Session 8: pathology and clinicopathological correlations

077 THE RELIABILITY OF IMMUNOHISTOCHEMICAL ANALYSIS OF TUMOR MICROENVIRONMENT IN FOLLICULAR LYMPHOMA; A VALIDATION STUDY FROM THE LUNENBURG LYMPHOMA BIOMARKER CONSORTIUM (LLBC)

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Introduction: Cell populations of the tumor microenvironment have been implicated for prognostic relevance in follicular lymphoma (FL) The results of immunohistochemistry (IHC) based studies are contradictory, possibly due to differences in treatment protocols and patient selection. In addition, the reproducibility of scoring of cell densities and infiltration patterns has not been studied thus far and can be expected to be of major impact. A validation study was performed to 1) estimate the variation and reproducibility of manual scoring, 2) determine if a systematic difference between manual and computer-assisted scoring exists, 3) evaluate if computer-assisted scoring is more reliable assuming that flow cytometry (FACS) is the gold standard.

Materials and methods: TMAs from 25 FL and 2 tonsils with FFPE- material and FACS data for CD3, CD4 and CD8. IHC for MIB1, CD10, CD3, CD4, CD8, FoxP3, CD68, CD21 and CD34 were scored according to strictly set guidelines to evaluate cellular densities and patterns by 7 pathologists and by 2 independent image analysis (Ariol SL-50). Distribution of markers was analyzed to evaluate scoring effects using agreement (kappa, concordance coefficient), analysis of variance (scoring laboratory effect) and rank analysis. Correlation was evaluated for manual scores between labs and for correlations with image analysis and flow.

Results: Agreement among labs for manual scoring was consistently low (kappa 0.25-0.19). Image analysis versus manual scoring showed a significant correlation for all markers. However, manual scoring consistently underestimated in lower and overestimated in higher scoring ranges, therefore overestimating the variation. Comparison with flow data for CD3, CD4 and CD8 underlined this. Image analysis showed a far better correlation with FACS data with moderate correlation levels (concordance estimates 15-29) with consistently higher values by image analysis that could potentially be corrected by calibration.

Conclusions: Manual scoring of cell densities in the tumor microenvironment is poorly reproducible and systematically overestimates the variation. This may in part explain the contradictory results of studies on the prognostic impact of the tumor microenvironment in FL. Computer-assisted scoring may resolve these problems to a considerable extent and may be a better choice for future studies.

078 DEVELOPMENT AND APPLICATION OF A NEW IMMUNOPHENOTYPIC ALGORITHM FOR MOLECULAR CLASSIFICATION OF DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL): REPORT FROM AN INTERNATIONAL DLBCL RITUXIMAB-CHOP CONSORTIUM PROGRAM STUDY

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Background: Gene expression profiling (GEP) has divided diffuse large B-cell lymphoma (DLBCL) into molecular subgroups that originate from different stages of lymphocyte development, namely germinal center B-cell-like (GCB), and activated B-cell-like. This classification has prognostic significance, but GEP is expensive and not applicable in daily practice. Immunohistochemical algorithms have been proposed as a surrogate for GEP analysis.

Methods: We assembled tissue microarrays (TMAs) of 475 patients with de novo DLBCL who were uniformly treated with rituximab-CHOP. All cases were successfully studied by GEP. Sections were stained with antibodies to CD10, GCET1, FOXP1, MUM1, and BCL6 and classified in this order according to a rationale of subsequent steps of differentiation of malignant B-cells. Cutoffs for each marker were obtained using receiver operating characteristic (ROC) curves, which obviated the need to use any arbitrary method.

Results: An algorithm based on the expression of three markers, CD10, FOXP1, and BCL6, was developed, that had 92.6% concordance with GEP. The predictivity of this algorithm compared favorably with the Hans' algorithm (87.3%), and the more recent Hans* (86,3%), Choi (90.2%), Choi* (81.2%), and Tally algorithms (90.5%, without LMO2 analysis). The algorithm we developed has a simpler structure compared with other recently proposed algorithms, favoring its use in daily practice. According to our algorithm both overall survival and progression-free survival were significantly longer for the GCB group compared with the non-GCB group. In multivariate analysis, both the International Prognostic Index and the algorithm we propose were significant independent predictors of survival.

