did not correlate significantly with overall survival, it appeared to show an association (KMS, $p = 0.14$ log rank test). No association between nuclear NF-κB expression and overall suvival is identified. There was also no significant correlation between IPI scores and cytoplasmic NF-κB expression or nuclear NF-κB expression.

Conclusions: NF-κB expression, both nuclear and cytoplasmic, is relatively common in DLBCLs. Furthermore, our data suggests that cytoplasmic (but not nuclear) NF-κB expression by IHC appear to be associated with worse outcomes in DLBCL patients. Additional studies are needed to substantiate these findings and determine if NF-κB inhibitors may be useful in DLBCL patients with NF-κB expression.

1159 Downregulation of MCl-1 and Induction of Apoptosis in Mantle Cell Lymphoma by Flavopiridol (Pan CDK4 Inhibitor)

G Venkataraman, T Maududi, F OzPuyan, HI Bahar, J Qin, KF Izban, S Alkan. Loyola University Medical Center, Maywood, IL; Elmhurst Memorial Hospital, Elmhurst, IL.

Background: Typical Mantle Cell Lymphoma (MCL) is a distinct B-cell Non-Hodgkin's Lymphoma associated with over-expression of cyclin D1 following translocation between the IgH and BCL-1 genes. MCL has an aggressive clinical course when compared to other low-grade lymphomas. Due to the important functional interaction between cyclin D1 and cyclin dependent kinases, cyclin dependent kinase inhibitors such as Flavopiridol are under consideration for treatment of patients with MCL.

Design: We investigated the in vitro effects of Flavopiridol on the MCL cell line (JeKo-1). Cytotoxicity assays, morphologic and flow cytometry examination for assessing apoptosis was performed. Western blotting for analysis of cell cycle (namely p16, p19, p21, p27, pRb, phospho-pRb and CDK4 and cyclin D1) and apoptosis (bcl-2, mcl-1, procaspase-3 and PARP) related proteins was performed. In addition, five mantle cell lymphoma patient samples were also evaluated for cytotoxicity with Flavopiridol.

Results: Flavopiridol at 10 nmol/L induced apoptosis by 6 hours of treatment as noted by flow cytometric analysis, morphologic examination and Western blotting. The cleavage of procaspase-3 and PARP and the decrease of Flavopiridol-induced apoptosis by pan-caspase inhibition suggested that the caspase pathway serves an important role in the apoptotic process. This was associated with downregulation of mcl-1, a key anti-apoptotic protein. Furthermore, MCL cells exposed to Flavopiridol showed downregulation of key cell cycle proteins acting at the restriction point control between the G1 and S phases. There was dramatic downregulation of cyclin D1 with concomitant downregulation of p16, p27, pRb and phospho-pRb at 6 hours of treatment and a progressive upregulation of p19 after 6 hours. In the analysis of MCL patient samples, there was a significant cytotoxicity (>50% inhibition).On the other hand, cytotoxicity studies further demonstrated that the combination of Flavopiridol and Bortezomib (an inhibitor of the proteasome machinery licensed currently for use in multiple myeloma patients) causes significant cytotoxicity in MCL cells.

Conclusions: Collectively, our data indicates that Flavopiridol may have significant therapeutic potential in the context of MCL when used synergistically with drugs targeted against complementary cellular pathways.

1160 Role of CXCL13 in the Pathogenesis of Bartonella Henselae Monocytoid-B-Cell-Rich, Suppurative Granulomas

W Vermi, F Facchetti, F Gentili, S Sozzani, E Riboldi, R Badolato, M Chilosi, C Doglioni, T Musso. University of Brescia, Brescia, Italy; University of Verona, Verona, Italy; San Raffaele Hospital, Milan, Italy; University of Turin, Turin, Italy.

Background: The immune response against infections strictly relies on the interaction of pathogens with professional antigen-presenting cells in peripheral tissues. Among them, dendritic cells (DCs) have the unique ability to induce a potent antigen-dependent stimulation and play a central role in the initiation of the primary immune response.

Design: The B cell-rich suppurative granulomas occurring in cat-scratch disease (CSD)(Facchetti F, et al. Am J Surg Pathol 1992; 16:955-961) suggest a peculiar organization of the immune response to B. henselae. In this study we analyzed the effects of B. henselae infection on monocyte-derived DCs in vitro, and studied the cell composition and chemokine/cytokine production in CSD granulomas on tissue sections. All granulomas were proven to contain B. henselae by staining with a specific monoclonal antibody (clone H2A10, Abcam, Cambridge, UK).

Results: In vitro DC infection by B. henselae results in internalization of bacteria, phenotypic maturation with increased expression of HLA-DR and CD86, and induction of CD83, CD208 and CCR7. B. henselae-infected DCs produce high amounts of IL-10, as well as CXCL8 (IL8) and CXCL13, two chemokines active respectively on neutrophils and B lymphocytes. Remarkably, CSD granulomas in vivo contain CXCL13-producing DCs; using double immunofluorescence these DCs were shown to be negative for follicular dendritic cell markers (CD21, CD23), but to express antigens of extra-follicular DCs (such as CD11c and CD14). In keeping with the in vitro data, these granulomas contain high amounts of IL8 and IL10. We demonstrate that the B-cells in CSD granuloma are represented by CD20+TCL1- monocytoid B-cells; noteworthy, these cells express T-bet, a transcription factor able to induce a T-independent immunoglobulin (Ig) class switch in B lymphocytes.

Conclusions: These findings suggest that the humoral immune response to B. henselae initiates in the extrafollicular areas of infected lymph nodes and is regulated by DCs. These results provide the molecular basis for the morphogenesis of CSD granulomas.

1161 Utility of PAX-5 Immunohistochemistry To Assess Bone Marrow Involvement by B-Cell Malignancy in the Setting of Anti-CD20 Therapy and Its Expression in a Variety of Bone Marrow Disorders

DM Vlasoff, WG Morice, JD Hoyer. Mayo Clinic, Rochester, MN.

Background: The use of CD20 immunohistochemistry in assessing bone marrow (BM) involvement by B-cell lymphoproliferative disorders (B-LPDs) is often compromised by anti-CD20 immunotherapy. PAX-5 is a B-cell antigen that is expressed both in early and late stages of B-cell differentiation; however, its utility as a marker for B-LPD involvement in BM biopsies (BM Bx) in the setting of anti-CD20 therapy has not been studied. Also, recent reports of PAX-5 expression in some non-B-cell neoplasms raise the possibility that this antigen may not always be lineage specific.

Design: We retrospectively studied PAX-5 expression in 150 B5-fixed, paraffin-embedded BM Bx involved by a variety of primary and secondary bone marrow disorders. We also tested BM Bx from 80 patients with various mature B-LPD which had both pre- and post-anti-CD20 therapy BM Bx. Immunoperoxidase staining was performed in all cases using antibodies to PAX-5, the B-LPD BM Bx were also stained with antibodies to CD3, CD20 and CD79a. PAX-5 staining was graded on a 0 to 3+ scale.

