session 7 – pathology and clinical/pathological correlations

067 CD5 POSITIVE SPLENIC MARGINAL ZONE LYMPHOMA: CYTOLOGICAL, IMMUNOLOGICAL, CYTOGENETICAL AND MOLECULAR FEATURES OF A SERIES OF 35 CASES

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Splenic marginal zone B-cell lymphoma (SMZL) is an indolent lymphoma showing some characteristic cyto-histological, cytogenetical and molecular features. Phenotypically it is defined by a negative profile with no CD5, CD10, CD23, CD43 expression. A CD5 positivity has been reported in some typical MZL cases and proposed as a marker for a more aggressive and disseminated disease. The differential diagnosis between CD5+ SMZL and B-CLL or MCL is still controversial in peripheral blood without histological control. Here we compared a series of 35 CD5 positive SMZL histologically proven in 26 cases and without t(11;14) detected by FISH, with 44 typical CD5 negative SMZL. From a clinical, cytological and immunological point of view, the CD5+ SMZL presented many similarities with the CD5- SMZL. The main differences consisted in a higher lymphocytosis at diagnosis (7,9 G/L in CD5+ cases and 3,9 G/L in CD5- cases, respectively *p=0.0006*) and a more frequent IgM isotype of the monoclonal component (76% vs 59%). No CD5 negative B-cells were detected in spleen specimen and the tumoral mass assessed by spleen weight seemed similar between both groups. These data suggest that CD5 expression gives B-cells a higher propensity to recirculate from spleen. Karyotypic changes were similar in both groups and included the chromosomal abnormalities previously described in SMZL. However, the trisomy 18 was more frequent in the CD5+ group (29%) than in the CD5- group (11%). Most CD5+cases were mutated in contrast to the CD5- cases (86% vs 54%; *p=0.024*). The usage of V_H4 was more represented in the CD5+ cases (5/21; 24%) than in the CD5- cases (2/24; 8%) No difference in outcome and overall survival was found between both groups. In conclusion, this study allowed to confirm the existence of CD5 positive SMZL (25% of our SMZL series), closely related to classical CD5- SMZL and distinct from CLL and MCL. The differences observed with CD5- cases, especially the V_H usage, suggest that this entity may arise from a distinct cell in the marginal compartment.

068 PERIPHERAL T-CELL LYMPHOMAS WITH A FOLLICULAR GROWTH PATTERN: A PECULIAR LYMPHOMA SUBTYPE DERIVED FROM FOLLICULAR HELPER T CELLS (T_{FH}) WITH A POSSIBLE LINK TO ANGIOIMMUNOBLASTIC T-CELL LYMPHOMAS

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Rare cases of peripheral T-cell lymphomas with follicular growth pattern (PTCL-F) have been recently reported, and their associatioin with t(5;9)(q33;q22) involving ITK and SYK has been suggested. However, the clinicopathologic aspect of PTCL-F is poorly described and their cell of origin is still unknown. The aim of the present study is to analyse the pathological, phenotypic and cytogenetic features of a series of 43 patients which showed histologic features of PTCL-F in at least one biopsy. Forty-six biopsies with histologic features of PTCL-F were classified into 3 categories according to predominant aspect at low magnification: (1) follicular lymphoma-like (n=8), (2) progressive transformation of germinal center-like (n=22), and (3) follicular pattern with AITL-like features (n=16). Neoplastic cells were medium-sized clear cells that were CD4+ (38/45), CD10+ (34/48), BCL6+ (17/28) and expressed PD-1 (39/39) and CXCL13 (39/40), markers of T_{FH}. Rare EBV+ cells (19/28) were frequently observed, as well as a population of small IgD+ B cells surrounding neoplastic cells. Seven out of 30 patients within the 3 patterns had t(5;9)(q33;q22) detected by fluorescent in situ hybridization. At presentation with PTCL-F, patients had polyadenopathy (23/25), stage III-IV disease (22/28), B symptoms (7/24) and skin lesions (5/19). Among the 8 patients with sequential biopsies, 4 disclosed clinical and histological features of AITL in 1 episode. Our results show that this rare form of PTCL-F (1) has an immunophenotype indicative of T_{FH} origin, (2) is associated with t(5;9) in a proportion of cases, and (3) shows some overlapping features with AITL, raising the question of a possible relationship.

