Engineered human IL-2/IL-2Rβ orthogonal pairs selectively enhance CAR T cells to drive complete responses in hematological and solid epithelial tumor models

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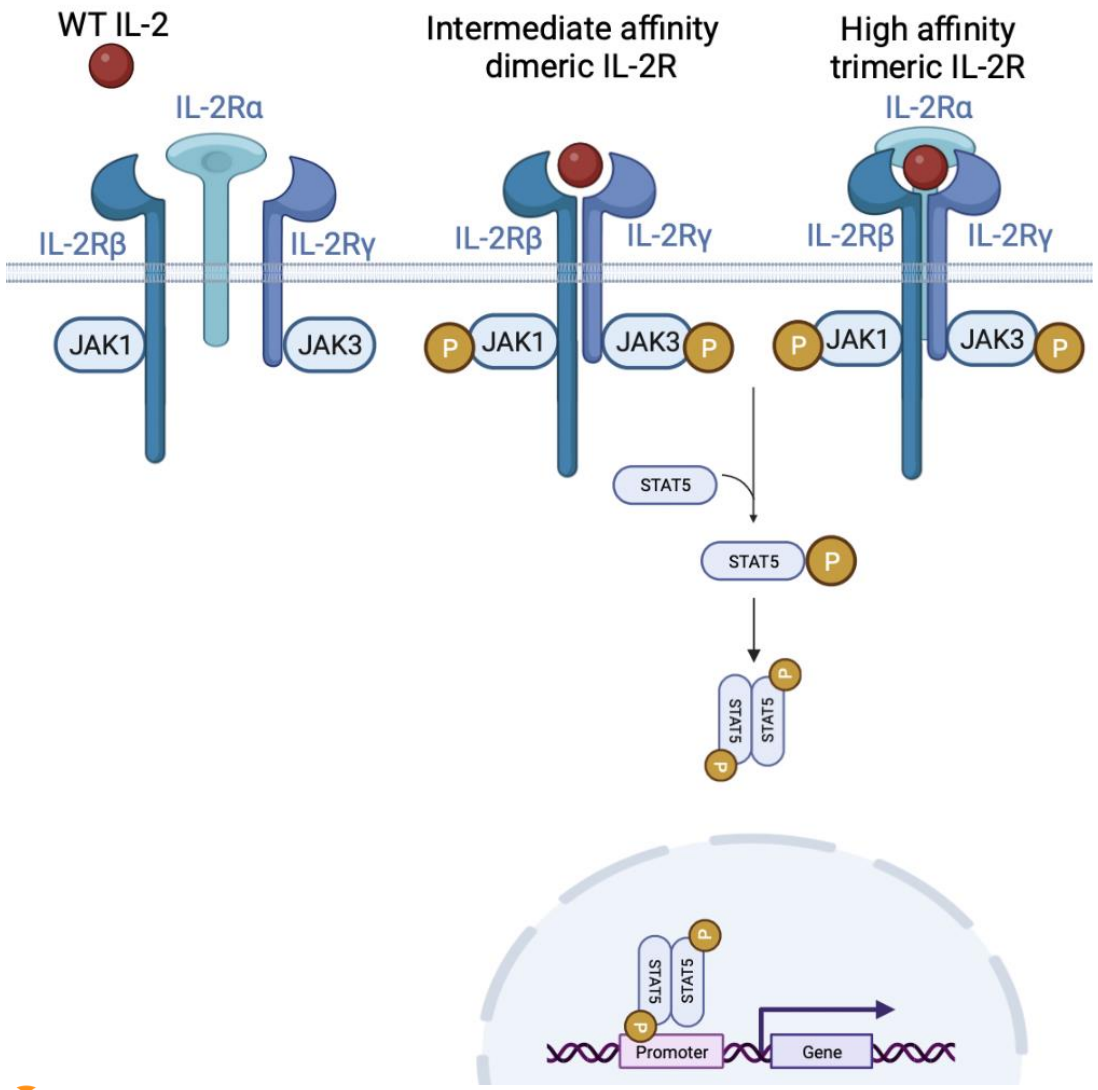
Synthekine

Menlo Park, California

IL-2: A Potent Cytokine to Armor Adoptive T Cell Therapy



- IL-2 is a pleiotropic cytokine that positively influences the homeostasis and development of different T cell lineages and other immune cells (e.g. NK cells and eosinophils)

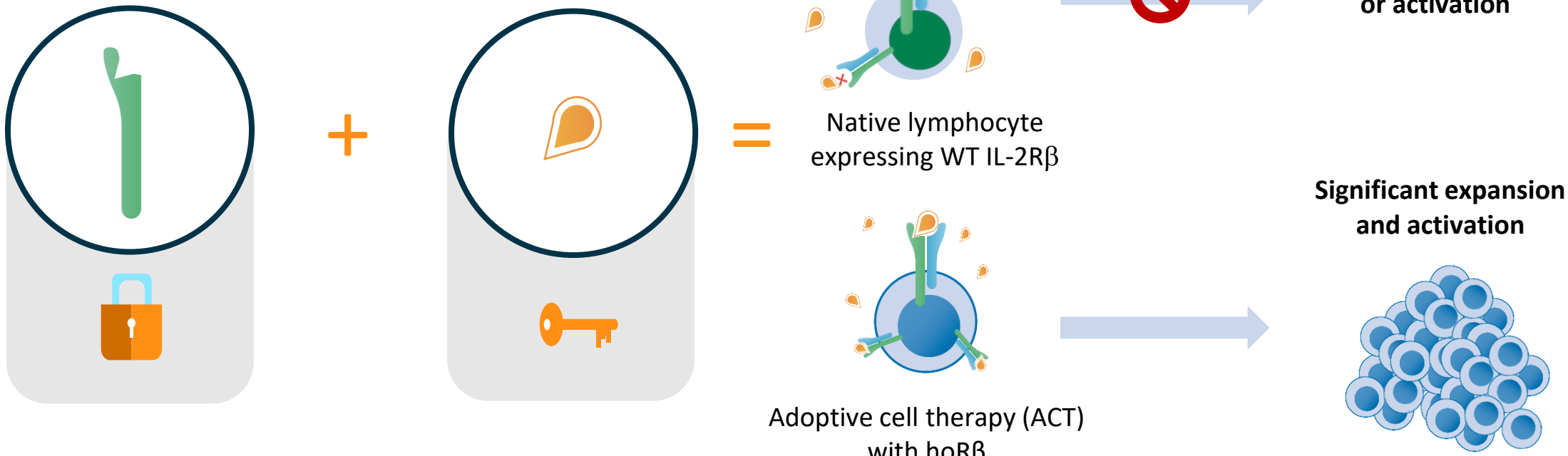
- IL-2 signals through the stepwise assembly of the IL-2R complex to primarily activate the JAK/STAT5 pathway
  - IL-2Rα increases IL-2 affinity towards IL-2Rβ, then binds to the common gamma chain IL-2Rɣ

- Recombinant IL-2 (Proleukin) is used as a monotherapy and in combination with TCR and TIL therapies
  - Limited by significant, life-threatening toxicity (small therapeutic window)
    - Capillary leak syndrome (CLS) and hypotension are mediated by non-selective activation of immune cells

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

hoRß, an engineered IL-2 receptor beta subunit

STK-009, an engineered IL-2 cytokine



Potential to be incorporated into a wide range of ACTs, including CAR-Ts (SYNCAR-001, SYNCAR-002), TCRs, TILs, and Tregs

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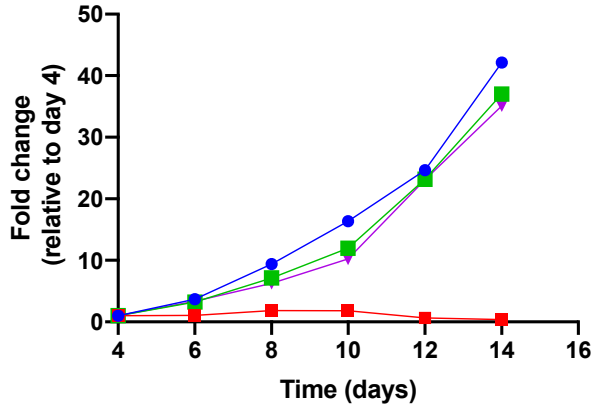
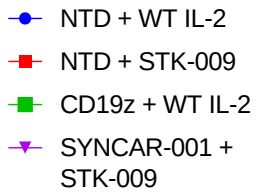
Sockolosky et al. Science 2018, Zhang et al. Sci. Trans. Med. 2021, Aspuria et al. Sci. Trans. Med. 2021

CAR Manufacturing in Ortho IL-2 (STK-009) and Specific Expansion of hoRβ Expressing CARs

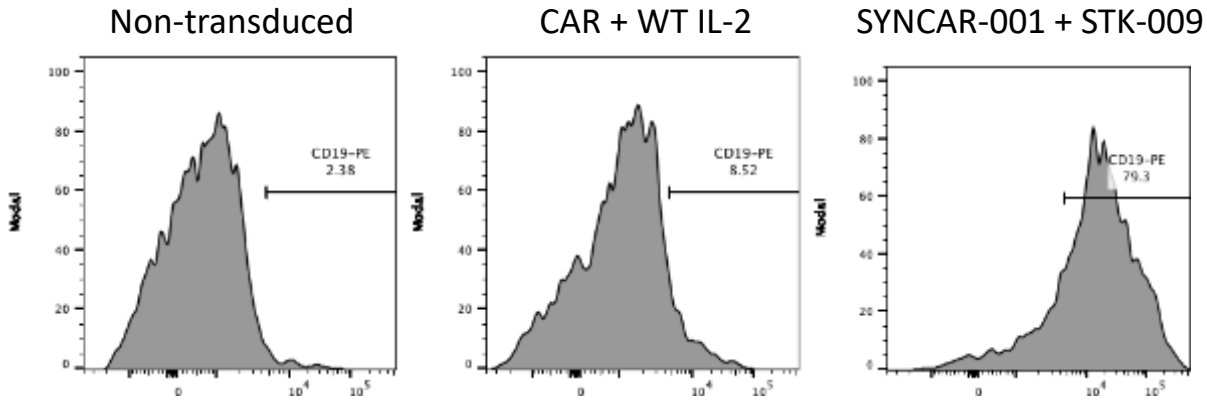
SYNCAR manufacturing in STK-009 has equivalent growth to conventional CAR manufacturing with WT IL-2





SYNCAR-001 switch to STK-009 @ Day 4 of manufacturing

SYNCAR manufacturing in STK-009 significantly enriches for CAR transduction vs traditional CAR manufacturing methods



CAR transduction

@ Day 14 (last day of manufacturing)

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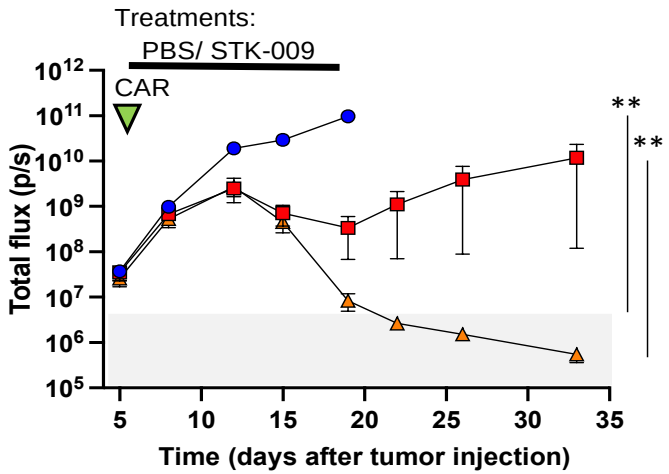
STK-009 + SYNCAR-001 Demonstrates Improved Activity Versus the CD19 CAR Alone

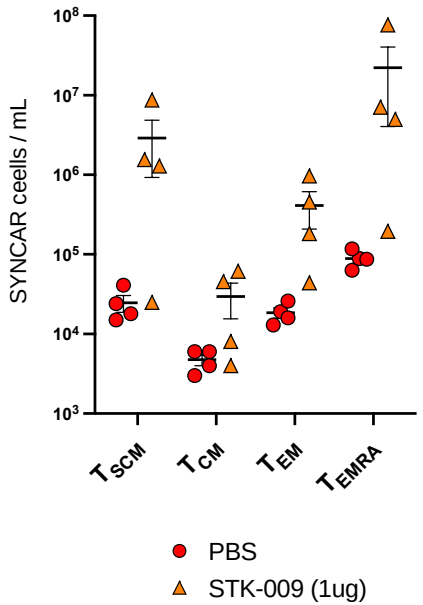
RAJI disseminated tumor model in mice, dosed with suboptimal SYNCAR-001 dose -/+ STK-009