Results: PAX-5 expression was detected in all cases of pre-B (9) and mature B-LPD (37). No staining was seen in any of the following disorders: Hodgkin lymphoma (5), multiple myeloma (10), pre-T-cell (4) and various mature T-cell (3) neoplasms, various myelodysplastic (9), myeloproliferative (16), or systemic mast cell (4) disorders, metastatic carcinomas (5), Ewing's sarcoma (1), melanoma (1), and neuroblastoma (2). Only 1 of 29 cases of acute myeloid leukemia (AML minimally differentiated, M0), and 2 of 6 cases of metastatic small cell carcinoma were positive with PAX-5. PAX-5 staining of normal B-cells was confirmed in 9 cases with benign lymphoid aggregates. In pre- and post-anti-CD20 therapy BM Bx from patients with various mature B-LPD, PAX-5 expression in the majority of cases strongly correlated with CD20 and CD79a and was in some cases superior. However, in 17 of 80 (21%) post-anti-CD20 therapy BM Bx CD20 expression was either lost or markedly reduced in the residual disease, while PAX-5 was strongly preserved and lacked non-specific cross-reactivity routinely observed with CD79a.

Conclusions: These studies confirm PAX-5 as a sensitive and specific marker of BM involvement by a variety of B-LPDs. Furthermore, they demonstrate that PAX-5 immunostaining is a useful tool highlighting the presence of disease after anti-CD20 immunotherapy.

1162 Non-Neoplastic Bone Marrow Pathology Associated with Autoimmune Disorders

DM Vlasoff, WG Morice. Mayo Clinic, Rochester, MN.

Background: Bone marrow (BM) examination is often utilized in the evaluation of patients with autoimmune diseases. There have been anecdotal reports describing abnormal BM findings in these diseases, however, little is known regarding the spectrum of changes associated with these conditions.

Design: BM aspirate and biopsy specimens from 82 patients with various autoimmune diseases (39 rheumatoid arthritis, 24 systemic lupus erythematosus, 4 juvenile rheumatoid arthritis, 4 adult Still's disease, 8 mixed connective tissue disorder, 1 dermatomyositis, 1 psoriatic arthritis, 1 Tokayasu's arteritis) from the Mayo Clinic Files were reviewed. None of the cases were involved by malignancy to prevent bias due to treatment or paraneoplastic effects. The results of cytochemistry, paraffin immunhistochemistry, flow cytometry, and cytogenetic analyses were reviewed when available.

Results: The histopathologic BM features are summarized in the Tables 1 and 2. A wide spectrum of changes in hematopoiesis were present. Of note, in several cases maturation was left-shifted, in a minor subset there were changes mimicking myelodysplasia. A variety of patterns of increased lymphoid and plamsacytic elements were observed, clonality was not detected in any of the cases by either immunophenotyping or molecular genetic analyses. In addition to these findings, in single cases each of the following were present: extensive BM necrosis, extensive BM infiltration by foamy histiocytes, and BM replacement by pauci-cellular stromal myxoid change. In none of these cases extensive clinical and pathologic evaluation revealed evidence of BM or systemic malignancy.

Conclusions: A wide spectrum of non-neoplastic BM changes can be observed in autoimmune diseases, many of which may mimic malignant changes either in hematopoetic or lympoid elements. In these chronically ill patients it is difficult to discern if these effects are attributable to the disease process or effects of prolonged immunosuppressive therapy. Regardless, it is important that they be recognized so that a malignant diagnosis is not inappropriately rendered.

Table 1

	cellularity	red cell recursors	granulocyte precursors	megakaryocytes
increased	28 (34%)	30 (37%)	21 (26%)	23 (28%)
decreased	22 (27%)	19 (23%)	24 (29%)	13 (16%)
normal	32 (39%)	33 (40%)	37 (45%)	46 (56%)
immature precursors	-	27 (33%)	15 (18%)	-
dysplastic morphology	-	6 (7%)	3 (4%)	6 (7%)

n=82

Table 2

increased lymphoid aggregates	increased interstitial cytotoxic T-cells	granulomatous lymphohistiocytic proliferations	increased plasma cells
25 (30%)	11 (13%)	6 (7%)	9 (11%)

n=82

1163 Comprehensive Proteomic Analysis of Reed-Sternberg Cells

JC Wallentine, KK Kim, C Seiler, DK Crockett, SR Tripp, KSJ Elenitoba-Johnson, MS Lim. University of Utah School of Medicine, Salt Lake City, UT; ARUP Institute for Clinical and Experimental Pathology, Salt Lake City, UT.

Background: Mass spectrometry-based proteomics in conjunction with liquid chromatography and bioinformatic analysis provide a highly sensitive and high-throughput approach in the identification of proteins in complex mixtures. Comprehensive analysis of the proteins expressed by Reed-Sternberg (RS) cells would assist in the discovery of potential biomarkers and improve our understanding of its pathogenesis. We have utilized a proteomic approach to identify a comprehensive list of proteins expressed by Hodgkin lymphoma (HL)-derived cell lines.

Design: The subcellular proteome of three fractions (cytosol, membrane, and nuclear) from L428 and KMH2 cell lines were fractionated using 1 dimensional SDS-PAGE, and analyzed by reverse-phase liquid chromatography coupled with electrospray ionization tandem mass spectrometry (MS/MS). Peptide spectra were searched using TurboSEQUEST® against the UniProt protein database. Protein validations were performed by immunohistochemistry using tissue microarray, western blot analysis and immunofluorescence microscopy.

Results: A total of 1,420 proteins were identified with 760 from the cytosolic fraction, 305 from the membrane fraction and 364 from the nuclear fraction using an error rate of < 5.0%. Identification of proteins from diverse functional groups such as protein kinases (B-RAF, JAK3, TRKB, WNK1-2, MEKK1, SNK), transcription factors (NFX1, ZNF85, SOX-18), and signaling proteins (Axin1, IL12B, CCR-4, CCR-1) reflected the functional complexity of the RS proteome. Proteins with previously reported oncogenic function in other cancers were identified (BCL-11A, B-RAF, PIM-1, LCK, N-RAS). Furthermore proteins from signaling pathways implicated in HL such as Notch1-4, MEK kinase 1,4 and CD30 were also identified. Selected proteins without previously demonstrated expression in HL were validated by western blot analysis (B-RAF), immunofluorescence microscopy (Tenascin-x, Mucin-2) and immunohistochemistry using a tissue microarray (B-RAF, PIM1).

Conclusions: This study represents the first and largest comprehensive inventory of proteins expressed by HL-derived RS cells and demonstrates the utility of combining cellular subfractionation, MS/MS, and bioinformatics analysis for comprehensive identification of proteins which may be potential biomarkers of the disease.

1164 Relative Effects of Signal Transduction Pathway Inhibitors on NPM-ALK-Induced Changes in Gene Expression

Q Wang, L Sims, MA Thompson. Vanderbilt University Medical Center, Nashville, TN.