069 INDUCTION OF A HODGKIN-LIKE PHENOTYPE IN AGGRESSIVE B-CELL LYMPHOMA CELLS BY EPIGENETIC REPROGRAMMING

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Background: The tumour cells of aggressive B-cell lymphoma are either derived from germinal centre B cells or activated B cells. They consistently show an immunophenotype of mature B cells with expression of all B-cell typical and transcription factor genes. In contrast, the tumour (HRS) cells of classic Hodgkin lymphoma (CHL), which are also derived from germinal centre B cells, have almost completely lost their B-cell identity. In addition, HRS cells acquired the expression of several antigens which are regarded as B-cell lineage inappropriate. The purpose of our study is to investigate whether epigenetic mechanisms play a role in the development of the unique phenotype of HRS cells.

Material and methods: Hodgkin and aggressive B cell lines were treated with DNA-demethylation (5-aza-dC) and histone-acetylation (TSA) reagents. Treated and untreated cell lines were analysed by Affymetrix GeneChips. Numerous up- and down-regulated genes were identifed and subsequently verified by quantitative RT-PCR and Western blot analysis. Chromatin-immunoprecipitation was carried out to determine the epigenetic modifications in the promoter region of the corresponding genes.

Results: The treatment of Hodgkin cell lines with 5-aza-dC and TSA had no significant effects on the reactivation of the B-cell expression program and the down-regulation of Hodgkin-characteristic genes. Instead, the treatment of aggressive B-cell lymphoma cell lines resulted in a complete loss of their B-cell phenotype and – in parallel – to an up-regulation of Hodgkin-characteristic genes. Our data clearly demonstrate that DNA-demethylation and histone-acetylation is able to re-program B cells into cells with a Hodgkin-like phenotype.

Conclusions: These findings suggest that the same genes which can be switched on in B cells by DNA-demethylation and histone-acetylation and which down-regulate the B-cell program in B cells are constitutively active in HRS cells. Studies are progress to identify these genes.

070 HISTIOCYTIC/ DENDRITIC SARCOMAS CLONALLY RELATED TO FOLLICULAR LYMPHOMA: EVIDENCE FOR LINEAGE PLASTICITY IN MATURE HUMAN B-CELLS

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Rare cases of histiocytic and dendritic cell neoplasms (H/DC) have been reported in patients with follicular lymphoma (FL), but the biologic relationship between the two neoplasms is unknown. We studied eight patients with both FL and (H/DC) neoplasms using immunohistochemistry, fluorescence in situ hybridization (FISH) for t(14;18), and PCR/sequencing of Bcl2 and IgHrearrangements. There were 5 men and 3 women (median age, 59y). All cases of FL were positive for t(14;18). The H/DC tumors included 7 histiocytic sarcomas, 5 of which showed evidence of dendritic differentiation and 1 interdigitating cell sarcoma. Five H/DC tumors were metachronous, following FL by 2 mos to 12 yrs; tumors were synchronous in 3.7/8 of the H/DC tumors showed presence of the t(14;18) by FISH. In one case FISH was unsuccessful, but the BCL2/JH was confirmed by PCR. PCR for IgH gene rearrangement and the BCL2/JH translocation and sequencing identified identical IgH gene rearrangements or BCL2 gene breakpoints in all patients tested. All H/DC tumors lacked PAX5 and upregulation of CEBPbeta and PU. 1 was seen in all cases tested. These results provide evidence for a common clonal origin of FL and H/DC neoplasms when occurring in the same patient. Moreover, they provide evidence for transdifferentiation of the FL B-cell clone, possibly without an intermediate step associated with de-differentiation. This report is the first to document lineage plasticity in a mature human B-cell system, and indicates that lineage plasticity is not restricted to immature hematopoietic neoplasms. As previously shown by others in murine systems, the loss of PAX5 appears to be a critical event in alteration of the B-cell program.

071 CUT-OFF VALUE DETERMINATION FOR PROGNOSTIC MARKERS IN DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL)

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Background: A large number of biomarkers, particularly proteins, that contribute to prognosis in DLBCL have been identified. However translation into generally accepted standards to predict survival has not yet been accomplished mainly because of contradictory reports in the literature resulting from arbitrary methodologies used to set cut-off values for determining positivity. Thus interobserver reproducibility can not be retraced and comparison between reports is difficult. Some of these problems might be resolved by standardized high throughput analysis methods such as tissue microarrays (TMA), rational statistical methods for determination of cut-off scores, and consideration of both tumor- and patient-specific parameters on thoroughly characterized study collectives.

