Similarities and Differences in Immune Modulation by Acalabrutinib and Zanubrutinib in Chronic Lymphocytic Leukemia

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INTRODUCTION

Covalent BTK inhibitors (cBTKi) have transformed the treatment of CLL. Acalabrutinib and zanubrutinib are next-generation BTKi with improved safety compared to ibrutinib, but their long-term immunomodulatory effects remain incompletely characterized. We conducted a two-year comparative study of their impact on immune cell subsets and plasma biomarkers.

METHODS

Thirty-four patients with CLL received either acalabrutinib (n=18) or zanubrutinib (n=16). Peripheral blood samples were collected at multiple time points up to 24 months (mo). Immune cell subsets cells were assessed by flow-cytometry. Plasma levels of 92 inflammatory proteins were quantified by proximity extension assay (Olink). BTK hotspot mutations were assessed by ddPCR. Age- and sex-matched healthy donors served as controls.

RESULTS

CD19⁺ B cells declined earlier with acalabrutinib (4 mo) than with zanubrutinib (6 mo). Baseline CD4⁺ and CD8⁺ T cells were elevated, more pronounced in the zanubrutinib group. Both subsets decreased significantly, with faster normalization on acalabrutinib. CD4⁺ levels reached those of healthy donors, while CD8⁺ remained elevated at two years. Th1 cells declined significantly; Th2 and Th17 changes were modest. Regulatory T cells normalized (Figure 1). T-cell memory subsets distribution, initially skewed toward effector subsets, shifted toward restoration, however, naïve CD4⁺ T cells declined below HD levels by 2 years (Figure 2). Exhausted T cells (PD-1⁺/TIGIT⁺) decreased in both cohorts but exhausted CD8⁺ cells remained higher than in healthy donors. Overall, immune normalization occurred earlier with acalabrutinib, paralleling its faster reduction in tumor burden.

Seventeen plasma proteins changed significantly in both cohorts, mostly decreasing, including the CLL-associated markers CCL3, CCL4, CD5, and CD6. Distinct biomarker signatures were observed: a transient rise in MMP1 and AXIN1 with acalabrutinib, and MCP2/MCP3 upregulation with zanubrutinib (Figure 3).

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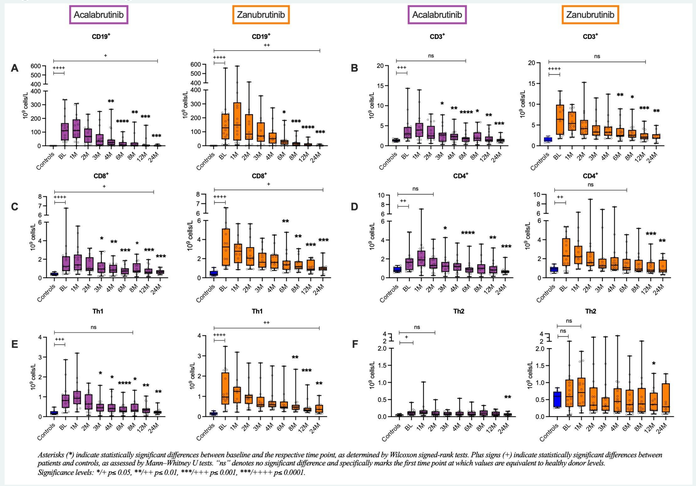
Figure 1. Changes in B cells and T-cell subsets



*Asterisks (*) indicate statistically significant differences between baseline and the respective time point, as determined by Wilcoxon signed-rank tests. Plus signs (+) indicate statistically significant differences between patients and controls, as assessed by Mann–Whitney U tests. "ns" denotes no significant difference and specifically marks the first time point at which values are equivalent to healthy donor levels. Significance levels: */+ ≤ 0.05, **/++ ≤ 0.01, ***/+++ ≤ 0.001, ****/++++ ≤ 0.0001.*

