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[213] Breast Implant Capsule-Associated Anaplastic Large Cell Lymphoma (BIC-ALCL)

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- **Background:** The US-FDA recently issued preliminary findings of an analysis to assess a possible association between anaplastic large cell lymphoma (ALCL) and breast implants. The analysis was prompted by a small (~30), but growing, number of cases of a rare form of lymphoma in women with breast implants, typically arising within the capsule and causing a clinically-evident peri-implant fluid accumulation. We describe 3 new cases of breast implantassociated anaplastic large cell lymphoma (BIC-ALCL) that highlight its characteristic clinical and pathologic features.
- **Design:** We studied the histopathologic characteristics, molecular pathology and clinical course of 3 cases of BICALCL.
- **Results:** The patients were 46, 67 and 67 years old, respectively, and all had breast implant reconstruction following mastectomy for cancer. All 3 presented with peri-implant fluid accumulation occurring 5 to 13 years after reconstruction. Gross exam showed the affected peri-implant capsules were thickened. Microscopy showed noncohesive, enlarged, atypical-appearing cells, some with reniform or horseshoe-shaped nuclei, in eosinophilic material adherent to the inner capsule surface; the atypical cells also infiltrated the inner capsule layers. In all 3 cases, the ALCL cells were ALK-negative and positive for CD30 and EMA; CD3 and CD4 were positive in 1 case each and both of these showed a monoclonally rearranged T-cell receptor !-chain gene *(TRG@)*. Flow cytometry analysis of periimplant fluid from the third case detected a predominance of T cells, but molecular studies on the fluid did not detect a *TRG@* gene rearrangement. All 3 had stage I lymphoma, confined to the breast. Two patients were treated with chemotherapy; one also had a stem cell transplant. All 3 are alive with neither breast cancer- nor lymphoma recurrence 36, 12 and 7 months after diagnosis of BIC-ALCL.
- **Conclusions:** Breast implant exchange with capsule resection prompted by peri-implant fluid accumulation should be carefully examined for BIC-ALCL. Clues to diagnosis are the unusual clinical presentation, a thickened scar capsule and histologic sections showing an atypical cellular infiltrate. These findings should prompt appropriate immunohistochemical stains and molecular analysis, where feasible.

Category: Breast

[1556] Nodal Involvement by Transformed Cutaneous CD30-Positive T-Cell Lymphoma Mimicking Classical Hodgkin Lymphoma

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- **Background:** Classical Hodgkin lymphoma (cHL) following mycosis fungoides (MF) or lymphomatoid papulosis (LyP) in the same patient has been debated in the literature. There is considerable morphologic and immunophenotypic overlap between cHL and nodal involvement of CD30-positive T-cell lymphomas (TCL). Whether such cases represent TCL with Hodgkin-like cells or cHL is often difficult to resolve.
- **Design:** Biopsies from patients with a prior history of cutaneous TCL or primary cutaneous CD30-positive T-cell lymphoproliferative disorder and lymph node biopsies reported as either CD30-positive TCL with Hodgkin-like cells or cHL were retrieved from the authors' institution. We performed immunophenotypic and T-cell receptor gene rearrangement studies (*TRG*) in order to clarify the diagnosis. Laser capture microdissection (LCM) was performed in one case.
- **Results:** Of 11 cases identified, 10 were considered CD30-positive TCL with Hodgkin-like cells, while one was confirmed as cHL upon review. Four cases originally diagnosed as cHL were revised as CD30-positive TCL. The CD30- positive TCL showed a male predominance (M:F, 4:1) with a median age of 53 years (range 44-72 years). 9/10 patients initially presented with skin lesions and later developed nodal involvement, although in some cases lack of knowledge of the cutaneous lesions led to a misdiagnosis of cHL In 8/10 patients the draining lymph node was involved, whereas in 2 cases generalized skin disease was present. Tumor cells morphologically resembled Hodgkin/Reed-Sternberg (HRS) cells, and were strongly positive for CD30 and negative for B-cell markers (i.e. PAX5, CD20) in all cases. Expression for CD15 was observed in the majority of cases (9/10). Also, 7/10 cases of CD30- positive TCL with Hodgkin-like cells had tumor cells that expressed at least one T-cell marker and all (9/9) cases studied revealed a clonal rearrangement by *TRG*. LCM in one case showed identical clones in skin and LN. In situ hybridization studies for EBV were negative for all studied cases. In one case the diagnosis of cHL followed by LyP was confirmed, with HRS-cells expressing PAX5, CD30 and CD15.
- **Conclusions:** In some cases of transformed MF/LyP with nodal involvement, the distinction from cHL can be challenging, but combined morphologic, immunophenotypic, and molecular studies together with careful clinical correlations help to differentiate these lesions. Misdiagnosis as cHL remains a diagnostic pitfall.

