Off-the-Shelf DLL3/CD70 Dual CAR T Cells With Optimized Site-Specific Integration for SCLC and Neuroendocrine Tumors

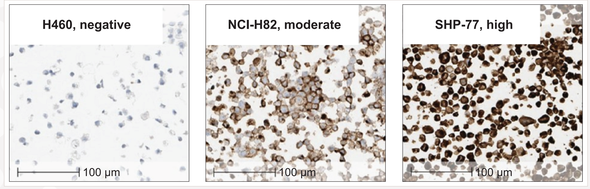
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INTRODUCTION

- DLL3 is a clinically validated target in SCLC and other neuroendocrine tumors (**Figure 1**)[1-4]
- Allogeneic DLL3 CAR T cells with high sensitivity, specificity, and activity have been previously described[5]
- Compared with autologous therapies, off-the-shelf allogeneic DLL3 CAR T cells offer immediate availability and the convenience of scalable production; however, cell expansion and clinical efficacy may be limited by premature immune rejection
  - Targeting CD70-expressing alloreactive T cells has been shown to mitigate rejection in preclinical and clinical studies,[6,7] providing a rationale for incorporating an anti-rejection CD70 CAR to enhance the persistence and activity of allogeneic DLL3 CAR T cells
- We therefore developed allogeneic DLL3/CD70 dual CAR T cells with optimized SSI to maximize antitumor efficacy and improve resistance to immune-mediated rejection

Figure 1. DLL3 IHC Expression in Lung Cancer Cell Lines[1]



Microphotographs show different levels of DLL3 IHC expression in lung cancer cell lines: negative in H460, an NSCLC cell line; moderate in NCI-H82, an SCLC cell line; and high in SHP-77, an SCLC cell line.

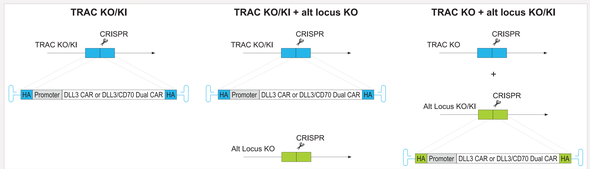
METHODS

- TRAC KO DLL3 and DLL3/CD70 dual CAR T cells were produced using SSI with CRISPR gene editing technology and an AAV
- In addition to SSI at the TRAC locus, multiple other integration sites were evaluated
- Cytotoxic activity and CAR T-cell expansion were assessed *in vitro* and *in vivo* using functional assays and SCLC xenograft models
- Persistence of DLL3/CD70 dual CAR T cells in the presence of alloreactive host T cells was evaluated in MLR assays

RESULTS

- TRAC KO DLL3 and DLL3/CD70 dual CAR T cells engineered via SSI at the TRAC locus or alternative loci were successfully produced from healthy donor-derived T cells
- Across KI designs, differences were observed in CAR expression, phenotype, and *in vivo* efficacy
- Compared with SSI at TRAC, SSI at other loci associated with T-cell function enhanced CAR T-cell performance
- Among the loci tested, SSI at SOCS1 emerged as the lead design and was associated with earlier CAR T-cell expansion and improved tumor control across DLL3-high (SHP-77) and DLL3-moderate (NCI-H82) xenograft models
- Matched KO/KI studies showed that the improved function is associated with KI rather than KO alone
- Compared with control T cells, DLL3/CD70 dual CAR T cells eliminated CD70$^+$ alloreactive T cells and showed resistance to rejection

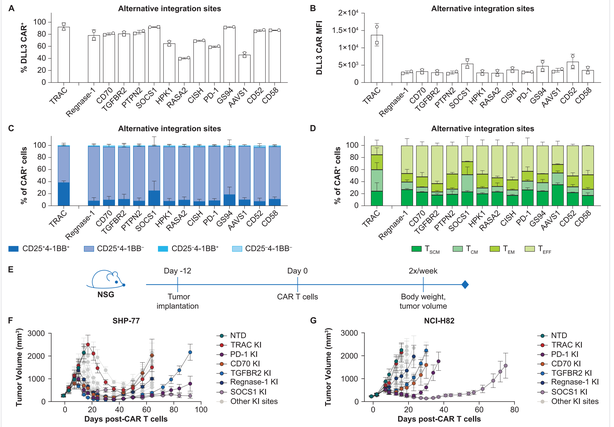
Figure 2. SSI Strategies for CAR T-Cell Engineering



Schematic depicts 3 SSI approaches for DLL3 single or DLL3/CD70 dual CAR integration. In addition to the TRAC locus, alternative integration sites were assessed to enhance CAR T-cell function through an additional gene KO and/or by altering CAR expression dynamics through locus-specific integration. Unlike the DLL3 CAR, which contains a targeting scFv, hinge and transmembrane domains, a costimulatory domain, and a CD3ζ signaling domain, the CD70 CAR lacks a costimulatory domain.

