(253) eCAR™-T: Dual-Inactivation of SOCS1 and Regnase-1 by CRISPR/Cas9 Gene Editing Enhances CAR-T Function Against Solid Tumors

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Abstract

CAR-T immunotherapies have shown great success against hematological cancers, though efficacy has been limited against solid tumors. Using our CRISPRomics® platform, we demonstrated that inactivation of SOCS1 and Regnase-1 (Reg1) by CRISPR/Cas9 gene editing greatly enhanced transgenic TCR cells in a syngeneic melanoma mouse model. Applying this insight, we developed the dual-edited TIL therapy KSQ-004EX, currently in a phase 1/2 clinical trial (NCT06598371). We demonstrate here that loss of SOCS1 and Regnase-1 similarly improves the anti-tumor activity of CAR-T cells against solid tumors. To establish proof-of-concept of a dual-edited CAR-T program, we employed an anti-mesothelin CAR (mesoCAR) system including developing a manufacturing process to produce eCAR-T cells with high CAR expression and editing efficiency. In functional assays, loss of SOCS1 and Regnase-1 enhanced cytolysis against tumor cells and antigen-dependent proliferation of mesoCAR-T cells. Crucially, compared to control cells, we observed robust *in vivo* anti-tumor activity and sustained tumor control with edited mesoCAR-T cells. These results indicate edits of SOCS1 and Regnase-1 in mesoCAR-T cells can greatly improve their anti-tumor efficacy.

Methods

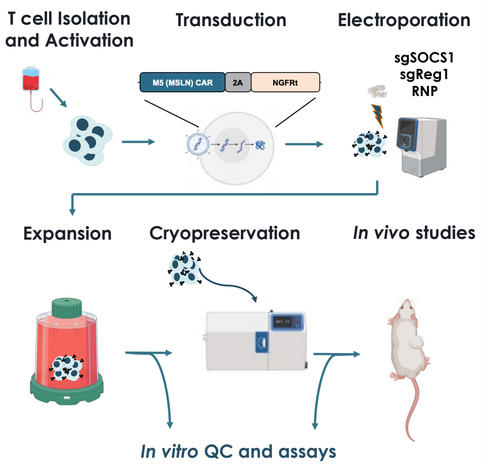

Figure 1| eCAR-T manufacturing and testing. Outline of engineering process to produce mesoCAR-T cells enhanced by gene editing of SOCS1 and Regnase-1. Quality control (QC) is conducted to assess editing and transduction efficiency.

eCAR-T Manufacturing Process Supports High CAR Expression and Editing Efficiency

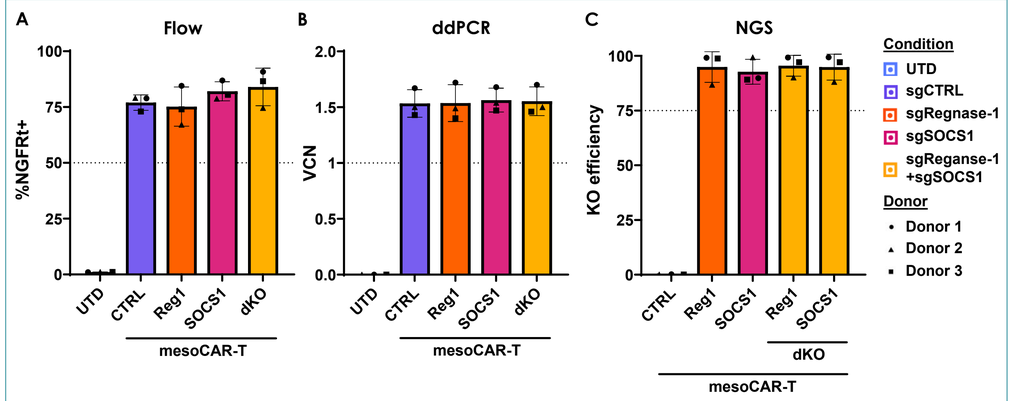

Figure 2| QC assays demonstrate eCAR-T cells have high transduction and editing efficiency. (A) NGFR reporter positivity of CAR-T cells was observed by flow 7 days after transduction. **(B)** Vector Copy Number was observed via ddPCR 7 days after transduction. **(C)** Knockout efficiency was observed via NGS 6 days after electroporation. **(A-C)** Bars represent average across three separate donors with symbol denoting different donors. UTD = Untransduced; CTRL = non-target gRNA; dKO = SOCS1 and Regnase-1 dual knockout.