Category: Hematopathology

[1362] Quantitative Immunoshistochemistry Identifies B-Cell Receptor Signaling and AKT Activity in Diffuse Large B-Cell Lymphoma

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- **Background:** Diffuse large B cell lymphoma (DLBCL) is the most common type on non-Hodgkin lymphoma. The prognosis is poor indicating the need for more individualized therapeutic approaches. B cell receptor (BCR) mediated signaling and PI3K/AKT signaling are implicated in the pathogenesis of DLBCL. Recently, the BCR-downstream kinases SYK and BTK and AKT have emerged as potential therapeutic targets. Application of these targeted therapies requires quantitative assessment of the activity of distinct signal transduction networks in clinical specimens.
- **Design:** We employed quantitative immunohistochemical (qIHC) analysis in formalin-fixed paraffin embedded DLBCL cell lines and a cohort of 60 patient specimens on a tissue microarray using antibodies to phosphorylated forms of proximal BCR related kinases LYN, SYK, BTK. We also evaluated the subcellular localization of AKT-regulated transcription factor FOXO1, and examined signal integration between the BCR and AKT signaling.
- **Results:** We identified a robust protein signature underlying BCR signaling in DLBCL patient specimens. Active BCR signaling was successfully detected in more than 50% of the examined tumors. Further analysis of distal BCR signaling via PI3K/AKT pathway revealed a survival-associated signal: cytoplasmic localization of the forkhead transcription factor FOXO1 was seen in 52% of all tumors and was associated with active BCR signature in 60-70% of positive specimens. Nuclear exclusion of FOXO1 was independent of BCR signaling in a subset of DLBCL tumors suggesting that constitutive AKT activation may mediate survival in these cases. Cytoplasmic localization of FOXO1 correlated with evidence of AKT activation in the majority of the DLBCL cell lines, providing a robust surrogate marker for AKT activity in patient specimens.
- **Conclusions:** These results reveal that a large proportion of DLBCLs manifest active BCR signaling that translates to AKT activation and cytoplasmic FOXO1 localization. We conclude that qIHC provides a framework for assessing the integrity and activity of the BCR pathway in DLBCL biopsy samples that will assist in patient selection for appropriate targeted therapy.

Category: Hematopathology

[1483] Pediatric-Type Follicular Lymphoma Occurs in Both Children and Adults and Is Characterized by a High Proliferation Index and the Absence of a BCL2 Gene Rearrangement

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- **Background:** Despite its excellent prognosis, pediatric-type follicular lymphoma (PFL) remains challenging to define; it is uncertain if this indolent lymphoma is merely defined by age or may also occur in adult patients. In an effort to better define the morphologic, immunophenotypic, and genetic characteristics of PFL, we performed a comprehensive retrospective analysis of clonal B-cell proliferations with follicular pattern (CFPs) occurring in patients <40 years of age.
- **Design:** We reviewed 42 cases of CFPs, in patients ranging from 8 to 38y of age (median 17y, 35M/7F). 36 were Stage 1, and 6 were Stage 3 or 4. We evaluated histologic and immunohistochemical features (including Ki67 proliferation index, PI) as well as FISH and/or molecular genetic analysis for BCL2, BCL6, MYC, and MUM1 rearrangements. We then used parameters associated with Stage 1 disease to interrogate an independent group of 60 similarly characterized FLs occurring in patients ≥ 18 y of age.
- **Results:** None of the 36 stage 1 CFP cases had progressive/recurrent disease; 5/6 of the stage 3 or 4 cases had progressive/recurrent disease, all of whom were treated with chemotherapy.

Table 1: Features of Stage 1 vs. Stage 3/4 Clonal Follicular Proliferations

	Stage 1 (n=36)	Stage 3-4 (n=6)	P-value
Age (median)	18	25	p=0.02
M:F	31:5	4:2	NS
LN size (median)	2.2 cm	2.3 cm	NS
BCL2 rearrangement	0/27	3/4	p=0.0009
BCL2 protein expression	4/33	5/5	p=0.0003
PI >40% (Ki-67)	28/28	0/4	p=0.0005
Complete Architectural effacement	7/30	4/5	p=0.03
Follicles > 2mm	27/34	0/5	p=0.001
Grade †	22:6:7	3:2:0	NS

† Grade 1: Grade 2 : Grade 3

No patients had BCL6, MYC, or MUM1 rearrangement. Several features, in particular both lack of BCL2 gene rearrangement (BCL2R-) and high (>40%) PI (HPI), correlated with stage 1 disease. Applying these criteria to a separate cohort of 60 FL cases, 3 cases (ages 18, 49 and 61 y) were both BCL2R- and had HPI. All 3 BCL2R-/HPI cases were Stage 1 and had no evidence of residual disease at the latest followup (median 135 months). In contrast, only 3/57 of the 'adult-type' FL patients (with BCL2 rearrangement and/or low PI) had no residual disease at latest followup (p=0.001).