Material and methods: We analyzed by immunohistochemistry 301 clinically well documented primary DLBCL on a previously validated TMA in the light of receiver operating characteristic (ROC) curve analysis, to determine the optimal cut-off values of the biomarkers bcl-2, bcl-6, CD10, FOXP1, MUM1, cyclin E and Ki-67 for efficient outcome prediction respecting disease-specific survival (DSS). DSS was analyzed by the Kaplan-Meier method.

Results: The prognostic performance of the analyzed markers, corresponding to areas under ROC (AUROC) closer to 1, the optimal cut-off scores in % as suggested by ROC and the respective p-values in the Kaplan-Meier analysis were as follows:

negative prognostic markers
bcl-2: 0.552, 58%, p=0.082
cyclin E: 0.606, 20%, p=0.021
FOXP1: 0.583, 48%, p=0.014
MUM1: 0.513, 65%, p=0.007
positive prognostic markers
bcl-6: 0.542, 14%, p=0.122
CD10: 0.538, 8%, p=0.184
Ki-67: 0.537, 73%, p=0.122

Comparison of the results linked to DSS by the Kaplan-Meier method unequivocally showed the superior discriminating power of the cut-off levels calculated by ROC, compared to arbitrary cut-off values from the literature.

Conclusions: ROC curve analysis can be used as a rational method for determination of clinically relevant cut-off levels for prognostic biomarkers in DLBCL.

072 COMPOSITE FOLLICULAR (FL) AND DIFFUSE LARGE B CELL LYMPHOMA (DLBCL): A GENE EXPRESSION PROFILING (GEP) STUDY

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Background: FL is an indolent lymphoma with heterogeneous clinical behaviour. Transformation (Tr) into an aggressive disease, most commonly DLBCL is associated with short survival. The molecular mechanisms involved in Tr are largely unknown. Reported GEP studies performed using sequential fresh biopsies have dichotomized transformed cases based on a proliferation signature. Early steps of Tr have proven difficult to study. Composite lymphoma (CL) with concurrent FL & DLBCL in the same lymph node biopsy are rare, but likely represent Tr in its early stages.

Methods: Using 44k Agilent® DNAarrays, we analyzed gene-expression patterns of formalin-fixed paraffin-embedded tissue (FFPET) biopsies with CL. A pilot study using 5 pairs of fresh vs FFPET reactive lymph node were arrayed using the same platform and showed a moderate correlation of 0.6 (range 0.46-0.65). We evaluated 14 (8M/6F) newly diagnosed CLs. Lymphoma cell purity (LCP) was assessed by light microscopy and B cell stains and areas of FL & DLBCL were dissected and arrayed separately. Global sample profiles and differentially expressed genes in FL vs DLBCL were analyzed. Reported profiles of transformed FL (Davies, BJH 2006) and DLBLC (Rosenwald, NEJM 2002) were used.

Results: 11 samples were successfully arrayed: 5 grade 1, 3 grade 2 and 3 grade 3a FLs. Median LCP was 76 % (FL) and 93% (DLBCL). A small subset of genes were significantly differentially expressed between FL vs DLBCL. Clustering of patients using global expression profiles of FL vs DLBCL showed 3 clusters with 2 (#1), 4 (#2) and 5 (#3) cases. Profile #1 was characterized by up-regulation of cell cycle and proliferation (*MYC* and *MAPK*) pathway genes and both cases were FL3a. Profiles #2 and #3 showed up-regulation of intracellular signal transduction & cell growth (*FAK*) and cell survival (*PPARY* pathway), respectively. Overall, clustering did not correlate with grading or LCP. A similar 3 cluster structure was observed using various reported signature gene profiles.

Conclusion: Three distinct clusters were observed in CLs. They show no correlation with currently used histological grading and non-malignant cell content. As reported, one cluster correlated with increased proliferation. Importantly, this study shows 3 probable pathways of early histological transformation in FL.

073 NEW METHODS FOR FORMALIN-FIXED PARAFFIN-EMBEDDED TISSUE (FFPET) SAMPLE MICROARRAY ANALYSIS PERMIT ACCURATE PREDICTION OF GCB AND ABC SUBTYPES OF DLBCL

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Background: FFPET samples are routinely required for pathological examination and provide a rich source of material for biomarker discovery. FFPET samples may have degraded RNA and have proven difficult for robust analysis by microarrays.We tested the ability of a new method of amplification and labeling from FFPET, at predicting cell of origin (COO) in diffuse large B cell lymphoma (DLBCL) subtypes using gene expression profiling (GEP).

