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POSTER

702. CAR-T CELL THERAPIES: BASIC AND TRANSLATIONAL

Venetoclax enhances T cell fitness and CAR T-cell manufacturing potential in chronic lymphocytic leukemia (CLL)

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Abstract IntroductionChronic lymphocytic leukemia (CLL) is marked by profound immune dysregulation, which compromises both the numerical and functional integrity of the T cell compartment, posing significant challenges for chimeric antigen receptor (CAR) T-cell therapy. CLL patients often exhibit an elevated expression of inhibitory receptors such as PD-1 and CTLA-4 on their T cells. Additionally, the immunosuppressive microenvironment in these patients is marked by the predominance of effector and effector memory T cells, which are less capable of sustaining long-term immune responses. Prior studies, including work from our group (Fraietta JA et al., 2018), have highlighted the importance of early memory T cell subsets in promoting CAR T cell expansion, persistence, and anti-tumor efficacy. However, these subsets are intrinsically rare in CLL, limiting the success of manufacturing strategies that rely on their enrichment or ex-vivo expansion. Studies by Ludwig LM et al. (2021) and van Bruggen JAC et al. (2022) demonstrated that short-term Venetoclax treatment selectively depletes naive T cells, but prolonged therapy led to their numerical recovery along with upregulation of OXPHOS and glycolysis pathways. However, the effects of Venetoclax on T-cell functionality and its implications for CAR T-cell manufacturing remain uncertain. We aimed to study if treatment with Venetoclax could improve the qualitative and quantitative defects in the T cells in CLL patients ensuring better CAR T-cells and identify the ideal timepoint following treatment to collect T cells for CAR T manufacturing.
MethodsWe analyzed peripheral blood samples from discovery (n=14) and validation (n=23) cohorts of CLL patients, with one or more prior lines of therapy, treated with Venetoclax monotherapy or in combination with the anti-CD20 antibody Obinutuzumab. Deep phenotyping of T cells and their response to activation with PMA and Ionomycin was performed using a spectral flow cytometry panel. CD19-directed CAR T cells (incorporating the 4-1BB costimulatory domain) were manufactured, and their proliferation and anti-tumor efficacy were assessed by flow cytometry and restimulation assays in vitro. Statistical analyses were performed using the Friedman test, 2-way ANOVA or Kruskal Wallis test, as appropriate.
ResultsTreatment with Venetoclax improved the qualitative and quantitative T cell defects with a significant increase in the proportion of naïve (CD45RA+CD27+CCR7+) T cells (both CD4+ and CD8+) and decrease in the effector memory (CD45RA-CD27-CCR7-) compartment. Both manual gating and unsupervised clustering analysis showed a significant decrease in the frequency of regulatory T cells (Tregs) and T cells expressing markers of effector differentiation (KLRG1, CD244, EOMES) and inhibition/exhaustion markers (PD1, CD39, CTLA4, TIGIT). The differences were more pronounced after 1 year of treatment and the findings were confirmed in an independent validation cohort. Upon activation, the treatment-naive samples showed a significant increase (percentage or absolute) in the expression of activation-exhaustion markers (ICOS, PD1, TIM3, TIGIT, CTLA4) while the increase was more restrained after Venetoclax treatment. CAR T-cells were manufactured and functionally assessed through co-culture and repeated stimulation with the aggressive, MHC-deficient CD19+ leukemia cell line NALM6. CAR T-cells manufactured with samples one year post treatment with Venetoclax exhibited greater fold expansion and population doublings on repeat stimulation with NALM6 cells compared to their paired treatment-naive samples. Cytokine analysis and cytotoxicity assays are currently being performed to strengthen our findings.

ConclusionOur findings strongly suggest that the BCL2 inhibitor Venetoclax exerts beneficial immunomodulatory effects beyond their primary anti-tumor activity. By reducing tumor burden and potentially directly influencing T cell biology, Venetoclax facilitates not only quantitative T cell recovery but also qualitative improvements. Such improvements include phenotypic shifts away from exhaustion and towards less differentiated states (potentially enriching for early memory precursors) and the restoration of key T cell functions like proliferation and cytokine production - factors that may significantly improve CAR T cell manufacturing and therapeutic efficacy.

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