975 CHARACTERIZATION OF SIGNALING AND METABOLIC DIFFERENCES BETWEEN γδ AND αβ CAR T CELLS

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Background γδ T cell-based immunotherapies have emerged as an alternative to the traditional αβ T cell-based products. For example, our group has shown that γδ CAR T cells can reduce tumor burden and mitigate tumor-induced bone deterioration in a preclinical model of bone metastatic prostate cancer. In the present study, we investigated the signaling events triggered by a second-generation CAR (originally designed for αβ T cells) in γδ T cells, to test the hypothesis that CAR-induced signaling varies depending on the T cell subset. The ultimate goal of our study is to gain mechanistic insight into the biology of γδ T cells and to inform future CAR design for improved γδ-based adoptive cell therapies.

Methods We designed our analysis as a side-by-side comparison of phosphorylation events, resulting from CAR activation, between γδ and αβ T cells. These were expanded in parallel from a healthy donor and transduced with a retroviral vector (MSGV1) to express a second-generation anti-PSCA CAR (PSCA-8t28z). CAR-T cells were then cocultured independently with metabolically labelled C4-2B-PSCA tumor cells, for 1 hour, and protein phosphorylation was quantified using **L**iquid **C**hromatography–**T**andem **M**ass **S**pectrometry (LC/MS-MS). Statistically significant differences in phosphorylation were defined using a Welch's *t*-test (fold-change $\geq$ 1.5 and *p*-value $<$ 0.05). Using Qiagen's Ingenuity Pathway Analysis software, we identified canonical pathways that were significantly over-represented in the population of proteins that displayed differential phosphorylation in γδ CAR T cells relative to αβ. We used the SeaHorse XF kits for Glycolytic Rate Assay and T Cell Metabolic Profiling for functional characterization of the metabolic properties of CAR-T cells. Finally, flow cytometry was used to analyze Glut-1 expression (anti-Glut1, clone 202915), glucose intake (2NBDG), mitochondrial mass (Mito-Tracker Green), and mitochondrial membrane polarization (TMRE).

Results We identified 323 phosphorylation events that were differentially abundant between T cell subsets. Within this group, glycolysis and gluconeogenesis were within the top overrepresented canonical pathways. Stimulated γδ T cells showed significantly lower glycolytic rate compared to αβ. CAR expression was accompanied by higher glycolytic rate and expression of Glut-1 receptor in both T-cell types. Finally, oxidative phosphorylation (OXPHOS) was lower in γδ CAR T cells, potentially related to their also lower mitochondrial mass.

Conclusions CAR-induced signaling varies among T cell subsets, and γδ T cells display lower glycolytic and OXPHOS rates upon activation. Ongoing efforts are focused on delineating molecular causes and functional consequences of the metabolic differences between αβ and γδ T cells.

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Ethics Approval All animal experiments were performed under University of South Florida IACUC approval (R1762; R7429) and in accordance with the Guidelines for the Care and Use of Laboratory Animals manual published by the National Institutes of Health.

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