Methods: RNA was extracted from 49 DLBCL samples taken from fresh/frozen (FF) tissue and matching FFPET.5 ug of FF RNA was amplified, labeled and hybridized on Affymetrix U133-Plus-2 arrays using the 1 cycle amplification protocol.COO was assigned using the probe set ids published by G. Wright (PNAS 2003). 10ng of FF and 50ng of FFPET RNA was amplified using a new method of amplification (Nugen WT-Ovation FFPE System) and analyzed on U133-Plus-2 arrays. 24 samples (12 ABC and 12 GCB based on the Wright signature (WS)) were randomly assigned to a training group. Differential gene expression between ABC and GCB training sets of FF and FFPET samples were used to create new COO classification models. These new models and the WS were used to predict COO on the remaining 25 blinded samples.

Results: The quality of RNA extracted from FFPET was inferior to RNA extracted from FF.The % present calls from the FFPET arrays were also lower than the FF arrays (25% FFPET Nugen; 42% 1 cycle FF; 55% FF Nugen). The WS classified the Nugen amplified FF and FFPET samples with 92% and 88% accuracy. The new model derived from Nugen FF samples predicted the blinded FF and FFPET samples with 96% and 84% accuracy. Whereas the model derived from FFPET samples predicted the FF and FFPET samples with 96% and 92% accuracy.The concordance between all models was low for gene probe sets but high for biological pathways and gene function.

Conclusion: We have demonstrated that a new method of amplification of FFPET samples can accurately predict COO in DLBCL by GEP. The data produced by the Nugen method only misclassified samples that would have been classified as ''unclassifiable'' or type 3 according the WS. This important advance may permit valuable biological insights to be extracted from GEP of FFPET archival samples.

074 CLINICAL IMPACT OF *BCL2*, *BCL6* AND *C-MYC* REARRANGEMENTS IN DIFFUSE LARGE B CELL LYMPHOMA PATIENTS TREATED WITH R-CHOP

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Introduction: The prognostic impact of *BCL2*, *BCL6* and *c-MYC* rearrangements has been investigated in heterogeneous series of diffuse large B-cell lymphoma (DLBCL) patients treated with variable regimens prior to Rituximab. The aim of this study is to evaluate the prognostic impact of these gene alterations in a homogeneous cohort of DLBCL patients treated with CHOP or R-CHOP.

Material and Methods: Three hundred ninety nine patients with DLBCL, age 60 to 80, were enrolled in the LNH98-5 clinical trial that aimed to compare the efficacy of CHOP versus R-CHOP (Coiffier, NEJM 2002). Tissue microarrays from 185 patients with available pathological material were studied for *BCL2*, *BCL6* and *c-MYC* rearrangements by interphase fluorescence in situ hybridization (FISH) usingsplit-signal DNA probes (euro-fish.org). DLBCL were classified in germinal center B-cell-like (GCB) and non-GCB-like subtypes using the immunohistochemical algorithm with CD10, BCL6 and MUM1 (Hans, Blood 2004).

Results: One hundred three patients displayed interpretable FISH signals: 18q21/*BCL2*, 3q27/*BCL6* and 8q24/*c-MYC* rearrangements were detected in 14,6% (15/103), 29,7% (30/101) and 5% (5/101) of the cases, respectively. A high correlation between genes breakpoints and DLBCL subtypes was observed: 100% of DLBCL with *BCL2* rearrangement were of GCB subtype (p<0.0001) and 91% of DLBCL with *BCL6* rearrangement were of non-GCB subtype (p=0.004). In the R-CHOP treatment arm, *BCL2* rearrangement had a favorable impact on overall survival (OS) (82% versus 50% at 5 years, p=0,05) whereas *BCL6* rearrangement tended to be associated with an unfavorable outcome (OS=33% versus 66% at 5 years, p=0.07).

Conclusions: The frequency of *BCL2*, *BCL6* and *c-MYC* rearrangements were similar to previous reports. *BCL2* and *BCL6* alterations correlated with the GCB-like and non-GCB-like profile, respectively. This study suggests a prognostic impact of *BCL2* and *BCL6* rearrangements in patients treated with R-CHOP which needs to be confirmed in a larger series of patients.

075 LMO2 PROTEIN EXPRESSION, *LMO2* GERMLINE GENETIC VARIATION, AND OVERALL SURVIVAL IN DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL)

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Background: Both LMO2 mRNA and LMO2 protein expression in DLBCL have been associated with superior survival; a role for germline genetic variation in LMO2 has not been previously reported.