Dual-Editing of SOCS1 and Regnase-1 Enhances CAR-T Activity

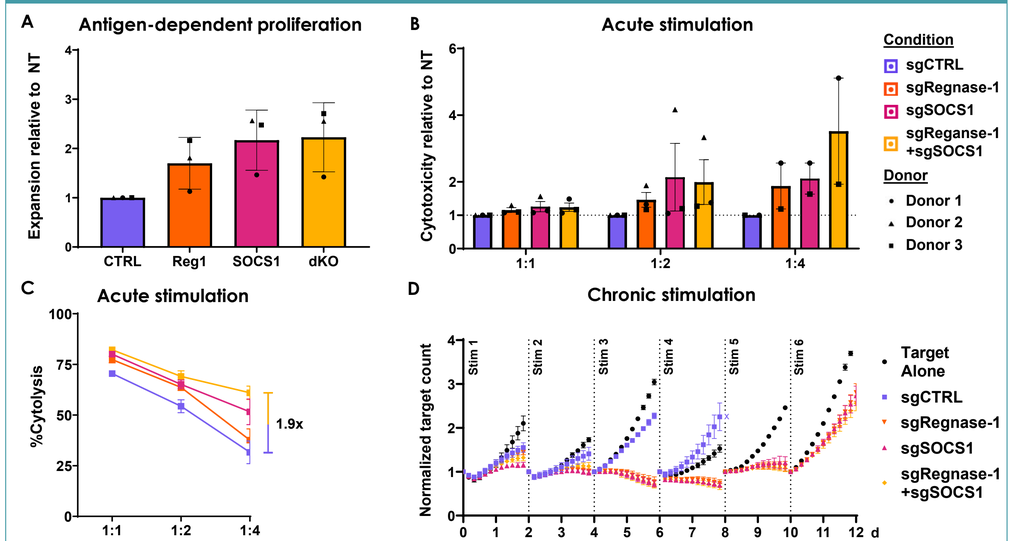

Figure 3| Anti-MSLN eCAR-T cells enhanced acute cytotoxicity, chronic cytotoxicity, & antigen-dependent proliferation against HCT116 (MSLN^{hi}) tumor cells. (A) Antigen-dependent proliferation of eCAR-T cells at 1:2 E:T ratio and 96 hr timepoint **(B-C)** Cytotoxicity of anti-MSLN eCAR-T cells against HCT116 (MSLN^{hi}) cells at 96 hr across multiple E:T ratios (1:1,1:2, and 1:4). **(C)** Representative killing curve of one donor from **(B)**. **(D)** Chronic stimulation of anti-MSLN eCAR-T cells for 6 stimulations. Initial set up as 1:2 E:T and follows a global fractional reseeding regime. **(A-B)** Data normalized to NT with bars representing average across three separate donors with symbol denoting different donors. **(B-D)** Incucyte-based live cell imaging was utilized.