Background: Anaplastic lymphoma kinase (ALK) is over-expressed in 40-60% of anaplastic large cell lymphomas (ALCL), usually as a result of the t(2;5)(p23;q35) translocation encoding a fusion between nucleophosmin (NPM) and ALK. ALK is a receptor tyrosine kinase; fusion with NPM results in the constitutive activation of ALK. Studies on the transforming activity of NPM-ALK have implicated downstream involvement of several signal transduction pathways. These studies were designed to determine the effect of NPM-ALK on gene expression by microarray analysis, and to determine the relative dependence of the changes in gene expression on these implicated signal transduction pathways.

Design: The murine lymphoid Ba/F3 cell line was stably transfected with a pcDNA3-NPM-ALK construct constitutively expressing NPM-ALK, kindly provided by Dr. Stephan Morris. A control cell line stably transfected with the empty pcDNA3 vector was also created. Microarray analysis using RNA from Ba/F3-NPM-ALK and Ba/F3-pcDNA3 cells was performed in triplicate using the mouse NIA 22.4K cDNA clone set. Subsequently a panel of genes confirmed by quantitative RT-PCR (QRT-PCR) to be over-expressed in Ba/F3-NPM-ALK cells was used to determine the relative effects of 4 different kinase inhibitors on gene expression induced by NPM-ALK.

Results: Microarray analysis demonstrated 82 genes up-regulated greater than 2 fold in RNA from Ba/F3-NPM-ALK cells compared to Ba/F3-pcDNA3 cells. Overexpression of 9 of these genes, whose products are involved in apoptosis (CTLA-2a, CTLA-2b), cytokine signalling (osteopontin, SOCS3), signal transduction (FRAG1), transcription (Nupr-1, RPT-1, ID-2), and matrix interactions (TIMP-1), in the Ba/F3-NPM-ALK cells was confirmed by QRT-PCR. Expression of this panel of genes in Ba/F3-NPM-ALK cells treated with each of 4 kinase inhibitors was assayed by QRT-PCR. Genes inhibited greater than 2 fold in at least 2 experiments by the listed inhibitors are summarized in Table 1.

Table 1

Inhibitor	Path inhibited	Genes inhibited
WHI-P154	JAK/STAT	osteopontin, Nupr-1, TIMP-1, CTLA-2b, SOCS3
PD98059	MEK/MAP kinase	CTLA-2a, osteopontin
Ly294002	PI3-kinase	Nupr-1
U73122	PLC-gamma	No consistent inhibition

Conclusions: The JAK/STAT inhibitor WHI-P154 reduced expression of five out of nine genes, more than the other inhibitors. These results indicate that the JAK/STAT pathway is important in effecting changes in gene expression in response to NPM-ALK.

1165 Significance of Cytogenetic and Immunophenotypic Findings in Patients with Acute Myeloid Leukemia Evolving from Myelodysplastic Syndrome

JS Warrington, MJ Routbort, PJ Buckley, JZ Gong, BK Goodman, AS Lagoo. Duke University Medical Center, Durham, NC.

Background: Cytogenetic abnormalities represent important prognostic indicators in patients with myelodysplastic syndrome (MDS) and acute myeloid leukemia (AML). Prognostic significance of cytogenetics and its correlation with immunophenotype of leukemic blasts in patients who develop AML from MDS has not been examined.

Design: From the pathology reports of patients diagnosed at our institution from 1996 to 2003, we identified 78 cases of AML arising from MDS among the 366 acute leukemia patients in whom flow cytometric findings were available. Cytogenetic features were examined for possible correlations with demographics, early response to treatment and the percentage of disease-free individuals at endpoint (death or study termination). Multicolor flow cytometry was used to evaluate the immunophenotype of the blasts and the possible heterogeneity in antigen expression.

Results: Cytogenetic profiles for 82% of these cases were available, out of which 61.3% demonstrated chromosomal alterations. Of these, 69% displayed two or more chromosomal abnormalities ("complex cytogenetics"). In comparison to subjects with normal karyotype, complex cytogenetics were associated with a higher proportion of patients with disease at closure of the study or death (relative risk 4.4; $p<0.001$) and with a shorter follow-up interval after diagnosis of AML (141 vs. 471 days; Mann-Whitney Rank Sum test, $p<0.005$). There was no association between complex cytogenetics and early response to treatment, age, gender or race. The most frequent cytogenetic alterations were monosomy 7, trisomy 8, and del(5q), all of which were identified both as single abnormalities and as components of complex cytogenetics. Immunophenotypic analysis showed that 40.3% of cases displayed a homogeneous immunophenotype, 13% exhibited two distinct abnormal populations, and 46.8% had one or more antigens expressed only on a subset of the myeloblasts. CD33, CD34, and CD117 were the antigens most commonly expressed on only a fraction of the myeloblasts. There was no correlation between the immunophenotypic homogeneity of the blasts and the complexity of the cytogenetic abnormality.

Conclusions: In patients with AML arising from MDS, complex cytogenetics are associated with a poor outcome; however, these chromosomal alterations do not predict early response to treatment, and are not associated with demographic differences. Immunophenotypic heterogeneity of leukemic blasts does not correspond to the cytogenetic findings.

1166 Peripheral T-Cell and NK/T-Cell Lymphomas: An International Study of 1,179 Cases

DD Weisenburger. University of Nebraska Medical Center, Omaha, NE.

Background: Peripheral T-cell lymphoma (PTCL) and NK/T-cell lymphoma (NKTL) are uncommon and consist of a heterogeneous group of neoplasms with a generally poor clinical outcome. The recent classification proposed by the WHO includes new categories and diagnostic criteria, but has not been formally evaluated. Therefore, a group of hematopathologists and clinicians undertook a large international study to assess the clinical applicability and reproducibility of this new classification.

Design: We collected 1,179 adult cases with previously-untreated PTCL or NKTL from 20 centers around the world. Consecutive cases representative of the geographic region and presenting between January 1, 1990, and December 31, 2002, were included. Tissue biopsies with a detailed phenotype and clinical data with followup were required. All cases were reviewed by panels of four expert hematopathologists and diagnostic disagreements were resolved by consensus.

Results: The frequencies of various lymphoma types were significantly different in North America (NA), Europe (EU) and the Far East (FE). PTCL unspecified was most common in NA (34.5%), angioimmunoblastic type in EU (30.4%), adult T-cell leukemia/

lymphoma (ATLL) in southern Japan (50%), and NKTL in the FE excluding Japan (35.6%). Overall agreement of the experts with the consensus diagnosis was only 81%, but varied widely by type (range 66% - 98%). Overall agreement of five experts with their own diagnoses after re-review of a subset of the cases was only 79% (range 67% - 93%). Review of the clinical data resulted in a change in the final diagnosis in 10.2% of the cases. The median overall survival (OS) for all cases was less than two years, and the 10-year failure-free survival was only 15%. However, survival was relatively good for all three types of anaplastic large cell lymphoma and the subcutaneous panniculitic type, very poor for ATLL, enteropathy and hepatosplenic types, and extranasal NKTL, and intermediate for the other types. Pathologic features predictive of survival in the major types included the percentage of transformed cells, Ki67 proliferation rate, cytotoxic (TIA1+) phenotype, and the percentage of CD8+ background T-cells.
Conclusions: Diagnostic criteria need to be refined in order to improve the accuracy and reproducibility of the diagnosis of these disorders. Although the WHO classification delineates disease entities and syndromes with different presentations and survival outcomes, further work is needed to classify these diseases based on specific cell types and molecular mechanisms.

