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POSTER ABSTRACTS

641.CHRONIC LYMPHOCYTIC LEUKEMIAS: BASIC AND TRANSLATIONAL

Investigation into Intraclonal Heterogeneity of CXCR4 ^{Dim}CD5 ^{Bright} Chronic Lymphocytic Leukemia Cells Identifies Distinct Activation Signatures

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Chronic Lymphocytic Leukemia (CLL) is a heterogeneous disease associated with diverse clinical and molecular profiles, suggesting long-term clonal evolution driven by genetic diversification from a small, often undetected, subset of leukemic cells endowed with superior growth capacities. More importantly, this diversification process enhances the opportunity of CLL subclones to survive selective pressure, such as BTK or PLCG2 mutations that can abolish the effect of BTK inhibitors. While this evolutionary process is inevitable in leukemic cells, therapeutic intervention targeting the cells that have just replicated their DNA, and hence could have accumulated new genomic mutation, would be an effective strategy to significantly delay or abort the emergence of deleterious mutations.

In CLL, a series of studies involving consumption of $^{2}H_{2}O$ determined that CXCR4 ^{Dim}CD5 ^{Bright} cells (proliferative fraction, PF) of CLL clones contain most of the recently divided cells, identified by the presence of ^{2}H-DNA. This conclusion was supported by finding elevated numbers of Ki67 $^{+}$ and MCM6 $^{+}$ cells in the same fraction, when compared to other CLL cells. Furthermore, PF is superior in: lymphoid tissue homing in xenograft models; activating T cells; the capacity to alter tumor microenvironment (TME) by Th2 skewing; and expression of AICDA which can increase the likelihood of off-target genomic mutations.

To permit a focused analysis of PF that would allow us to intercept this evolutionary loop, we characterized the single cell transcriptomes of PF from 4 U-CLL and 4 M-CLL patients immediately prior to ibrutinib treatment. Consistent with previous reports, the majority of PF (herein APC-PF) cells express certain antigen presentation molecules with a gene signature suggestive of T cell regulation and cell-cell interactions. Additionally, we discovered a small subpopulation with an upregulation of *MIR155HG, HSP90AB, CCL3, CCL4, MYC* and a concomitant MYC activation signature (herein MYC-PF). Since miR-155 expression can be induced by CD40L or B-cell-activating factors, this PF subset may reflect prior BCR ligation and/or T cell co-stimulation. However, the strongest BCR activation signature was found in another PF subpopulation (herein BCR-PF) with an enrichment of germinal center-related genes (e.g., *BACH2, PAX5, FOXP1*) and CLL-associated molecules (*BCL2, ROR1, MSI2, PLCG2*). Furthermore, the BCR-PF cells are least likely to be resting (in G1/G0) compared to others. Collectively, these findings suggest effective BCR engagement, either from the TME or cell-autonomously. Corroborating these findings with our previous studies documenting the CXCR4/CD5 fractions and the expression of protein proliferation markers, our transcriptomic analyses suggest that the BCR-PF may be the most immediate emigrant from the proliferation centers (PC) within CLL lymph nodes (LN). To probe this possibility, spatial transcriptomic analysis was performed on FFPE LN tissues from surgical biopsies from 3 U-CLL and 1 M-CLL patients. Single cell deconvolution was used to determine the relative abundance of cells within the anatomically defined PCs that resemble the three PF subpopulations transcriptomically. Our results suggest that PCs are more enriched in cells resembling BCR-PF, followed by cells bearing the APC-PF signature. To extend on these observations, we have documented that the unique surface markers expressed in these PF subfractions positively correlate with our transcriptomic results.

Taken together, these results not only highlighted the molecular heterogeneity of CLL, but also lead to the identification of two PF subpopulations that may provide novel actionable therapeutic targets to significantly halt CLL clonal evolution.

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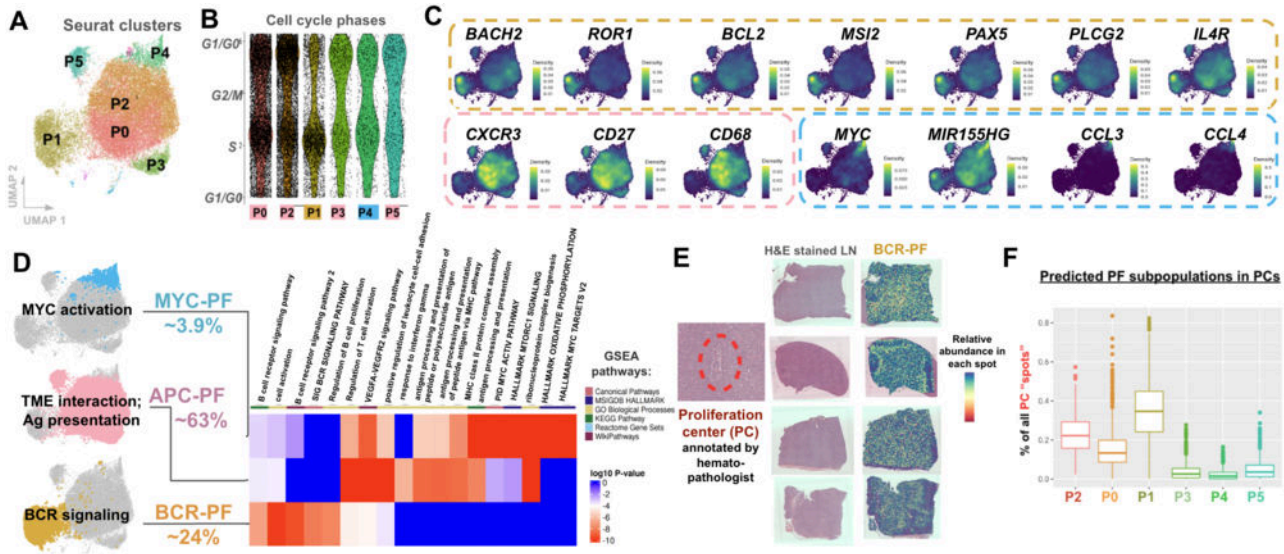

Fig. 1 UMAP clustering of 27890 PF cells (n=8). **B.** Cell cycle distribution determined by Tricycle. **C.** Density plots of genes associated with major clusters. **D.** Gene signature pathways enrichment determined by ssGSEA. **E.** H&E stained CLL LN Visium slide (n=4) with a representative PCs annotated in red circle and the relative abundance of PC cells similar to BCR-PF cluster from scRNA-seq. **F.** Predicted enrichment of PF subsets in PCs.

Figure 1

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