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 AITL 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 (11/14) detected by FISH, with 44 typical CD5 negative SMZL. From a clinical, cyto-logical 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, respectively 3.9 G/L in CD5- cases, respectively p=0.0006) and a more frequent IgM isotype of the monoclonal component (26% vs 59%, p=0.0006). No CD5 negative B-cells were detected in spleen specimen and the tumor 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 found 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 IGVH was more represented in the CD5+ cases (5/21; 24%) than in the CD5- cases (2/24; 8%) with 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 (15% of our SMZL series), closely related to classical CD5- SMZL and distinct from CLL and MCL. The differences observed with CD5- cases, especially the usage, suggest that this entity may arise from a distinct cell in the marginal compartment.

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Rare cases of peripheral T-cell lymphomas with follicular growth pattern (PTCL-F) have been recently reported, and their association with (5;19)(q33;q22) involving ITK and SYK has been suggested. However, the clinicopathological 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 ATL-like features (n=16). Neoplastic cells were medium-sized clear cells that were CD4+ (34/43), CD10+ (34/48), BCL6+ (17/28) and expressed PD-1 (39/39) and CXCL13 (34/40), markers of TFH. Rare EBV+ cells (18/28) 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;19)(q33;q22) detected by fluorescence 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 ATL in 1 episode. Our results show that this rare form of PTCL-F (1) has an immunophenotype indicative of TFH origin, (2) is associated with t(5;19) in a proportion of cases, and (3) shows some overlapping features with ATL, raising the question of a possible relationship.
073 NEW METHODS FOR FORMALIN-FIXED PARRAFFIN-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. Knight (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 86% 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. There was a strong correlation 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.
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 expression was detected in 44% 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=0.63; 95% CI 0.36-1.15). 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) 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 (p=0.002), while LMO2 protein expression trended toward better survival (p=0.05). Coexpression of LMO2 was associated with superior survival; a role for germline genetic variation in LMO2 has not been previously reported.

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.