247 FOXO1 IS A MASTER REGULATOR OF CAR T MEMORY PROGRAMMING

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Background CAR T cell therapy is a promising therapeutic modality for cancer treatment, but poor CAR T cell persistence limits efficacy in patients.[1] CAR T cells with a memory-like phenotype are associated with durable persistence in patients and response to therapy,[2] thereby implicating memory as an important therapeutic axis. Thus, strategies to stably promote CAR T memory differentiation are urgently needed. Here, we demonstrate that FOXO1 is required for the development of memory CAR T cells and that FOXO1 overexpression maintains memory gene expression programs and enhances CAR T cell antitumor activity in liquid and solid tumor models.

Methods T cells were co-transduced to express a CAR and a transcription factor, and in relevant experiments were CRISPR-edited using Cas9. CAR T cell function was assessed *in vitro* using tumor co-culture assays wherein cytokine secretion, killing, and metabolic fitness (via Seahorse) were measured. *In vivo* studies were performed using murine xenograft tumor models in NSG mice. CAR T phenotyping experiments were performed using flow cytometry, RNA-seq, and ATAC-seq. Public datasets were reanalyzed to evaluate FOXO1 activity in patients treated with CAR T cell therapy.

Results FOXO1 knockout in human CAR T cells prevented the development of a memory-like phenotype, and instead, promoted an exhausted phenotype and gene expression profile and attenuated antitumor activity *in vitro* and *in vivo*. FOXO1 overexpression in CAR T cells dramatically enhanced functionality *in vitro* and *in vivo*, especially in models of chronic antigen stimulation, and promoted a memory-like phenotype via flow cytometry, RNA-seq, and ATAC-seq. A FOXO1 regulon consisting of 41 putative FOXO1 target genes, which were unbiasedly identified in our knockout and overexpression studies, highly correlated with long-term persistence and positive outcomes in patients treated with CD19 CAR T cells.

Conclusions Our results demonstrate that FOXO1 is a master regulator of CAR T cell memory programming and that overexpression of FOXO1 triggers both transcriptional and epigenetic changes in CAR T cells that enhance memory differentiation, persistence, and potency. Our findings further demonstrate the potential for transcription factor engineering as an approach to generate highly effective CAR T cell products for antitumor therapy.

REFERENCES

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Ethics Approval All animal studies were undertaken under Stanford University APLAC #31287 and Children's Hospital of Philadelphia ACUP-approved protocols.

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