

# “focus on...” session: CLL

## 021 POLYMORPHISMS IN APOPTOSIS AND IMMUNOREGULATORY RELATED GENES ARE ASSOCIATED WITH AN INCREASED RISK OF CHRONIC LYMPHOCYTIC LEUKEMIA (CLL)

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Family and epidemiologic studies have suggested an inherited susceptibility for CLL. To identify low penetrance susceptibility alleles for CLL risk, we genotyped 768 SNPs in 689 cases of CLL and 723 controls from Northeast Spain. We have performed an approach selecting non-synonymous SNPs in 618 genes involved in cancer biology, and a second approach focused on potentially functional and marker SNPs in 50 candidates. Genotyping was carried out using Illumina Platform. To test the hypothesis of association between SNPs and CLL risk, multivariate methods based on logistic regression analyses were used. To quantify the degree of the association, odds ratios (OR) and 95% confidence intervals (95% CI) were calculated for each group under the four inheritance models. All analyses were adjusted for age, gender and center of recruitment. We assessed the robustness of the findings by calculating FDR. We found a strong association with CLL risk after stringent adjustment for multiple testing for six variants: *CCNH* rs2266690 (OR, 0.63; 95% CI, 0.53-0.74), *APAF1* rs17028658 (OR, 0.42; 95% CI, 0.29-0.61), *IL16* rs4505265, *CASP8* rs1045485 (OR, 0.56; 95% CI, 0.44-0.72), *NOS2A* rs2779251 (OR, 0.70; 95% CI, 0.57-0.84) and *CCR7* rs3136687 (OR, 0.40; 95% CI, 0.24-0.67). Moreover, we found association with CLL risk for 22 haplotypes in 17 candidate genes related to apoptosis and immunoregulation. Finally, we evaluated *in silico* the potential influence of these SNPs on the mRNA expression. Minor allele for *APAF1* rs17028658 and *IL16* rs4505265 in the germ-line may be associated with lower mRNA levels, no expression differences were observed for *CCR7* rs3136687, whereas *NOS2A* could not be assessed. Common genetic variants in apoptosis and immunoregulation related genes are associated with an increased CLL risk.

## 022 DOUBLING TIME OF SOLUBLE CD23 (sCD23DT): A POWERFUL INDEPENDENT PROGNOSTIC FACTOR FOR STAGE A UNTREATED CHRONIC LYMPHOCYTIC LEUKEMIA PATIENTS (CLL)

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**Background:** We have previously reported that sCD23 level is correlated with stages, prognosis and overall survival (OS) of patients (pts) with CLL. We evaluated prospectively the sCD23DT as a prognostic factor for time to treatment (TTT) and OS in untreated Binet stage A CLL pts and we compared it to the most commonly used biological prognostic factors lymphocytes DT (LDT), IgVH mutational status, ZAP-70, CD38 and LPL expression.

**Methods:** sCD23 level was prospectively evaluated by a commercially available enzyme-linked immunosorbent assay (ELISA). ZAP-70 and CD38 expression were determined in leukemic cells by flow cytometry, and positive expression was respectively defined as >20% and >7% positive cells. LPL expression was measured by real time PCR on cDNA from CD19 purified cells and optimal cut-off was determined by ROC curve analysis.

**Results:** Untreated stage A CLL pts were monitored for more than 15 years. 56 are evaluable for correlation between sCD23DT and new prognostic factors. Median age is 63 (43-89) years. The median follow-up is 64 (6-176) months. The sCD23DT is clearly correlated with a progression of the disease: median TTT and OS were respectively 20 and 83 months in pts with sCD23DT less than one year comparing to 141 and 177 months in pts with sCD23 DT greater than one year (P<0.0001). Mutational status, ZAP-70 and LPL expression were also strong predictors for TTT (P<0.0001) and OS (respectively P=0.0004, P=0.0001, P=0.0025) but CD38 and LDT had only a significant impact on TTT (respectively, P=0.0338 and P<0.0001). More interestingly, among poor prognostic pts (Unmutated, ZAP-70+, LPL+ and CD38+), sCD23DT allows to isolate 2 different populations: one with a very aggressive evolution (TTT <15 months) and another with a more indolent evolution (TTT above 5 years). In a Cox multivariate regression analysis, sCD23DT is the sole independent prognostic marker for TTT (P=0.0027).