Complete responses with STK-009

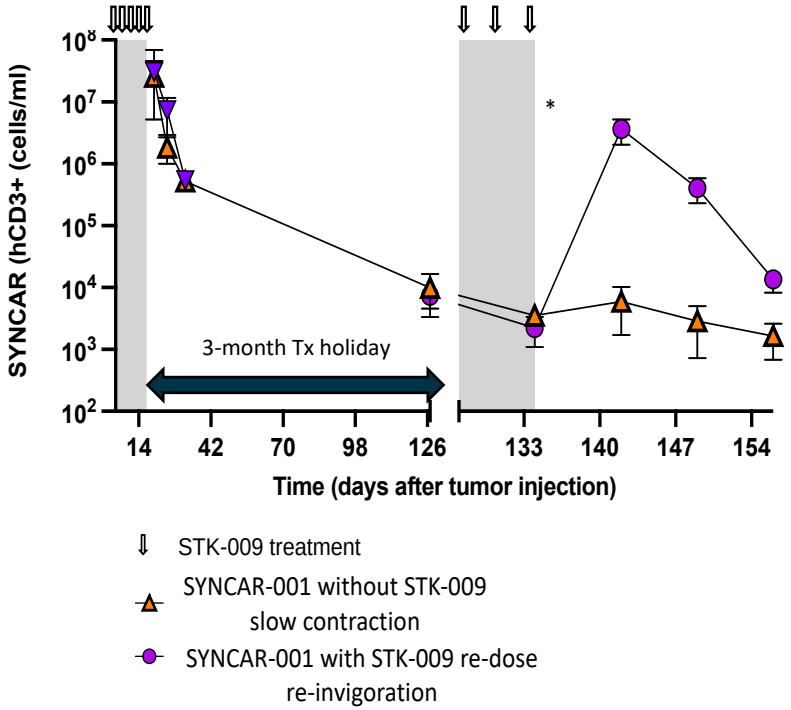


	Treatment	CR
	PBS	0 %
	SYNCAR + PBS	50 %
	SYNCAR + STK-009 1∝g	100 %

Unbiased expansion of immunophenotypes



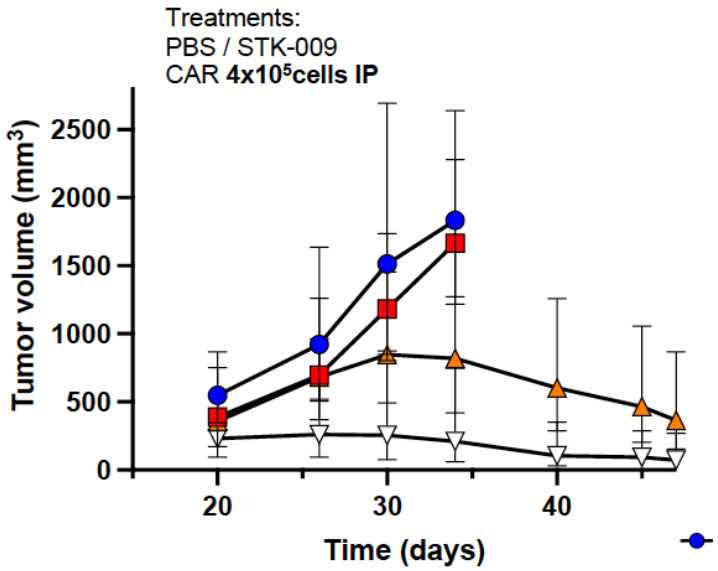
STK-009 re-treatment drives T cell re-expansion



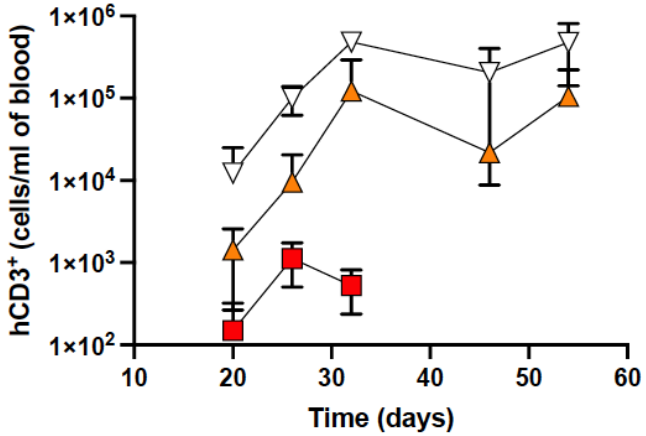
STK-009 + SYNCAR-001 Induce Responses in a Subcutaneous RAJI NSG Model Characteristically Resistant to CAR Ts

RAJI subcutaneous tumor model in mice, dosed with 400,000 SYNCAR-001 cells and with STK-009

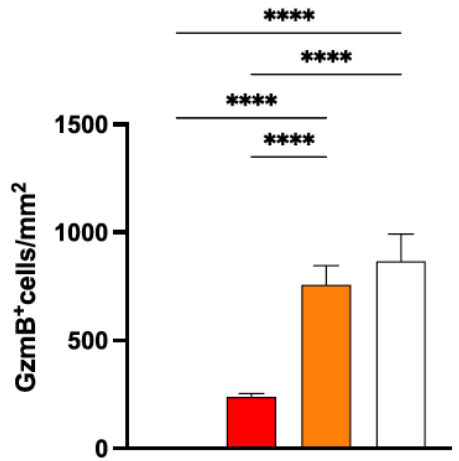
SYNCAR-001 + STK-009 induces tumor shrinkage

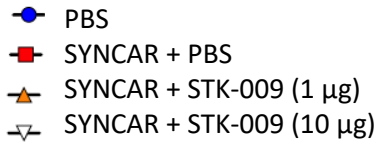


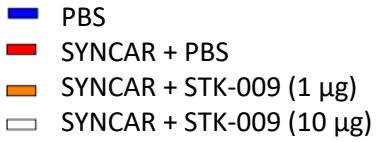
STK-009 expands SYNCAR-001 in systemic circulation



STK-009 activates SYNCAR-001 in the tumor



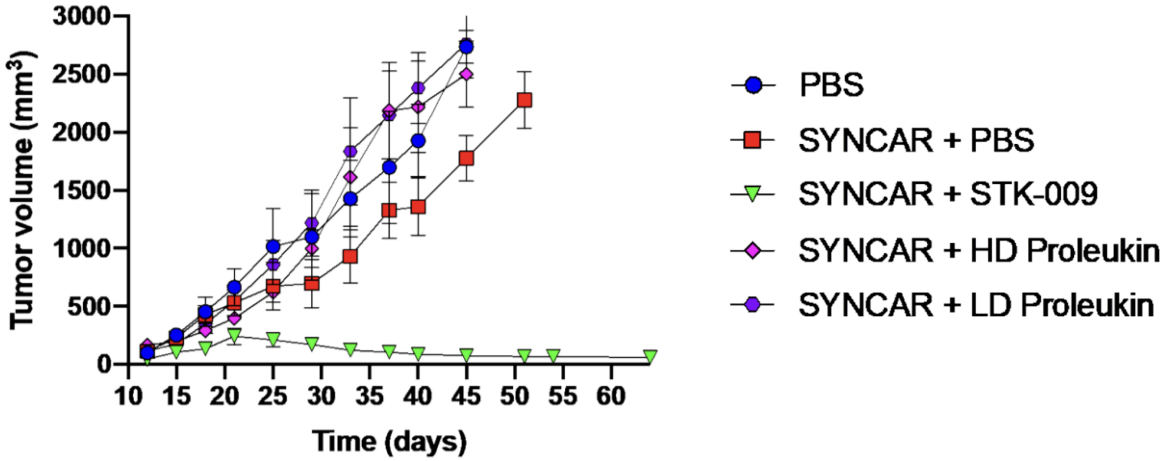




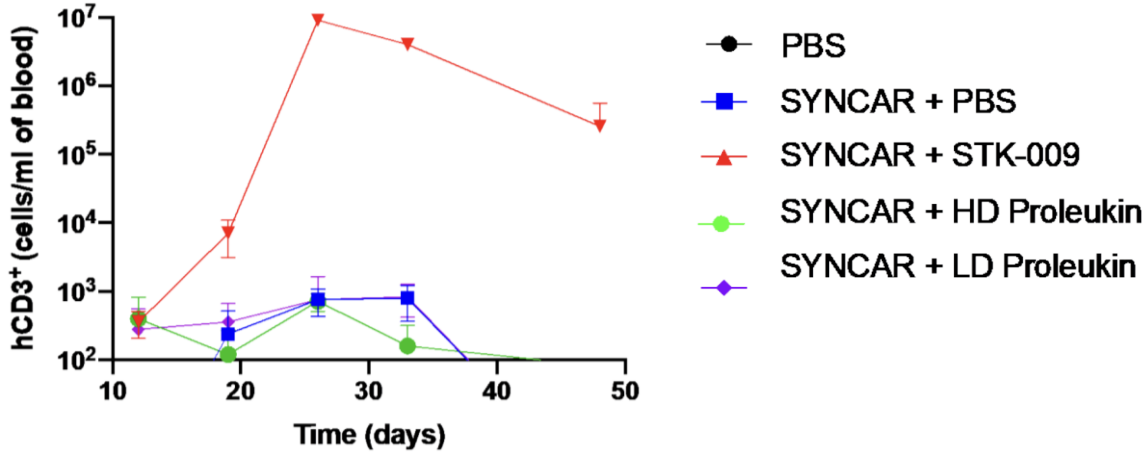
STK-009 But Not Proleukin Enables Tumor Rejection and Eliminates Liver Metastases

RAJI subcutaneous tumor model

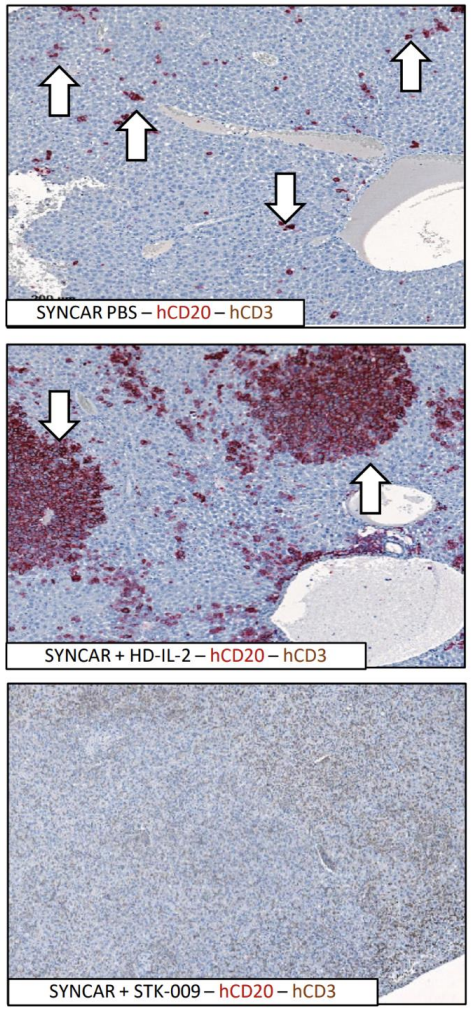
Tumor volume



hCD3 cells



Liver IHC



⇩ *Liver Metastasis*

Proleukin enhances liver met growth

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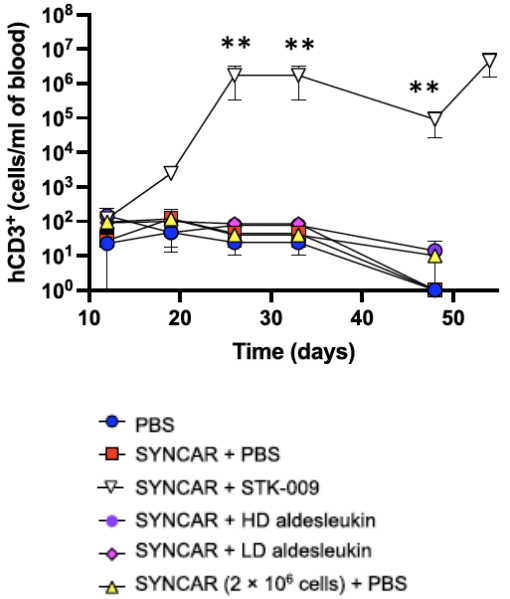
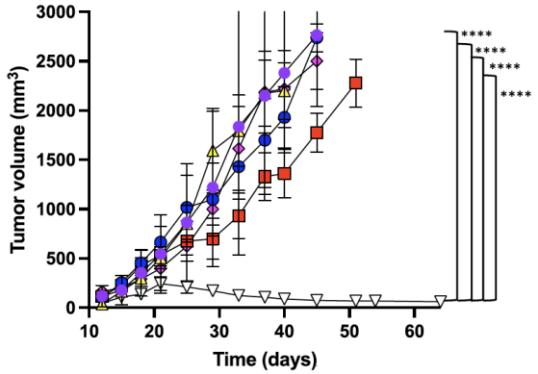
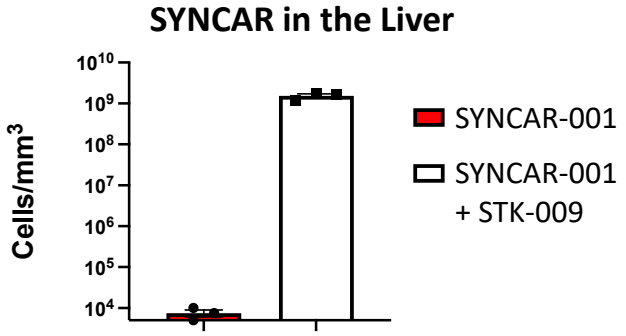
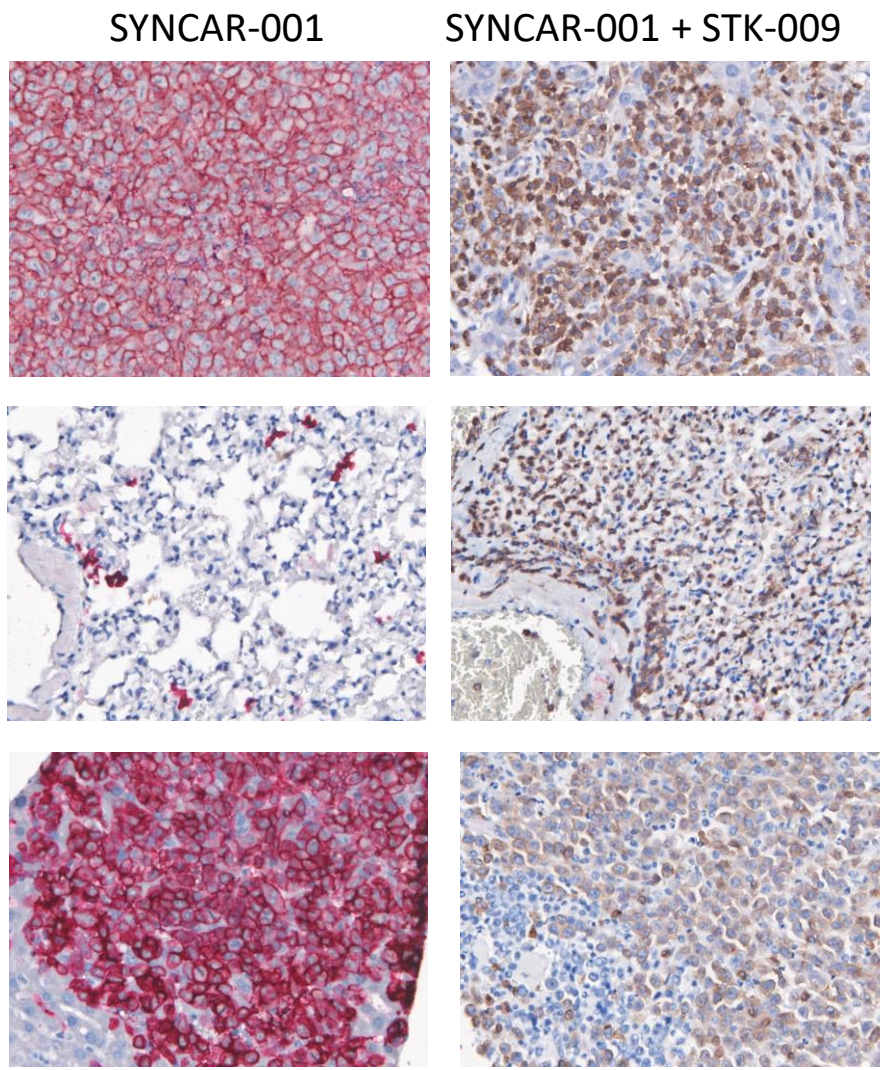
SYNCAR-001 Cells Are in the Tissue in Preclinical Cured Animals