- **Conclusions:** PFL may be defined as an indolent clonal proliferation with a follicular pattern and variable histologic grade and BCL2 protein expression, but that both lacks BCL2 gene rearrangement and has HPI (>40%); large expansile follicles and lack of complete architectural effacement are morphologic clues to identify PFL. Similar indolent clonal follicular proliferations can occur in adults.

Category: Hematopathology

[1516] Follicular Lymphoma (FL) like B-Cells of Uncertain Significance (In Situ FL) Has a Low Rate of Progression, but Is Very Frequently Present in Biopsies Preceding Overt FL and a Moderate Proportion Is Associated with Other Lymphoid Neoplasms

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- **Background:** It has been proposed that in situ FL be called FL like B-cells of uncertain significance (FLBUS) because of growing concern that it is not an overt lymphoma. However, variable proportions of coexistent or subsequent FL and other lymphomas (ML) have been reported in a limited number of studies. Parameters predictive of progression are not established. In addition, the proportion of overt FL preceded by FLBUS is unknown.
- **Design:** 26 biopsies with FLBUS were reviewed including assessment of BCL2+ follicles, associated ML & clinical followup. In addition, prior "benign" lymph node (LN) biopsies from 6 patients who developed overt FL were retrieved & stained for BCL2, CD10, CD20 & CD3.
- **Results:** 11/26 (42%) patients with FLBUS had concurrent ML–FL-2, DLBCL-2, CLL/SLL-2, MALT-1, in situ mantle cell ML (MCL)-1, periph T-cell ML (PTCL)-2 & classical HL-1. 2/26 (8%) had subsequent ML (FL with prior PTCL, DLBCL) at 0.5-1 mo. 14/26 (54%) did not develop any ML (median followup 27 (1-68) mo.). Most cases had a high proportion of BCL2+ follicles with most of these having >25% BCL2+ cells.

Clinicopathologic Features, median (range)					
	All_cases*	Concurrent FL/DLBCL	Subsequent FL/DLBCL	Concurrent other B_cell_ML	No Lymphoma
Cases, #	26	4	2	4	14
Age, yr	75(30-87)	66(30-81)	75(71-78)	80(74-87)	69(40-86)
M:F	18:8	2:2	1:1	3:1	11:3
BCL2+ Foll, Abs #	11(1-207)	20(3-31)	14(10-17)	7(1-11)	11(4-207)
BCL2+ Foll, %	79(2-100)	72(5-97)	88(85-91)	44(2-75)	88(6-100)
% of BCL2+ Foll with <25% BCL2+ cells	27(0-86)	37(0-43)	26(10-41)	39(0-86)	21(0-73)
% of BCL2+ Foll with >50% BCL2+ cells	43(0-100)	47(32-100)	61(53-70)°	15(0-45)°	50(5-100)
% of BCL2+ Foll with >95% BCL2+ cells	10(0-100)	10(10-33)	13(6-20)	0(0-14)	12(0-100)
Follow-up, mo	29(1-68)	37(12-58)	32(1-63)	37(2-66)	27(1-68)
Known Rx	8	3	1	1	2
Alive	22	4	1	2	13
Dead	4	0	1-DLBCL	2-CLL/SLL	1-No ML

*Includes 1 PTCL & 1 HL not in other columns;°p=0.03

No significant differences were identified for the various BCL2-related parameters between the major groups of patients with 1 exception. 8 patients were treated and 4 died. 5/6 (83%) "benign" LN biopsies obtained 1-108 mo. prior to diagnosis of an overt FL (median 56 mo.) had FLBUS.

- **Conclusions:** Although isolated FLBUS appears to have a low rate of progression, most FL are preceded by FLBUS, analogous to the situation with CLL and MCL. In addition, almost half of the FLBUS had potentially related or often apparently unrelated lymphomas. The extent of FLBUS did not have significant implications.

