SEPARATING THE WHEAT FROM THE CHAFF: ENGINEERING CARTS WITH SUPERIOR METABOLIC ATTRIBUTES AGAINST SOLID TUMORS

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Background For cellular immunotherapies, clinical outcomes depend on the proliferative potency and metabolic fitness of the therapeutic product. For their most successful indications, CAR T cells are effective ‘serial killers,’ each T cell recognizing and eliminating many target cells. What fuels CAR T cell serial killing is unknown. Two critical events define the efficiency of T cell serial killing: migration and immune synapse formation/cytolysis. Importantly, each event is influenced by the local metabolic milieu.

Methods Using a specialized CAR T cell conditioning regimen, the goal of this research to is determine the relative energy cost of migration versus cytolysis using innovative eSIGHT RTCA technology. Our project will reveal how the spare respiratory capacity (SRC), supports CAR T cell migration and/or cytolysis, founded on the hypothesis that cells that can replenish their SRC in repetitive antigen stimulation models, are more efficient serial killers.

Results Our data sheds light on critical metabolic states that impair CAR T cell cytolytic activity. We also use multi-omic approaches to identify arginosuccinate synthase 1 (ASS1), a gene distinguishable at the metabolic (Seahorse), transcriptional (RNAseq), epigenetic level (ATAC seq), and functional (tumor clearance in vivo) in CAR T cells. Supporting the premise of our work, we show that ASS1 supports high SRC levels despite frequent antigen encounter in repetitive stimulation models in vitro. In parallel work, we provide data that reductive glutamine metabolism is enhanced in 28zCARTs, suggesting mechanisms for why 28zCARTs outperform BbZ CARTs in some hypoxic tumor models (figure1B).

Conclusions Our findings reveal unique conditioning and genetic strategies to arm CAR T cells with unique metabolic attributes against solid tumors.