Lymphoma biology

373 B-CELL LYMPHOMA CELLS OVEREXRESSING BMI-1 ARE CORRELATED WITH DRUG RESISTANCE THROUGH ENHANCED EXPRESSION OF SURVIVIN AND ARE MORE EFFECTIVELY ELIMINATED BY T CELLS WITH CHIMERIC RECEPTOR AGAINST CD38 THAN CD19

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BMI-1 is essential for self-renewal and proliferation of leukemic and hematopoietic stem and progenitor cells. The expression of BMI-1 is well correlated with disease progression and poor prognosis in cancer patients. We showed that ectopic expression of BMI-1 in B-cell lymphoma cell lines, HT and RL, conferred resistance to etoposide and oxaliplatin, known to enhance sensitivity by targeting the survivin gene, but not to irinotecan, which is not relevant to the downregulation of survivin expression. Survivin has been reportedly implicated in resistance to chemotherapeutic agents. The expression of survivin was not only augmented in cells transduced with BMI-1, but persisted in the presence of etoposide in cells overexpressing BMI-1. By contrast, the mock-transduced cells succumbed in the medium with anti-cancer drugs with an accompanying decrease in BMI-1 and survivin expression. BMI-1 overexpression stabilized survivin post-translationaly without an accompanying rise in the mRNA, suggesting survivin to be a potential target for BMI-1. Knockdown of either BMI-1 or survivin restored sensitivity to etoposide in the BMI-1-overexpressing lymphoma cells. An analysis of 6 patients with B-cell lymphoma showed that in the drug-resistant patients, levels of BMI-1 and survivin were maintained even after drug administration. Downregulation of both BMI-1 and survivin expression was, however, observed in the drug-sensitive patients. Thus, BMI-1 may facilitate drug resistance in B-cell lymphoma cells through the regulation of survivin. Next, we examined whether BMI-1 by itself or BMI-1 CR, which is a more specific and sensitive approach, could be used as a marker of drug resistance. BMI-1 and survivin expression levels were significantly higher in the drug-resistant patients than in the drug-sensitive patients. We also found that the expression of BMI-1 and survivin was inversely correlated with the expression of CD38, a marker of drug resistance. These results suggest that the BMI-1/survivin pathway plays a crucial role in drug resistance and could be a potential target for the development of new drugs.

Conclusions: It is feasible to derive short-term cell cultures from native lymph node biopsies of patients with lymphomas. Immunophenotyping by flow cytometry may help in the diagnostic process and may reveal the pathophysiology of lymphoma cells under conditions of in vitro cultivation.

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375 WITHDRAWN

376 U-DCS, THE FIRST HUMAN CELL LINE DERIVED FROM AN INTERDIGITATING DENDRITIC CELL SARCOMA

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Introduction: Human dendritic cells are a phenotypically diverse group of antigen-presenting cells found in fractions in tissues throughout the body. The low numbers of dendritic cells present in blood mononuclear cells has hampered a detailed molecular analysis of dendritic cell function. No stable human dendritic cell lines could be established so far. Interdigitating dendritic cell sarcoma (IDCS) is an extremely rare neoplasm that mostly occurs in the lymph nodes. Only 184 cases have been reported worldwide.

Materials and Methods: Here we describe a novel permanent human IDCS cell line, U-DCS, derived from an interdigitating dendritic cell sarcoma of a male patient. Serial passaging of the cells gave rise to a fast proliferating cell line, which was characterized by immunocytochemical, molecular, cytogenetic and Elisa techniques.

Results: The cell line maintained stable phenotypic characteristics during the last one year in vitro. U-DCS is growing adherent with typical dendritic morphology, expresses MHC-I and MHC-II proteins, CD68, CD80, CD1c, HLA protein, and, in a subset, CD163 and CD205 but not CD40. mRNA expression analysis revealed transcripts for the toll-like receptors TLR3, -4, -9 and RIG-I (DDX58) but not for TLR2. The cells react with T and B lymphocytes. Karyotype analysis, m-FISH and FACs analysis revealed chromosomal instability, a hypotetraploid karyotype with about 130 chromosomes and multiple aberrations. CGH analysis showed more than three-fold loss of chromosome 4q and more than three-fold gain of chromosome Y. In contrast, short tandem repeat (STR) analysis revealed that U-DCS is stable on the molecular level. U-DCS forms slow growing tumors in the mouse NOD.