Category: Hematopathology

[1586] Phospho-ERK$^{Thr202/Tyr204}$ Is Overexpressed in Hairy Cell Leukemia and Is a Useful Diagnostic Marker in Bone Marrow Trephine Sections

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- **Background:** *BRAF* V600E mutations are present in virtually all cases of hairy cell leukemia (HCL) and absent in other small B-cell leukemias and lymphomas. This activating mutation results in constitutive *BRAF* signaling, which is manifested by phosphorylation of ERK. We hypothesized that detection of phospho-ERK (pERK) in tissue sections may be a specific and useful marker for diagnosis of HCL.
- **Design:** We performed a pERK/CD20 double-stain in 89 bone marrow samples involved by B-cell lymphoproliferative disorders of small lymphocytes, including 27 cases of HCL confirmed by flow cytometry. Immunostaining was performed on decalcified formalin-fixed paraffin embedded bone marrow sections with an automated immunostainer (Ventana Medical Systems). The double staining protocol used a rabbit monoclonal antibody to pERK1/2$^{Thr202/Tyr204}$ (Cell Signaling Technology, clone D13.14.4E) and mouse monoclonal antibody for CD20 (Dako, Carpinteria, CA, clone L26). Allele-specific PCR for the *BRAF* V600E mutation was performed on 11 cases of HCL with available DNA and in 1 non-HCL case where pERK staining was positive.
- **Results:** pERK staining (cytoplasmic and nuclear localization) in at least 70% of B-cells was observed in all 27 cases of HCL tested. B-cells in all cases of CLL/SLL (n=12), follicular lymphoma (n=7), splenic marginal zone lymphoma (n=9), mantle cell lymphoma (n=9), CD5- B-cell leukemia/lymphoma not otherwise specified (NOS, n=10), lymphoplasmacytic lymphoma (n=11), and hairy cell leukemia variant (n=2) were negative for pERK. One of two cases of atypical CLL, likely representing mixed-cell CLL with 17p deletion, was pERK-positive. Allele-specific PCR on 11 cases of HCL contained the *BRAF* V600E variant in all 11 cases. The one non-HCL case that was positive for p-ERK also contained the BRAF V600E. Overall, while 100% of HCL cases expressed pERK, only 1 of 64 (1.6%) of other small B-cell lymphoma/leukemias in bone marrow expressed pERK. Thus, the sensitivity and specificity of pERK for diagnosis of HCL in our hands is 100 and 98%.
- **Conclusions:** *BRAF* V600E mutations are present in all HCL cases. Immunohistochemistry for pERK can be reliably applied on routinely processed bone marrow trephine sections and is highly sensitive and specific for HCL. It appears to be a useful tool in the differential diagnosis of small B-cell leukemias/lymphomas, and pERK is a surrogate marker for *BRAF* V600E in the rare non-HCL leukemias with this mutation.

Category: Hematopathology

[1406] Annexin A1 (ANXA1), Key Confirmatory Marker Discriminating Hairy Cell Leukemia from Variant Hairy Cell Leukemia and Other Morphologically Similar B-Cell Neoplasms

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- **Background:** Differentiating hairy cell leukemia (HCL) from variant hairy cell leukemia (vHCL), splenic marginal zone lymphoma (MZL), mantle cell lymphoma (MCL) or chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) can be problematic especially when evaluating core biopsy material. Distinguishing HCL is of clinical importance as treatment differs markedly. As morphology and immunophenotype can overlap, a marker specific for HCL would be advantageous. Annexin A1 (ANXA1), a gene upregulated in HCL, has been reported in a single study to provide highly sensitive and specific immunocytochemical detection of HCL. We evaluated ANAX1 and TRAP, a classic stain for HCL, to determine their usefulness in distinguishing HCL from morphologically similar entities.
- **Design:** Tissue microarrays were constructed with duplicate paraffin-embedded tissue cores from 11 HCLs, 2 vHCLs, 22 MZLs, 14 MCLs, and 23 CLL/SLLs. Immunostaining was performed on all specimens using antibodies against ANXA1 and TRAP. Each core was evaluated for the percentage of tumor cells staining and recorded for Proportion [P(0): 0%, P(1): 1-25%, P(2): 25-50%, and P(3): >50%] and Intensity [I (0-3+)]. A score was then calculated P+I=total. Scores of 0 were considered negative and >0 positive.
- **Results:** Our study showed 11/11 (100%) of classical HCL cases strongly positive for ANXA1 while both (100%) of the vHCL cases were negative (P+I=0). All of the other B-cell neoplasms tested were also negative for staining with ANXA1; taking into account that ANXA1 can stain myeloid cells and T-lymphocytes. TRAP staining showed 11/11 (100%) HCL cases were strongly positive (P+I=6) while 2/2 (100%) of vHCL cases were negative (P+I=0). 22/22 (100%) of MZL cases were positive for TRAP with P+I scores ranging from 3-5. 13/14 (93%) of MCL cases were positive for TRAP with P+I scores ranging from 2-3. 18/23 (78%) of CLL/SLL cases were positive for TRAP with P+I scores ranging from 2-6 with most cases P+I=2-3.
- **Conclusions:** ANXA1 paraffin immunomorphologic detection of hairy cell leukemia demonstrates 100% specificity and sensitivity, with no staining seen in vHCL. While TRAP staining is highly sensitive, it is non-specific with significant staining seen in other B-cell neoplasms, though it is negative in vHCL. Thus TRAP is a supportive but not diagnostic stain in the diagnosis of hairy cell leukemia. Due to its high sensitivity and specificity, ANXA1 should be used as the key confirmatory marker in discriminating hairy cell leukemia from vHCL and morphologically similar B-cell neoplasms.

