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# POSTER ABSTRACTS

## 641.CHRONIC LYMPHOCYTIC LEUKEMIAS: BASIC AND TRANSLATIONAL

### B-Cell Receptor Function and Gene Expression Profiling at Single-Cell Level across the Spectrum from Normal to Neoplastic CD5-Positive B-Cell Compartments in Humans

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Antigen-independent, autonomous signalling of the B-cell receptor (BCR) is an oncogenic driver common to all cases and subtypes of chronic lymphocytic leukemia (CLL). We have recently shown that the dominant clonal population of low-count (<500 cells/μL) monoclonal B lymphocytosis (MBL) with CD5 $^{+}$CD20 $^{low}$CD79B $^{low}$ CLL phenotype identified prospectively in healthy siblings of CLL patients also exhibit autonomous BCR signalling, albeit with lower signalling strength than CLL BCR (Quinten *et al., Haematologica* 2023). To gain further insight into the mechanisms of clonal expansion within the CD5 $^{+}$ B-cell compartment as the possible origin of CLL, we performed single-cell gene expression profiling and exemplary analyses of BCR function in normal CD5 $^{+}$ B cells (CD20 $^{hi}$CD79B $^{hi}$) and in CLL-phenotype B cells (CD5 $^{+}$CD20 $^{low}$CD79B $^{low}$) of healthy siblings of CLL patients in comparison to CLL and high-count MBL cells.

Screening of blood samples of 16 sibling stem cell donors of CLL patients by flow cytometry revealed the presence of CD5 $^{+}$CD20 $^{low}$CD79B $^{low}$ B cells at low counts in 5 donors. These cells and normal CD5 $^{+}$CD20 $^{hi}$CD79B $^{hi}$ B cells were sorted in parallel, pooled at equal ratios, and analyzed together with CLL B cells from their sibling stem cell recipients and 2 unrelated high-count MBL B cells on two 10X Genomics chips for single-cell 5' gene expression and BCR sequencing. Data deconvolution per individual was performed based on expressed SNPs and on the individuals' HLA types. In a combined 2-dimensional t-SNE plot based on the 2000 most variably expressed genes, the normal CD5 $^{+}$CD20 $^{hi}$CD79B $^{hi}$ B cells from all 5 donors clustered distinctly from CLL-phenotype cells from all sources (dotted lines, Figure) and indeed showed significantly higher expression of CD20 and CD79B. Gene set enrichment analysis revealed significant upregulation of the hallmark oxidative phosphorylation gene set in all CLL samples compared to non-CLL B-cell populations.

BCR analysis demonstrated clonal dominance of >99% for both Ig heavy and light chains in the 5 CLL samples. In accordance with the indication for allogeneic stem cell transplantation, these CLL BCR were unmutated. In marked contrast, normal CD5 $^{+}$CD20 $^{hi}$CD79B $^{hi}$ B cells from the healthy SC donors were also unmutated but completely polyclonal, i.e. no identical BCR was detected in any two cells of this compartment. In 4/5 donors, the CLL-phenotype CD5 $^{+}$CD20 $^{low}$CD79B $^{low}$ compartment contained multiple clonal expansions of various degrees. In one high-count MBL, 17 other B-cell clones with various degrees of low-level expansion coexisted with the >90% dominant MBL clone.

BCR from the most dominant and one or more of the less expanded CD5 $^{+}$CD20 $^{low}$CD79B $^{low}$ clones of the healthy donors were tested for autonomous signaling as quantified by BCR-induced calcium flux without experimental cross-linking in murine TKO pre-B cells and compared to BCR from nonexpanded CD5 $^{+}$CD20 $^{hi}$CD79B $^{hi}$ cells and from their CLL siblings. Autonomous BCR signaling strength was calculated from the fraction of cells with spontaneous calcium flux above test background and calibrated against calcium flux after BCR crosslinking. CLL and high-count MBL BCR had robust median autonomous BCR signaling of 0.326. Expanded CD5 $^{+}$CD20 $^{low}$CD79B $^{low}$ clones of the healthy donors showed significantly weaker BCR signalling strength of 0.163 ($p<0.01$; Scatter Plot). BCR signalling in nonexpanded CD5 $^{+}$ B cells appeared weaker than in expanded clones but was clearly above the background of BCR from mature B cells isolated from lymph node biopsies expressing a mutated IgG.

