CAR-Macrophages with custom intronic shRNA exhibit enhanced efficacy against solid tumors

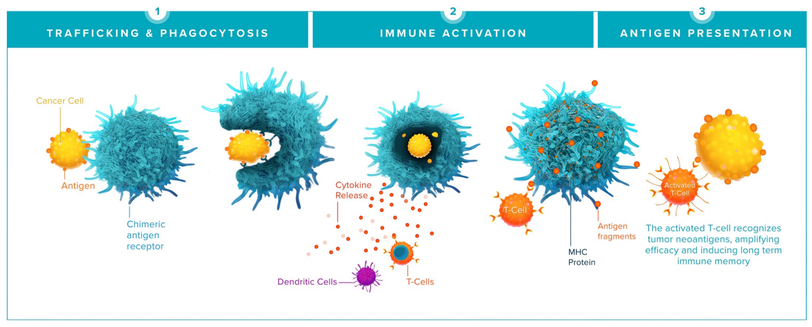
Chris Sloas, Rashid Gabbasov, Nicholas Anderson, Yuhao Huangfu, Karan Nagar, Kerri Ciccaglione, Tierra Tobin, Nicholas Minutolo, Thomas Condamine, Michael Klichinsky, Yumi Ohtani

Carisma Therapeutics Inc., Philadelphia, PA

Introduction

CAR-Macrophages (CAR-M) for targeting solid tumors

CAR-M harness macrophage effector functions to clear solid tumors [1-3] via phagocytosis, TME activation, and epitope spreading

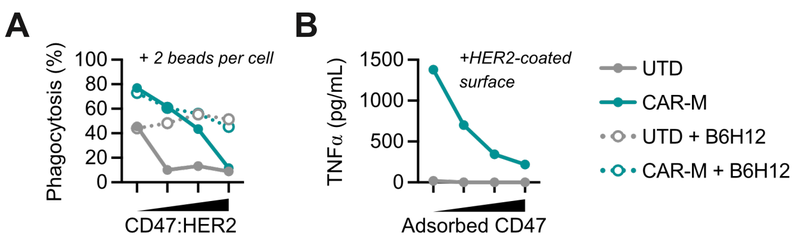


SIRPα-CD47 checkpoint signals can inhibit CAR-M

CD47 sends a "don't eat me" signal to macrophage-expressed SIRPα.

Tumors can overexpress CD47 to evade macrophage phagocytosis.

In vitro assays show that CD47 can inhibit CAR-M activity. In a bead-based phagocytosis assay, CD47 reduced CAR-M phagocytosis of antigen-coated beads. CD47-mediated inhibition could be circumvented with CD47 blocking antibody B6H12 (**A**). CD47 reduced cytokine release from CAR-M cultured on antigen-coated surfaces (**B**).



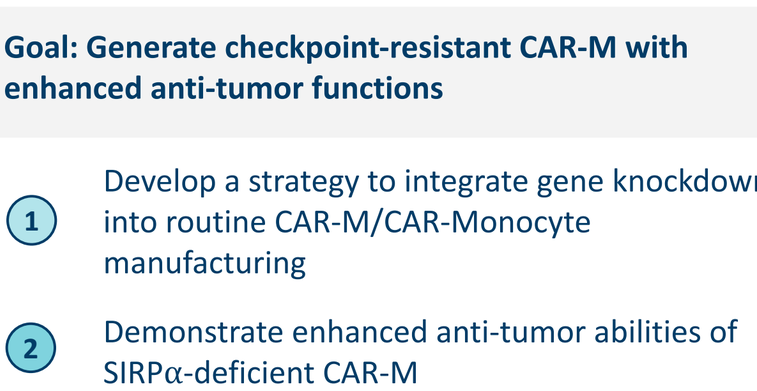
Objectives

Goal: Generate checkpoint-resistant CAR-M with enhanced anti-tumor functions

1. Develop a strategy to integrate gene knockdown into routine CAR-M/CAR-Monocyte manufacturing
2. Demonstrate enhanced anti-tumor abilities of SIRPα-deficient CAR-M