- STK-009 overcomes CAR resistance of bulky lymphoma
- STK-009 induces relocation of T cells into the tumor, and various peripheral tissues including the lung and the liver, containing tumor metastasis
- STK-009 leads to tumor clearance in the liver and lung (and the tumor) and survival of the host



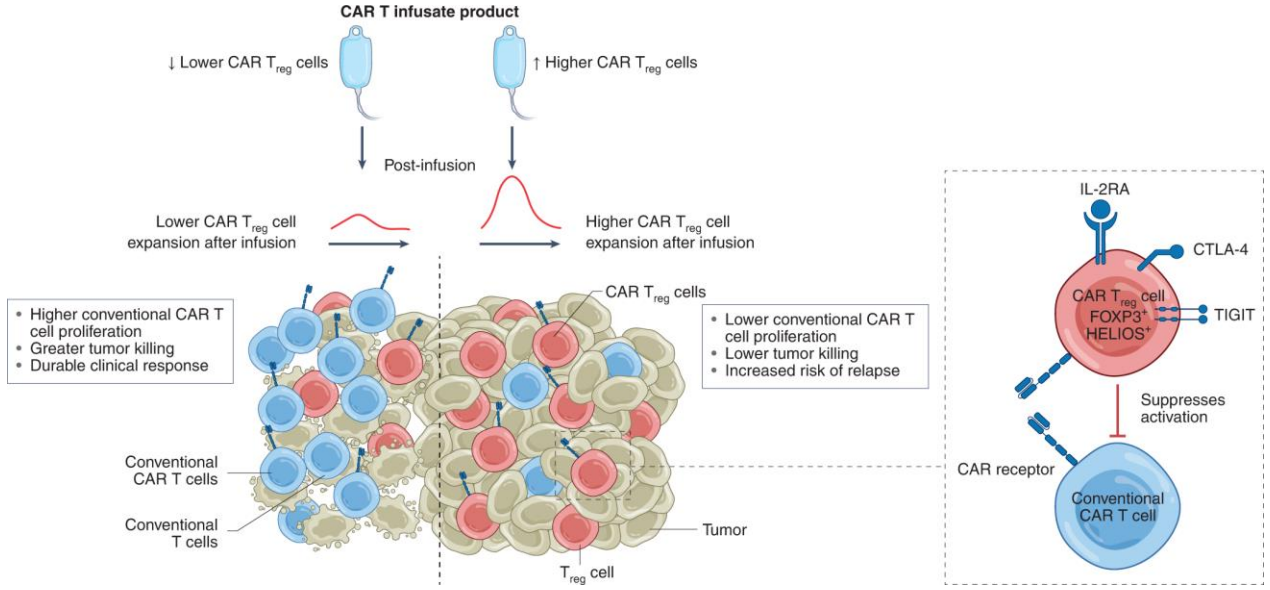




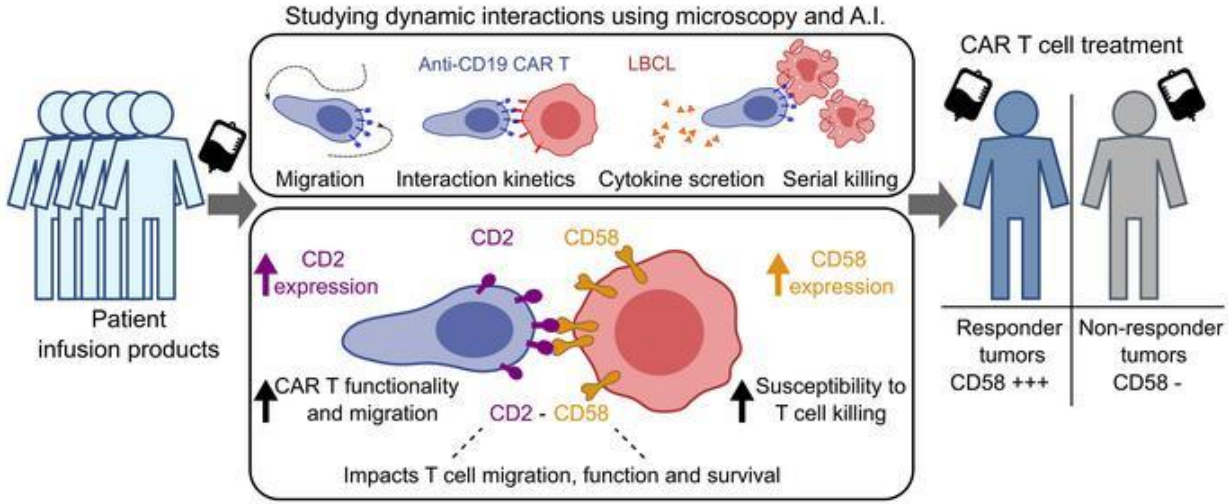


Key Mechanisms of CD19 CAR T Resistance Outside of Antigen Loss

CAR Tregs during manufacturing



CD58 loss on target cells



Saini & Neelapu Nature Med 2022
Romain et al. JCI 2022
Majzner et al. Blood 2020

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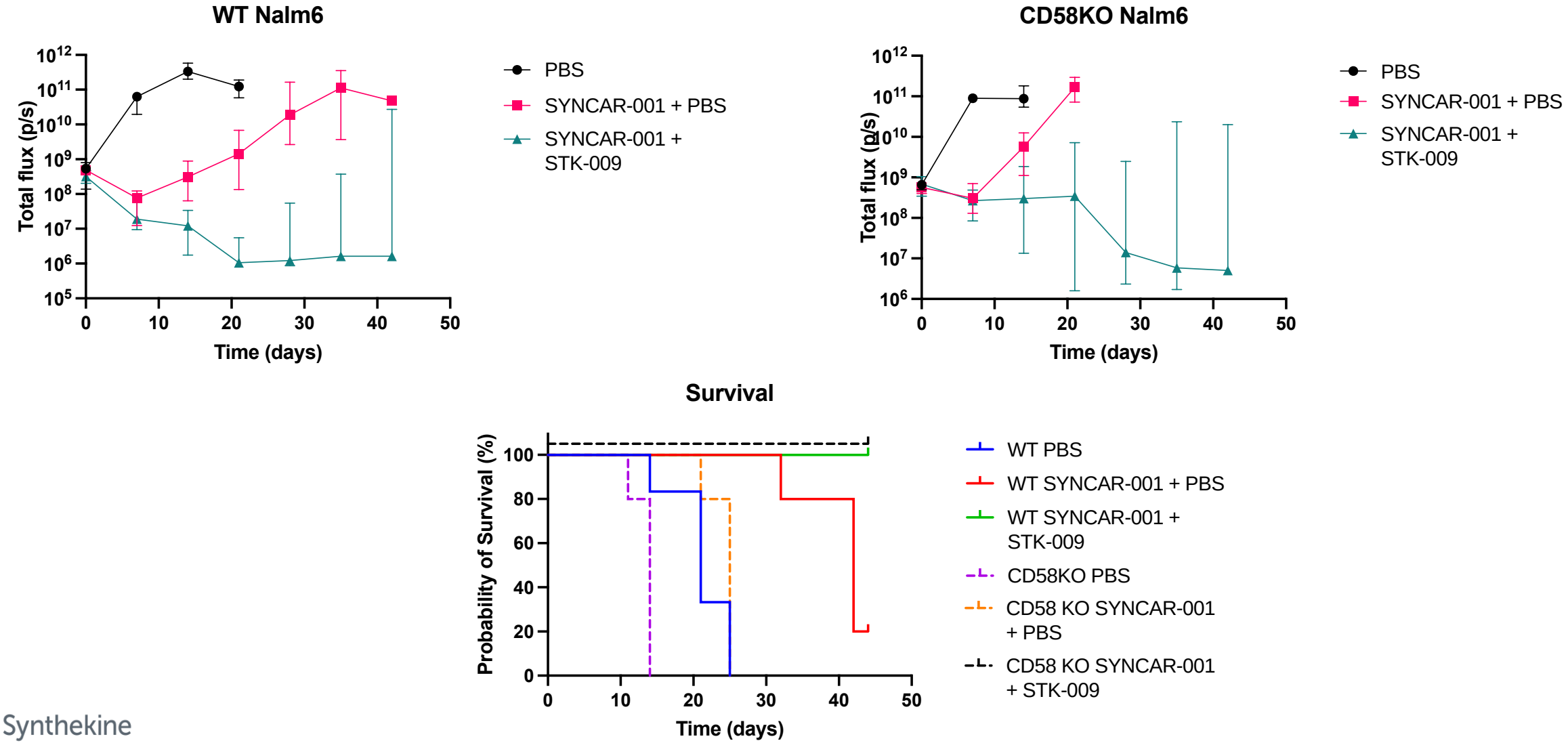
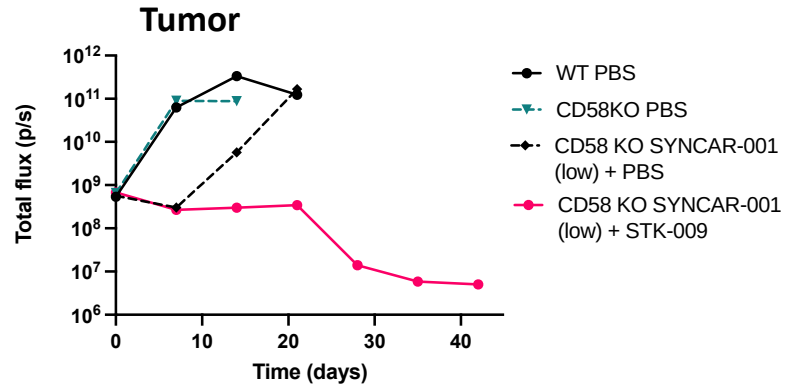
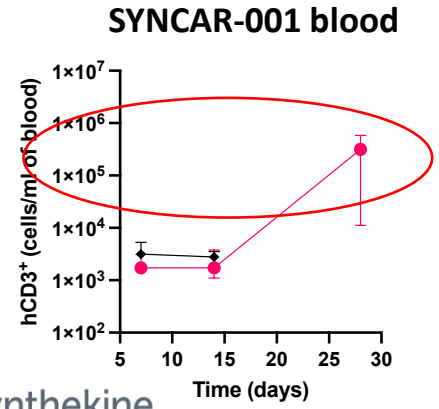
STK-009 + SYNCAR-001 Overcomes CD58 KO Mediated CAR T Resistance in a Nalm6 Model

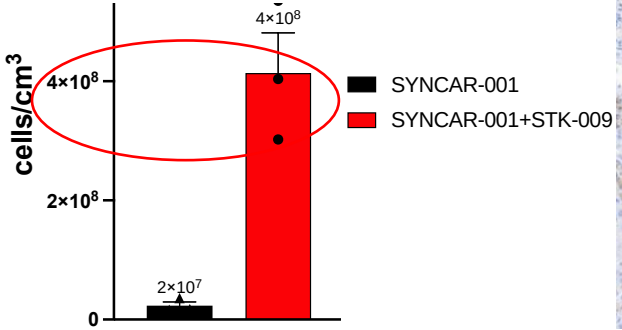


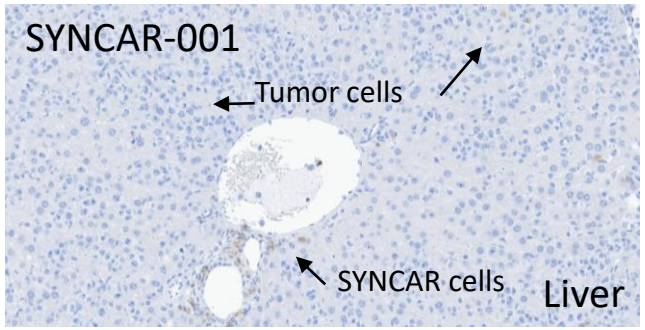
STK-009 Expands SYNCAR-001 in the Tumor and Peripheral Tissues in Preclinical Lymphoma Model