Category: Hematopathology

[1392] *BRAF* V600E Mutations in Low Grade B-Cell Lymphomas

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- **Background:** Activating mutations in the Serine/threonine-protein kinase b-raf *(BRAF)* including the common Valine to Glutamine substitution at coding position 600 (V600E) have been described in a variety of solid tumor types. The V600E mutation was recently reported to be present in all tested cases of Hairy Cell Leukemia (HCL), but not other low-grade B-cell lymphomas, suggesting it is both a sensitive and specific marker for HCL. We sought to validate these findings in low grade B-cell lymphomas including histologically similar lymphomas such as Splenic Marginal Zone Lymphoma (SMZL).
- **Design:** We identified a set of low grade B-cell lymphomas including 5 HCLs, 5 SMZLs, 23 low-grade Follicular Lymphomas (FL), and 19 nodal small lymphocytic lymphomas (SLL). DNA was extracted from formalin-fixed tissue blocks and PCR amplified using primers targeting *BRAF* exon 15. DNA was then digested using the tspRI restriction enzyme that cuts DNA harboring the c1799 T->A sequence corresponding to *BRAF* V600E mutations. Select cases were then sequenced by both Sanger sequencing, *BRAF* exon 15, and Next Generation Sequencing (NGS), all *BRAF* exons, for confirmation.
- **Results:** 5/5 HCL cases, 2/5 SMZL cases, 0/23 FL cases, and 0/19 SLL cases were positive for the BRAF V600E mutation by PCR and enzyme digest. However, when exon 15 was sequenced in the SMZL cases there was no evidence of a V600E mutation. We further sequenced all *BRAF* coding exons in the 2 PCR-positive SMLZ cases and saw no evidence of BRAF V600E or additional *BRAF* mutations. A histologic review of the PCR-positive SMZL cases revealed the spleen to show lymphomatous infiltration of the white and red pulp with no reactivity for Annexin A1 or CD103, CD25 and CD11c. These ancillary studies confirmed the diagnosis of SMZL.
- **Conclusions:** We found the *BRAF* V600E mutation in all cases of HCL, but not in cases of SLL or FL, confirming what has been previously reported. In addition we found 2/5 cases of SMZL that were PCR-positive for *BRAF* V600E mutations, however we could not verify these findings by sequencing. Given the increased sensitivity of PCR-based detection methods over Sanger sequencing and NGS (20% and 10% respectively) it is unclear if the two *BRAF* V600E PCR-positive cases represent low level mutation frequency, tumor cell dilution, or false positive results. However, these finding suggest caution when using PCR-based BRAF V600E testing to classify cases as HCL if SMZL is included in the differential diagnosis.

Category: Hematopathology

[1359] Correlation of *MYC* Gene Translocation Status with MYC Protein Expression in Burkitt Lymphoma and Diffuse Large B Cell Lymphoma

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- **Background:** *MYC* proto-oncogene is rearranged in several aggressive B cell lymphomas, including Burkitt Lymphoma (BL) and Diffuse Large B cell Lymphoma (DLBCL). In cases of BL, *MYC* translocation to immunoglobulin (IG) gene loci is seen in 100% of cases and it represents a primary event. In contrast, only 8 – 10% of DLBCL show *MYC* translocation, often to non-IG partners, and usually within a complex karyotype, suggesting it is a secondary event. *MYC* translocation correlates with inferior survival in patients who concomitantly have *BCL-2* translocation. This study tested whether *MYC* expression by immunohistochemistry (IHC) can predict *MYC* translocation and be used as a prognostic IHC marker.
- **Design:** 41 HIV+ DLBCL, 76 HIV- DLBCL, 12 HIV+ BL and 19 HIV- BL patients were enrolled in a clinical trial. Of these cases, there was archival material sufficient to perform additional interpretable studies in 19 HIV+ DLBCL, 30 HIV- DLBCL, 7 HIV+ BL and 7 HIV- BL cases. FISH and IHC were performed using Vysis LSI MYC break apart probe and MYC antibody (clone Y69, Epitomics). IHC slides were scored based on stain intensity and percent positive cells and results were correlated with presence of *MYC* translocation.
- **Results:** All BL cases, regardless of HIV status (14 of 14), had *MYC* translocation which correlated with strong (2- 3+/3) *MYC* expression in 75-100% of cells. In contrast, 14% of HIV- DLBCL (4 of 30) and 21% of HIV+ DLBCL (4 of 19) showed *MYC* translocation while 63% of HIV- DLBCL (19 of 30) and 84% of HIV+ DLBCL (16 of 19) showed MYC expression. In addition, both within translocation positive and translocation negative cases of DLBCL, irrespective of HIV status, there was a wide range of expression of *MYC*, from no expression at all (0/3) to strong expression (2- 3+/3) in 75-100% of cells.
- **Conclusions:** The above data demonstrates that although strong *MYC* expression correlates with *MYC* translocation in BL cases, strong *MYC* expression by IHC is not predictive of *MYC* translocation status in DLBCL. In addition, this data suggests that a mechanism other than *MYC* translocation is responsible for *MYC* expression in a large fraction of DLBCL patients.