Methods: We evaluated 187 DLBCL patients who participated in a population-based study conducted from 1998-2000 in the United States. Immunohistochemistry for LMO2 was conducted on tumor tissue from diagnostic biopsies, and 20 single nucleotide polymorphisms (SNPs) from LMO2 were genotyped from germline DNA. Cox Regression was used to estimate Hazard Ratios (HRs) and 95% Confidence Intervals (CIs).

Results: The median age at diagnosis was 60 years (range, 24-74), and 54 (29%) patients died during follow-up. LMO2 protein was expressed in 45% of patients. Expression was associated with superior survival (HR=0.55; 95% CI 0.31-0.97), although this attenuated somewhat after adjustment for clinical factors (HR=0.65; 95% CI 0.37-1.16). A joint test of all SNPs in LMO2 was strongly associated with survival (p=0.001) after adjustment for clinical factors. The most informative SNP was rs941940 (HRCT=0.65; 95% CI 0.35-1.16; HRTT=0.23; 95% CI 0.07-0.67), which is located upstream of the transcription initiation site. Patients with LMO2 protein expression were 2.8 times more likely to carry the homozygous variant genotype (TT) of this SNP compared to patients with no expression (95% CI 1.31-6.11). In a model that included clinical factors, the collection of LMO2 SNPs remained significant predictors of survival (p=0.002), while LMO2 protein expression trended toward better survival (HR=0.61; 95% CI 0.32-1.14).

Conclusions: We confirm that LMO2 protein expression is associated with superior survival. Further, genetic variation in LMO2 correlated with LMO2 protein expression and predicted overall survival in DLBCL. These data suggest that genetic variation in *LMO2* provides additional prognostic information in DLBCL beyond tumor LMO2 protein expression and clinical factors.

076 DIM CD20 OR CD5+ EXPRESSION BY FLOW CYTOMETRY IS ASSOCIATED WITH A POOR SURVIVAL IN PATIENTS WITH DIFFUSE LARGE B CELL LYMPHOMA (DLBCL) TREATED WITH EITHER CHOP OR CHOP-R

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Background: CD19 and CD20 antigens are membrane bound proteins that are expressed by virtually all B cell NHL. A subset of pts with DLBCL has shown dim CD20 expression by flow cytometry (FC) and may not benefit by the addition of rituximab (R) to CHOP chemotherapy. We determined the outcome of DLBCL pts who expressed dim CD20 or dim CD19 by FC, treated with CHOP +/- R, and correlated CD20 protein expression with the presence of mutations in the CD20 gene.

Methods: Pts with primary DLBCL who had FC performed at the BCCA using their diagnostic biopsy between 1997 and 2007 were included in the study. Quantitative expression of CD20, CD19, CD3, CD4, CD5, CD8 and CD10 was assessed using FC. CD20 expression was defined as dim if it was < 80% that of CD19. Similarly, CD19 was defined as dim if it was < 80% that of CD20. We determined cytoplasmic expression of CD20 by immunohistochemistry (IHC) using the L26 antibody and sequenced exon 5 of the CD20 gene (coding for the extracellular domain) in CHOP-R treated pts.

Results: Of the 310 pts in the study, 6% had dim CD20, 11% had dim CD19, 8% were $CD5^+$ and 30% were $CD10^+$. 82% of the dim CD20 cases by FC were $CD20^+$ by IHC. 106 patients were treated with CHOP and 204 pts were treated with CHOP-R. Dim CD20 and $CD5^+$ each correlated with a significantly inferior survival (OS) when compared to CD20 normal $CD5^-$ cases in both CHOP +/- R treated patients. Dim CD19 correlated with an inferior OS only in CHOP but not CHOP-R treated patients. $CD10^+$, %CD3 and CD4/CD8 ratio had no impact on OS. Dim CD20 pts and $CD5^+$ pts tended to have higher IPI scores and dim CD20 cases were more likely to be $CD5^+$ despite having no prior history of NHL. None of the 11 dim CD20 cases treated with CHOP-R had mutations in the CD20 gene.

Conclusion: Dim CD20 and $CD5^+$ de novo DLBCLs are associated with an inferior OS in both CHOP +/- R treated pts, suggesting that these antigens may be important in the pathogenesis. Flow cytometry can identify a group of high-risk pts who would otherwise be missed using IHC alone. Finally, mutations in the exon coding for the extracellular domain of the CD20 antigen are not a cause of dim CD20.