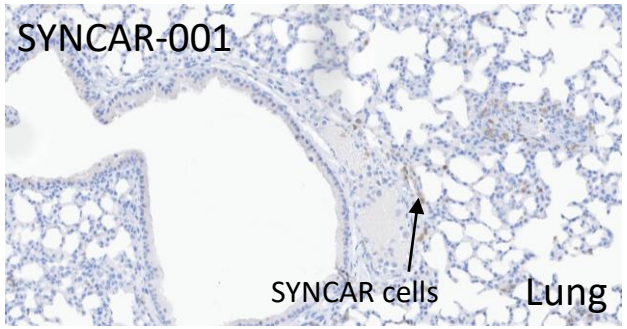
- STK-009 overcomes CAR resistance of CD58 deficient NALM6 lymphoma cells
- STK-009 induces relocation of T cells into the tumor, and various peripheral tissues including the lung and the liver
  - Equates to $4x10^8$ SYNCAR/ml liver tissue vs $2.5x10^5$ in the blood (1000x SYNCAR levels in the blood)
- Correlates with tumor clearance in the liver and lung and survival of the host
- Analysis of systemic SYNCAR activity markers – Granzymes, cytokines

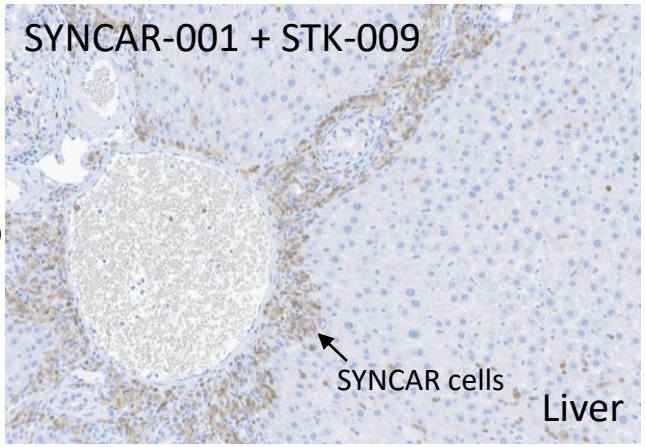


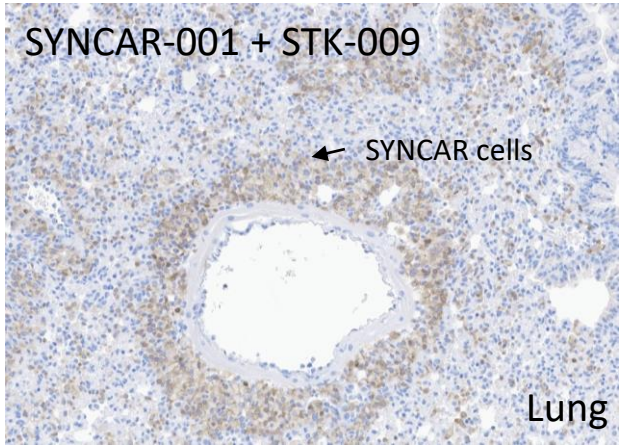












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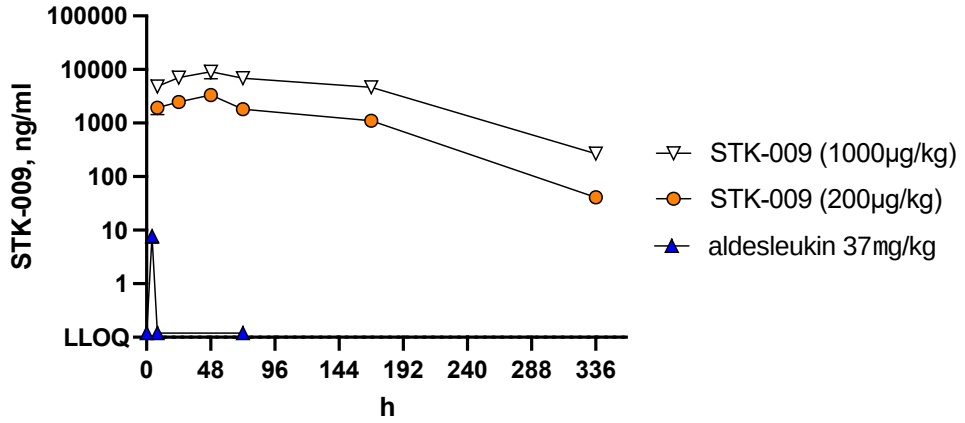
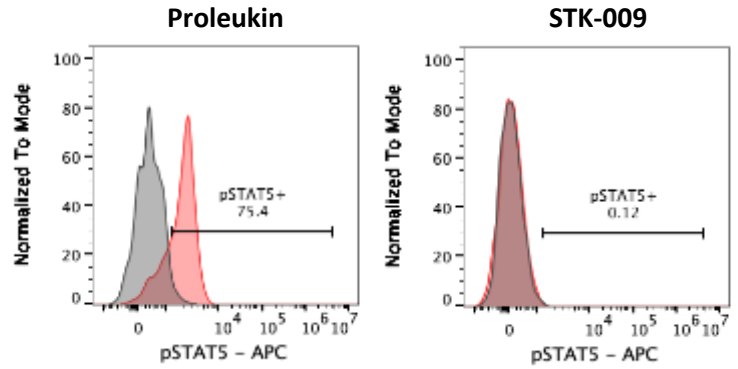
STK-009 Demonstrates Extended PK and No Native Lymphocyte Activation in NHPs

- **STK-009 had high, sustained exposures in NHP**
  - PEG: decreased renal clearance
  - Ortho: decreased target mediated clearance
- **No clinical or pathological changes observed**
  - No increase in eosinophils
  - No change in lymphocyte / white blood cells
- **STK-009 treatment for up to 2 weeks did not induce IL-2 related cellular or cytokine/chemokine changes**
  - No induction of STAT-5 phosphorylation
  - No NK cell proliferation
  - No change to T cell populations
- **STK-009 does not activate the host IL-2 pathway in the absence of ortho-IL-2Rβ**

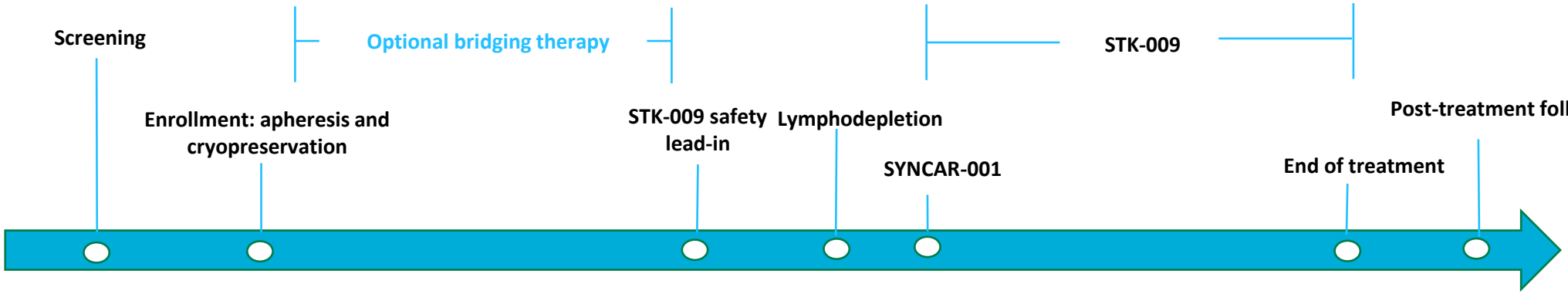
Single dose of STK-009 in NHPs has >2 week exposure



WT IL-2 but not STK-009 activates T cells in NHPs



STK-009 + SYNCAR-001 Phase 1 Study Schema



Key eligibility criteria

- Age ≥18 years
- Histologically confirmed B cell malignancy (CLL/SLL, DLBCL, FL, MZL and MCL)
- CAR-T naive
- Measurable disease at enrollment
- Relapsed/refractory disease
- ECOG PS 0-2
- Adequate organ function

Study treatment

- A safety lead-in dose of STK-009 will be administered prior to lymphodepletion in the first patients.
- Lymphodepletion (Day -5 to -3): Cyclophosphamide 300 mg/m^2/day and fludarabine 30 mg/m^2/day, administered ×3 days.
- SYNCAR-001 treatment (Day 0) consists of a single intravenous infusion
- After SYNCAR-001 initiation, STK-009 is dosed SC weekly for 12 weeks and then monthly for 3 months

Key end points

- **Primary**: Incidence of DLTs and safety to determine a recommended dose
- **Secondary**: Cellular kinetics, immunogenicity, ORR, DOR, PFS, OS

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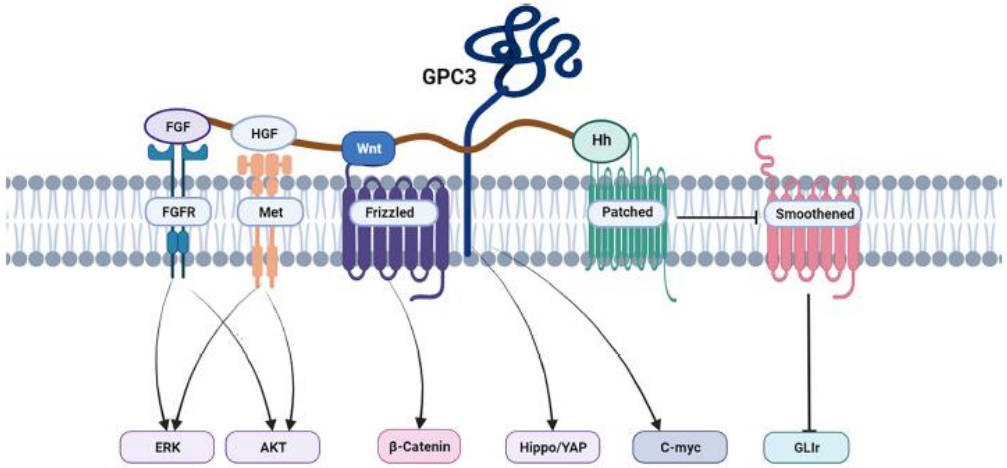
ClinicalTrials.gov Identifier: NCT05665062

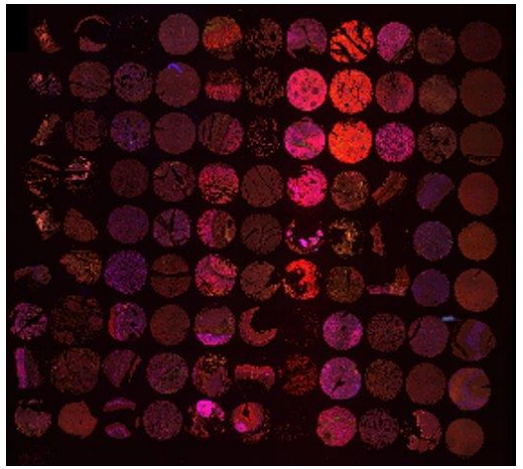
GPC3 is an Attractive CAR T Target in Hepatocellular Carcinoma



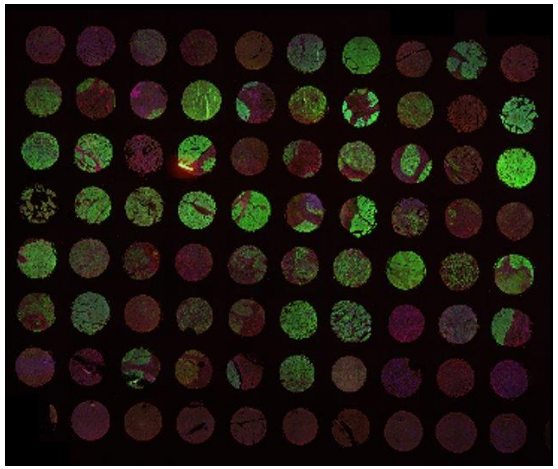
- Glypican 3 (GPC3) is a heparan sulfate proteoglycan and cell surface oncofetal protein
- It is implicated in a variety of processes, including cell growth, differentiation, and migration
- Significant expression on normal adult tissue limited to placenta and largely devoid in other tissues
- GPC3 is expressed in approximately 2/3 of HCC
- Numerous anti-GPC3 CAR T programs are currently in clinical trials