Category: Hematopathology

[1514] Immunostains for C-MYC and BCL2 Protein Predict Survival in Patients with Diffuse Large B-Cell Lymphoma Treated with Rituximab

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- **Background:** Diffuse large B-cell lymphoma (DLBCL) is a heterogeneous disease, and the addition of rituximab to standard chemotherapy has confounded the use of prognostic markers such as Ki67 and BCL2. The impact of C-MYC expression on the prognosis of DLBCL is also unclear, especially when coupled with Ki67 and BCL2. Therefore, we investigated the expression of these three proteins in paraffin-embedded tissue, including their interaction and influence on the survival of DLBCL patients.
- **Design:** Tissue microarray slides from 105 cases of *de novo* DLBCL treated with rituximab and CHOP or CHOP-like therapies were stained using antibodies against GCET1, CD10, BCL6, MUM1, FOXP1, BCL2, C-MYC and Ki67. The tumors were assigned a germinal center B-cell-like (GCB) or non-GCB subtype according to the Choi algorithm for cell of origin. Positivity for BCL2, C-MYC and Ki67 was graded in 10% increments by two pathologists. The Kaplan-Meyer method was used to estimate overall survival (OS) and event-free survival (EFS), and the log-rank test was used to compare the survival distributions. Cox regression analysis was used to compare OS and EFS in multivariate analysis after adjusting for the International Prognostic Index (IPI) and cell of origin.
- **Results:** Among the 105 patients, 58 (55%) were male and 47 (45%) were female, with a median age of 62 years. By univariate analysis, the IPI, cell of origin, and BCL2 and C-MYC expression were significant predictors of OS and EFS, whereas Ki67 was not predictive. In multivariate analysis, C-MYC was an independent predictor of OS (p=0.013), whereas BCL2 was a significant predictor of OS and EFS in the low IPI group (p=0.015 and p=0.0007, respectively). Survival analysis showed that patients who had both BCL2<30% and C-MYC<50% had the best prognosis, whereas the patients with BCL2≥30% and C-MYC≥50% had the worst outcome. In multivariate analysis, the combination of the BCL2 and C-MYC was an independent predictor of OS and EFS (p=0.016 and p=0.006, respectively). The risk of death was 8.7 times greater in cases with BCL2≥30% and C-MYC≥50% as compared to those with BCL2<30% and C-MYC<50%.
- **Conclusions:** In patients with DLBCL, high expression of C-MYC and BCL2 is a predictor of poor survival. Immunohistochemistry for C-MYC and BCL2 is a useful method for risk stratification of patients with DLBCL.

Category: Hematopathology

[1515] High-Grade B-Cell Lymphoma with Features Intermediate between Burkitt Lymphoma and Diffuse Large B-Cell Lymphoma (Grey Zone Lymphoma): A Clinicopathologic Analysis of 39 Cases

