Background

Signaling cascades triggered by intracellular domains (ICDs) of Chimeric Antigen Receptor (CAR) in immune cells drive cell behaviors that correspond to different therapeutic outcomes. While canonical CAR-T ICD combinations 4-1BB-CD3z (BBz) and CD28-CD3z (28z) have achieved clinical success in a limited number of hematologic indications, CAR-based cell therapies have not yet been successful in more challenging indications. Systematic discovery of novel ICD combinations that drive more favorable T cell phenotypes has been onerous due to technical constraints in high-throughput screening and scaling.

Methods

Here, we developed a versatile platform that allows parallel comparison of 10,000 different 2nd generation CAR ICD combinations in primary CD8+ T cells. We utilized combinatorial genetics en masse (CombiGEM) to assemble multiple DNA-barcoded lentiviral libraries of CAR ICDs distinguished by the affinity of extracellular binder and the length of hinge/spacer. We screened CAR ICD libraries for T cell persistence and fitness in a serial tumor rechallenge assay in a pooled manner.

Results

Precisely tracking enriched barcodes over the course of 20 days allowed us to identify novel CAR clones with superior T cell proliferation and survival relative to conventional BBz and 28z CARs. Hits were validated in downstream arrayed studies with additional functional characterization including cytotoxicity, cytokine secretion, and T cell memory and exhaustion phenotypes in the serial tumor rechallenge assay.

Conclusions

We believe our approach is readily applicable to diverse immune cell types, including T cell subsets, NK cells and macrophages, as well as different CAR domains including binders, structural components, and armoring components in addition to ICDs. Rapid and systematic generation and comparison of synthetic molecules at scale in pooled libraries via our platform will further elucidate design principles to guide next generation cell therapies engineering strategies.