255 FUNCTIONAL AND MULTI-OMIC CHARACTERIZATION OF CAR T CELLS ACROSS HEALTHY DONORS

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Background The utilization of Chimeric Antigen Receptor T cells (CAR-Ts) has demonstrated exceptional efficacy in the treatment of cancer, revolutionizing the field of drug development through the use of genetically modified T cells as a therapeutic approach. However, the development of these therapies presents distinct challenges due to the inherent variability among individuals. The precise mechanisms that drive clinical outcomes remain inadequately understood. In this study, we exploit the variability observed in healthy donors and employ multi-omics analysis to discover proteomic and genomic indicators linked to enhanced functionality of CAR-T cells.

Methods Human CD8+ T cells were isolated from unique healthy donors Peripheral Blood Mononuclear Cells (PBMC) and simultaneously stimulated using anti-CD3/28 and transduced with lentiviral particles containing CD19 CAR (CD3z, 28z, or BBz) P2A GFP. 7 days post transduction CAR T cells were sorted to >95% purity and cocultured with the NALM6 cells at various ratios. Live cell imaging was used to count target cells over a 48h period and determine a cytotoxicity score. Purified naïve CD8+ T cells and resultant CAR T cells were subjected to extensive DNA Methylation Array and Proteomic analysis. Multi-omic analysis on the top and bottom 25% performing CAR T cells was conducted.

Results Functional analysis revealed a wide range of CAR T cell variability that does not correlate with age (28z, BBz, CD3z respectively R^2 =0.01578, 0.08983, 0.06378, p-value= 0.6194, 0.2425, 0.3281). However, we did find a significant positive correlation in DNA methylation informed Age(mAge) and with expression of inhibitory receptors during T cell activation, specifically CLTA-4 (R^2 = 0.33, p-value= 0.025) and TIGIT (R^2 = 0.29, p-value= 0.036). Further we found a negative correlation of CAR T cell cytotoxicity and the expression of the exhaustion-associated transcription factor TOX2 (28z, BBz, CD3z respectively R^2 = 0.4271, 0.2776, 0.2847, p-value= 0.0033, 0.0298, 0.0274). Full exploratory proteomic analysis or naïve CD8+ T cells and CAR T cells is presented comparing the top and bottom 25% performing CAR T cells.

Conclusions A large amount of variability exists in healthy donor CAR T cells that cannot be explained by chronological age. Here we leverage this variability to identify new proteomic and genomic biomarkers associated with CAR T cell function. These findings have the potential to inform CAR T cell monitoring and engineering strategies.

http://dx.doi.org/10.1136/jitc-2023-SITC2023.0255