GPC3/CD3 multiplex IHC

Normal Tissue



Hepatocellular carcinoma



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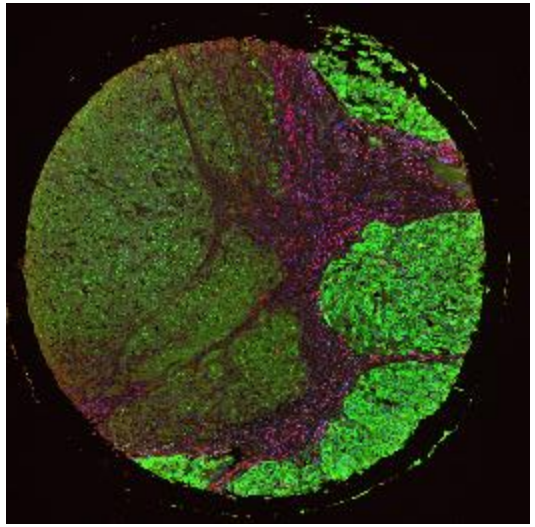
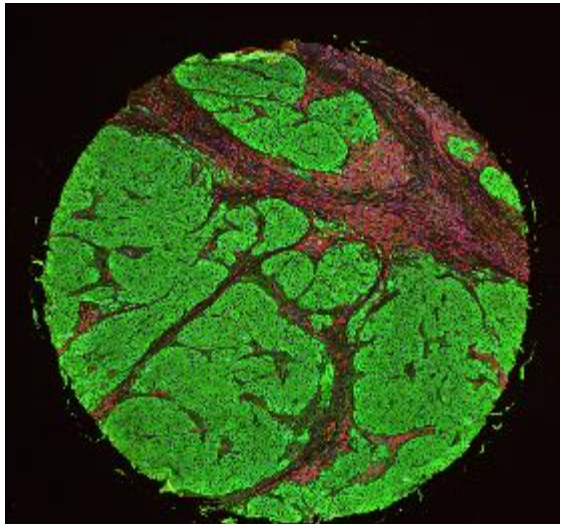
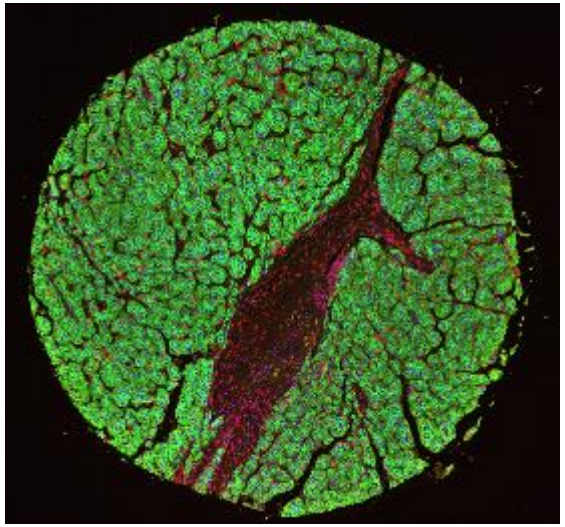
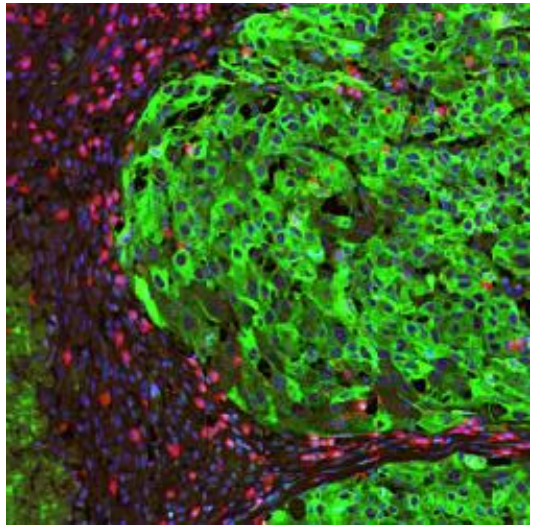
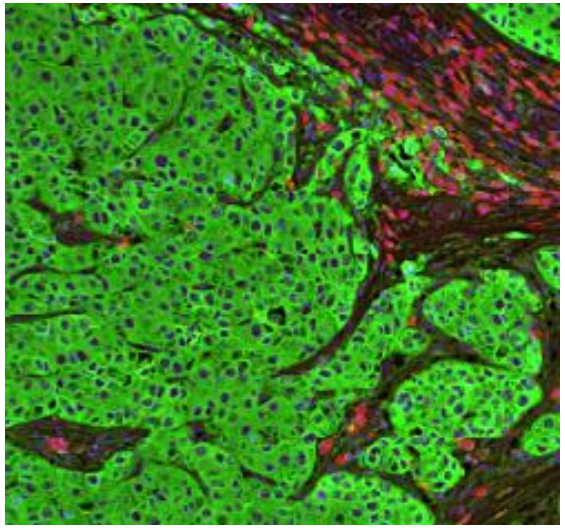
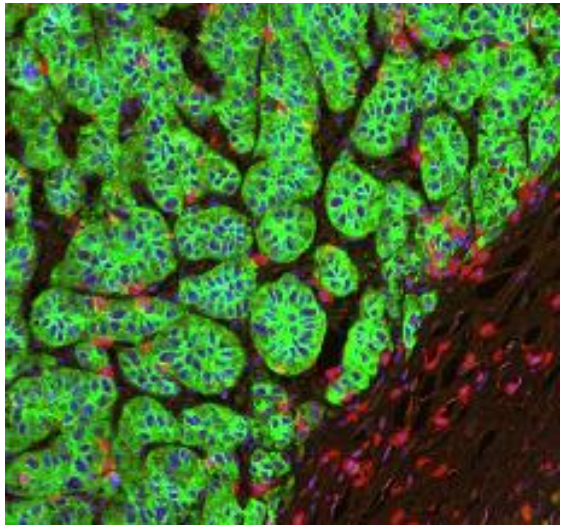
GPC3 Localized to the Cell Membrane While T Cells Mostly Localized in the Stroma in HCC Samples

GPC3/ CD3 multiplex IHC

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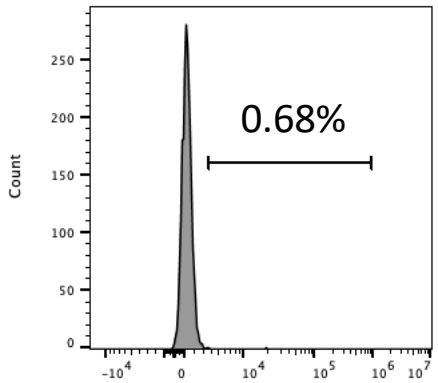
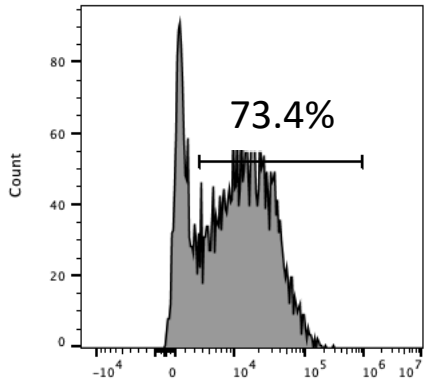
STK-009 Specifically Enriches and Stimulates SYNCAR-002 Ex Vivo

GPC3 CAR

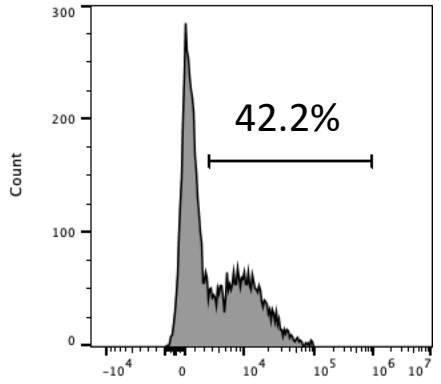
Non-transduced WT IL-2



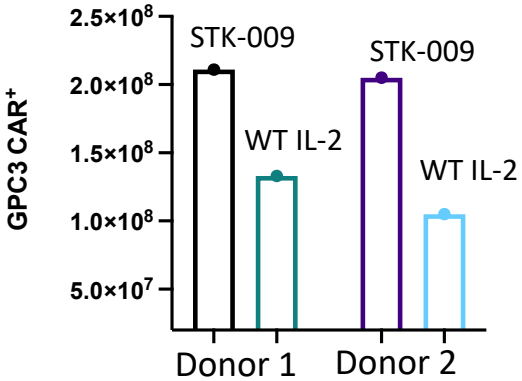
SYNCAR-002 STK-009



SYNCAR-002 WT IL-2

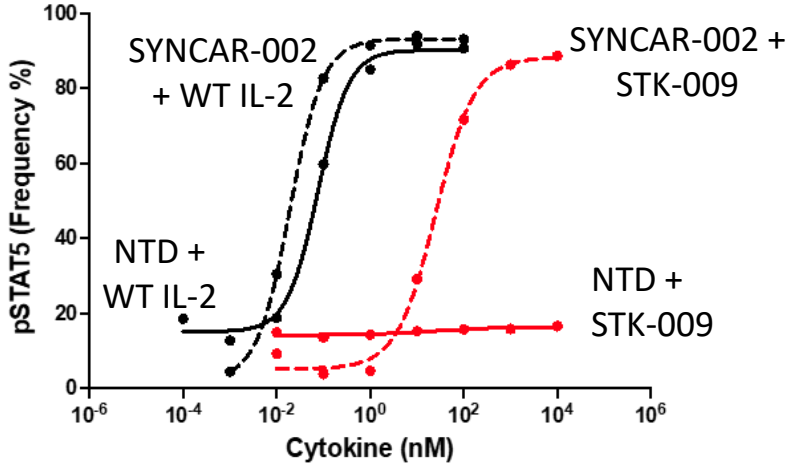


Total GPC3 CAR⁺ cells on harvest



Cells transduced in WT IL-2 and switched to STK-009 or maintained in WT IL-2 on Day 4 until end of manufacturing

pSTAT5 assay



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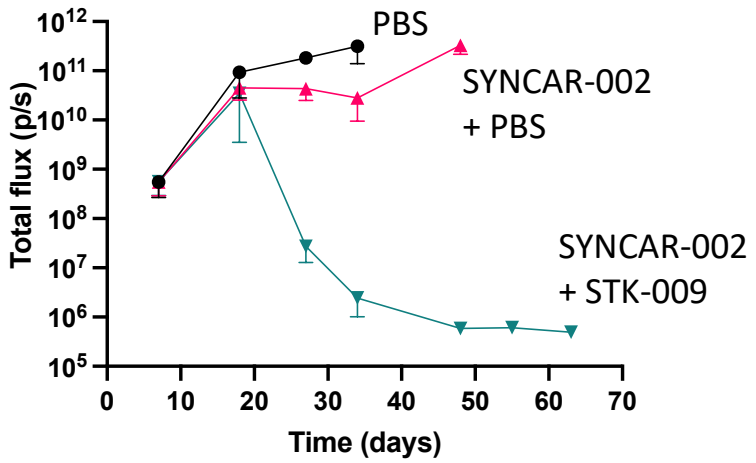
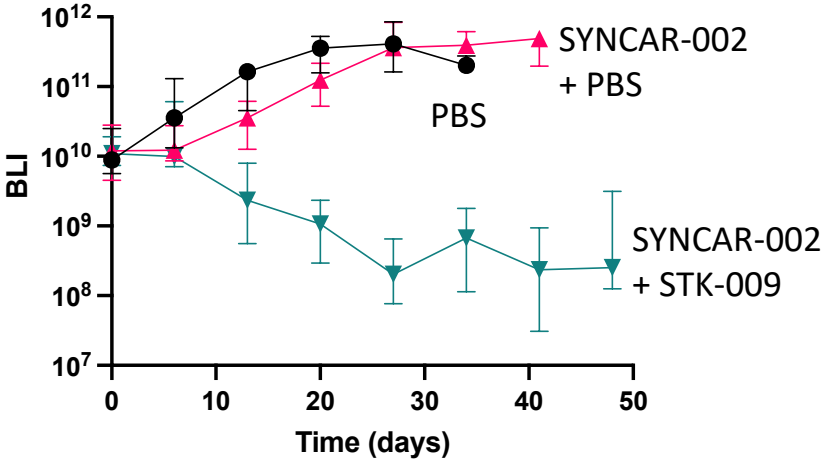
STK-009 + Low Dose SYNCAR-002 Treatment of Intraperitoneal HCC Xenograft Models Results in Tumor Control

HUH-7 IP

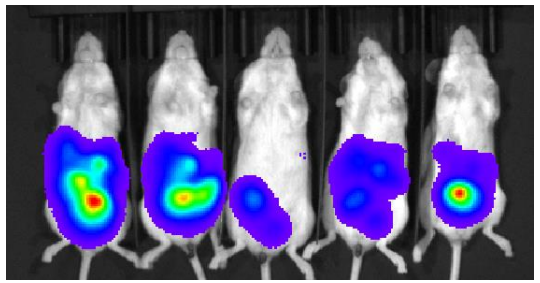


HEP3B IP

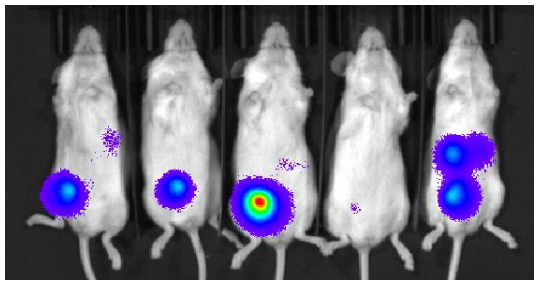


HEP3B IP (Day 20)