1167 MUM1 and BCL6 but Not HGAL Are Independent Prognostic Indicators of Outcome in Nodal Diffuse Large B-Cell Lymphoma

NT Wongchaowart, E Segota, B Pohlman, IS Lossos, Y Natkunam, R Levy, T Jin, ED Hsi. Cleveland Clinic Foundation, Cleveland, OH; University of Miami, Miami, FL; Stanford University School of Medicine, Stanford, CA.
Background: Diffuse large B-cell lymphoma (DLBL) can be divided into germinal center (GC) and non-GC immunophenotypes (IP) based on expression of CD10, BCL6, and MUM1. Human germinal center-associated lymphoma (HGAL) protein may be associated with favorable prognosis in DLBL. We set out to determine if HGAL protein expression provided prognostic information in addition to the GC IP.
Design: We studied initial diagnostic biopsies from 57 patients (pts) with primary nodal DLBL. These pts received CHOP or CHOP-like chemotherapy, and variably received rituximab, radiation therapy, and/or high dose therapy with stem cell transplantation. Cases were tested for immunohistochemical expression of HGAL, CD10, BCL6, and MUM1 using a tissue microarray (30% cutoff). GC and non-GC IP were defined using previously described criteria (Hans et al. Blood 2004, 103:275-82). Clinical endpoints were disease progression and death. Data were analyzed using Cox proportional hazards testing.
Results: There were 32 men and 25 women (median age of 60 years). Survivor median follow-up was 69.9 months. 62%, 64%, 82%, and 58% of cases expressed HGAL, CD10, BCL6, and MUM1. 69% had a GC IP. Univariate analysis showed high International Prognostic Index (IPI) and MUM1 predicted higher progression risk (P<.001 and P=.027) and death (P<.001 and P=.031). Non-GC IP predicted death, but not progression (P=.023 and .19). HGAL predicted neither progression nor death (P=0.23 and 0.37). Multivariate analysis for progression showed high IPI (HR 7.07, P<.001), MUM1 (HR 3.88, P=.005), and BCL6 (HR 0.29, P=.010) as significant predictors. Multivariate analysis showed only high IPI (HR 6.48, P<.001) and MUM1 (HR 3.21, P=.015) as significant predictors of death.
Conclusions: High IPI, MUM1, and BCL6 were independent adverse predictors of progression and/or death. In this series, limited by small sample size and heterogeneous treatment, HGAL provided no added prognostic information, but analysis of a larger cohort is warranted.

1168 Expression of N-cadherin in B-Cell Non-Hodgkin and Hodgkin Lymphomas

Q Xie, L Chen, J Albanese, R Shaknovich. Montefiore Medical Center, Bronx, NY.
Background: N-cadherin is a Ca^{2+}-dependent cell-cell adhesion molecule, which plays important roles in maintaining the hematopoietic stem cell niche in the bone marrow and in the interaction of CD19+ CD34+ lymphoid progenitor cells with N-cadherin+ osteoblasts lining the bony trabeculae. N-cadherin is not expressed on normal mature lymphocytes, but is overexpressed on some T-cell lymphomas and possibly targets those to N-cadherin+ mesenchymal tissue in the skin and CNS. Whether N-cadherin is aberrantly expressed in B-cell non-Hodgkin or Hodgkin Lymphomas and if its expression plays a role in targeting those lymphomas to the bone marrow compartment is not known.
Design: We performed immunohistochemichemistry with anti-N-cadherin antibody (Abcam, MA) followed by Envision+ development system (Dakocytometion, CA) on paraffin-embedded antigen retrieved slides. We analyzed N-cadherin expression in 84 B-cell non-Hodgkin and Hodgkin Lymphomas and in control normal hematopoietic tissues.
Results: No expression of N-cadherin in the lymphoid compartment was identified within reactive tonsils or lymph nodes. 84 lymphomas, involving lymph nodes or extranodal soft tissue were tested, including DLBCL, FL, CLL/SLL, MCL, MZL, HCL and cHL. N-cadherin was aberrantly expressed in DLBCL: 53% (N=62), FL:80% (N=10), CLL/SLL: 75% (N=4), MCL: 0% (N=2), MZL: 50% (N=2), HL: 100% (N=2), HCL:100% (N=2).
Conclusions: N-cadherin is aberrantly expressed in B-cell non-Hodgkin lymphomas and Hodgkin lymphomas. N-cadherin-positive lymphomas tend to be associated with more fibrosis and have higher grade morphology (immunoblastic).Validity of these findings is supported by gene expression profiling data by Alizadeh et al., which revealed higher N-cadherin expression in transformed cell lines and an activated subgroup of DLBCL.

1169 BCL-6 Negativity, Female Gender, and Morphologic Type Predict Poor Overall Survival in Primary Cutaneous Large B-Cell Lymphomas with a Diffuse Architecture

X Xie, U Sundram, S Kohler, I Lossos, Y Natkunam, JR Cook, SH Swerdlow, J Guitart, M Smith, D Bosler, C Listinsky, J Hammel, ED Hsi. Cleveland Clinic Foundation; Stanford University School of Medicine; University of Pittsburgh School of Medicine; William Beaumont Hospital; Northwestern University; University Hospital of Cleveland; University of Miami.
Background: The classification of primary cutaneous large B-cell lymphomas (PCLBCL) with a diffuse architecture has been the source of some confusion. In the WHO-EORTC classification, such lymphomas may be classified based primarily on clinical and morphologic features as 1) follicle center lymphoma (FCL); 2) LBCL, leg type; or 3) LBCL, other. The primary reason for such classification stems from an aggressive course for some cases of leg type LBCL compared to FCL. We studied a series of diffuse PCLBCLs with markers shown to be of importance in classification and prognosis in nodal diffuse large B-cell lymphoma.
Design: 32 cases of PCLBCL with a diffuse architecture were included. 11 cases were previously reported. Cases were classified as FCL or LBCL, leg type according to the WHO-EORTC classification. Immunostains were performed for CD20, HGAL, BCL2, BCL6, CD10, and MUM1. Cases were scored as negative (<5%) or positive (≥5%). Germinal center B-cell (GCB) type (as defined by Hans et al Blood, 2004, using 30% threshold) was also determined. Overall survival (OS)and event-free survival (EFS) were the clinical endpoints.
Results: There were 18 women and 14 men, median age of 62 years. Morphologically, 22 were FCL and 10 LBCL, leg type. Older age, female gender, BCL2 expression, BCL6 negativity, and location on the leg were associated with LBCL, leg type (P<.001, P=.001, P=.002, P=0.001, and P<.001, respectively, Fisher Exact). HGAL was expressed in all cases. CD10 and MUM1 were not associated with subtype. Univariable log rank testing showed only female gender was associated with EFS (P=.007). Factors associated with shorter OS were female gender (P=.030) and BCL6 negativity (P=.008). Leg type LBCL was of only marginal significance for OS (P=0.052). Expression of HGAL, BCL2, MUM1, CD10, and GCB-type were not associated with outcome.
Conclusions: Female gender and lack of BCL6 expression predict for poor OS, while histologic subtype is of marginal significance. CD10, MUM1, or GCB-type do not appear to be useful prognostic factors. Interestingly, HGAL was seen in all cases, suggesting germinal center origin in the vast majority of diffuse PCLBCLs.

