Orthogonal IL-2/IL-2Rβ Signaling Selectively Enhances and Sustains a Synthetic Effector State And Outperforms Constitutive Armoring Approaches

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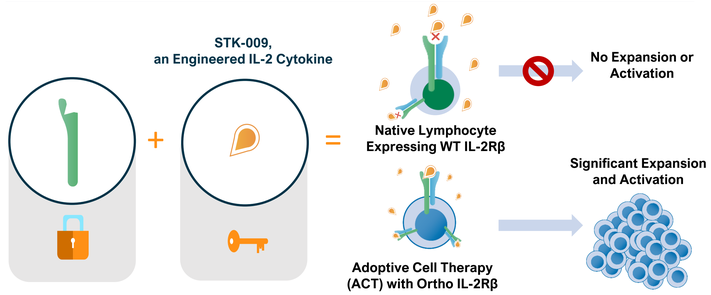
Abstract

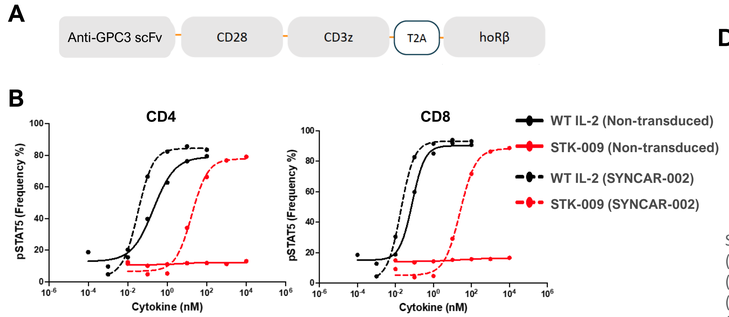
CAR T-cell therapy shows promise against hematological malignancies, yet its efficacy is often hampered by limited proliferation, persistence, and effector function. We demonstrate that orthogonal IL-2 signaling enhances antitumor potency of CAR T-cells in resistant cancer models and outperforms existing CAR-T armoring strategies in both efficacy and toxicity. Orthogonal IL-2 drives an unconventional effector cell state characterized by enhanced cell cycle progression and persistence and a diminished stress response. Mechanistically, orthogonal IL-2 promotes MYC expression by dampening proteasome activity, thereby fostering effector differentiation. These findings offer novel mechanistic insights into how IL-2 regulates T cell fate and provide an actionable armoring strategy to reprogram T-cells into a favorable effector state.

Orthogonal Cytokine + Cell Therapy: A Lock and Key System to Stimulate ACTs Selectively In Vivo



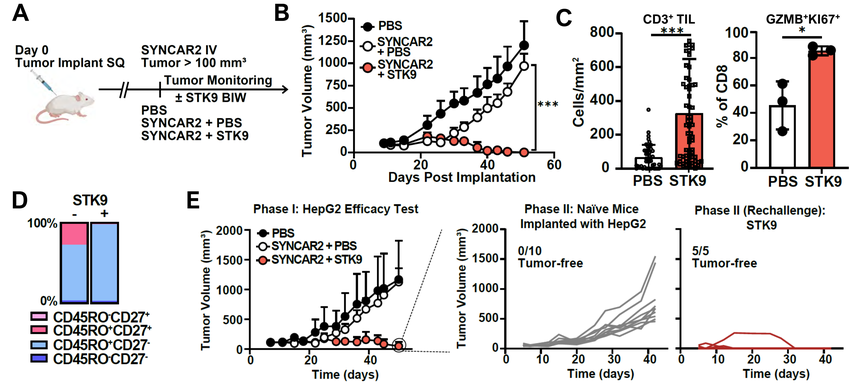
OrthoIL-2Rβ receptors (hoRβ) exhibit significant preference for their cognate ligand, STK-009, a pegylated orthogonal IL-2. Therefore, engineered T cells expressing the ortho receptor will respond selectively to STK-009, thereby allowing specific expansion and enhancement of engineered T cell activity.