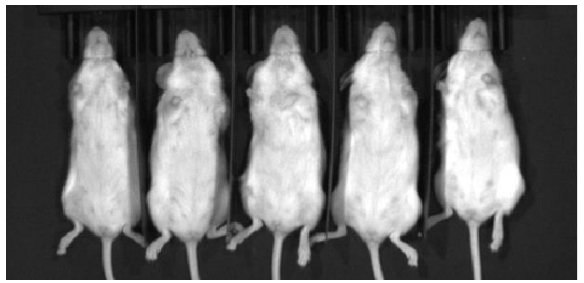
PBS

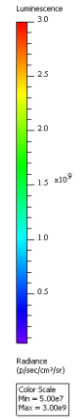


SYNCAR-002 + PBS



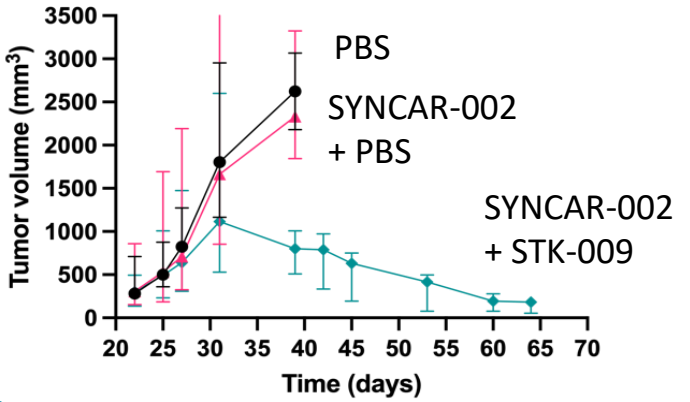
SYNCAR-002 + STK-009



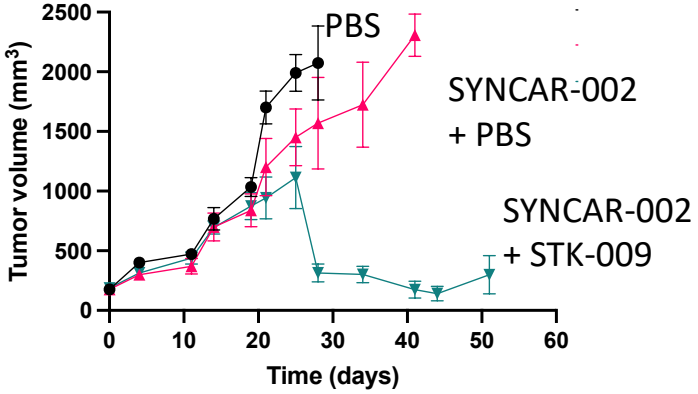


STK-009 + Low Dose SYNCAR-002 Treatment of Various Subcutaneous HCC Xenograft Models Results in Tumor Control

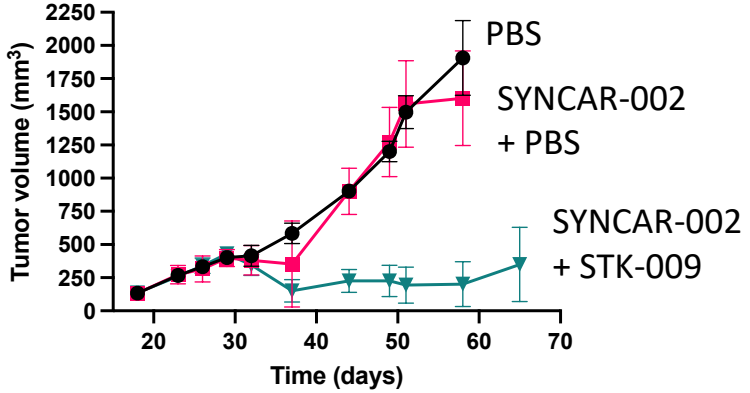
HUH-7 SQ (GPC3^{med})



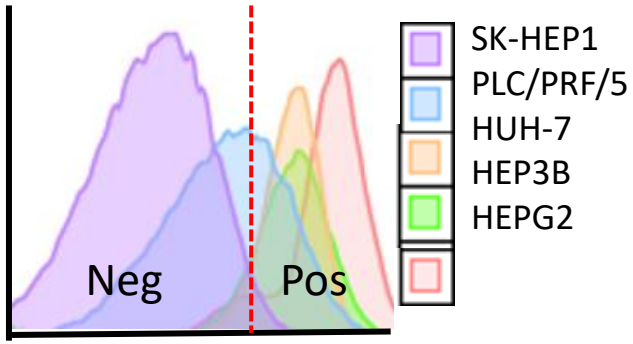
HEP3B SQ (GPC3^{med})



HEPG2 SQ (GPC3^{high})