**Conclusion:** sCD23DT is a powerful prognostic factor for TTT and OS in pts with untreated stage A CLL and refine the high risk population defined by widely accepted

prognostic factors. These observations support the introduction of sCD23 evaluation into the routine assessment of CLL pts.

## 023 QUANTITATIVE GENE EXPRESSION ANALYSIS OF SURROGATE MARKERS FOR GENETIC RISK GROUPS AND SURVIVAL IN CLL

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**Introduction/Background:** In CLL, numerous surrogate markers for genetic features such as VH mutation status have been described. Their detailed relation to the most important genetic subgroups, i.e. VH mutation status, V3-21 usage, del11q22-q23 (11q-), del17p13 (17p-), and their value as prognostic markers in the context with established prognostic factors is largely unknown.

**Material and Methods:** Transcript levels of 18 candidate genes (*ADAM29*, *ATM*, *CLLU1*, *DMD*, *GLO1*, *HCSL1*, *KIAA0977*, *LPL*, *MGC9913*, *PCDH9*, *PEG10*, *SEPT10*, *TCF7*, *TCL1*, *TP53*, *VIM*, *ZAP70*, *ZNF2*) were determined by real-time quantitative RT-PCR (RQ-PCR) and investigated regarding their predictive value for genetic subgroups and survival in 151 CD19 purified patients samples.

**Results:** Best assignment of VH mutation status was achieved by LPL and ZAP70, followed by TCF7, which was overexpressed VH mutated CLL. Patients at genetic risk (VH unmut. or V3-21 usage or 11q- or 17p-) were best assigned by ZAP70, in which a determination by RQ-PCR yielded better results compared to the FACS method. When applying a hierarchical risk model (risk 17p-> 11q-> VH unmut. or V3-21 usage>VH mutated) high misclassification rates occurred with any individual marker, which could be improved using a marker combination of 5 genes. Still, a reliable discrimination of 11q- or 17p- from VH unmutated patients without these abnormalities was not achieved. In multivariate survival analysis, LPL was the strongest survival predictor among the candidate genes; however, when including genetic factors, the surrogate markers lost their independent prognostic significance.

**Conclusions:** Screening for patients at genetic risk can be performed using ZAP70 or LPL, a marker combination reduces misclassifications. A distinction of 11q- or 17p- from other risk patients is not possible. The prognostic impact of the surrogate markers is inferior compared to the established genetic factors.

## 024 B CELL RECEPTOR-DEPENDENT INDUCTION OF CCL3 AND CCL4 BY CHRONIC LYMPHOCYTIC LEUKEMIA B CELLS IS ASSOCIATED WITH ZAP-70 AND INDEPENDENT OF SYK INHIBITION

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Nurselike cells (NLC) provide survival- and drug resistance-signals to CLL cells *in vitro*, and NLC can be detected *in situ* in lymphoid tissues from CLL patients. High level expression of CD68 makes NLC comparable to CD68<sup>+</sup>lymphoma-associated macrophages in follicular lymphoma, which are associated with an aggressive clinical course and poor outcome. To dissect the molecular cross talk between CLL and NLC, we examined gene expression profiles of CLL cells before and after co-culture with NLC. NLC induced a homogeneous gene expression response with high-level expression of B cell maturation antigen (*BCMA*, *TNFRSF17*) and two chemoattractants, *CCL3* and *CCL4*. Levels of *CCL3* expression and *CCL4* expression correlated with the ZAP-70 expression. Supernatants from CLL cells co-cultured with NLC revealed high *CCL3* and *CCL4* protein levels (up to >10 ng/ml). BCR triggering also induced a rapid (starting after 2h) and robust *CCL3* and *CCL4* secretion by CLL cells with a maximum response at 10 µg/mL anti-IgM or higher. In contrast anti-CD40 mAbs had no effect, and *CCL3* and *CCL4* induction was not affected by the spleen tyrosine kinase (Syk) inhibitor piceatannol. The correlation to ZAP-70 expression and lack of inhibition using piceatannol suggest that BCR-induced secretion of these chemokines depends upon ZAP-70 rather than Syk. Conditioned supernatants from CLL cells containing ~10 ng/ml of *CCL3* and *CCL4* stimulated chemotaxis and actin polymerization in normal lymphocyte, indicating bioactivity of the secreted chemokines. CLL patients displayed higher *CCL3* and *CCL4* plasma levels than healthy donors, suggesting that these chemokines are secreted in relevant