Materials and Methods

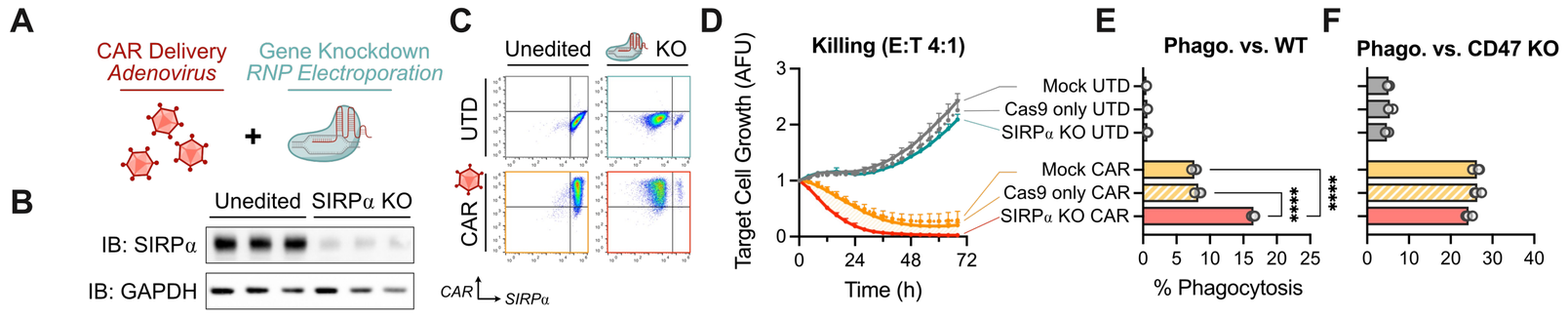
Approach: Knockout (KO) of SIRPα in CAR-M using CRISPR/Cas9 or intronic shRNA



- CAR-M are generated via adenoviral transduction of primary human monocytes or macrophages
- CRISPR-mediated editing is performed using CRISPR/Cas9 ribonucleoproteins (RNPs) that are delivered via electroporation
- shRNA-mediated editing is achieved using a CAR-containing adenoviral vector modified with custom shRNA sequences nested in a synthetic intron
- Killing and phagocytic capacities of macrophages are evaluated using *in vitro* co-culture assays with HER2-expressing SKOV3 tumor cells
- *In vivo* tumor models are performed in immunodeficient NSGS mice
- All *in vitro* data shown are representative of at least three independent donors and/or experiments