Cg-Prkdc(+/-)22g+/-7/2 which are deficient in mature lymphocytes and NK cells, thereby conserving its phenotype after recultivation in vitro. Conclusion: U-DCS is the first permanent human dendritic cell line and could be useful and helpful in future dendritic research.

377 CONCENTRATION OF SELECTED CYTOKINES IN BONE MARROW AND PERIPHERAL BLOOD OF PATIENTS WITH LYMPHOMA

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Introduction: Cytokines participate as important mediators in immune responses. They influence development and survival of many cells and they could play a critical role in progression of cancer. Some of them are thought to be useful markers of hematological malignancies, particularly lymphomas.

Material and Methods: In our study, we investigated the plasma concentration of following cytokines, their receptors and co-stimulatory molecules: IL-1b, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-13, IL-18, TNF-a, VEGF, IFN-g, G-CSF, sIL-2R, sIL-6R, sCD23 and sCD40L using an enzyme-linked immunosorbent assay (ELISA).

Results: Plasma levels of IL-4 and IL-6R were significantly higher in DLBCL (p=0.0015, p=3×10^-10) and MZL patients (p=0.0022, p=0.00014), as compared to healthy controls. In DLBCL patients, levels of sCD40 were elevated (p=0.018). On the other side, MZL patients showed significantly higher levels of IL-2 (p=0.019). Patients with CLL/SLL diagnosis embodied increment in IL-2 (p=0.025), sIL-2R (p=0.00014) and IL-18
378 WITHDRAWN

379 THE ROLE OF AID FOR THE PROGRESSION OF FOLLICULAR LYMPHOMA

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Background: Follicular lymphoma (FL), a heterogeneous entity with a cell origin in germinal center B (GC) lymphocytes, may develop into a progressive or transformed disease in some patients. Secondary genetic events have been associated with this transformation such as c-myc amplification or mutation. Activation induced cytidine deaminase (AID) is required for somatic hyper mutation and class switch recombination transformation such as c-myc amplification or mutation. However, the role of AID in transformed FL has not been established. Here we tried to identify the significance of AID associated with c-myc in the progression of FL.

Materials and Methods: A total of thirty-six clinical samples obtained from the patients with FL were divided into three groups: patients with FL in grade 1 or (n=15), patients with FL in grade 3 who could survive more than two years (n=14), and patients with rapidly progressed FL in grade 3 (RPFL) who died within two years after diagnosis (n=7). AID and c-myc expression among those patients with FL were examined with RT-PCR and quantitative real-time PCR. In order to examine the role of AID expression for RPFL, the full length of AID transcription was transfected into cell lines established from the patients with RPFL, and their cell proliferation and survival were compared to the controls in vitro.

Results: Tissues from the patients with FL in grade 3 and the patients with RPFL expressed relatively higher levels of c-myc than those from the patients with FL in grade 1 or 2 (p = 0.02 and p = 0.01 respectively). Also, high levels of AID expression was observed among the tissues from the patients with FL in grade 3, but not significantly. Interestingly, six of seven samples with RPFL expressed low levels of AID or did not express AID even though they expressed high levels of c-myc. Moreover, transfection of AID full length into AID-negative cell lines, which were established from three patients with RPFL, reduced cell proliferation and survival in vitro.

Conclusions: Our results indicate that AID may play a role of negative regulator on cell survival of FL when sufficient c-myc is expressed in the tumor. Switch off or low expression of AID after c-myc amplification may correlate to rapid progressive and clinical outcomes of FL.