1170 Nodular Lymphocyte Predominant Hodgkin's Lymphoma: A Possible Subset Occurring in Older Individuals at Atypical Locations

DT Yang, CH Dunphy, S Tripp, SL Perkins. The University of Utah, Salt Lake City, UT; University of North Carolina, Chapel Hill, NC; ARUP Laboratories, Salt Lake City, UT.
Background: Nodular lymphocyte predominant Hodgkin's lymphoma (NLPHL) is characterized by a nodular, or a combination of nodular and diffuse, proliferation of scattered large, neoplastic, Bcl-2 negative B-cells in a background of small, non-neoplastic, reactive B-cells or, in the diffuse areas, T-cells. NLPHL typically presents in 30 to 50 year old men as early-stage disease with localized cervical, axillary, or inguinal adenopathy and demonstrates indolent behavior with excellent outcomes. There is evidence that a small fraction (3% to 6%) of NLPHL may progress to diffuse large B-cell lymphoma (DLBCL). We describe a series of NLPHL cases where a subset occurred in older individuals at atypical sites and demonstrated significantly higher rates of progression towards DLBCL.
Design: A series of 56 consecutive tissue biopsies diagnosed as NLPHL from 2 institutions were reviewed and diagnoses were confirmed by consensus based on World Health Organization criteria. Progression was defined as the presence of sheets of large cells (>25 cells). Clinical data from the cases were tabulated and summarized. Immunohistochemical staining for Bcl-2 was performed on 4 cases with atypical sites of involvement and a random sample of 10 cases with typical sites of involvement.
Results: Of the 56 cases of NLPHL, 6 occurred at atypical sites (2 retroperitoneal, 2 mesenteric, 1 choledocal, and 1 splenic) and 2 of these (33%) demonstrated progression while only 2 of the 50 cases (4%) occurring in typical sites showed progression. The average and median age of patients with atypical sites of involvement were 65 and 71 years respectively, while they were 35 and 33 years respectively for those with involvement of typical sites. Bcl-2 expression was found in both cases from atypical sites that demonstrated progression to DLBCL and not in any of the cases without progression.
Conclusions: Progression from NLPHL to the more aggressive lymphoma, DLBCL may affect both disease behavior and management. We identified a possible subset of NLPHL with distinct clinical features, occurring in older individuals at atypical sites, associated with an increased rate of progression to DLBCL. Interestingly, the cases that demonstrated progression also demonstrated Bcl-2 expression, which is unexpected in NLPHL. Identification and subsequent follow up of such cases may lead to a modification in management and improvement in outcomes.

1171 Large-Scale Identification of Proteins Expressed by Follicular Lymphoma-Derived Cells in Formalin-Fixed Paraffin-Embedded Sections

DT Yang, DK Crockett, CP Vaughn, SR Tripp, MS Lim, KSJ Elenitoba-Johnson. University of Utah, Salt Lake City, UT; ARUP Laboratories, Salt Lake City, UT.
Background: Recent advances in mass spectrometry (MS) instrumentation and the completion of the human genome sequence have triggered an explosive growth in the application of MS to high-throughput and large-scale analysis of complex proteomes. However, this technology has not been applied to formalin-fixed paraffin-embedded (FFPE) material because the cross linking effect of formalin severely limits recovery of intact proteins. MS-based proteomic analysis on FFPE samples can be a powerful tool for the identification of biomarkers and therapeutic targets through exploiting the vast

repositories of archival tissue that are associated with well-documented clinical follow-up. We employed a "bottom-up" approach involving enzyme digestion for protein extraction and subsequent liquid chromatography (LC) followed by tandem mass spectrometry (MS/MS) for protein identification in FFPE specimens.

Design: Proteins were extracted in parallel by enzyme digestion with trypsin and glutamic C endopeptidase from two sample types, a three-year old FFPE block, and fresh cell lysates, both from a follicular lymphoma-derived cell line (SUDHL-4) and analyzed by LC-MS/MS. Protein identifications were scored using Xcorr criteria of 1.5, 2.2, and 3.5 for +1, +2, and +3 ions respectively, and false positivity rate at <5% by PeptideProphet. Orthogonal validation of selected proteins identified in both sample types was performed by western blot analysis, immunofluorescence microscopy, and immunohistochemistry on a follicular lymphoma (FL) and diffuse large B-cell lymphoma (DLBCL) tissue microarray.

Results: Of the 324 and 514 proteins identified in the FFPE material and fresh cell lysate respectively, 263 were in common. 52% of the proteins identified in the fresh cell lysate were also identified in the FFPE material. B-Raf, JAK1, PKC, and STAT1 was observed by immunoblot analysis and PLCγ1 was confirmed by immunofluorescence microsopy on SUDHL-4 cells. Immunohistochemical staining for B-Raf, PKC, and STAT1 was positive in 3/9 (27%), 9/9 (100%) and 3/11 (27%) of FL cases and 18/28 (64%), 27/29 (93%) and 9/29 (31%) of DLBCL cases.

Conclusions: Large-scale identification of proteins in specimens is feasible using FFPE material. This development creates tremendous opportunities for the discovery of diagnostically and prognostically relevant biomarkers as well as proteins involved in the pathogenesis of several disease processes including cancer.

1172 CD7 Expression Predicts Poor Disease Free Survival and Post-Remission Survival in Patients with Acute Myeloid Leukemia and Normal Karyotype

J Yeung, QL Yi, H Chang. University Health Network, University of Toronto, Toronto, ON, Canada.

Background: Several established prognostic factors are used in the assessment of patients with acute myeloid leukemia (AML), with cytogenetic abnormalities being the most significant. However, AML patients with normal karyotype comprise the largest subgroup (~50%) and have a highly heterogeneous clinical course. Additional prognostic markers are needed to predict their clinical outcomes.

Design: By multi-parameter flow cytometry we analyzed CD7 expression in 185 patients with normal karyotype AML and correlated with their clinical features.