Conclusions: Our new algorithm can predict prognosis of DLBCL patients according to GEP subgroups in the era of rituximab therapy.

079 DIFFERENTIAL GENE EXPRESSION PATTERNS IN EXTRANODAL SUBSETS OF NON-GCB DIFFUSE LARGE B-CELL LYMPHOMA

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Introduction/Background: Diffuse Large B- Cell Lymphoma (DLBCL) is a heterogeneous disease which can arise in virtually any organ or anatomical site. Based on genetic profiling studies DLBCLs are divided into GCB and non-GCB types, with GCB having a more favorable prognosis. Some DLBCLs such as those arising in the testis have a particularly aggressive clinical course with a tendency to spread to the central nervous system. This could partly be due to the finding that all testis DLBCLs are of the non-GCB subtype, but additional patterns in gene expression may explain the different biological behavior in this subset of DLBCL and suggest a more targeted treatment approach. The aim of this study was to find out if testicular DLBCLs show a different gene expression pattern from other non-GCB tumors.

Materials and methods: 78 *de novo* DLBCLs, classified as non-GCB using the Hans algorithm were included in this study. RNA from all tumors had been purified from formalin fixed, paraffin embedded diagnostic biopsies from assumed primary site of presentation and categorized as either primarily non-testis (n=68) or testis (n= 8). Two tumors could not be categorized and were excluded. The expressions of 29 potentially prognostic genes and three normalizer genes (*GUSB, TBP, ABL*) were studied by qRT-PCR using Taqman Arrays®. Results were analyzed for differential gene expressions using dedicated qRT- PCR software (RealTime StatMiner®). A fold change $\geq$1.5 in gene expression was considered a "real" difference. P-values $\leq$0.05 were considered significant.

Results: Relative to non-GCB tumors as a whole, DLBCLs arising in the testis showed a 3.1 fold overexpression of *CyclinD2* (P=0.02) and a 2.9 fold overexpression of *TLE1 (P=0.05)*. *B4GALT* was 1.8 fold overexpressed (P=0.03). Relative to testis lymphomas, other non-GCB tumors were found to overexpress COL3A1 (3.4 fold, P<0.01), FN1 (3.1 fold, P=0.02), PLAU (2.3 fold, P=0.05), and SOD2 (2.1 fold, P=0.02).

Conclusions: Within the non-GCB subset of DLBCL we found distinct differences in gene expression. The testis lymphomas were characterized by significant overexpression of genes that are markers of poor prognosis as well as low expression of good prognosis genes. These findings are in line with clinical observations and highlight the fact that even within the GCB/non-GCB subtypes of DLBCL there are marked differences in gene expression that could justify specific treatment approaches.

080 GENE EXPRESSION PROFILING (GEP) AND NOT IMMUNOCHEMICAL ALGORITHMS PREDICTS PROGNOSIS IN PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA (DLBCL) TREATED WITH R-CHOP

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Background: In patients with DLBCL, GEP distinguish two groups with different origin: germinal-center (GC) and activated (ABC). However, GEP is a technique not available in current clinical practice. For this reason, attempts to reproduce GEP data by immunohistochemical algorithms (IHCA) have been made. The aim of this study was to apply different IHCA in a series of patients with DLBCL treated with R-CHOP to assess the correlation with GEP and their usefulness to predict response and outcome.

Material and Methods: We constructed tissue microarrays in 157 patients (80M/77F; median age 65 years) with DLBCL of the *GELCAB* treated with R-CHOP. We applied the most popular algorithms using CD10, bcl6, GCET1, LMO2, MUM1, FOXP1 and bcl2: Colomo (Blood 2003, 101); Hans (Blood 2004, 103); Muris (J Pathol 2006, 208); Choi (Clin Cancer R. 2009, 15), and Tally (Meyer., JCO 2010). GEP studies were performed in 62 patients.