380 INTEGRATIVE GENOMIC PROFILING OF HIGH-RISK DIFFUSE LARGE B-CELL LYMPHOMA PATIENTS LESS THAN 65 YEARS OLD TREATED WITH DOSE-ENSEMBLED CHEMOIMMUNOTHERAPY AND CNS PROPHYLAXIS

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Background: Several gene-expression signatures with prognostic significance in DLBCL have been identified. Here, we have integrated the high-resolution array comparative genomic hybridization (aCGH) data with transcription profiles to define novel prognostic markers for homogeneously treated DLBCL patients.

Patients: 59 de novo high-risk DLBCL patients less than 65 years were treated with six courses of R-CHOP14 followed by two courses of systemic CNS prophylaxis.

Methods: 31 DLBCL cases were subject to RNA-seq and BCL2 variants were determined using SNVmix. The BCL2 gene (exons 1-3) was resequenced by Sanger method in 542 samples: 348 primary DLBCL, 30 small lymphocytic lymphoma (SLL), 26 follicular lymphoma (FL), 25 mantle cell lymphoma (MCL), and 25 peripheral T cell lymphoma (PTCL) patient samples; 8 purified centroblasts; 51 germline DNAs from MCL or FL patients in this study; and 24 DLBCL cell lines.

Results: t(14;18) translocation, promoter hypermethylation, gene amplification, and as a target gene of NF-kB signaling. BCL2 deregulation also affects DLBCL prognosis.

Conclusions: This work was supported by grant IGA MZCR NS 9671-4 and MSM 0021622430.

381 BCL2 MUTATIONS IN DIFFUSE LARGE B-CELL LYMPHOMA

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Background: BCL2 is deregulated in diffuse large B-cell lymphoma (DLBCL) by the t(14;18) translocation, promoter hypermethylation, gene amplification, and as a target gene of NF-kB signaling. BCL2 deregulation also affects DLBCL prognosis.

Methods: 31 DLBCL cases were subject to RNA-seq and BCL2 variants were determined using SNVmix. The BCL2 gene (exons 1-3) was resequenced by Sanger method in 542 samples: 348 primary DLBCL, 30 small lymphocytic lymphoma (SLL), 26 follicular lymphoma (FL), 25 mantle cell lymphoma (MCL), and 25 peripheral T cell lymphoma (PTCL) patient samples; 8 purified centroblasts; 51 germline DNAs from MCL or FL patients in this study; and 24 DLBCL cell lines.

Results: t(14;18) translocation, promoter hypermethylation, gene amplification, and as a target gene of NF-kB signaling. BCL2 deregulation also affects DLBCL prognosis.

Conclusions: This work was supported by grant IGA MZCR NS 9671-4 and MSM 0021622430.
The GOELAMS 075 is a phase 3 trial focusing on patients between 18 and 60 years old with advanced de novo DLBCL. Our project aims at understanding the gene expression disturbances which take place at diagnosis in the blood of patients included in this trial.

**Material and methods:** Peripheral blood samples were collected in PAXGene™ tubes from 95 patients with DLBCL at diagnosis and 93 sex- and gender-matched blood donors as controls. After RNA isolation and globin reduction, cDNA was hybridized onto Affymetrix GeneChip® Human Exon 1.0 ST arrays. After quality check, core-annotation of the same array was performed using the Probeset Multi-Average (RMA) method and filtered using detection above background. Unsupervised analysis was performed using the Principal Component Analysis (PCA). Differential expression between controls and DLBCLs was determined with Mann-Whitney U tests corrected with a Bonferroni and Hochberg false-discovery rate <5%. Genes were further selected according to the absolute Fold Change. Biological data were interpreted using the Broad Institute MSigDBv3.0 database.