Results: CD7 was expressed in 68 (37%) patients. CD7 expression was not associated with age, sex, white blood cell (WBC) count, or extramedullary disease. The complete remission rate was similar in CD7+ and CD7- groups (71 vs 72%). However, patients expressing CD7 had significant shorter disease free (DFS) and post-remission survivals (PRS) than patients without CD7 (DFS of 12 vs. 42 months, P = 0.005; PRS of 15 vs. 33 months, P = 0.013). We also found that expression of CD34 or HLA-DR was associated with lower CR rate (P = 0.0007 and P = 0.019 respectively) but did not affect DFS or OS. Furthermore, as for all AML patients, we demonstrated that in the normal karyotypic subgroup, patients with higher WBC counts (>$50x10^9$/l) and older age (>60 years) had lower CR rate (P = 0.003 and P = 0.0157 respectively) and shorter OS (P = 0.0035 and P = 0.007 respectively). Multivariate analysis of age, WBC counts, CD34, HLA-DR and CD7 showed that CD7 expression was an independent risk factor for DFS (p= 0.01) and PRS (p= 0.02).

Conclusions: Aberrant CD7 expression is an independent prognostic factor adversely affecting DFS and PRS for AML patients with normal karyotype at diagnosis. If confirmed, CD7 expression may facilitate prognostic stratification of those normal karyotype AML patients who achieve first remission for alternative therapy to improve their outcomes.

1173 Sequence Analysis Proves Clonal Identity in Five Patients with Typical and Blastoid Mantle Cell Lymphoma

CC Yin, LJ Medeiros, P Lin, C Cromwell, D Jones, R Luthra, LV Abruzzo. UT MD Anderson Cancer Center, Houston, TX.

Background: Mantle cell lymphoma (MCL) is typically composed of small irregular lymphoid cells. Blastoid variants, composed of small immature (classic) or large (pleomorphic) cells, can arise *de novo* or occur in patients with typical MCL. In the latter group, the clonal relationship between typical and blastoid MCL has rarely been assessed at the molecular level.

Design: We identified 5 patients with typical MCL who subsequently developed blastoid MCL. Immunophenotypic analysis was performed by flow cytometry and/or immunohistochemistry. DNA extracted from paraffin-embedded tissue was assessed for IgH gene rearrangements by PCR using two consensus oligonucleotide primers directed to framework II (5'-TGGRTCCGVCAGSCYCCNGG-3') and JH segments (5'-AACTGCAGAGGAGACGGTGACC-3'). The VDJ sequences in each set of paired samples were then compared, and the V region was also compared to germline.

Results: There were 2 men and 3 women with a median age of 65 years (range 64-72) at diagnosis of typical MCL. The median interval between typical MCL and onset of blastoid MCL was 32 months (range 11-66). All patients presented with typical MCL in lymph nodes. Subsequent blastoid MCL was identified in lymph nodes (2), soft tissue (2), or rectum (1). The immunophenotype of typical and blastoid MCL in all cases was identical (positive for cyclin D1, CD5, CD20 and monotypic surface light chain). The MIB1 antibody demonstrated a median proliferation rate of 10% in the typical and 70% in the blastoid MCL. Sequence analysis proved clonal identity in each set of paired samples in all 5 cases. The differences between the V region and germline sequences ranged from 1.6% to 2.7%, with 3 cases >2% (Table 1).

Conclusions: VDJ sequence analysis of the rearranged IgH allele in paired samples of 5 patients with typical and blastoid MCL revealed clonal identity. These results support the concept that blastoid MCL arising in patients with typical MCL represents histologic progression.

Sequence analysis of paired typical and blastoid MCL

ID	Type	CDRIII sequence	Mutation rate (%)
1	T	GTGCGACGGGCCTCAGCCCGCGGGGCGGGAGCTACA-JH5	1.7
	B	GTGCGACGGGCCTCAGCCCGCGGGGCGGGAGCTACA-JH5	1.7
2	T	GTGCGAGAGTTTACTATGATAGTAGTGGTTATTAT-JH4	2.2
	B	GTGCGAGAGTTTACTATGATAGTAGTGGTTATTAT-JH4	2.2
3	T	GTACCACATCCCATTACTATGGTTCGGGGAGTTACCCTTTT-JH4	1.6
	B	GTACCACATCCCATTACTATGGTTCGGGGAGTTACCCTTTT-JH4	1.6
4	T	GTGCAAAAGTGGCTGGTACAACCGAAAACTACTTT-JH4	2.2
	B	GTGCAAAAGTGGCTGGTACAACCGAAAACTACTTT-JH4	2.2
5	T	GTGCGAGAGGCGAAGTGGGAGCTACTACATCGGC-JH6	2.7
	B	GTGCGAGAGGCGAAGTGGGAGCTACTACATCGGC-JH6	2.7

T, typical; B, blastoid

1174 Detection of t(11;18) API2/MALT1 Translocation Specifically Associated with MALT Lymphoma by Robust Real-Time RT-PCR

W Zhang, J Garces, HY Dong. Genzyme Genetics, New York, NY; Genzyme Genetics, Westborough, MA.

Background: The t(11;18) API2/MALT1 translocation is specifically associated with MALT lymphoma, particular those occurring in the lung and stomach. The presence of t(11;18) in gastric MALT lymphoma also strongly predicts resistance to *H. Pylori* eradication therapy. Early detection of t(11;18) may help for diagnosis and stratification of treatment options.

Design: Total RNA was isolated using the High Pure™ RNA Paraffin Kit (Roche Diagnostics Corp.) with modifications and reversely transcribed. The cDNA was amplified by real-time PCR in an ABI PRISM® 7700 Sequence Detection System using one forward primer in the API2 gene and two reverse primers in the MALT1 gene. *In vitro* transcribed RNAs from plasmid templates containing the targeted breakpoints were used as positive controls. This single-tube multiplex RT-PCR method covers three common breakpoints in the MALT1 gene, and is expected to detect 84% of all t(11;18) in the literature or 99% of those detected by RT-PCR reported by others. The analytical sensitivity was consistently at levels of 100 copies or better.

Results: We blindly tested 40 biopsy samples from 38 patients with gastric MALT lymphoma; all but two were FFPE small endoscopic biopsies in a typical size range of 0.1-0.5cm. Of these, 10/40 of the samples (25%) or 8/38 of the patients (21%) were positive for t(11;18). For two patients, the assay was performed on two separate specimens either at the time of initial diagnosis or before and after treatment, all were consistently positive. There were 9/10 specimens involving the breakpoints at A1446M1123/1150 by RT-PCR; 8 were at A1446M1123 and 1 at A1446M1150, proven by DNA sequencing. The other case had the breakpoint at A1446M814. We also tested 7 samples of pulmonary MALT lymphoma; 3/7 (43%) were positive with 2 involving A1446M814 and 1 at A1446M1123/1150. In contrast, t(11;18) was absent in all 11 cases of gastric large B cell lymphoma and 11 cases of chronic active gastritis (including seven with dense reactive lymphoid infiltrates). In addition, 18 known negative samples unrelated to lymphoma were also consistently negative.