In conclusion, the low-count CD5 $^{+}$CD20 $^{low}$CD79B $^{low}$ CLL-phenotype B-cell compartment in healthy adults is composed of multiple, predominantly mutated B-cell clones. Therefore, the term "monoclonal B lymphocytosis" is highly misleading for these B-cell populations and should be avoided. Normal CD5 $^{+}$CD20 $^{hi}$CD79B $^{hi}$ B cells are exclusively unexpanded and express unmutated BCR, indicating a naïve status. Unexpectedly, these nonexpanded CD5 $^{+}$ B cells express BCR with rela-

tively weak but quantifiable autonomous signalling. Globally, autonomous BCR signalling strength across the CD5 $^{+}$ B-cell compartment appears to be correlated to the degree of clonal expansion. Fully malignant CLL cells overexpress genes of the oxidative phosphorylation pathway, presumably to meet increased energy demand from alternative sources.

**Disclosures** No relevant conflicts of interest to declare.

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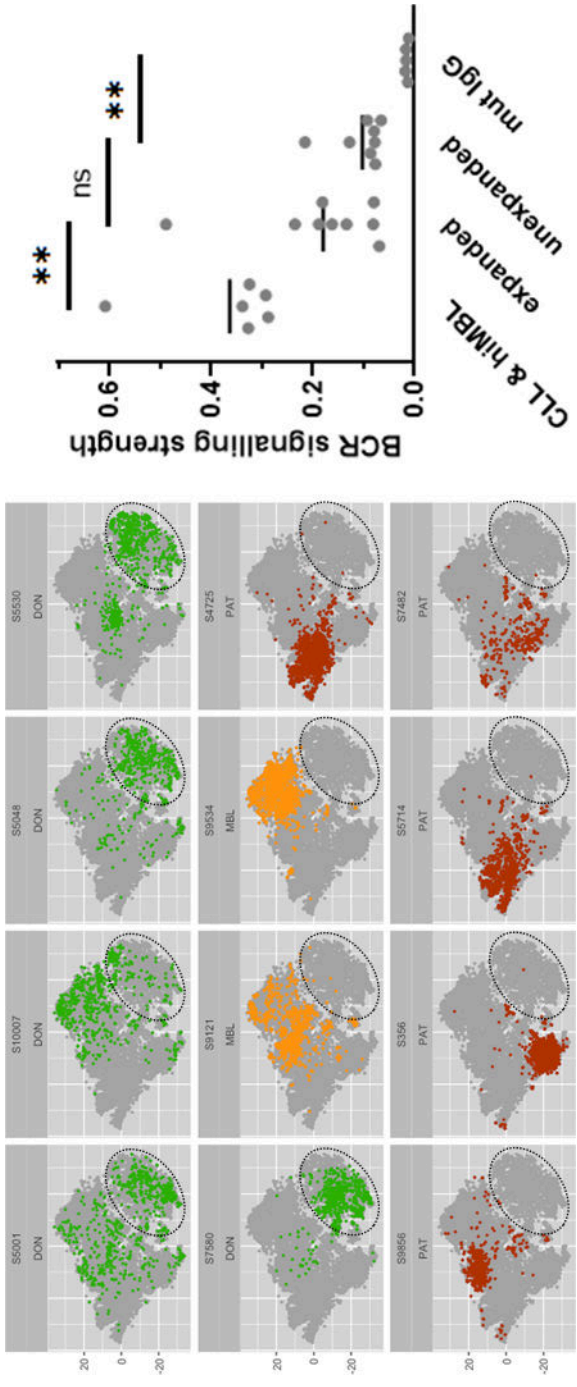


**Figure 1**