SYNCAR-002: Anti-GPC3 CAR T cells Co-expressing hoRβ that Specifically Respond to STK-009 and Maintain Response to WT IL-2



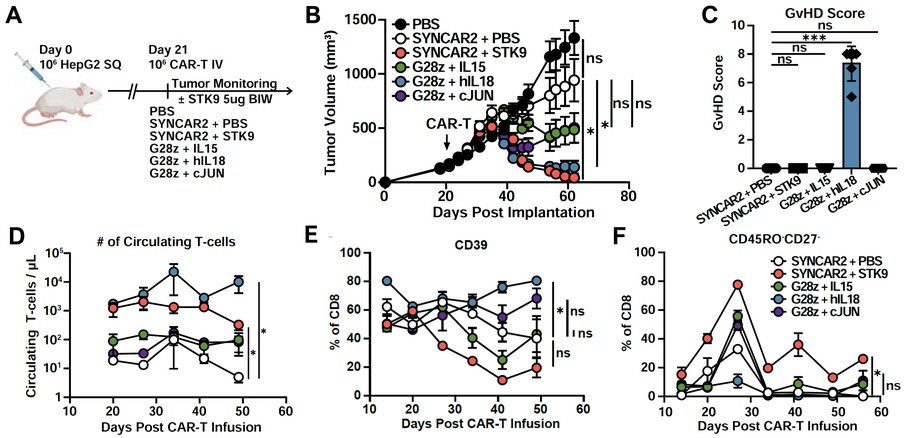
SYNCAR-002 architecture and specific response to STK-009. (A) Lentiviral construct (B) Phospho-STAT5 signaling assay. Non-transduced and SYNCAR-002 cells were treated with either WT IL-2 or STK-009 for 20 min and processed for pSTAT5 analysis.

Orthogonal IL-2 Signaling Induces A Durable Effector Cell State and Enhances Antitumor Potency of CAR T-cells



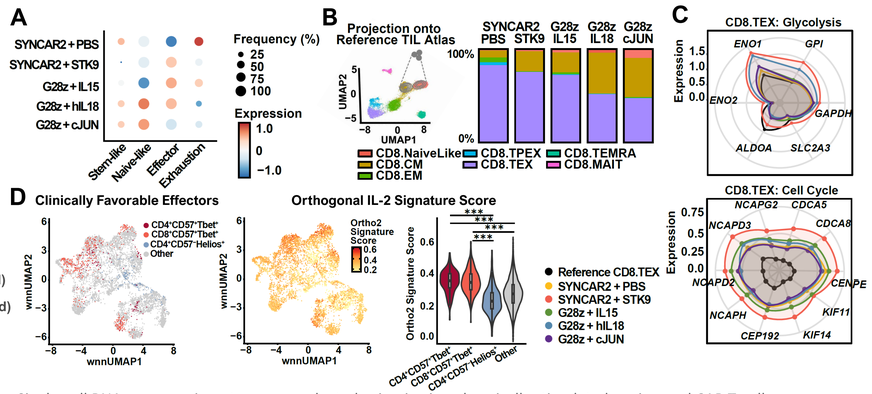
(A) Schematic of the SQ HEPG2/NSG mouse model study design.
(B) Longitudinal tumor growth.
(C) Quantitation of tumor infiltrating CD3⁺ (left) and GZMB⁺KI67⁺ (right) via IHC.
(D) STK9 improves effector differentiation of SYNCAR T-cells in the tumor.
(E) Mice with HepG2 tumors that received SYNCAR-002 T-cells and STK-009 were cured by Day 50. The cured and tumor-naïve mice were rechallenged with HepG2 tumor and treated with STK-009.