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- **Background:** High-grade B-cell lymphoma with features intermediate between Burkitt lymphoma (BL) and diffuse large B-cell lymphoma (DLCBL) represents a heterogeneous and poorly-characterized entity. Therefore, we investigated 39 cases of this grey zone lymphoma.
- **Design:** We searched our database for the period of 1985 to 2010 for potential cases and these were reviewed by two hematopathologists. The following immunohistochemical stains were performed: CD3, CD10, CD20, BCL2, BCL6, MUM1, GCET1, FOXP1, C-MYC and Ki67. The tumors were assigned a germinal center B-cell-like (GCB) or non-GCB subtype according to Choi algorithm for cell of origin. Immunostains for BCL2, C-MYC and Ki67 were graded in increments of 10%, and the cutpoints of 30% and 50% were used for BCL2 and C-MYC, respectively. Fluorescence in situ hybridization (FISH) for C-MYC and BCL2 gene rearrangements were also performed. The Kaplan-Meier method was used to estimate overall survival (OS).
- **Results:** Among the 39 patients, 21 (54%) were male and 18 (46%) were female, with a median age of 69 years. The median OS was only 9 months and the 5-year OS was only 30%. The majority of patients presented with advanced stage (III/IV) disease (62%), high LDH levels (63%), and high (3-5) International Prognostic Index scores (54%). Treatment regimens were aggressive, but only 41% of the patients had a complete remission. Morphologically, the tumors were composed predominantly of medium-sized, centroblast-like cells with high proliferation and numerous tingible-body macrophages. Seventy percent of the cases had Ki67 expression !80%. Twenty-nine cases (74%) had a GBC phenotype. The majority of cases (77%) expressed BCL2 protein, but only 44% of these had a BCL2 gene rearrangement. High C-MYC protein expression was seen in 41% of the cases, and 85% of these had C-MYC rearrangement. Seven cases were “double hit” lymphomas with rearrangement of both C-MYC and BCL2. However, none of the immunohistochemical or FISH markers were predictive of survival.
- **Conclusions:** High-grade B-cell lymphoma with features intermediate between BL and DLBCL is a morphologically recognizable entity with an extremely poor prognosis. Most cases fall into the GCB category, with high proliferation, and high BCL2 and C-MYC expression. However, only a subset of cases with BCL2 and C-MYC expression have rearrangement of these genes, suggesting other mechanisms of gene deregulation in this entity.

Category: Hematopathology

[1550] Co-Expression of MYC and BCL2 Protein in R-CHOP Treated *De Novo* Diffuse Large B-Cell Lymphoma Predicts Poor Outcome

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- **Background:** *De novo* diffuse large B-cell lymphoma (DLBCL) is a heterogeneous disease with a variable response to R-CHOP combination therapy. Dual rearrangement of *MYC* and *BCL2* in DLBCL ("double-hit" lymphoma) is an established predictor of aggressive behavior and poor response to therapy. The significance of dual *MYC* and BCL2 protein expression in R-CHOP treated *de novo* DLBCL is not established.
- **Design:** 178 cases of formalin-fixed paraffin-embedded R-CHOP treated *de novo* DLBCL in a tissue microarray were independently evaluated by two pathologists for expression of MYC, BCL2 (Dako 124 and Epitomics E17), CD10, BCL6, MUM1, GCET1, FOXP1 and LMO2 by immunohistochemistry. MYC-positivity was defined as ≥40% cells with nuclear staining. BCL2-positivity was defined as ≥30% cells with cytoplasmic staining using either 124 or E17 antibody. MYC and BCL2 expression were correlated with overall (OS) and progression free survival (PFS), the International Prognostic Index (IPI) score, cell of origin (COO) immunophenotype, and MYC and BCL2 gene rearrangement. Clinical data were available for all patients. The COO immunophenotype was determined using Hans, Choi, and Tally algorithms. MYC and BCL2 status was successfully determined by FISH analysis in 155 cases.
- **Results:** The patient population consisted of 112 males and 66 females ranging from 16-90 years in age (median 65 years). 62 cases were MYC+, 129 cases were BCL2+, and 50 cases were MYC+/BCL2+ (28%). Kaplan-Meier univariate analysis showed MYC+/BCL2+ DLBCL, compared with MYC+/BCL2- and MYC- DLBCL, was associated with inferior 5-year OS (40% vs. 74% vs. 84%, p=0.001) and PFS (32% vs. 64% vs. 85%, p=0.002). MYC+/BCL2+ DLBCL was significantly associated with a non-GCB COO immunophenotype by Choi (p=0.004), Hans (p=0.020), and Tally (p=0.002) criteria. *MYC* was rearranged in 20 (13%) total cases and 13 (24%) MYC+ cases (p=<0.001). 10 cases were dual *MYC /BCL2* rearranged, 6 of which were MYC+/BCL2+ (p=0.019). Cox regression multivariate analysis showed MYC+/BCL2+ expression maintained prognostic significance in OS (p=0.008) and PFS (p=0.035), independent of IPI, COO immunophenotype, and dual *MYC/BCL2* rearrangement.
- **Conclusions:** In R-CHOP treated *de novo* DLBCL co-expression of MYC and BCL2 occurs in 28% of cases. MYC expression occurs in a sizable proportion of patients independent *of MYC* translocation and importantly, co-expression of MYC and BCL2 independently predicts inferior OS and PFS.