SIRPα-deficient CAR-M exhibit enhanced anti-tumor functions

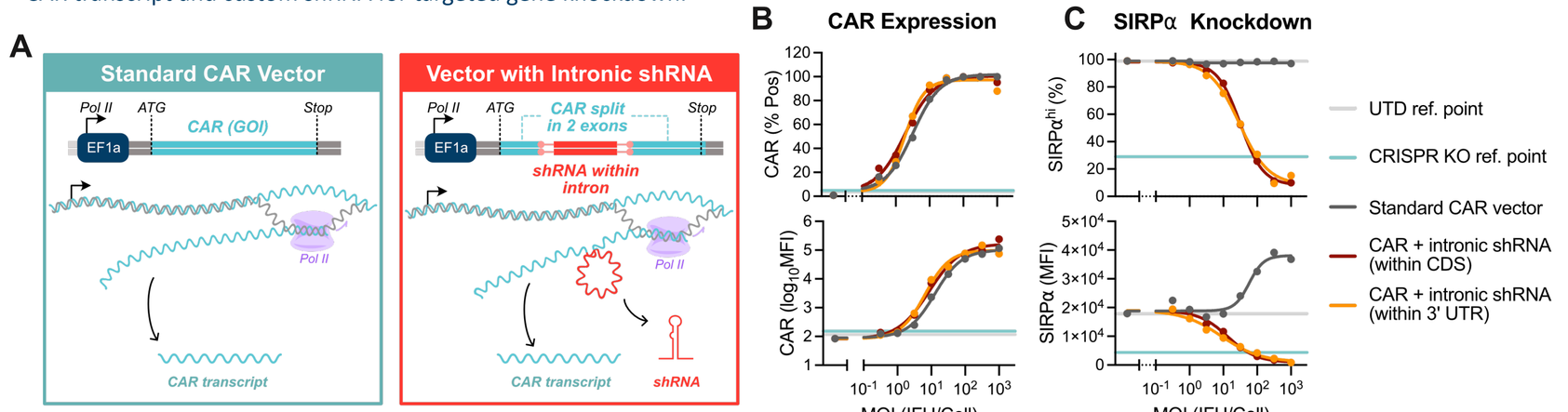
SIRPα-KO CAR-M can be generated through sequential adenovirus transduction and CRISPR/Cas9 RNP electroporation (**A-C**). SIRPα-deficient CAR-M exhibit enhanced killing and phagocytosis of SKOV3 tumor cells, which express the CAR antigen HER2 (**D, E**). Enhancements to CAR-M are specific to the CD47-SIRPα signaling axis (**F**). In UTD cells lacking CAR expression, SIRPα knockdown alone is insufficient to enhance anti-tumor activity.



Intronic shRNA enable one-step CAR delivery and SIRPα knockdown

To circumvent multi-step manufacturing necessitated by CRISPR/Cas9, we introduced intronic shRNA to our adenoviral vector (**A**). Transcription from a shared promoter and subsequent splicing yields both an intact CAR transcript and custom shRNA for targeted gene knockdown.

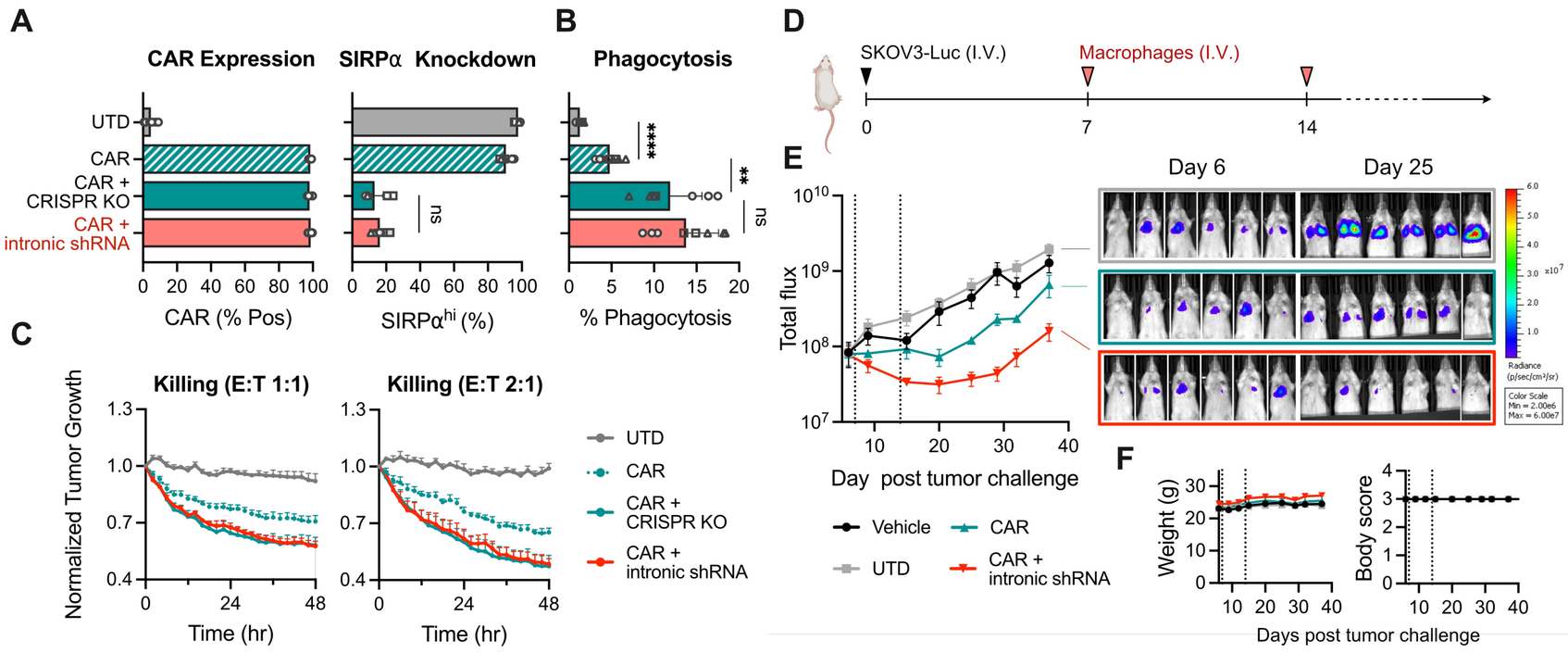
Intronic shRNA were introduced to the CAR CDS or the 3' UTR. Both designs enabled concomitant CAR expression (B) and SIRPα knockdown (**C**), exceeding levels achieved by CRISPR electroporation.