**Results:** 80 patients and 87 samples met quality criteria. 6270 transcript-1D were found differentially expressed among the 8680 filtered transcripts. PCA revealed a clear cut between controls and DLBCL samples in a two-genes ional scatter plot. With an absolute FC > 2, 96 genes were upregulated including TNFFS13B, interferon-induced proteins. 50 genes were downregulated including IL2RB and lymphoid-related transcription factors. With an absolute FC > 1.5, the number of genes increased reaching 501 upregulated and 529 downregulated genes.

**Conclusions:** This GEP study reveals profound disturbances in the gene expression in whole blood of patients with DLBCL compared healthy controls. They are mainly related to inflammation and inhibition of lymphoid processes. Ongoing analyses focus on characterizing specific pathways and cell origin that drive this DLBCL whole blood pattern. Specificity and sensitivity of this pattern will be analyzed as well as the prognostic value in DLBCL of specific genes.

**384 INTEGRATED MOLECULAR PROFILE OF BURKITT LYMPHOMA**

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**Background:** Endemic Burkitt lymphoma (BL) is the commonest pediatric cancer in Africa. Recently, we showed that Endemic BL differs from the sporadic variant of BL, which occur in developed countries, for a series genes involved in relevant functional programs, including immune response, BCR signaling, and cell cycle regulation. However, the exact contribution of EBV (which is currently considered a main etiopatogenetic factor of the disease) to the molecular profile of the disease has not been clearly established.

In this study we aimed to further explore the molecular profile of BL subtypes, by focusing on the possible roles of EBV and other possible pathogenic events (i.e. chronic immune stimulation) as determinants of the differences in GEP among the different BL subtypes.

**Material and Methods:** We studied 48 BL cases, including endemic (N=24), sporadic (N=12) and HIV-related forms (N=12). For the latter two subgroups, cases with or without evidence of EBV infection were enrolled. We performed whole genome GEP by using the Illumina DASL technology, and extended miRNA profiling by using the Affymetrix Human miRNA arrays. Results were validated by immunohistochemistry (IHC) and in situ hybridization (ISH) on a large series of cases, as well as by functional assays on BL cell lines. To characterize the function of RelB in B cell lymphoma, we first determined the activation of JNK and MAPK pathways in S107 and S107 RelB cells.

**Results:** We specifically focused in differences between EBV positive vs. EBV negative differences in both gene and miRNA expression among the different BL types. In addition, we specifically focused in differences between EBV positive vs. EBV negative cases. Finally, we validated our findings by IHC, ISH and functional assays. All the results will be preliminary presented at the ICLM in Lugano.

**Conclusions:** BL subtypes differ for their molecular profiles. High throughput techniques allowed a identify specific pathways deregulated in each groups.

**385 DLEU1 SIRNA GENE KNOCKDOWN IN PEDIATRIC BURKITT LYMPHOMA (PBL) IS ASSOCIATED WITH A SIGNIFICANT DECREASE IN DRUG INDUCED APOPTOSIS: IMPLICATION OF DLEU1 AS A TUMOR SUPPRESSOR GENE**

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**Background:** PBL represents 40% of pediatric NHL and is characterized by an 8q24 (c-myc) gene rearrangement. Most PBL patients (70%) also have a secondary chromosomal aberration (Poirel/Cairo et al, Leukemia, 2009). Children with PBL who have a 13q14.3 deletion have a significantly poorer survival (Nelson/Cairo/Perkins/Sanger et al Blood 2010). DLEU1, a Burkitt classifier (Dave/Storm et al, NEJM, 2006), is located at 13q4.3. cMYC binds to the promoter of DLEU1 to initiate the expression of DLEU1, DLEU1 in turn negatively regulates CMYC. Thus, DLEU1 may act as a tumor suppressor, and if deleted in children with 13q- PBL may allow the deregulation of several signaling pathways leading to drug/immune therapy resistance and inhibit apoptosis. We investigated the effects of DLEU1 on chemotherapy (cyclophosphamide; CY) and antibody (rituximab) -induced B apoptosis.