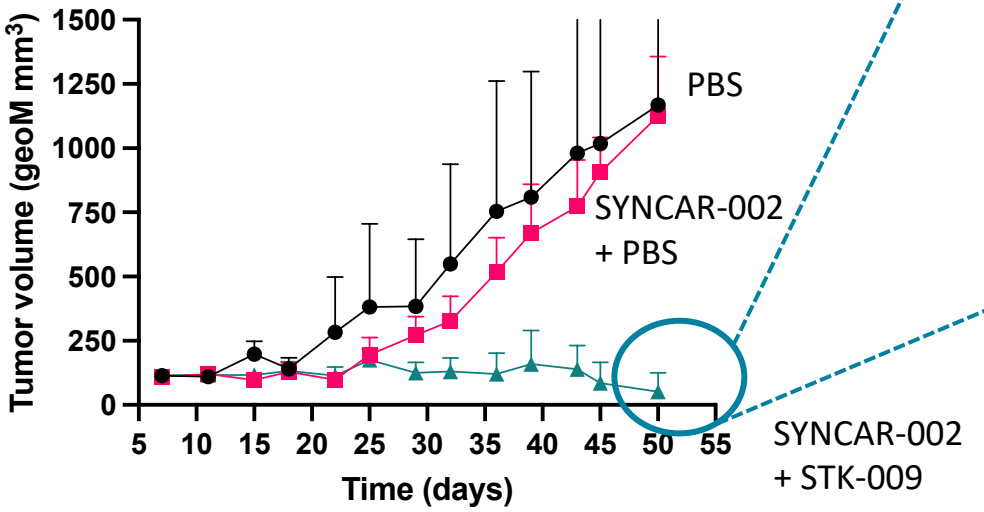
GPC3 expression on HCC cell lines



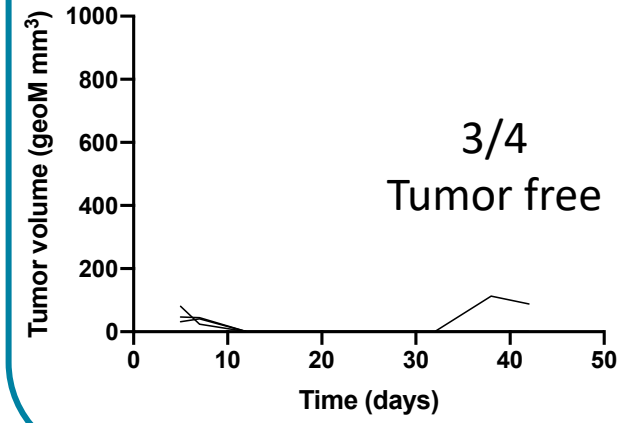
Synthekine

Mice Previously Cured by STK-009 + SYNCAR-002 Withstand HEPG2 Tumor Rechallenge

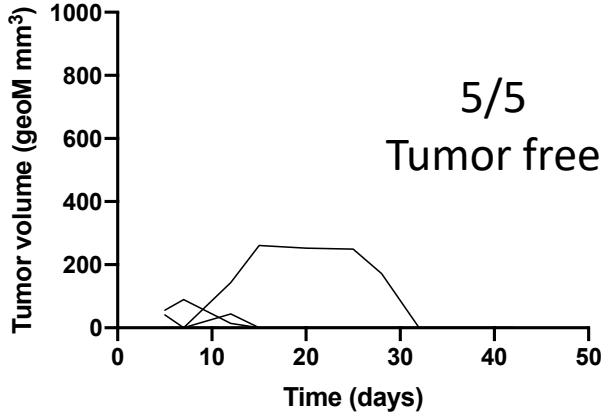
Phase I: HEPG2 efficacy study



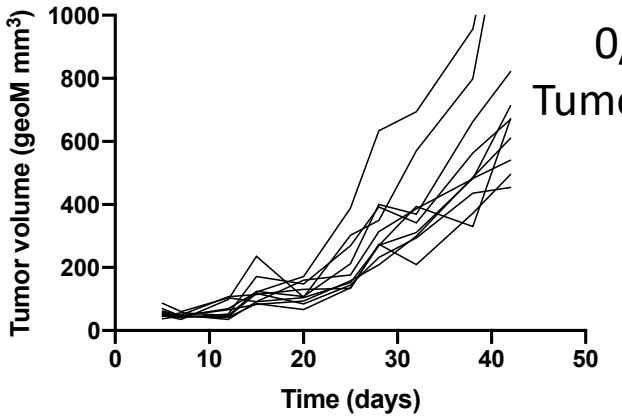
Phase II (rechallenge): PBS



Phase II (rechallenge): STK-009

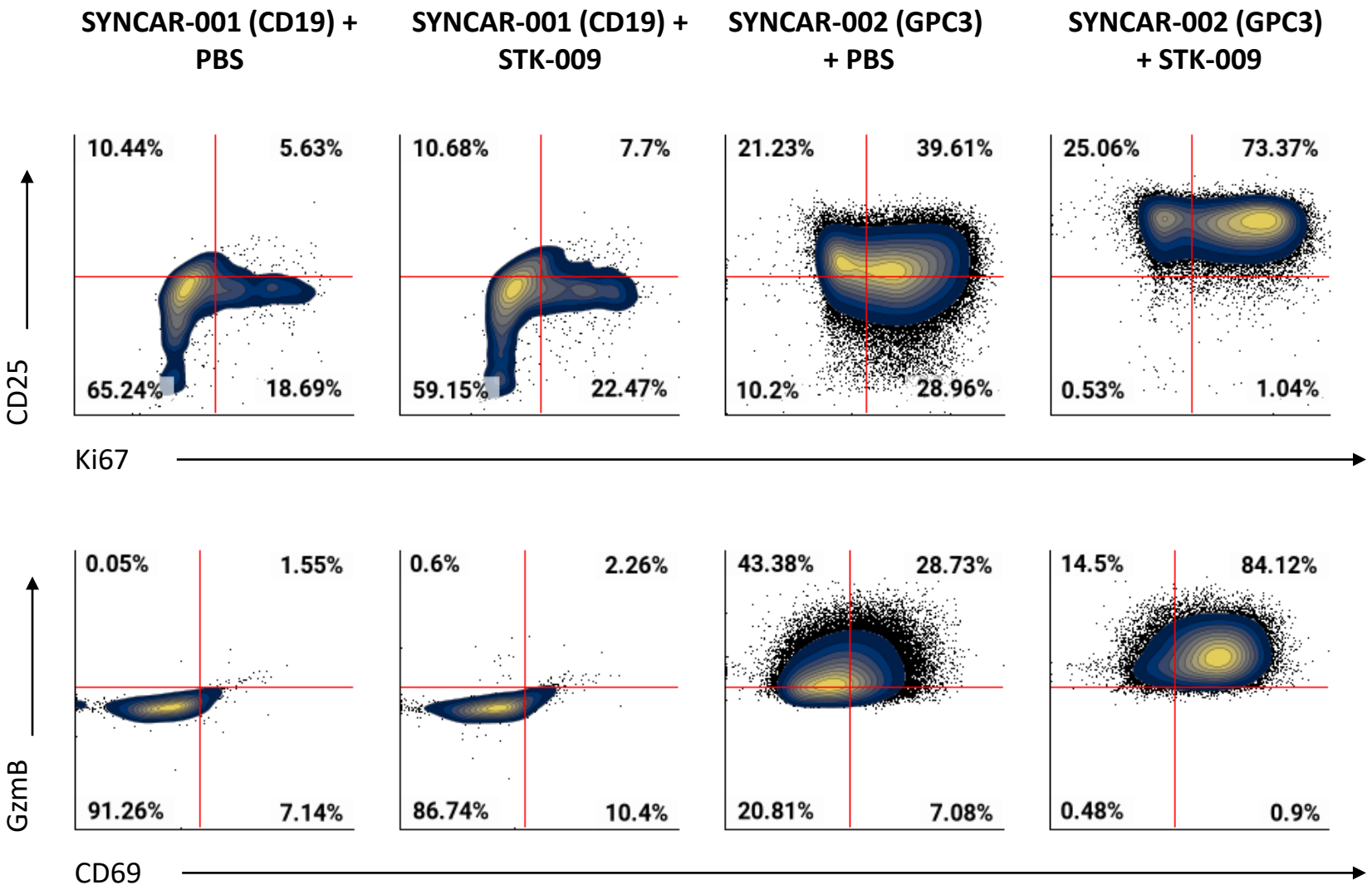


Phase II: Naïve mice implanted with HEPG2



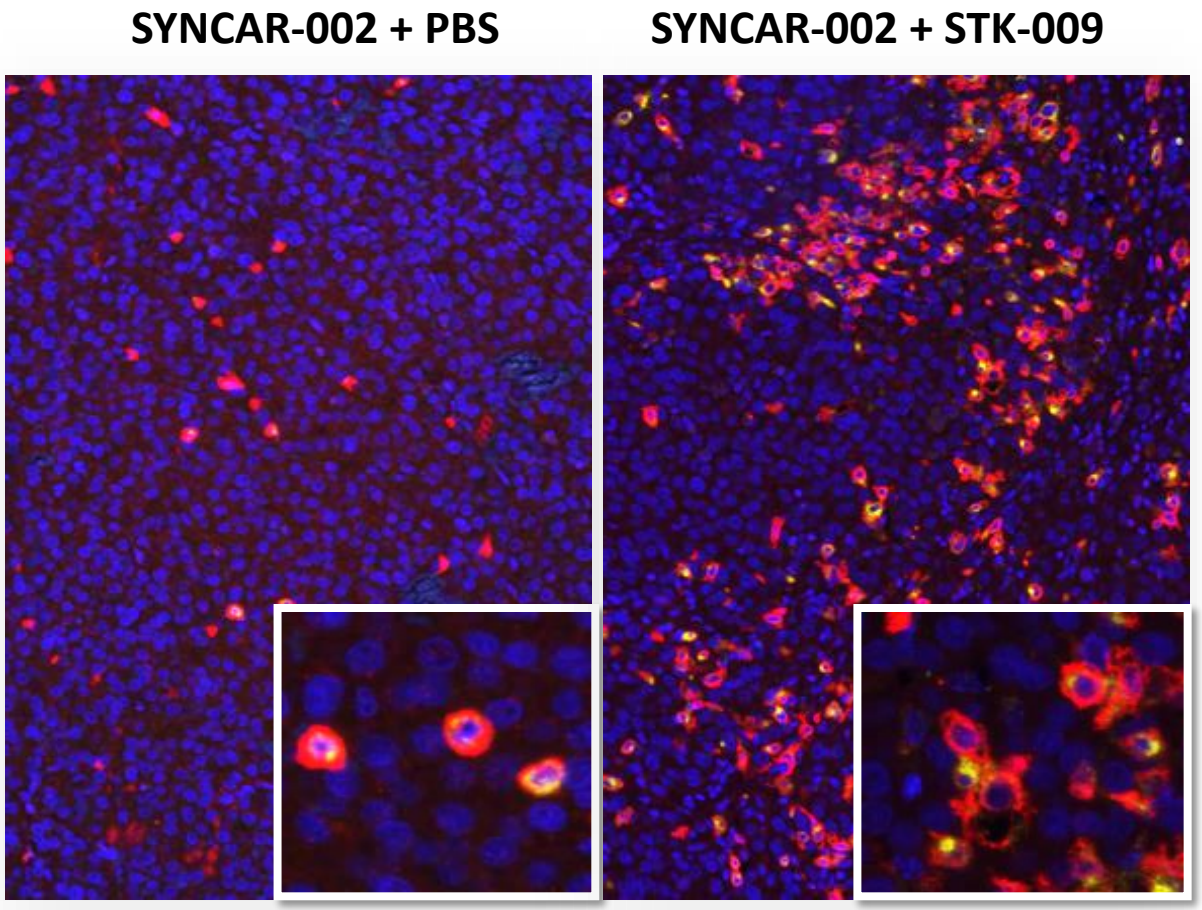
Synthekine

STK-009 Significantly Increases Intratumoral SYNCAR-002 Activation in Solid HEPG2 Tumors

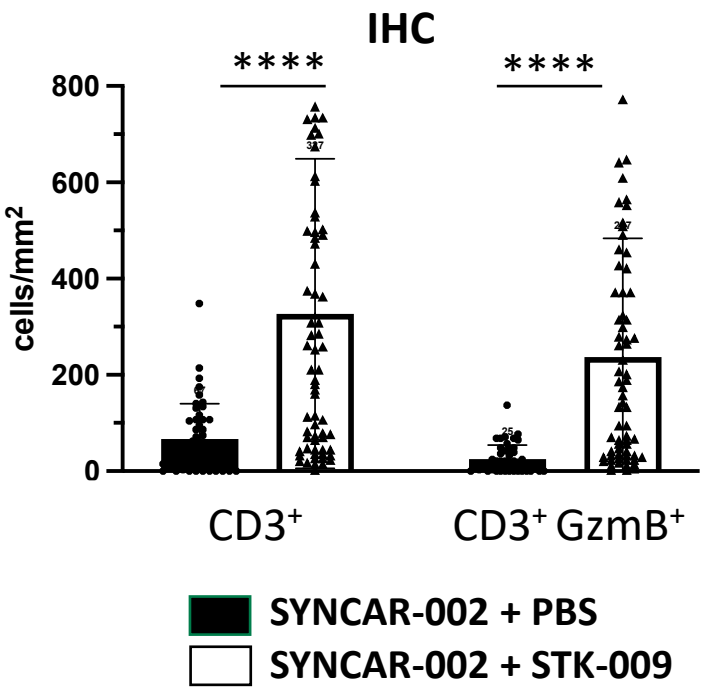


Synthekine

STK-009 Significantly Increases Intratumoral SYNCAR-002 Expansion and Activation in HEPG2 Tumors



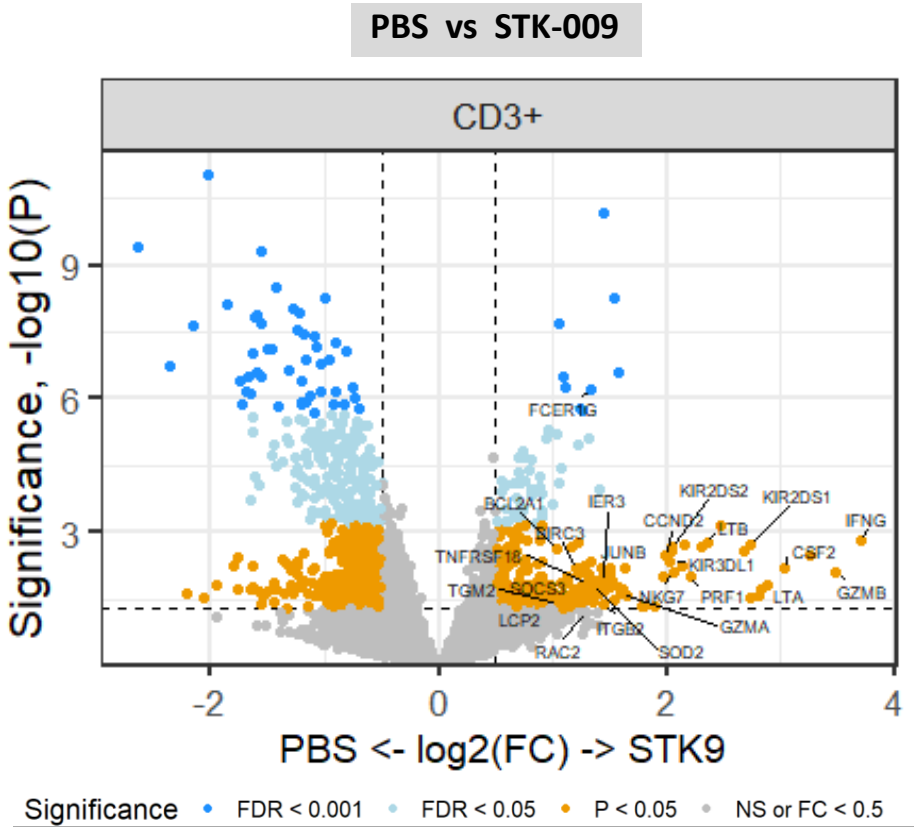
CD3/GranzymeB: Day 14 post-ACT

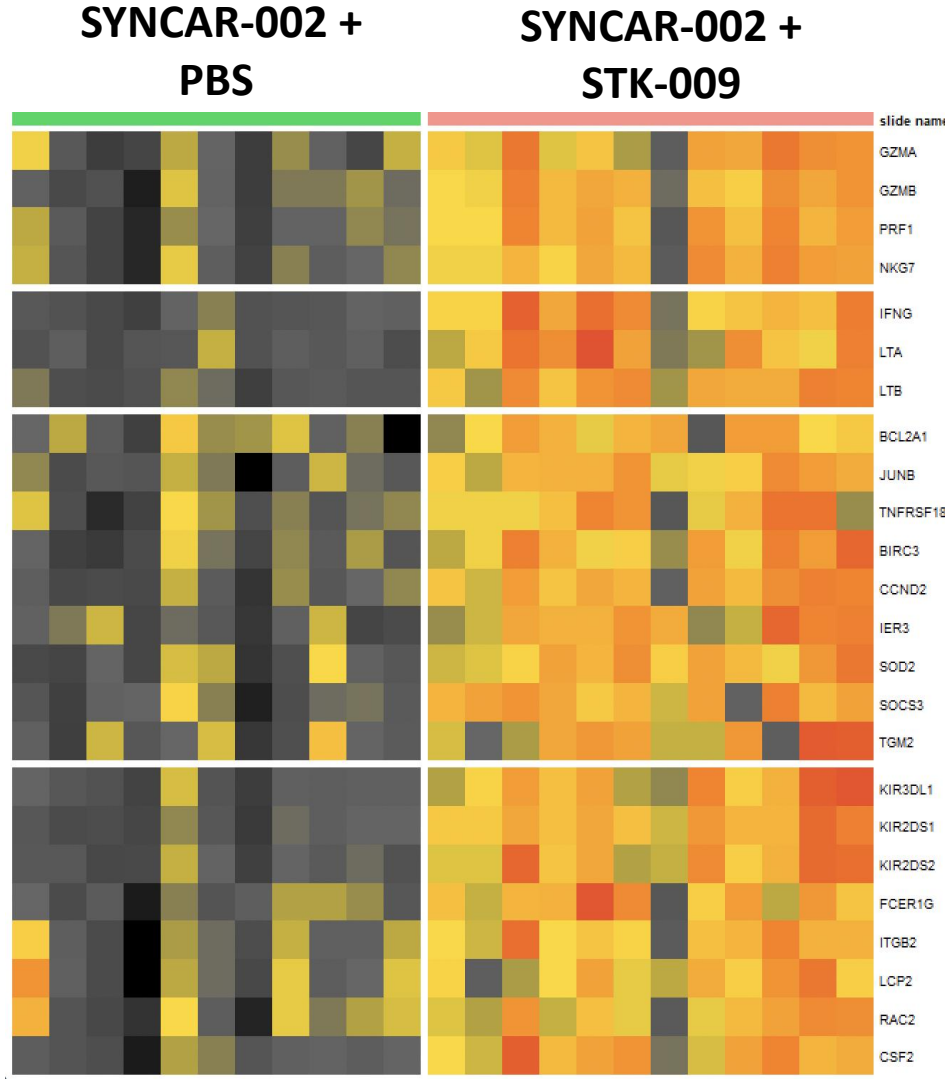


Synthekine

STK-009 Induces Effector Molecules, Pro-Survival, and NK Cell Associated Markers in Intratumoral SYNCAR-002 Cells

Spatial Transcriptomics (GeoMx) performed on HEPG2 tumors treated with SYNCAR-002 -/+ STK-009