Conclusions: Our assay was highly sensitive and reproducible, and the detection rate was consistent with those of other large series in the literature using standard RT-PCR. Our results suggest that real-time RT-PCR for MALT lymphoma associated t(11;18) is a useful tool for assisting in diagnosis, predicting treatment response, and monitoring persistent/recurrent tumors.

1175 Primary Bone Large B-Cell Lymphoma: Morphologic and Immunohistochemical Characteristics of 7 Pediatric Cases

X-F Zhao, JK Choi. University of Maryland School of Medicine, Baltimore, MD; The Children's Hospital of Philadelphia; University of Pennsylvania School of Medicine, Philadelphia, PA.

Background: Four percent of pediatric non-Hodgkin lymphoma (NHL) are restricted to the bone/bone marrow and are often designated as primary lymphoma of bone (PLB). PLB consists of lymphoblastic lymphoma, Burkitt lymphoma, and large cell lymphoma. The last has a favorable prognosis but is poorly characterized and is the focus of this study.

Design: NHLs (n=306) at the Children's Hospital of Philadelphia from 1980 to 2003 were reviewed retrospectively and 16 cases of PLB were identified, of which 9 were classified as large cell lymphomas. Seven of the 9 cases had sufficient material for review and paraffin immunohistochemical studies.

Results: In all 7 cases, the tumor cells were paratrabecular or completely replaced the bone marrow and consisted of medium to large sized cells with round to irregular nuclei, dispersed chromatin, small to indistinct nucleoli, abundant cytoplasm, and poorly defined cytoplasmic borders. Mitotic figures were rare to absent. More variable morphologic features included varying levels of necrosis, cytoplasmic retraction, and associated reactive T lymphocytes, neutrophils, and histiocytes. Occasional cases were associated with necrosis or myeloid hyperplasia. Paraffin immunohistochemistry showed that the tumor cells were strongly positive for CD20, CD79a, and PAX5 while negative for CD34, TdT, CD3, CD5, CD30, CD21, ALK1, and CD68 (7/7). Most were positive for CD45 (6/7), CD10 (5/7), BCL6 (6/7), and p53 (5/7) while negative for BCL2 (4/7), MIC2 (6/7), and O13 (6/7). Ki67 staining was variable, ranging from <5% to >75% positive tumor cells. No small, cleaved B cell component was identified.

Conclusions: Pediatric primary large cell lymphoma of bone is a mature B-cell neoplasm, possibly of follicular center origin, with a favorable prognosis. The tumor cells lack the single to multiple prominent nucleoli of typical centroblasts or immunoblasts that are often seen with metastatic large B-cell lymphomas. These findings suggest that primary bone large B-cell lymphoma (PBLBCL) is a distinct pathologic entity in children. Additional studies are needed to determine if our findings can be generalized and used to distinguish the adult PBLBCLs with favorable prognosis from those with poor prognosis.

1176 FISH Suggests That MIB-1 Labeling Index Is Not a Reliable Distinguisher of Atypical Burkitt Lymphoma from Diffuse Large B-Cell Lymphoma

X-F Zhao, A Perry, Y Ning, A Hassan, SA Stass, LP Dehner. Washington University School of Medicine, St. Louis, MO; University of Maryland School of Medicine, Baltimore, MD.

Background: Atypical Burkitt lymphoma (ABL) is defined as a highly aggressive B-cell neoplasm of predominantly medium-sized cells with non-classic Burkitt morphology, such as increased atypia, a nearly 100% growth fraction, and consistent translocations involving the c-*myc* gene. Because of its pleomorphism, ABL is often confused with diffuse large B-cell lymphoma (DLBCL), which has abundant cytoplasm, vesicular nuclei and small or prominent nucleoli, and not infrequently a similarly high proliferation rate. Although the distinction of these two entities has significant clinical implications, the differential diagnosis is difficult in some cases.

Design: We have selected 7 challenging cases of B-cell lymphoma with atypical morphology. Six cases were nodal and one was derived from bone marrow. Patients ranged in age from 19 to 71 years (median age of 62 years), and included 3 males and 4 females. ABL and DLBCL was the differential diagnosis in each case. We studied these cases using immunohistochemistry, flow cytometry and fluorescence in situ hybridization (FISH) using c-*myc* break-apart and *IgH/BCL2* dual fusion probe sets.

Results: H&E sections showed that the tumor cells varied from medium to large in size with slightly clumped to vesicular nuclei and occasional prominent nucleoli. None of the cases showed a classic Burkitt morphology. All the cases were CD20+ except for case 3 that was CD20–/CD79a+. Proliferation indices as assessed by MIB-1 stain varied from 50% to 100%. A c-*myc* rearrangement was detected in 5 cases with labeling indices <90% whereas it was absent in 2 others with >90% labeling. In addition, a t(14;18) was additionally detected in 2 cases with a c-*myc* translocation. ABL was diagnosed in 5 cases with a c-*myc* translocation, and DLBCL was diagnosed in 2 cases with no c-*myc* translocation.

Conclusions: We conclude that the growth fraction is neither absolutely specific for the diagnosis of ABL, nor does it entirely exclude the possibility of DLBCL. The c-*myc* translocations are more reliable in resolving the diagnosis in cases with a confusing morphology. The significance of ABL cases harboring both c-*myc* rearrangement and a t(14;18) is unclear and this subgroup needs to be further defined.

1177 B-Cell Chronic Lymphocytic Leukemias with p53 Deletion Are Highly Resistant to Fludarabine *In Vitro*

Y Zhong, AC Bakke, G Fan, KM Gatter, R Braziel, JZ Huang. Oregon Health & Science University, Portland, OR.

Background: Individual patients with B-CLL demonstrate variable responses to standard induction and salvage therapeutic regimens. It would be highly desirable to develop a predictable and reproducible laboratory diagnostic strategy that guides the selection of appropriate drugs and/ or regimens based on the drug sensitivity and resistance profiles of leukemic cells for individual patients.

Design: A study was designed to investigate the differences of in vitro drug sensitivity profiles of leukemic cells with different cytogenetic abnormalities from CLL patients. CLL cells from 43 patients were incubated *in vitro* with four commonly used chemotherapeutic agents (fludarabine, chlorambucil, cladribine or prednisolone). Multiparameter flow cytometry was utilized to determine the decrease in leukemic cell viability after drug exposure. The *in vitro* drug sensitvity profile were correlated with the cytogenetic abnormalities and clinical responses retrospectively.

Results: The highest *in vitro* resistance to fludarabine, was seen in all seven cases of B-CLL cells with deletions of p53, a cytogenetic abnormality associated with poor clinical outcome and poor clinical response. A majority of cases highly resistant to fludarabine were also resistent with chlorambucil and cladribine, but not to prednisolone. In CLL cases without p53 deletion, a marked variability in drug sensitivity was observed *in vitro* but no significant difference was detected among cases with normal cytogenetics (n=13), ATM deletion (n=4), trisomy 12 (n=3), or 13q deletion (n=7).