**Methods:** Ramos PBL cell line was transiently transfected (24 hrs) with DLEU1 siRNA (5′-UAACUGGCAAUUGAUAUGU-3′) and 3′-UAUGAACCGCUCAUAUCUGAUA-5′) as previously described (Day/Cairo SIOP 2008). The siRNA transfected cells were then treated with various concentrations of CY or rituximab for additional 4 hrs. Cells were evaluated for apoptosis using Annexin V-FTC and Propidium Iodide followed by FACS using BD LSRII. Statistics was conducted by one-way ANOVA followed by Dunnett multiple comparisons test.

**Results:** There was a significant reduction in apoptosis in the CY treated transfected DLEU1 siRNA vs mock control cells (89.5 nM CY: 10.26±0.23% reduction, p=0.05 to negative control; 895 nM CY: 10.86±0.67% reduction, p<0.01; 8950 nM, 9.85±0.32% reduction, p<0.05. There was a similar significant reduction in rituximab induced apoptosis in the PBL transfected DLEU1 siRNA vs mock control cells (4 microgram/ml Rituximab: 25.3±2.55% reduction, p=0.01 to negative control; 40 microgram/ml Rituximab, 18.3±4.13% reduction, p<0.04; 400 microgram/ml. Rituximab, 32.33±1.77% reduction, p<0.02).

**Conclusion:** DLEU1 siRNA gene knockdown studies resulted in significantly less apoptosis in CY and rituximab treated PBL cells. Deletions of 13q14.3, which contains DLEU1, in PBL may confer a phenotype of drug resistance and suggests a tumor suppressor role of DLEU1.

**387 ANALYZING THE ROLE OF RELB IN B CELL LYMPHOMA**

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**Introduction:** NF-kB family member RelB is crucial for the development and function of normal B cells. Functionally, RelB acts as a repressor as well as activator of NF-kB-dependent gene-expression. RelB exerts its negative effect by a) sequestering RelA in inactive complexes and b) epigenetic silencing of NF-kB target genes. In contrast, the positive effect of RelB is mainly connected to the alternative NF-kB signalling pathway. This alternative pathway is characterized by a signal-induced processing of p100 and activation of RelB-p52 heterodimers. Importantly, a deregulated alternative NF-kB pathway was observed in several types of B cell lymphoma. For instance, an increased alternative NF-kB pathway had been reported in anaplastic large cell lymphomas cells as well as in H-RS cells. However, the exact role of RelB in B cell lymphoma biology remains largely unclear.

**Materials and methods:** Cell lines: Karposi 1106, MedB1, L450, L428, L1236, SupHD1, S107. Immune blot analysis, EMSA, qPCR, siRNA knock down, luciferase-reporter gene-assays.

**Results:** To characterize the function of RelB in B cell lymphoma, we first determined the activity of nuclear RelB in a panel of different B cell lymphoma cell lines (both Hodgkin and non-Hodgkin cell lines). Overall expression of RelB was similar in all cell lines analyzed. However, the nuclear localization was distinct with highest levels of nuclear RelB observed in L1236, SupHD1 and Karposi 1106. To identify RelB-target genes in B cell lymphoma cells, we generated a S107 murine plasmacytoma cell line stably overexpressing RelB as a model system. Analysis of a panel of apoptosis-regulating genes and genes down regulated by RelB as well as several genes induced by RelB. Whereas anti-apoptotic genes like Bax and Birc3 were only slightly induced, the expression of Traf1 was highly augmented. In contrast, expression of TRAF-family members Tra2, Tra4 and Tra5 was attenuated in S107 RelB cells.