Figure 2. Changes of T cell memory subsets

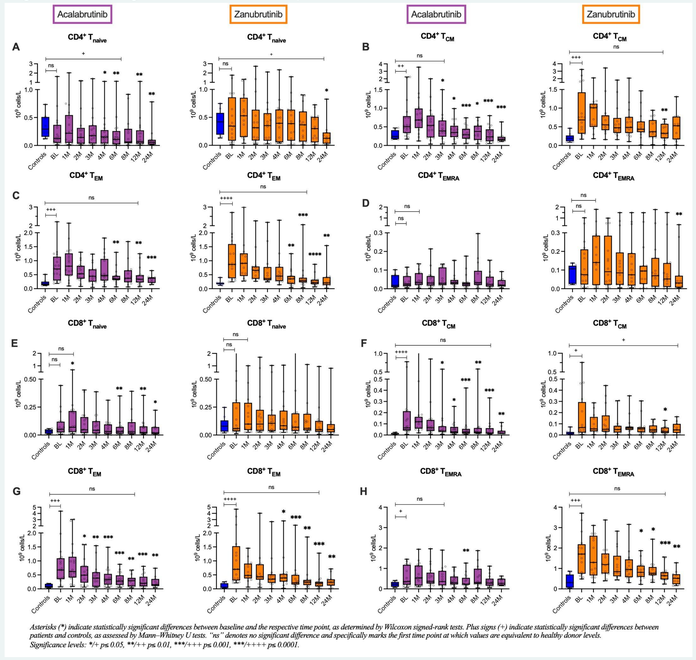


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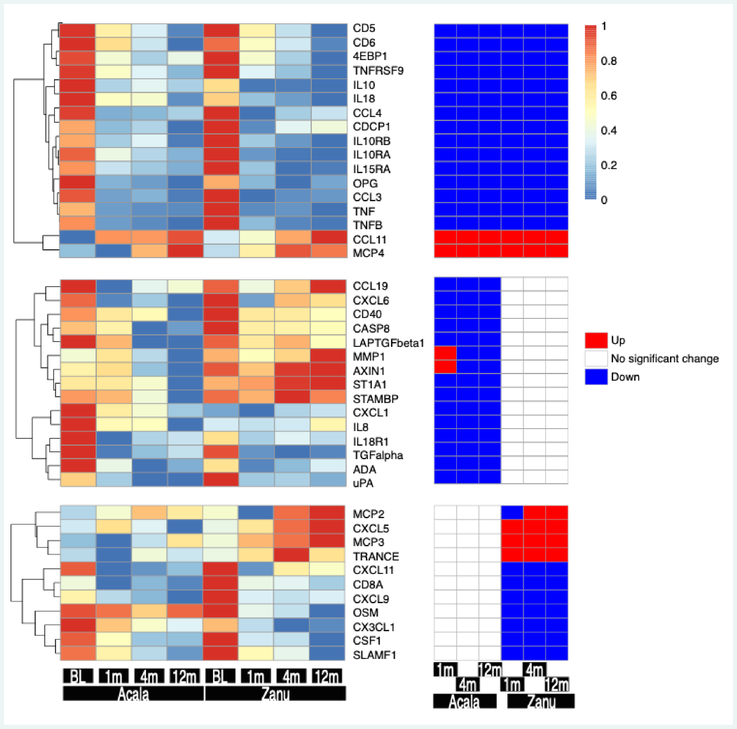
Figure 3. Plasma biomarker changes in patients treated with zanubrutinib or acalabrutinib, highlighting alterations observed in both cohorts and those specific to either treatment.



CONCLUSIONS

Immune restoration occurs during treatment with both cBTKi following disease control albeit with different time kinetics.

Such knowledge may guide on finding optimal timepoints regarding cell harvesting for cell-based therapies.

Despite similar immune reconstitution, distinct plasma protein profiles were observed with the two drugs suggesting differences in their off-target effects.