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641.CHRONIC LYMPHOCYTIC LEUKEMIAS: BASIC AND TRANSLATIONAL

Defining the Drivers of Idelalisib-Related Early Autoimmune Toxicity in Chronic Lymphocytic Leukemia

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Chronic lymphocytic leukemia (CLL) patients with disease resistant to BTK and BCL-2 inhibitors can potentially be treated with PI3K inhibitors to overcome resistance and improve outcomes. PI3K inhibitors such as idelalisib and duvelisib have shown promising efficacy in CLL patients, but severe toxicities remain a significant hurdle to their implementation in the clinic (PMID: 24615777). By investigating the molecular mechanisms that lead to severe early autoimmune toxicity with idelalisib, we hope to predict or prevent toxicity, thus expanding the use of this efficacious PI3K inhibitor class of drugs. To this end, the Brown lab showed that idelalisib-induced hepatitis is autoimmune. CyTOF analysis in CLL patients with early severe toxicity in a front-line idelalisib clinical trial cohort showed a significant decrease in CD4+ T regulatory cells (Treg) with a relative shift in favor of T effectors in peripheral blood. In addition, the Brown lab showed that the Th17 pathway is more active at baseline, with a further increase in patients with toxicity (PMID: 27247136, 36732326). Since the CYTOF data is restricted to fewer mostly T-cell markers in our studies, an unbiased single cell RNA sequencing (scRNA-seq) approach is warranted to explore the full effects of idelalisib on immune cells and CLL cells in patients with and without autoimmune toxicity. The question remains what molecular mechanisms drive the idelalisib-induced decrease in the Treg population, its effect on natural killer (NK) and CD8 T-cell function, and the activation of the Th17 pathway in CLL patients with early toxicity. We therefore carried out the first systematic effort to understand the mechanisms of idelalisib toxicity in molecular detail using combined scRNA and scTCR-seq.

In this study, we profile T-cell enriched PBMCs of matched pre-treatment and on-idelalisib treatment samples from CLL patients with early severe toxicity and without any toxicity (n=4 CLL patients from each group, a total of 16 samples) using 10X single cell transcriptomic and TCR profiling. Raw sequencing data files were processed using the Cell Ranger suite (version 7.1.0) from 10x Genomics. Sample identification based on SNP information was done using the Popscle suite. Seurat package (version 4.9.9.9) was used for the single cell analysis. After filtering cells with fewer than 600 features, less than 1000 UMIs, mitochondrial content higher than 15%, 51,487 cells were left for the downstream analysis. To explore transcriptional heterogeneity and perform initial cell clustering, principal component analysis and nonlinear dimensional reduction using UMAP were applied using 20 dimensions. Leiden algorithm was used for cluster identification. Cell types were identified using Azimuth with the PBMC reference dataset and confirmed with expression plots.

The initial observations of the cell proportions show that baseline samples from patients with early idelalisib toxicity have fewer Tregs, NK, and CD4+ cytotoxic T (CTL) cells compared to patients without toxicity. In addition, we observed a decline in Tregs, gamma-delta ($\gamma\delta$) T, and CD4+ CTL cells and a concurrent increase in proliferative CD4+ and CD8+ T-cells and proliferative NK on idelalisib treatment in the early toxicity group. On the contrary, CD4+ CTL and CD4+ T effector memory (TEM) cells are enriched with idelalisib therapy in patients without toxicities. At the same time, more CD4+ T-Cell Memory (TCM), $\gamma\delta$ T, CD8+ naive T, CD8+ TCM, and Mucosal-associated invariant T (MAIT) cells are present in pretreatment samples from patients with toxicity compared to patients without toxicity. Furthermore, proliferative T-cells are depleted with idelalisib therapy in patients without toxicity whereas they are enriched in patients with early toxicity. To further characterize idelalisib-induced depletion of Tregs, we reclustered them to identify distinct subgroups and found particular clusters of Treg were depleted in patients with early toxicity. In the non-toxicity group, more diversity of Tregs was identified with depletion of some clusters but

also an increase in a cluster. At present, we are integrating the scRNA & scTCR-seq data to characterize the cellular diversity and heterogeneous phenotypic states of the T-cell populations and updated results unraveling molecular mechanisms that drive early severe toxicity in CLL patients with idelalisib treatment will be presented at the meeting.

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