Results: The distribution of the cases according to the phenotype was similar for Colomo and Hans. In Choi and Tally the proportion of non-GC cases assigned was significantly higher (non-GC *vs.* GC, 67% *vs.* 33% and 63% vs. 37%, respectively). In Muris, the majority of the cases were allocated as GC phenotype (GC *vs.* non-GC, 57% *vs.* 43%). In 80 of 110 patients (72%) were allocated in the same group (either GC or non-GC). When the immunochemistry was compared with GEP data, the sensitivity in the GC group was 59%, 52%, 70%, 40% and 53% for Colomo, Hans, Muris, Choi and Tally, respectively. The sensitivity in the non-GC group was 81%, 85%, 62%, 84% and 80%, respectively. We observed a higher percentage of misclassified cases in the GC subset than in the non-GC. Differentiation profile as assessed by GEP (GC *vs.* ABC) showed a significant prognostic value for OS in the subset of patients with this GEP information, with a 5-year OS of 80% *vs.* 45%, respectively; P=0.03). By contrast, none of the profiles assessed by immunostaining were capable to predict OS.

Conclusions: In a homogeneous series of DLBCL patients treated with R-CHOP, the IHC algorithms (GC vs. non-GC) were not able to mimic the GEP information.

081 CONCURRENT BCL2 AND MYC TRANSLOCATIONS IN A PROSPECTIVE COHORT OF PATIENTS WITH DIFFUSE LARGE B-CELL LYMPHOMA

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Background: Concurrent chromosomal translocations involving the BCL2 and MYC protooncogenes, so-called double-hit, is found both in diffuse large B-cell lymphoma (DLBCL) and in a newly defined B-cell lymphoma category with features overlapping between DLBCL and Burkitt lymphoma (BCLU, 2008 WHO classification). Few studies have been published on series of double-hit lymphomas, and to our knowledge only retrospective series, reporting an average frequency of 5%. In addition to unanimous reports of highly aggressive clinical behavior; resistance to chemotherapy and poor outcome, double-hit lymphomas had varying morphology and mostly high proliferation rates. We conducted a prospective study of DLBCL/BCLU with the purpose of evaluating the frequency of doublehit BCL2/MYC translocations in DLBCL/BCLU and secondly if any pathological and/or clinical features correlated with the presence of double-hit.

Materials and methods: All patients diagnosed with DLBCL or BCLU at the Haematopathology Section and treated at Dept. of Hematology at Copenhagen University Hospital Herlev, were prospectively collected throughout 2009+2010. Tumors were classified according to morphology (2008 WHO), immunohistochemistry (CD10, BCL6, MUM1, BCL2, Ki-67) and FISH (BCL2, BCL6 and MYC translocations). Clinical data were obtained from patient files.

Results: Double-hit BCL2/MYC translocations were detected in 17 of 165 cases (10%). All double-hit DLBCL were GCB immunophenotype with varying morphology and Ki-67 index (15-95%). Interestingly, some double-hit cases were late relapses (primary double-hit). In the double-hit cohort there were significantly more transformed lymphomas. All other clinical features (IPI factors, sex, location) as well as treatment response, relapse and survival were not significantly different between the two groups.

Conclusion: In this prospective cohort of DLBCL/BCLU-patients the incidence of BCL2/MYC double-hit was 10%, which is more frequent than previously described. Double-hit lymphomas had GCB-immunophenotype but could not be identified by morphology or proliferation rate. Therefore if patients with double-hit DLBCL/BCLU should be identified, routine FISH-analysis for double-hit translocations should be performed in all patients with BCLU or DLBCL of GCB-subtype. We did not find an effect of double-hit on either clinical parameters, treatment response, relapse or survival, however the observation time was short.

082 THE COMBINATION OF STAT3, CSE1L AND MYC-TRANSLOCATION FORMS A NEW DIAGNOSTIC ALGORITHM WHICH DISTINGUISHES BURKITT FROM DIFFUSE LARGE B-CELL LYMPHOMA AND PREDICTS OUTCOME

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Background: A correct histological diagnosis of lymphoma is of crucial importance. In particular, in the case of aggressive lymphomas, the separation between BL and DLBCL is relevant for the treatment approach, and of prognostic impact. However, the diagnostic reproducibility of these aggressive B-cell lymphomas (BCLs) is poor and there is increased recognition of DLBCLs, which share features with BL but deviate with respect to one or more findings, currently classified in the provisional intermediate group DLBCL/BL in the last WHO classification.

Materials and Methods: A tissue micro-array (TMA) was established from more than 200 DLBCLs, BLs and DLBCL/BL, obtained from three Swiss Institutes of Pathology. These were cytogenetically defined (BCL2, BCL6, MYC translocations) and immunohistochemically analyzed for differential expression of new selected markers, based on recent transcriptional and gene-expression profile studies. Computational analysis of protein expression and FISH data led to predictive models for diagnosis (DLBCL and BL), trained as binary decision trees (RPART). OS and PFS were statistically analyzed, and different treatment protocols were taken into consideration.