**Conclusions:** The members of the TRAF family play a crucial role in the signal transduction pathways initiated upon engagement of surface receptors of the TNF receptor superfamily like TNFR1, CD40 or CD30. Enhanced TRAF1 expression is known to modulate the signalling through these receptors by affecting the formation of TRAF-trimers. Thus, RelB and therefore the alternative NF-kB signalling pathway- might attenuate signalling by these receptors by enhancing TRAF1 expression and suppressing TRAF2 expression. Experiments are under way to determine the activation of IKK in S107 and MAPK pathways in S107 RelB cells as well as after siRNA-mediated suppression of RelB in both Hodgkin and Hodgkin B lymphoma cell lines.
388 MONOCYTIC-MYELOID DERIVED SUPPRESSOR CELLS ARE INCREASED IN THE PERIPHERAL BLOOD IN DIFFUSE LARGE B CELL LYMPHOMA (DLBCL) BUT NOT IN INDOLENT B-CELL LYMPHOMAS OR CLL

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Background: Different tumors have evolved a variety of mechanisms to generate a suppressive network to evade the host immune response and counteract the anti-tumor effect of cytotoxic T cells thereby promoting tumor dependent angiogenesis, tumor invasion and metastasis. Myeloid derived suppressor cells (MDSCs) are an heterogeneous population of immature myeloid cells, macrophages, granulocytes, and other cells at different stages of maturation, found both in mice and humans which express Gr-1+ F4/80+ and CD11b+. MDSC suppress immune responses both in vitro and can in vivo be subdivided into 2 sub-populations: granulocytic and monocytic. Recently it has been demonstrated that M-MDSCs possess stronger suppressor activity.

Materials and Methods: Flowcytometry (FC) analysis was performed in order to quantify immune suppressive MDSCs in the peripheral blood (PB) from 12 patients with DLBCL, 10 with low grade lymphoma, 10 with CLL and 12 healthy volunteers. Surface staining for CD14+ HLA DRlow was used to define this population of MDSCs. In the patients with DLBCL, analyses were performed using two time points: before, and at the end of chemotherapy. Statistical analyses were done using the two-tailed Student t-test, and P< 0.01 was kept as a threshold for the level of significance.

Results: Patients with DLBCL had significantly more MDSCs in their peripheral blood (mean ± SD) than patients with low grade lymphoma (mean ± SD) and healthy volunteers (mean ± SD). Patients with chronic lymphocytic leukemia (CLL) who unexpectedly had a lower proportion of MDSCs (mean ± SD) were found to have a lower incidence of CD5+, CD23+ and FOXP3+ tumor cells and FOXP3+ infiltrating cells was measured using a micrometric ocular.

Conclusions: These preliminary but consistent results in a relatively small cohort of patients illustrate that at the time of initial diagnosis patients with DLBCL have significantly higher numbers of MDSCs in their peripheral blood than those with indolent lymphoma and CLL and normal individuals. Successful treatment of the lymphoma was accompanied by a decrease in the numbers of MDSCs to within the normal range. A larger study is ongoing in our institute to validate these results, to determine the functional significance of these findings in DLBCL, to establish whether the numbers of MDSCs present in the peripheral blood can be used as a marker of disease activity in DLBCL and to determine their more exact role in tumor surveillance.

389 REGULATORY T-CELLS IN GASTRIC MALIG NT LYMPHOMA

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Background: Gastric MALT lymphoma (gMALT) is an indolent lymphoma acquired in the course of H pylori infection. In this immune setting the regulatory T cells (FOXP3+) may play a role in the disease as a component of tumor microenvironment. The aim of this study was to analyze by immunohistochemistry the presence of these cells in gMALT at the time of diagnosis and during follow-up, and to evaluate their clinical significance in outcome as well as the role of treatment on the T cell type population.

Methods: Thirty-three patients with gMALT were included. Sections were immunostained for CD20 and FOXP3. The number of CD20+ tumor cells and FOXP3+ infiltrating cells were quantified using a micrometric ocular.

Results: The median age was 63 y (range 32-83) with 52% being male. Stage: I in 66%, II in 25% and IV in 9%. B-symptoms in 6%. At diagnosis, the mean (± SD) of MDSC infiltrating cells was 680 ± 232 and 30a29 ± 40 cells/cm2, respectively.

