(PF584) PTPN2 INHIBITION RESTORES T-CELL FUNCTION AND POTENTIATES T-CELL ENGAGER ACTIVITY IN CHRONIC LYMPHOCYTIC LEUKEMIA

Topic: 05. Chronic lymphocytic leukemia and related disorders - Biology & translational research

Liron Yitzhak[1], Yamit Shorer Arbel[1], Ben-Zion Katz[1, 2], Yair Herishanu*[1, 2]

[1]Tel Aviv Sourasky University Medical Center, Hematology, Tel Aviv, Israel;[2]Gray Faculty of Medicine and Health Sciences, Tel Aviv University, Tel Aviv, Israel;

Background

Chronic lymphocytic leukemia (CLL) is characterized by profound T-cell dysfunction that limits the efficacy of emerging immunotherapies, including CD20×CD3 bispecific antibodies. Protein tyrosine phosphatase non-receptor type 2 (PTPN2) is a central intracellular negative regulator of T-cell activation, restraining both proximal TCR signaling and cytokine-driven JAK/STAT pathways. Preclinical studies show that genetic deletion or pharmacologic inhibition of PTPN2 enhances T-cell activation, proliferation, cytokine secretion, effector differentiation, and antitumor activity.

Aims

This study aimed to investigate the potential of PTPN2 inhibition in combination with the bispecific antibody mosunetuzumab as a therapeutic approach for CLL. We hypothesized that inhibition of PTPN2 in CLL-derived T-cells will restore functional competence and synergize with mosunetuzumab to produce potent cytotoxicity against malignant B-cells.

Methods

To evaluate this hypothesis, we conducted several assays such as flow cytometry, confocal microscopy, ELISA and Incucyte Live-cell Analysis system. CLL patients-derived PBMCs were used in our experiments (n=18).

Results

Our results show that pretreatment with the PTPN2 inhibitor AC-484 enhances T-cell activation following stimulation with CD3/CD28 beads. T-cell activation in PBMCs was assessed by flow-cytometric analysis of surface CD69 and CD25 expression. AC-484 pretreatment led to increased CD69 expression in both $CD4^+$ and $CD8^+$ T-cell subsets and significantly upregulated CD25 expression in $CD8^+$ T cells. Using confocal microscopy, we show morphological changes reflected in an increased CD8+ T-cell size, induction and release of granzyme B, formation of large CLL-CD8+ T cells clusters and strong CD8+ T-cell: CLL immune synapses in samples treated with mosunetuzumab +/- PTPN2 inhibitor. In parallel, cytotoxicity and T-cell proliferation assays were conducted in co-cultures of purified T cells and B-CLL cells using Incucyte™ time-lapse imaging over 7 days. Our findings indicate a modest increase in T-cell mediated cytotoxicity (day 3) and enhanced proliferative responses following PTPN2 inhibition in combination with mosunetuzumab. Similar to the findings observed with the confocal microscopy, the treatment also induced CLL-T-cell clustering. Using ELISA-based assay platform, we show increased levels of granzyme B in sampled treated with AC-484 and mosunetuzumab. In comparisons between samples of immunoglobulin heavy chain gene (IGHV)-mutated CLL (M-CLL) and IGHV-unmutated CLL (U-CLL), the effects are more pronounced in the M-CLL group.

Summary/Conclusion

In conclusion, in this study we demonstrate the potential of combining PTPN2 inhibitor with the bispecific antibody mosunetuzumab to enhance antitumor T-cell activity in patient-derived CLL samples. Using this combination, we showed increased expression levels of surface activation markers,T cells proliferation, morphological changes that were compatible with T-cell activation, increased granzyme B secretion and improved anti-CLL T- cell cytotoxicity. The findings of our research may contribute to the development of novel approaches that use PTPN2 inhibition to enhance cellular therapies in CLL, potentially leading to better clinical outcomes.