Results: Expression of CSE1L and ID3 were associated with the diagnosis of BL ($p < 0.05$), whereas STAT3 was over-expressed in DLBCL. A computational algorithm proved the combined expression of CSE1L and STAT3 to be superior in predicting the diagnosis of BL and DLBCL when compared to the diagnostic markers used so far in the routine. Moreover, the combination of CSE1L and STAT3 expression as well as MYC translocation was able to identify patients, initially classified as intermediate between BL/DLBCL, who profited from intensive, BL-like regimens. The results were validated on 14 new aggressive BCLs.

Conclusions: We present a new immunohistochemical diagnostic algorithm easily applicable in the routine diagnostic of aggressive BCLs. This algorithm, in combination with FISH analysis for MYC, has positive predictive value for befit of intensive chemiotherapy regimens. These data need confirmation in prospective and larger studies.

083 OCCURRENCE OF THE T(14;18)(Q32;Q21) AND ITS VARIANTS IN CHRONIC B-CELL LYMPHOPROLIFERATIVE DISORDERS OTHER THAN FOLLICULAR LYMPHOMA

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The translocation t(14;18)(q32;q21) involving the immunoglobulin heavy chain locus (IGH) and the BCL2 gene, known as the genetic marker of follicular lymphoma (FL) has been reported incidentally in chronic lymphocytic leukemia (CLL)(2% in series of the literature). Among our consecutive series of chronic B-cell lymphoproliferative disease (CLPD) other than FL (n=993), we studied the occurrence of IG/BCL2 translocation (t(14;18)(q32;q21) t(2;18)(p11;q21) and t(18;22)(q21;q11)) by conventional cytogenetics and FISH in order to confirm the implication of BCL2. These translocations were observed in 37 cases that could be classified in 3 groups except one: CLL (n=22) chracterized by a Matutes score >3 in all cases but one with a score=1 but CLL diagnosis proven on LN biopsy, small lymphocytic lymphoma (SLL)(n=6) as defined by WHO classification, and CLPD unclassifiable (Matutes score<3, only a case with biopsy)(n=8). Interestingly the last case was identified as marginal zone lymphoma (MZL) on biopsy material. In our whole series of CLL/SLL, IG/BCL2 rearrangement was observed with the same occurrence that was previously reported (3%). Among this cohort of CLL/SLL with IG/BCL2 translocation, SLL appears particularly frequent (25%). Trisomy 12 was much more frequent than in conventional CLL series (59% vs 14-21%)($P<0.05$), while del13q was observed with a similar occurrence (33% vs 36-45%). Almost all CLL (95% of the cases) with t(14;18) expressed mutated IGHV gene (cut-off of 98%, median 92,5%), which is largely more than reported in common CLL (40%). No cases of our CLL with IG/BCL2 translocation used the VH3.21 segment in contrast to classical CLL. In these CLL, the immunological profile was atypical by the lack of CD5 (n=1) or CD23 (n=4) that was associated to the presence of trisomy12. The CLL with t(14;18) expressed CD38 (cut-off of 30%) with a frequnecy similar to common CLL. To conclude (1) we confirm that t(14;18) is a rare (2-3% of CLPD) but recurrent cytogenetic abnormality in SLL/CLL and is remarkably associated to trisomy 12 and mutated VH status; and (2) we describe the first case to our knowledge of non MALT MZL with a t(14;18) involving BCL2 gene.

084 INTEREST OF T(14;18) DETECTION FOR THE MANAGEMENT OF FOLLICLE CENTER LYMPHOMA PRESENTING AS SKIN LESIONS: 53 CASES STUDY

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Primary cutaneous B-cell follicle center lymphomas (PCFCL) are good prognostic diseases. However, skin relapse or extra cutaneous spreading occur in 40% and 10% of cases respectively. The hallmark of nodal FCL is the t(14;18)(q32;q21) translocation with *BCL2* breakpoint and *BCL2-IGH* fusion. We observed by FISH the absence of BCL2 split in PCFCL cases with positive BCL2-IGH detection showing that most cells do not carry the t(14;18). Alternatively, others observed BCL2-IGH fusion by FISH in 41% of PCFCL without BCL2-IGH breakpoint in PCR amplification. Our main goal was to study the prevalence and prognostic impact of t(14;18) in a series of PCFCL selected according to international diagnostic and staging criteria.