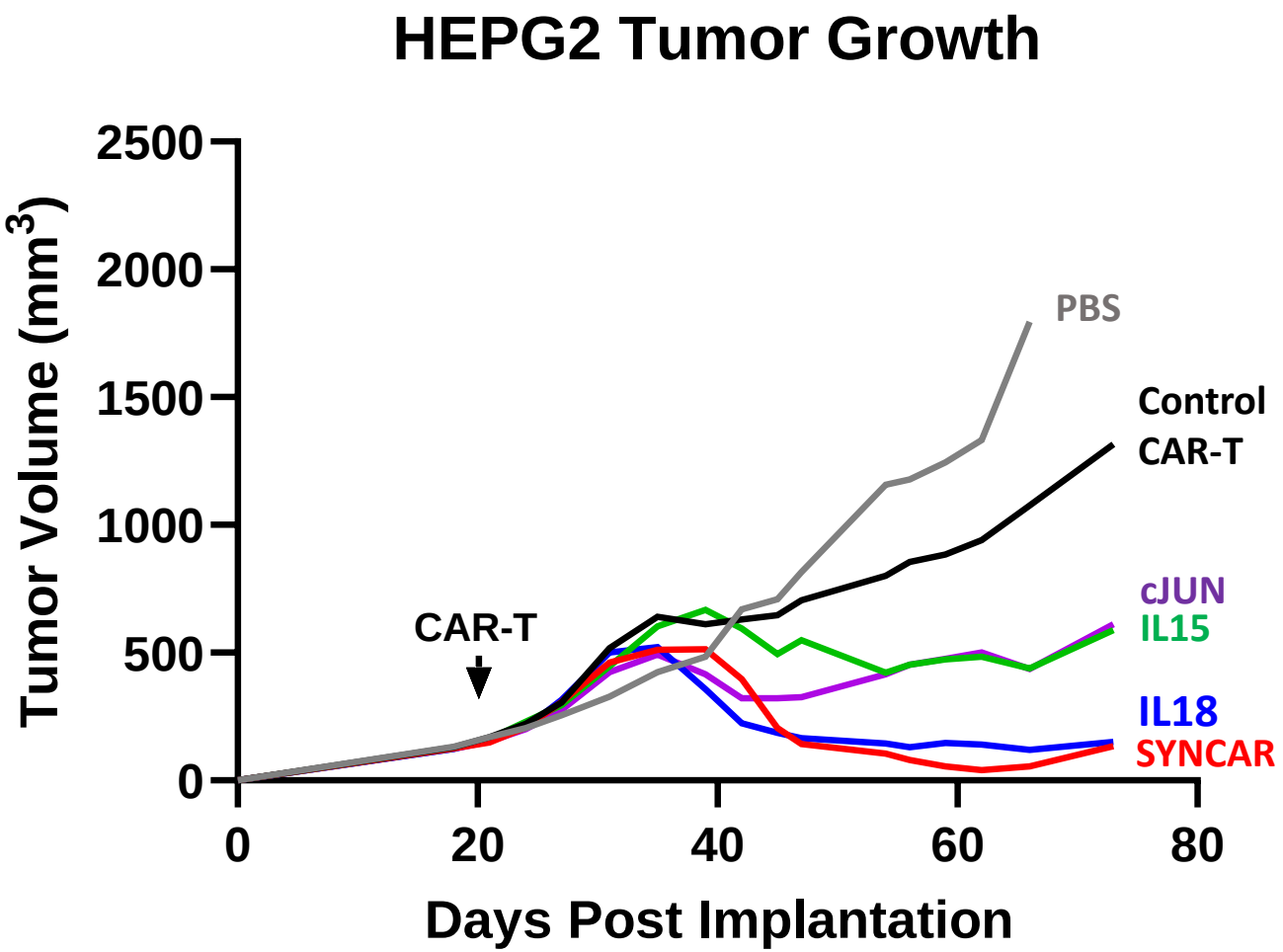
Cytotoxic machinery

Pro-survival

NK Cell associated

Synthekine

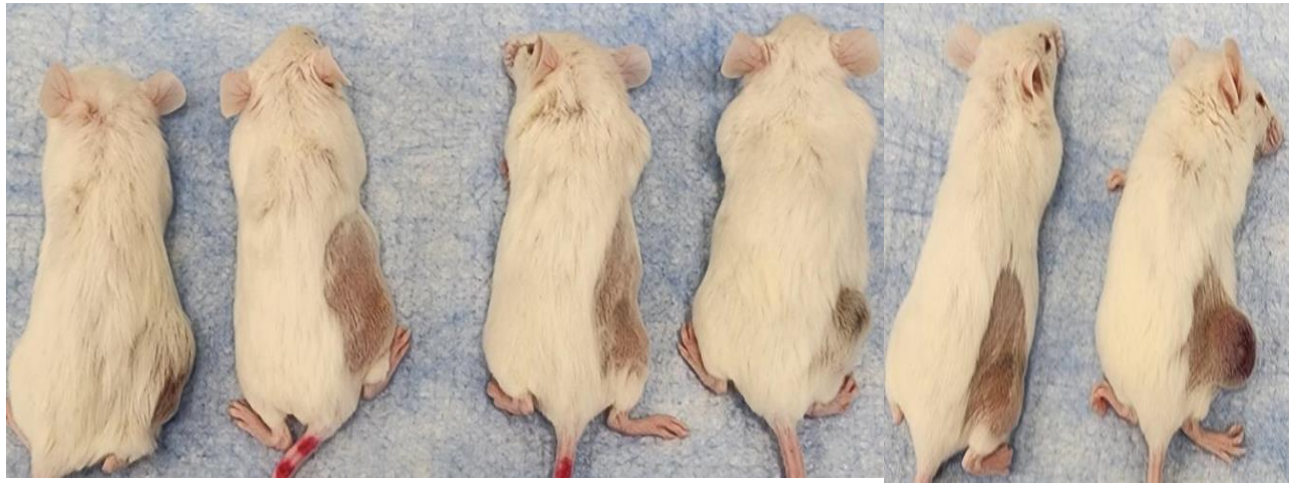
SYNCAR-002 + STK-009 Demonstrates Superior Antitumor Potency Compared to Other Armoring Approaches



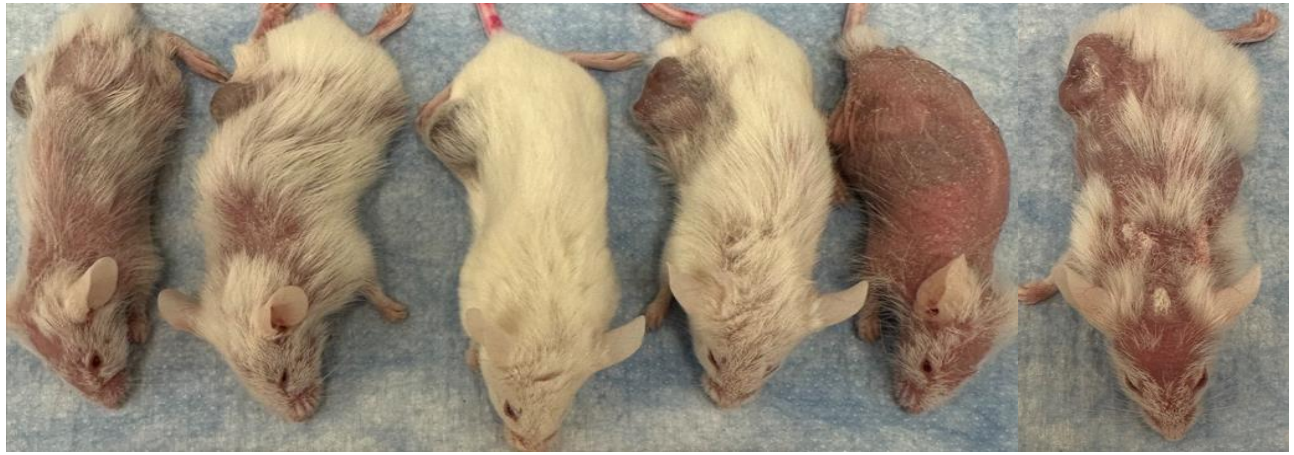
- PBS
- SYNCAR-002
- SYNCAR-002 (mfg. w/ STK-009) + STK-009
- GPC3 CAR-T with IL15/IL15Ra armoring
- GPC3 CAR-T with IL18 armoring
- GPC3 CAR-T with cJUN armoring

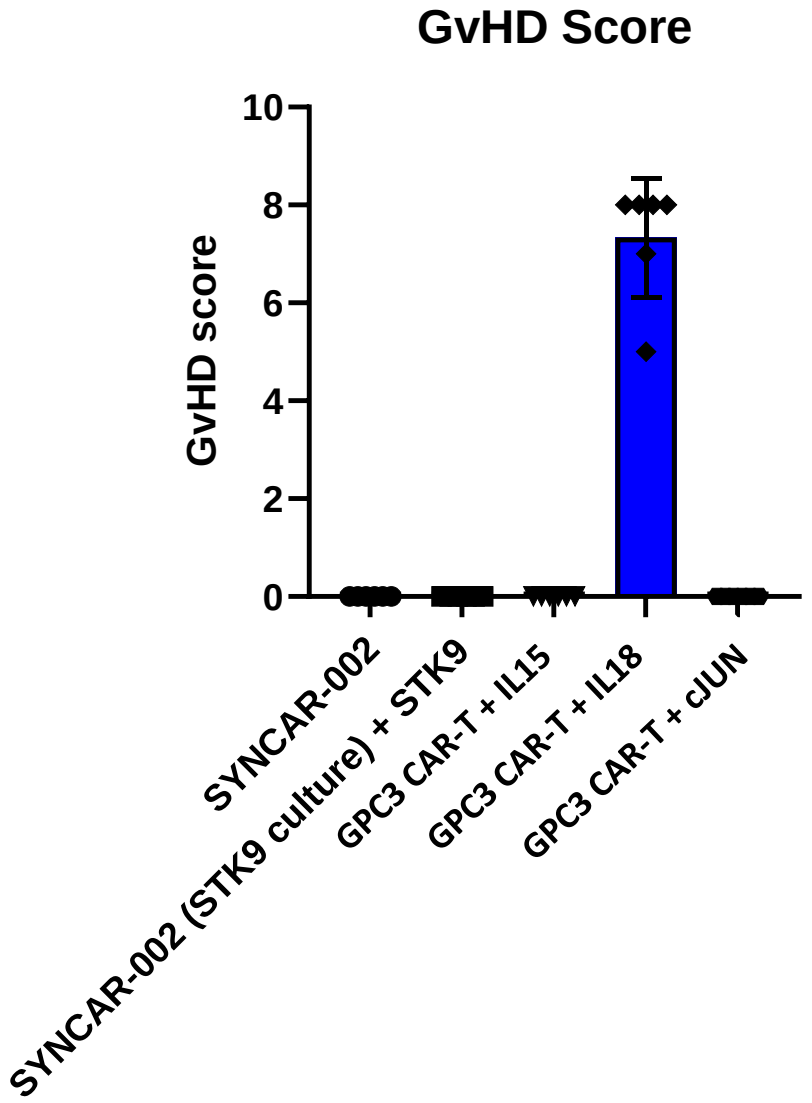
Constitutive IL18 Overexpression Leads to GvHD

SYNCAR-002 + STK9



GPC3 CAR-T + IL18 armoring

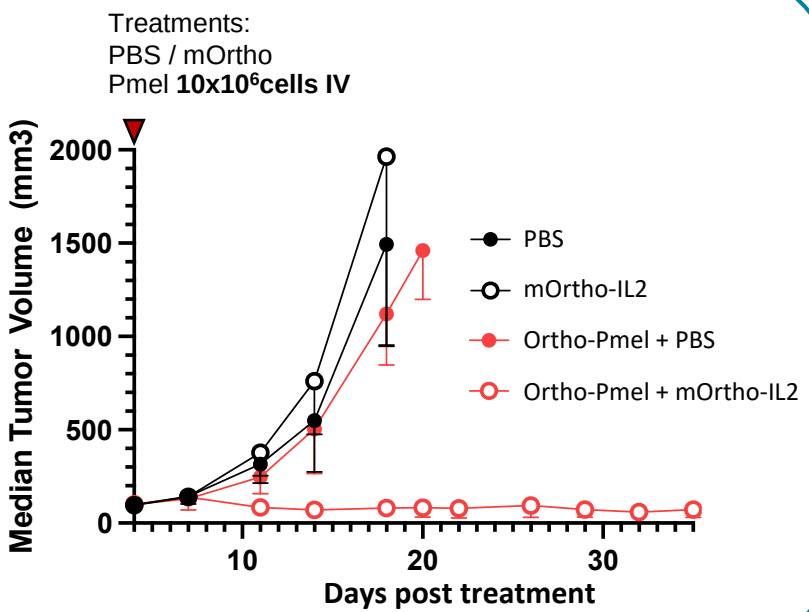




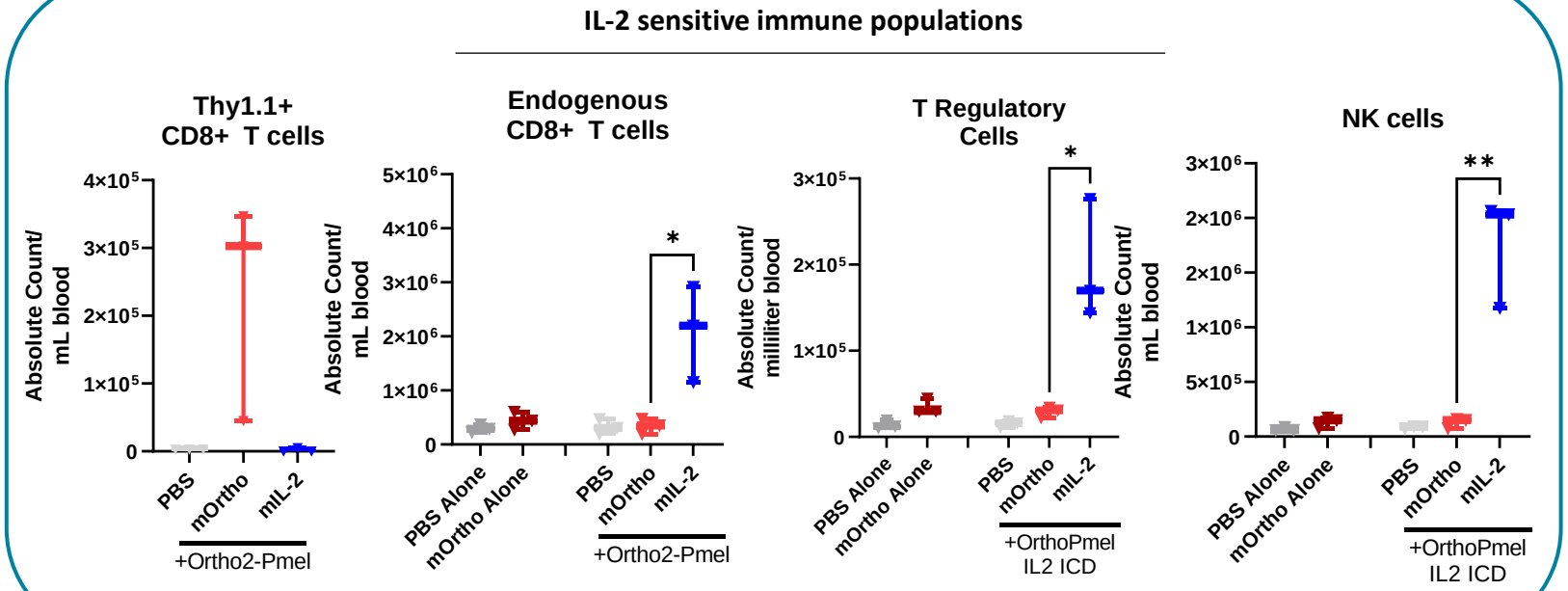
Murine Orthogonal IL-2 System Provides Adoptive Cell Therapy Efficacy Without Lymphodepletion

B16 mouse melanoma model with Pmel-TCR Transgenic T-cells and a mouse surrogate of STK-009

OrthoPmel + mOrtho shows tumor growth inhibition in a *non-lymphodepleted* model



mOrtho *specifically* expands orthoPmel T cells *in vivo*



STK-009 + SYNCAR Summary

- STK-009 provides a **private IL-2 signal** to orthoIL-2R expressing T cells **in vitro** and **in vivo**
- NHP study showed **sustained PK** of STK-009 and no activity on native lymphocytes
- SYNCAR T cells can be expanded **dramatically and at will** by STK-009 **in vivo**
- Potential to **obviate lymphodepletion** with STK-009
- STK-009 + SYNCAR T cells drive **deep and durable responses** in heme and solid epithelial tumor models