Combining Single Cell RNA Sequencing With Functional Analysis: An Analytic Pipeline to Optimize Selection of CAR T Cell Therapy vs. BiTEs

George, Anand (School: University School of Milwaukee)

Chimeric Antigen Receptor (CAR) T Cells and Bispecific T Cell Engagers (BiTEs) have revolutionized the treatment of relapsed/refractory hematologic cancers. However, there are no head-to-head comparisons testing differences in their ability to induce T cell activation - a surrogate for their efficacy and toxicity. It was hypothesized that differences in downstream signaling by anti-CD19 CAR-T cells and anti-CD3/CD19 BiTEs will lead to differential T cell fu??nctional capacity and transcriptional profiles. Once the CAR T cells and T cells co-cultured with BiTEs were activated with the target bead, the Beacon Optofluidic system and Singular Genomics G4 Sequencer were used to analyze differences in their phenotype, functional capacity, and transcriptional profiles. Signal seeking and validation experiments were performed to develop an analytic pipeline. Functional analysis demonstrated differential expression of cytokines (IFN-y, TNF-a, Granzyme B, Perforin) in CAR T cells compared to T cells with BiTEs. CAR T cells showed increased likelihood of exhaustion and greater polyfunctionality (both CD8+ and CD4+ T cells) in cytokine secretion than T cells with BiTEs. Single cell RNA sequencing demonstrated a transcriptional basis for the differential cytokine expression between CAR T cells and T cells with BiTEs, which was confirmed by concordant protein expression at single cell resolution. The distinct mechanistic differences between CAR T cells and BiTEs indicate the need to balance toxicity with therapeutic efficacy in treatment selection. These results demonstrate the feasibility of developing an analytic pipeline to analyze functional, transcriptional, and proteomic data at single cell resolution to help personalize cellular therapies.

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