53 patients with skin lesions of FCL without history of nodal FL were selected. Staging procedures identified 6 secondary cutaneous FCL (SCFCL). FISH test for BCL2 split was performed on skin sections biopsies. BCL2-JH amplification was conducted using the BIOMED2 protocol on FISH positive cases. Proportions were compared with Fisher exact test, survey curves (Kaplan Meier method) with Log Rank test.

FISH detected t(14;18) in 7 cases : 4/47 PCFCL, 3/6 SCFCL. The t(14;18) was associated with positivity of staging procedures with node and/or bone marrow involvement (p=0,02). PCR amplification was less sensitive, with only 4 out of 6 positive FISH cases.

Complete remission was obtained in 44 PCFCL. Skin relapse was observed in 41% of cases after a 36.8 median follow-up. The presence of small cells was associated with higher probability of skin relapse (p=0.01), contrary to others factors studied (age, T stage, first therapy, monoclonal rearrangement of IgH in skin, identical monoclonal rearrangement of IgH in skin and blood, presence of t(14;18). Two patients with PCFCL t(14;18) positive on initial sample had a secondary extracutaneous involvement after 29 and 55 months of follow-up.

The t(14 ;18) is rare in true PCFCL as it was significantly associated with positivity of initial staging and SCFCL diagnosis. t(14;18) detection would lead to perform an exhaustive staging including bone-marrow biopsy. While t(14;18) present in only 4 PCFCL was not associated with skin recurrences, it was associated with an higher risk of extracutaneous spreading. Therefore, detection of t(14;18) by FISH in PCFCL would lead to a closer monitoring of such patients.

085 *BCL2, BCL6, MYC, CCND1, MALT1* AND *BCL10* GENE REARRANGEMENTS IN PRIMARY TESTICULAR LYMPHOMAS DETECTED BY FLUORESCENT IN SITU HYBRIDIZATION WITH SPLIT SIGNAL PROBES

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Introduction: Primary testicular lymphomas are rare neoplasms, mostly represented by diffuse large B-cell lymphomas (DLBCL). Primary testicular DLBCL are clinically aggressive lymphomas and, despite the administration of intensive chemotherapeutic regimens, they still bear a poor prognosis, which is in contrast with other primary extranodal DLBCL, such as gastric and cutaneous DLBCL. Genetic alterations in DLBCL often encompass complex abnormalities including translocations, trisomies, amplifications and deletions. Several studies have highlighted specific chromosomal alterations in gastric DLBCL, while other primary extranodal sites, including testis, have not been studied extensively from a cytogenetic point of view.

Design: In this study, FISH analysis was performed on histological sections of a series of 13 primary testicular DLBCL. Probes for split signal FISH targeting BCL2, BCL6, MYC, CCND1, MALT1 and BCL10 (Dako, Copenhagen, Denmark) were used. The results were compared with those obtained in a previously studied series of 74 primary nodal DLBCL (Tibiletti et al., Hum Pathol, 2009).

Results: Gene rearrangements were identified in 8 out of 13 analysed cases (61%). All genes were involved in rearrangements. BCL6was the most rearranged gene (4/13 cases, 30%), analogously to what observed in primary nodal cases, followed by MYC (3/13 cases, 23%), with a higher frequency than in nodal lymphomas. Interestingly, in 2 cases (15%) we found a MALT1 rearrangement, which was never observed in primary nodal DLBCL. Multiple rearrangements were detected in 3 cases (23%). Molecular cytogenetic analysis revealed also polysomies of one or more of the investigated regions in all analyzed lymphomas (100%).

Conclusion: In conclusion, this study highlights a peculiar pattern of gene rearrangements detected by FISH analysis in primary testicular DLBCL, compared with primary nodal DLBCL.