amounts by CLL cells in vivo. These studies reveal a novel mechanism of cross-talk between CLL cells and NLC, namely the induction of BCMA, CCL3, and CCL4. Correlation of the expression with ZAP-70, and lack of inhibition using piceatannol provide further evidence for the importance of ZAP-70 in BCR signaling in CLL cells.

**025 ABNORMAL SERUM FREE LIGHT CHAIN RATIOS ARE ASSOCIATED WITH POOR SURVIVAL IN PATIENTS WITH CHRONIC LYMPHOCYTIC LEUKAEMIA**

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Serum free light chains (FLC) have prognostic significance in monoclonal gammopathy of uncertain significance, solitary plasmacytoma of bone, multiple myeloma, Waldenström's macroglobulinaemia and AL amyloidosis. The incidence of abnormal FLC in other lymphoid malignancies including chronic lymphocytic leukaemia (CLL) is unclear with only one published report which found 8/18 CLL patients with an abnormal FLC. There have been no studies correlating FLC with other biological variables and clinical outcomes in CLL or lymphoma. This was a retrospective study that analysed random serum FLC levels taken at varied time points in 226 CLL patients (183 Stage A, 18 Stage B, 16 Stage C, 9 unknown, mean age 74, male: female ratio 2.2) treated at 3 separate hospitals in the UK and correlated serum FLC with biological and clinical markers. Using Kaplan Meier survival hazards, abnormal FLC ratio is a significant indicator of poor survival (n=226, Log rank Mantel-Cox p=0.001) and also time to first treatment. Using Cox regression analysis (n=142 with complete data sets) we analysed stage, CD38, Zap 70, mutation status, FLC ratio,  $\beta$ 2M and age in a forward stepwise analysis and showed 4 independent prognostic variables Zap70 (p<0.001),  $\beta$ 2M (p=0.002), mutation status (p=0.003) and FLC ratio (p=0.009). An abnormal free light chain ratio contributes significantly and independently to the prediction of a worse outcome. Serum FLC at diagnosis needs to be studied prospectively in CLL patients and the biological rationale for its adverse impact needs investigating.

**026 CLINICAL CASPASE ACTIVATION IN CLL BY GCS-100, A NOVEL CARBOHYDRATE, IN A PHASE 2 STUDY**

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Chronic lymphocytic leukaemia (CLL) is characterised by deregulation of multiple cellular pathways. GCS-100, a modified citrus pectin, with the ability to bind the carbohydrate binding domain of Galectin 3. It has previously been demonstrated to have anti-CLL effects *ex vivo*, even in the context of fludarabine resistance (Lugano 2005). We have elucidated further the mechanisms of action of GCS-100 demonstrating inhibition of proliferation, induction of apoptosis and accumulation of cells in sub-G1 and G1 phases with reduction of cells in S and G2/M phase. Both the caspase-8 and -9 pathways were activated. Significantly a dose and time dependent decrease in Mcl-1 and Bcl-x<sub>L</sub> levels occurred. This was accompanied by a rapid induction of Noxa protein. Bcl-2, Bax, Bak, Bim, Bad, Bid and galectin 3 remained unchanged. Following GCS-100 treatment cell cycle proteins were examined and upregulation of the cell cycle inhibitor p21<sup>Cip1</sup> with concurrent reduction of the pro-cycling proteins cyclin E2, cyclin D1, cyclin D2 and CDK6 occurred. Furthermore, there is a reduction in signal transduction in the form of reduced activated IκBα, IKK and akt. In a clinical Phase 2 study (NCT00514696), patients with relapsed CLL were treated with GCS-100 by IV infusion and concurrent studies of protein expression of the CLL cells by Western blotting were performed. Activation of Caspase 8 and 9 were seen maximal 6 hours after infusion of GCS-100 and may correlate with clinical response. This study provides evidence for clinical and biological activity for GCS-100 in CLL in relapsed disease.