Conclusions: Our findings provide direct evidence of cellular resistance to fludarabine in CLL associated with p53 deletion, confirming prior clinical observations. *In vitro* drug sensitivity assay with routine clinical specimens containing leukemic cells admixed with normal cells. This method may prove useful in guiding choices for therapy for CLL patients based on the drug sensitivity profile of leukemic cells in individuals.

1178 Utility of Flow Cytometry in Detecting CNS Involvement by Hematopoetic Malignancies

DL Zynger, ND Dimov, D Variakojis, CL Goolsby. Northwestern University, Chicago, IL.

Background: Flow cytometry (FC) and morphology (M) are techniques implemented in the evaluation of cerebrospinal fluid (CSF). The purpose of the study was to assess the utilization and the diagnostic accuracy of FC in CSF evaluation in a large number of samples and to determine when FC would be a useful adjunct to routine M examination. Independent FC and M data were compared, with emphasis given to total cell count (TCC).

Design: Reports from 313 cases of CSF analyzed by 4-color FC were stratified as follows: insufficient hematopoetic cells, no abnormal population, and abnormal population detected. TCC was available for 102 of these cases. Clinical history was obtained for 227 cases from the past two years. Of these, there were 161 cases with independent M and FC reports of the same specimen, in which these two reports were signed-out without knowledge of the other report. Discordant reports were correlated with clinical history and TCC. Sixty-six of the 227 were eliminated as the reports were not independent or no M evaluation was performed.

Results: At this institution, FC analysis of CSF has increased more than 100% (86 samples in 2003, 109 in 2004, projected 177 in 2005). However, insufficient hematopoetic cells (26-31%) and abnormal populations detected (20-22%) have remained constant. Of those with an abnormal population, 48% had a TCC of less than 5000, including cases with less than 500 cells. Of the 227 cases, 80% had a history of a hematological malignancy. Of those with an abnormal population by FC, 6% (n=3) had no history of a hematopoetic disorder. Analysis of 161 cases by independent FC and M revealed 87% (n=140) concordance. Of these 161, over 50% of the cases in which FC detected an abnormal population not seen by M, occurred in specimens with a TCC of less than 5000. Abnormal populations were detected by M and not noted by FC in 2% (n=3). Abnormal populations were detected by FC but reported as negative by M in 11% (n=18). Interestingly, abnormal populations were detected by FC and not M in 62% (n=8) of mature T-cell neoplasms.

Conclusions: This comprehensive study revealed that FC can detect abnormal populations not evident by M in samples with low TCC. Thus, limiting the usage of FC based on TCC is not recommended. FC in conjunction with M examination yields enhanced detection of abnormal cellular populations in CSF. FC analysis of CSF in patients without history of hematological malignancy can detect lymphoproliferative disorders. Analysis of data at this institution reveals that the increased usage of FC in the analysis of CSF is warranted.

Infections

1179 Correlation of Cytokine Expression, Histologic Findings, and Clinical Outcome in the Placenta

I Aguilera-Barrantes, GJ Nuovo. Ohio State University Medical Center, Columbus, OH.

Background: Histological examination of placentas with in utero infections often lacks (on H&E sections) the typical lymphocytic or neutrophilic response associated with infection in other organs, and more fequently shows non-specific changes. Placental macrophages and trophoblasts are capable of producing many cytokines. These cytokines could be used as a marker for prenatal infections.

Design: We examined the histologic findings in 90 placentas and correlated this with the expression of cytokines (macrophage inflammatory protein alpha (MIP), IL-8, and tumor necrosis factor alpha (TNF)) as determined by immunohistochemistry, and clinical outcome. Of the 90 placentas, 40 were from either stillbirths (20) or from cases of severe morbidity (APGARs <5/5 with severe sequela) in which an infectious agent was identified (most cases were bacterial infection or coxsackie virus infection). The other 50 were the controls, all with APGARs >8/8 and no clinical problems. The 50 controls included cases of diabetes mellitus (10) and preeclampsia (10). Increased expression of a cytokine was defined by noting at least 5 positive cells per placenta section.

Results: Of the 50 controls, increased expression of a cytokine was evident in only 2 cases (4%). Of the 40 cases of severe morbidity/mortality of infectious cause, at least one cytokine was increased in 40/40 cases. Most cells expressing TNF were trophoblasts or macrophages, whereas MIP and IL-8 expression was noted mostly in macrophages. In comparison, we studied 20 cases of known neonatal morbidity/mortality that was not infectious (eg abruption, ruptured uterus, prolapsed cord). In 2/20 (10%) of these cases, there was increased expression of a cytokine. No histologic finding correlated with cytokine expression. Further, there was no correlation between the number of CD68 or CD45 positive cells with cytokine expression.

Conclusions: Marked increase in cytokine expression is seen in placentas with in utero infections associated with severe morbility/mortality. This marked increase in MIP, TNF, and/or IL-8 appears to be a specific and sensitive marker of severe in utero infection, as it was rarely noted in controls.

1180 Cytomegalovirus Gastrointestinal Disease in the Elderly

RM Angeles, ES Weisenberg. University of Illinois at Chicago, Chicago, IL; Advocate Illinois Masonic Medical Center, Chicago, IL.

Background: CMV is the most common viral cause of GI morbidity in immunosuppressed patients with AIDS and transplantation. It is also seen in refractory IBD patients. We have encountered CMV infections in the elderly without significant history. It is the objective of this study to determine the relative percentage of affected elderly patients diagnosed with CMV and to analyze clinical information for the presence of known risk factors.

Design: We identified 32 biopsies in 26 patients diagnosed with CMV infection from January 1998- March 2004 in two community hospitals, Advocate Lutheran General Hospital and Advocate Illinois Masonic Medical Center. Tissue blocks and slides were all available, retrieved, reviewed and stained for AFB (modified Kinyoun's), fungi (GMS), and immunostained for HSV I/II, CMV/clone DDG9/CCH2 and Adenovirus/clone 20/11/2/6 (Ventana Medical Systems, Inc., Tucson, AR). Cells were positive only if nuclear staining were present. Clinical and endoscopic data were collected if available.

Results: Seven of 26 patients (27%), 4 males and 3 females, were elderly. The clinical features, endocopic findings, biopsy site and follow up data are presented in Table 1. All cases showed varying degrees of acute and chronic inflammation, granulation tissue and focal ulceration with CMV inclusions. They were in endothelial cells in 6, epithelial cells in 2, and stromal cells in 4 cases. CMV IHC were positive in 4 cases. None had co-infection with other viral, mycobacterial, fungal and protozoal pathogens by H&E, special and IHC.

Conclusions: Our findings demonstrate that a significant number of biopsies with CMV GI disease occur in the elderly, most with history of immunosuppression. A significant minority will lack typical risk factors for CMV infection. We conclude that CMV should be included in the differential diagnosis of elderly patients with unexplained GI inflammation.