Conclusions: These preliminary but consistent results in a relatively small cohort of patients illustrate that at the time of initial diagnosis patients with DLBCL have significantly more MDSCs in their peripheral blood than those with indolent lymphoma and CLL and normal individuals. Successful treatment of the lymphoma was accompanied by a decrease in the numbers of MDSCs to within the normal range. A larger study is ongoing in our institute to validate these results, to determine the functional significance of these findings in DLBCL, to establish whether the numbers of MDSCs present in the peripheral blood can be used as a marker of disease activity in DLBCL and to determine their more exact role in tumor surveillance.

100 HIGH-RESOLUTION COPY-NUMBER ANALYSIS REINFORCES THE EXISTENCE OF A SPECIFIC MOLECULAR SIGNATURE IN PRIMARY CNS LYMPHOMAS (PCNSL)

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Introduction: Unlike nodal DLBCL, histogenetic origin and molecular pathogenesis of PCNSL are poorly understood. It is still a matter of debate if there is a genomic signature specific of PCNSL.

Materials and Methods: 17 EBV and HIV negative and immune competent PCNSL were studied by array-based comparative genomic hybridization (aCGH) with an average resolution of 38k. B-cell differentiation status was characterized for CD10, MUM-1, and BCL-6 immunostains. aCGH profile was compared with 59 nodal DLBCL.

Results: All 7 GCB and 10 non-GCB PCNSL analyzed have a complex karyotype with a median of 21 copy-number abnormalities (CNA) per case (range 10–49). Overall, 18 regions (10 losses and 8 gains) were affected in >20% of patients. Focal deletion of p21 (CDKN2A) was the most common CNA, found in 14 of 17 cases (82%). Other common CNAs were deletion of 6q23.3 (TNFAIP3; 59%), 6p21 (HLA genes; 53%), 6q11 PRDML; 47%) and gain of 12q12-q24 (53%), 7q11-q31 (35%) and 19q13 (35%). Five novel recurrent focal CNAs were found in PCNSL; loss of 3p12.3, 3p26.3 (TML1X1R), 10p14-p15, 12q43 (BC27A) and 16q12-q21 (28% of cases each). All the novel CNAs, excluding 16q12-q21, were only found in less than 5% or absent in nodal DLBCL, thus suggesting to be unique to PCNSL. Moreover, -3p21.3 and -1p14-p15 were mutually exclusively with -1q21-24-q4 (p=0.0004). At the gene level, recurrent homozygous deletions were found in CDKN2A, TNEM50A, CD58 and TOX, the latter two genes being T-cell development and activation. CD58 was also recurrently affected by monoallelic losses from 15 nt to 1-2 exons as was confirmed by DNA resequencing. Another 34 genes, including B2M, ETY6, HLA genes, PRDML, TNFSF10A and TNFRSF10B were also homozygously deleted. Pathway analysis shows an enrichment of genes associated with immune response, apoptosis and lymphocyte differentiation and proliferation.

Conclusions: We showed evidence of a highly complex genome and identified a subset of genes with potential relevance in PCNSL pathogenesis. The genomic profile described and published reinforces the existence of a specific molecular signature in PCNSL, thus helping to genetically differentiate this entity from the nodal DLBCL and related lymphomas.

391 WITHDRAWN

392 SPECULATE: SYNCHROTRON TECHNOLOGY IN CHRONIC LYMPHOCYTIC LEUKEMIA

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Background: Chronic lymphocytic leukemia (CLL) is the most prevalent form of leukemia. It is a diverse disease with prognosis ranging from a survival of two to eleven years despite modern therapy. Single genetic abnormalities do not always correlate well with disease outcomes. By the addition of synchrotron radiation to vibration spectroscopy, tissue may be analyzed at cellular and even cell structural level. By collecting these synchrotron infrared microscopy spectra or a "synchrotron fingerprint" and relating it to nuclear activity, it may be possible to predict more accurate clinical outcomes.