Anti-MSLN eCAR-T Cells with SOCS1 and Regnase-1 Edits Demonstrate Superior Tumor Control Compared to Non-Target Edited CAR-T Cells

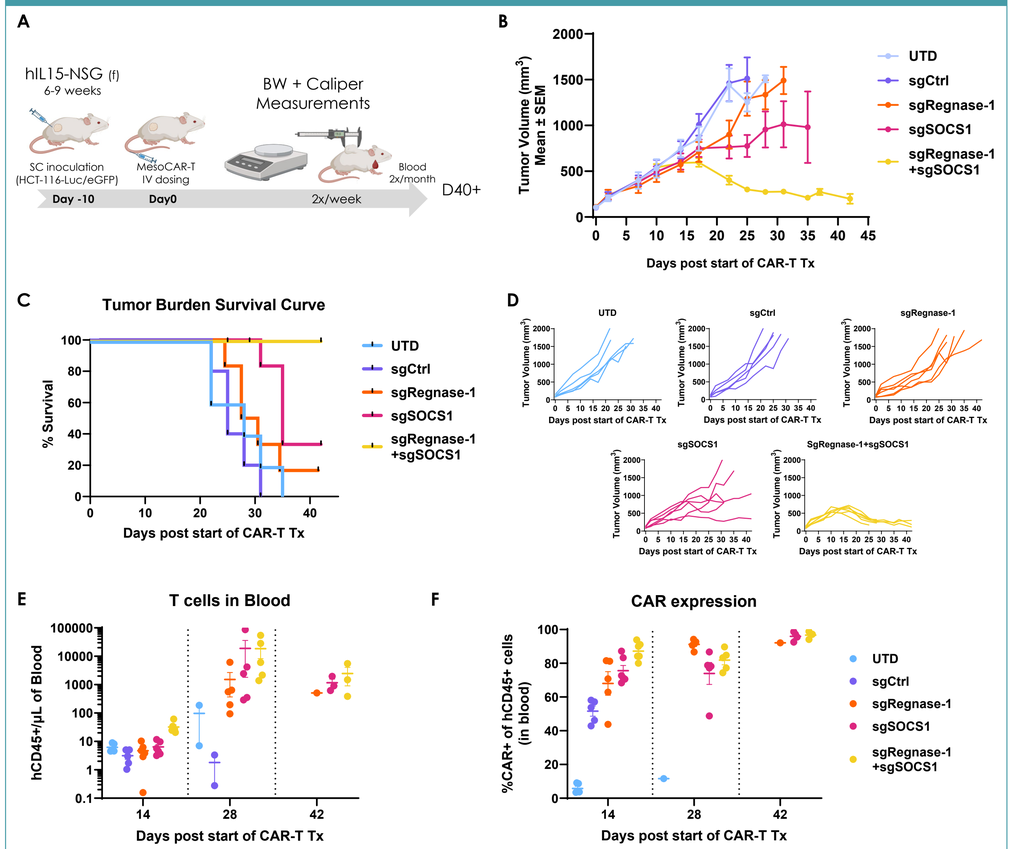

Figure 4| Anti-MSLN eCAR-T cells improved anti-tumor efficacy and survival in HCT-116 tumor-bearing mice. (A) Experimental outline of *in vivo* study utilizing eCAR-T cells against HCT-116 tumor-bearing mice. **(B)** Anti-MSLN eCAR-Ts were transferred into NSG-Hu-IL-15 Tg mice bearing HCT-116 tumors. Tumor volume is shown over time. **(C)** Tumor-burden based survival of **(B)**. **(D)** Tumor growth spider plots depicted data from **(B)**. **(E)** Levels of single and dual-edited mesoCAR-T cells by treatment group in the peripheral blood on Days 14, 28 and 42, as well as **(F)** proportion of CAR-expressing human CD45+ cells.

Conclusions

- Successfully developed eCAR-T manufacturing process to produce high-quality functional cells with editing efficiency >95% and CAR expression >70% via flow
- *In vitro*, inactivation of SOCS1 and Regnase-1 increased antigen-dependent proliferation and cytotoxic function of mesoCAR-T cells in acute and chronic stimulation settings, indicating improved function
- *In vivo*, dual-edited mesoCAR-T cells outperformed control cells, demonstrating robust regression and persistence in MSLN-expressing tumors
- Our results support editing Regnase-1 and SOCS1 as a promising strategy to extend the durability and potency of CAR-T therapies and to potentially overcome key barriers in solid tumor treatment