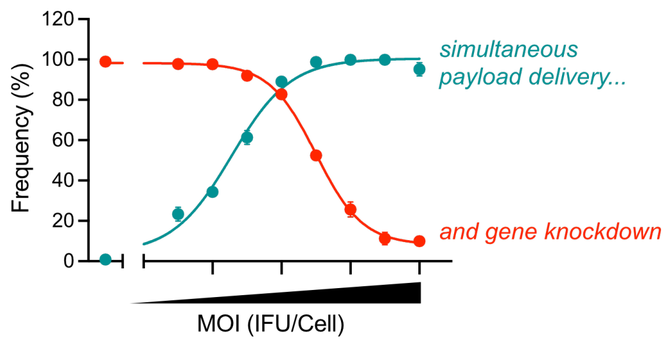
Gene knockdown with intronic shRNA improves tumor control by CAR-M

One-step engineering with intronic shRNA matches the functional enhancements provided to CAR-M by CRISPR/Cas9 electroporation. Both methods reduce SIRPα expression (**A**), augment phagocytosis (**B**), and enhance target cell killing by CAR-M (**C**).

The ability of intronic shRNA to improve tumor control *in vivo* was evaluated using a xenograft tumor model in NSGS mice (**D**). IV-administered CAR-M engineered with SIRPα-targeting shRNA achieved greater tumor control than standard CAR-M (**E-F**).



Conclusions



Gene-silenced CAR-M can be generated in a single step by integrating CAR delivery with custom intronic shRNA

Targeted SIRPα knockdown enhances the anti-tumor activity of CAR-M *in vivo*

The intronic shRNA design is a generalizable platform for multiplexed gene delivery and gene silencing for human monocytes and macrophages.

[1] Klichinsky, M. *et al.* Human chimeric antigen receptor macrophages for cancer immunotherapy. *Nat Biotechnol* 1–7 (2020).
[2] Anderson, N. R., Minutolo, N. G., Gill, S. & Klichinsky, M. Macrophage-Based Approaches for Cancer Immunotherapy. *Cancer Res* **81**, 1201–1208 (2021).
[3] Pierini, S. *et al.* Abstract 63: Chimeric antigen receptor macrophages (CAR-M) induce anti-tumor immunity and synergize with T cell checkpoint inhibitors in pre-clinical solid tumor models. *Immunology* 63–63 (2021).
Schematics of mouse experimental timelines created using Biorender.com

carisma THERAPEUTICS