Orthogonal IL-2 Armoring Outperforms Other CAR-T Armoring Strategies in Efficacy and Safety



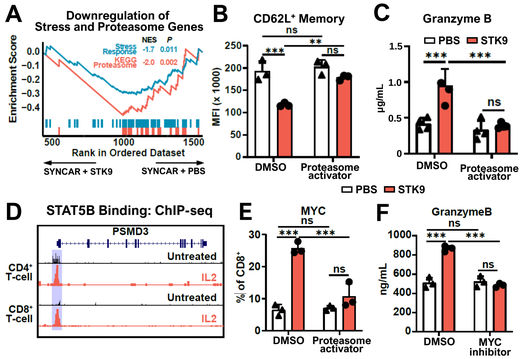
(A) Schematic of the SQ HepG2/NSG mouse model study design (B) Longitudinal tumor growth. (C) GvHD scores measured on day 62 post CAR T-cell infusion. (D) CAR T-cell expansion kinetics in peripheral blood. Frequencies of circulating CD3⁺CD8⁺ cells expressing (E) CD39⁺ and (F) CD45RO⁻CD27⁻.

Orthogonal IL-2 Signaling Promotes a Favorable Synthetic Effector Cell State Characterized by Enhanced Proliferation and Glycolysis Pathways



Single-cell RNA-seq experiments were conducted using *in vitro* chronically stimulated engineered CAR T-cells.
(A) Gene signatures scores associated with T-cell stemness, naive, effector, and CAR-T exhaustion.
(B) Projection of single cell data onto the reference TIL atlas. The bar charts indicate composition per sample.
(C) Expression of genes associated with glycolysis (top) and cell cycle progression (bottom) in exhausted cells.
(D) Circulating CAR T cells were sorted from large B cell lymphoma patients on day 7 after Axicabtagene ciloleucel infusion for CITE-seq (GSE168940). Left: CD4⁺CD57⁺Tbet⁺, CD8⁺CD57⁺Tbet⁺, and CD4⁺CD57⁻Helios⁺ populations are highlighted. Middle: Score of orthogonal IL-2 signaling gene signature enriched in tumor-infiltrating SYNCAR2 T-cells upon STK-009 treatment. Right: Orthogonal IL-2 gene signature scores across four CAR T-cell populations.

Orthogonal IL-2 Signaling Enhances Effector Differentiation by Suppressing Proteasome Gene Expression



(A) Gene set enrichment analysis of SYNCAR-002 T-cells treated with STK-009 versus PBS (gene set: Stress response, KEGG proteasome).
(B, C) Expression of CD62L and Granzyme B in CAR T-cells following chronic CAR activation with or without proteasome activator.
(D) Representative ChIP-seq tracks are shown for PSMD3 locus (CD8+ T-cell: PMID25992859, CD4+ T-cell: PMID21516110).
(E) Expression of MYC in CD8⁺ CAR-T cells after chronic CAR stimulation with or without STK-009 and a proteasome activator.
(F) Granzyme B production with or without STK-009 and a MYC inhibitor.

CONCLUSION

In addressing the challenge of limited antitumor potency in solid and resistant hematological malignancies, various CAR T-cell strategies have been explored. We conducted a thorough comparative analysis of our SYNCAR platform with existing CAR T-cell armoring strategies, exploring their distinct impacts on T-cell biology and antitumor effectiveness. We found that

- SYNCAR T-cells outperform existing armoring strategies which reprogram CAR T-cells toward memory.
- Orthogonal IL-2 signaling induces a **durable synthetic effector cell state** distinct from canonical effector and exhausted cells.
- Synthetic effector state of SYNCAR T-cells are characterized by **enhanced cell cycle progression and persistence**, diminished type I interferon and stress response, and resistance to dysfunction.
- The orthogonal IL-2-driven effector differentiation is mediated by suppression of proteasome activity which leads to upregulation of MYC.

Therefore, our study demonstrates superior antitumor activity of the SYNCAR platform in both efficacy and toxicity and provides novel mechanistic insights into how orthogonal IL-2 signaling drives a potent and persistent effector state.