086 TISSUE MICROARRAY IN DLBCL PATIENTS RECEIVING CHOP-R CHEMO-IMMUNOTHERAPY SHOWS SURVIVAL BENEFIT FOR COEXPRESSION OF LMO2/BCL6 AND POOR OUTCOME FOR EBER-ISH POSITIVE PATIENTS

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Introduction: Diffuse Large B-cell Lymphoma (DLBCL) is a heterogenous disease. The international prognostic index (IPI) is applicable following 'CHOP-R' chemo-immunotherapy, but variable outcomes in patients with identical IPI scores is still common. Also IPI does not permit identification of patients who based on biology, might benefit from alternative treatment strategies. Numerous markers either alone or in combination have been proposed. To our knowledge this is the first study to compare BCL6, BCL2, MUM1, GCET1, FOXP1, CD10, EBER and LMO2 and the Hans, Choi and Tally algorithms in CHOP-R treated patients.

Materials and methods: 102 sequential cases were identified in whom formulin fixed parrafin embedded (FFPE) tissue was available. Transformed lymphoma and follicular lymphoma grade 3B were excluded. All patients received CHOP-R but were otherwise unselected. Patients were identified from a prospective clinical database which includes IPI, treatment and outcome details, and was complete in >95% cases. Another nineteen frozen tissue-banked samples with demographic but no treatment / outcome details were available to extend the number of samples available for EBER-ISH incidence analysis. An extensive tissue microarray (TMA) was performed.

Results: In 121 immunocompetent DLBCL patients, 9.1% were EBER-ISH+, exclusively in those aged >50 years. In the 102 FFPE patients, EBER-ISH+ was associated with a much poorer overall (p=0.03) and event free survival (p<0.001). BCL-6 positive patients had improved outcome (EFS p=0.045, OS p=0.02), however a combination of the germinal centre (GC) markers LMO2 and BCL6 positivity further enhanced the prognostic value (EFS p=0.035, OS p=0.005). Neither Hans, Choi or Tally algorithms predicted for any difference in survival between GC and post-GC subtypes.

Conclusions: BCL6 positive patients had improved OS and EFS in our cohort confirming the benefit of CHOP-R in this subgroup In our group of uniformly treated patients the combination of LMO2 and BCL6 positivity correlated with an extremely good EFS and OS to CHOP-R chemo-immunotherapy. EBV+DLBCL is associated with an inferior outcome.

087 THE MOLECULAR HISTORY OF RICHTER SYNDROME

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Background: The mechanisms involved in chronic lymphocytic leukemia (CLL) transformation to Richter syndrome (RS) are poorly understood. We explored intraclonal diversification (ID) of immunoglobulin genes in RS in order to: *i)* follow the evolutionary history of the RS clone; *ii)* understand if interactions with antigen play a role in RS transformation.

Methods: RS (n=11) were clonally related to the paired CLL phase. Cases were scored positive for ID only in the presence of confirmed mutations. Phylogenetic analyses was performed with MEGA4.

Results: Most (10/11, 90.9%) clonally related RS directly stem from the original CLL clone observed at the time of CLL diagnosis. One single RS case had a transformation pattern compatible with sequential evolution from a secondary CLL subclone. Once RS transformation had occurred, all secondary CLL subclones disappeared and were substituted by the dominant RS clone with its own descendants. These observations suggest that the transforming genetic lesion might be acquired by a cell belonging to the original CLL clone, rather than being progressively accumulated by later CLL subclones. Accordingly, most (9/11, 81.1%) RS harbored a genetic lesion disrupting *TP53* that was already present, though at subclonal levels, in 5/11 (45.5%) samples of the paired CLL phase. Paired analysis of CLL/RS samples documented that RS transformation was accompanied by selection of a clone that did required no ID (4/11, 36.4%) or reduced ID levels (5/11, 45.4%) for interacting with antigen. This observation suggests that the RS clone has no or limited requirement to interact with antigens through further BCR affinity maturation by ID. Independence from antigen at RS transformation might result from the acquisition of new genetic

lesions subsidizing BCR activation by antigen. Screening of BCR pathway genes (*CD79A*, *CD79B*, *CARD11*) revealed the acquisition of a mutation within the coiled-coil domain of *CARD11* in 1/4 (25.0%) RS that switched off ID at transformation.

Conclusions: These data indicate that: *i)* the RS clone stems from a cell that is already present in the context of the initial CLL clone and gains selective advantage over the CLL subclones; *ii)* most RS have become independent of antigen stimulation for their sustainment.