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[1345] Characterization of Tissue Findings in Bone Marrow with Small Monoclonal B-Cell Populations

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- **Background:** Flow cytometry immunophenotyping (FC) allows detection of small populations of CLL type or CD5- monotypic B-cells (MBCs) in the blood. When MBCs are not associated with overt clinical disease or tissue involvement, and are <5000/uL, the term monoclonal B-lymphocytosis (MBL) is used. Most studies have focused on PB MBL, but low levels of MBCs are also found in BM. In this study, we evaluated BM pathology and clinical setting when small MBC populations similar to MBL were detected in the aspirate by flow cytometry.
- **Design:** BMs performed between 01/1999 and 12/2010 that showed MBCs <5% of total events by 4-8 color flow cytometry were selected for review. In BM biopsies done for lymphoma staging, only small clones with a phenotype different from the original lymphoma were included PB lymphocyte counts were <5000/uL. History and physical findings were retrieved from the EMR. H&E stained BM sections and immunostains (IHC) for CD3, CD20 or PAX5 or CD79a were reviewed.
- **Results:**

Clone Type (no. of cases)	Reason for BM				H&E/ IHC		
	Lymphoma staging	AML	Plasma cell dyscrasia	Cytopenias	lymphoid aggregates (LA)	B-cell rich LA	T-cell rich LA
CLL (11/24)	6	2	1	2	5/11	3	2
None CLL (13/24)	6	0	4	3	4/13	1	3

The majority of cases with either CLL or non-CLL type B-cell clones did not exhibit lymphoid infiltrates in the BM biopies. The cases that did have LA's were not considered diagnostic for lymphoma; all represented <5% of cellularity and were non- paratrabecular; 5 were T cell rich and 4 B cell rich and were insufficient to established lymphoma.

- **Conclusions:** In this study, small MBC clones were found in BM and were of both CLL type and non-CLL (CD5-) but the CD5- phenotype predominated, in contrast to those reported in the blood. Most BM biopsies with either type MBC had no lymphoid aggregates or no overt morphologic or IHC evidence for lymphoma. Recognition of these small B cell clones are important in evaluating BM biopsies for lymphoma as their presence, similar to PB, may not be equivalent to lymphoma.

Category: Hematopathology

[2152] Next-Generation Pathology: Deep DNA Sequencing and Targeted Therapy for Cancer

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- **Background:** Gene sequencing currently used to select therapy in non-small cell lung cancer (NSCLC), colorectal cancer (CRC) and melanoma (MM) employs traditional standard-of-care (SOC) "hotspot" single gene mutation analysis. Massively parallel (next-generation sequencing (NGS)) has now been adapted to formalin-fixed (FFPE) specimens to provide high sensitivity detection for point mutations, insertion/deletions, translocations and copy number alterations (CNA).
- **Design:** FFPE specimens from 83 solid tumors (50 CRC, 29 NSCLC, 4 MM) with results available from SOC genotyping by allele-specific PCR (KRAS codons 12/13, EGFR exons 17-20, or BRAF V600E) were fully sequenced for 145 genes by NGS. Hybridization-capture of 2574 exons across 145 oncogenes, tumor suppressor genes and ADME-related genes was performed to produce libraries appropriate for paired-end sequence analysis on the Illumina HiSeq2000 platform (Illumina, Inc., San Diego, CA).
- **Results:** NGS recapitulated the SOC test results in all cases. In-depth sequence analysis with median coverage averaging 213-fold (range 8 to 461) detected a per-sample average of 2 previously-described mutations, 7 novel mutations and 2 CNAs in the CRC, including frequent alterations in *TP53* (33), *APC* (27), *KRAS* (12) and *BRAF* (6). The NSCLC averaged 1 previously described mutation, 8 novel mutations and 1 CNA per sample, most frequently *KRAS* (10), *TP53* (7), *JAK2* (3), *EGFR* (2) and *BRAF* (2). The MM exhibited on average 1 previously described mutation, 7 novel mutations and 3 CNAs including *TP53* (4) and BRAF (2). In addition to validated clinically actionable mutations in *EGFR, KRAS*, and *BRAF*, and multiple alterations in well-known cancer genes *(TP53, STK11, APC, MLH1, BRCA2, SMAD4)*, a significant number of additional genomic alterations that have potential therapeutic implications were also detected including activating mutations in the PI3 kinase subunit gene *PIK3CA*; mutations in *MET, KIT, ERBB2* and *CDKN2A*; driver mutations not usually associated with solid tumors, such as the lymphoma-associated *JAK2 V617F* mutation in two NSCLCs; and in 1 CRC, a novel chromosome 2 rearrangement adjacent to the *ALK* kinase domain confirmed by analyzing a cDNA library constructed from extracted tumor RNA.
- **Conclusions:** NGS of hundreds of cancer-related genes can be reliably performed at a high level of sensitivity and specificity in clinical FFPE samples of solid tumors, can reproduce SOC single gene traditional sequencing results and shows great potential to inform on therapeutic decisions for patients with CRC, NSCLC and MM.

Category: Techniques