Methods: Blood samples were collected from 27 CLL patients at hematology clinics in Saskatoon, Saskatchewan and from 10 normal volunteers. Thirteen CLL samples were collected on low e- microscopy slides (Kevely Technologies, USA). Subsequently 14 CLL and ten normal samples were then processed via Histopapage-1077 (Sigma- Aldrich, USA) extraction and then mounted on low e. slides. Comparison between the synchrotron fingerprint of normal lymphocytes and lymphocytes of CLL patients were carried out with the mid Infrared (midIR) beamline at the Canadian Light Source, Saskatchewan. From each sample 14 cells were scanned and their spectrum’s averaged. Spectral analysis was performed using The Unscrambler® X Software. Charts from all SSL patients were reviewed to obtain their flow cytometry, cyogenetic characteristics and clinical course.
Results: The Mid-IR bands in the DNA regions (900-1300 cm\(^{-1}\)) and the protein bands (1300-1700 cm\(^{-1}\)) did not reveal any significant differences in cytogenetics, flow or lymphocyte count in the samples without histiopaque processing. Cluster analysis of the histiopaque slides in the protein regions (1300-1700 cm\(^{-1}\)) had a tendency to cluster into 2 groups. One predominately CLL (10/13) and the other predominately normals (6/10). By looking at the DNA band region (900-1300 cm\(^{-1}\)) via cluster analysis two groups emerged. The average of lymphocyte counts was 10x10\(^{9}\)/L in group 1 and 41x10\(^{9}\)/L in group 2 (P-value 0.0236).

Discussion: While current technology for predicting disease severity and clinical outcomes provides information for some CLL patients, many CLL patients have no known prognostic markers, making their clinical course unpredictable. The clustering analysis of the difference in lymphocyte count is promising and would be aided with a future look at cytogenetic profiles. By identifying patients with high levels of nuclear activity through infrared spectroscopy, it may be possible to predict severe disease and prognosis in patients with CLL.

393 DIFFERENT PROTEIN EXPRESSION PATTERNS MAY INDICATE DIFFERENT DISEASE MECHANISMS IN OLDER AND YOUNGER CHL PATIENTS

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Introduction: In western countries, the age distribution of Hodgkin lymphoma (HL) follows a characteristic bimodal curve showing the first peak at 15-20 years and a second peak in late life, 70-80 years. The mortality curve does not show the same bimodal pattern but increases with age. Is this observation just a function of age or does it also relates to different underlying biological processes for classical HL (cHL). HL shows heterogeneity in tumor cell number and morphology. A variable, but usually only minor proportion of cHL tumor tissue is composed of neoplastic Hodgkin/Reed-Stenberg cells, the surrounding cellular microenvironment are presumed to be important components in the development and course of the disease. Analysis using proteomic techniques may shed new light on the role of tumor and microenvironmental cells in key steps in HL-oncogenesis. Our aim is to provide hypothesis-generating data by comparing protein expression patterns in tumors from younger (<60yrs) and older (>60yrs) cHL patients in order to identify possible age-related differences in the biology of cHL.

Methods: Frozen tissue samples from 10 patients with stage I or II cHL were identified in the pathology archives and clinical data were obtained from the Danish Lymphoma Group database. Tissues were studied from 5 younger and 5 older patients. Tissues were subjected to high-resolution two-dimensional gel electrophoresis. Individual protein spots were visualized with silver staining and expression profiles in the younger and older groups were compared by computer analysis. Proteins with two fold or more differential expression between the two clinical groups were identified by liquid chromatography-tandem mass spectrometry and further studied by immunological methods.

Results: This study confirms the feasibility of using archival frozen tissues from cHL patients for proteomic analysis. The protein expression profiles of the two clinical groups analyzed showed significant and distinct differences. Preliminary results suggest differential expression among proteins belonging to functional families such as the MAPK signalling pathway, cell cycle regulation and cancer proto-oncogenes.

Conclusions: Proteomic analysis found significant differences in the protein composition of cHL tumor tissues from younger compared with older patients. Differential expression of a number of these proteins may provide further insights into the age-specific pathophysiology of cHL.