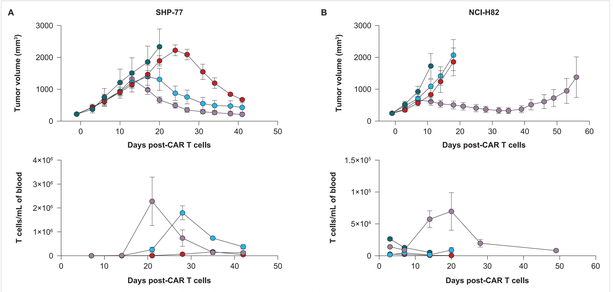
Presented at the Society for the Immunotherapy of Cancer (SITC); November 4-8, 2026; Phoenix, AZ, USA

Figure 3. DLL3 CAR T Cells Generated by SSI at Loci Other Than TRAC Show Superior *In Vivo* Efficacy in Two Models of SCLC



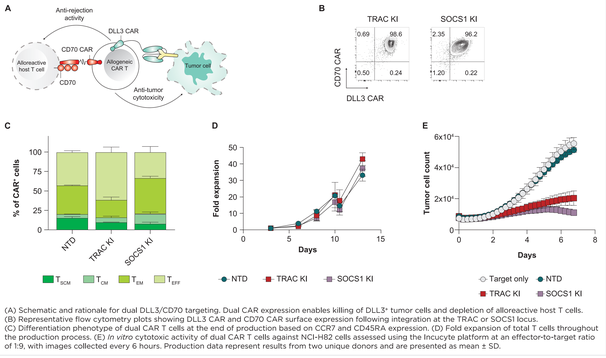
Flow cytometric characterization of DLL3 CAR T cells generated using different integration loci. (A) Frequency of DLL3 CAR-expressing cells at the end of production determined by intracellular staining. (B) DLL3 CAR surface expression at the end of production. Each symbol represents an individual donor. (C) Expression of activation markers CD25 and 4-1BB on Day 6 of production. (D) Distribution of memory and effector subsets based on CCR7 and CD45RA expression at the end of production. (E) *In vivo* efficacy study design. NSG mice were implanted subcutaneously with 5 × 10^6 tumor cells 12 days prior to CAR T-cell treatment. DLL3 CAR T cells generated using alternative integration loci delayed tumor progression in (F) SHP-77 and (G) NCI-H82 xenograft models. Data represent results from two unique donors. *In vivo* data are shown as mean ± SEM (n=3/group for SHP-77 and n=5/group for NCI-H82).

Figure 4. DLL3 CAR T Cells Generated via SSI at the SOCS1 Locus Show Earlier Expansion and Improved Tumor Control Compared With TRAC KI and TRAC KI/SOCS1 KO Controls



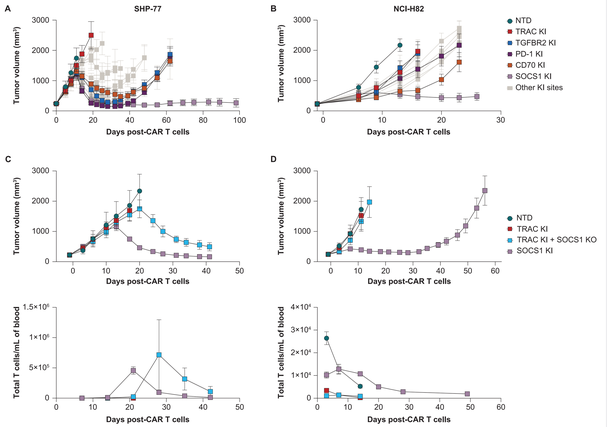
DLL3 CAR T cells generated using three SSI approaches were compared in two subcutaneous SCLC xenograft models for antitumor efficacy (top) and peripheral expansion (bottom). (A) SHP-77 and (B) NCI-H82 tumor-bearing NSG mice received a single intravenous dose of 5 × 10^5 and 5 × 10^6 CAR T cells, respectively. Tumor growth and peripheral CAR T-cell expansion were monitored longitudinally following treatment. Data represent results from two unique donors. Data are presented as mean ± SEM (n=3/group for SHP-77 and n=5/group for NCI-H82).

Figure 5. Efficient Co-Expression of DLL3 CAR With an Anti-Rejection CD70 CAR Following Integration at the SOCS1 Locus



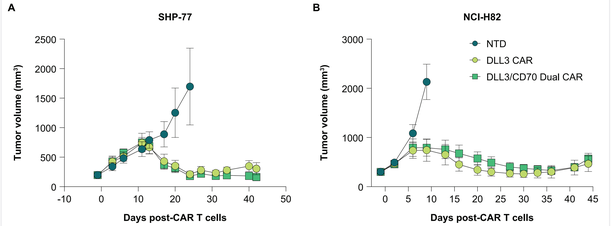
(A) Schematic and rationale for dual DLL3/CD70 targeting. Dual CAR expression enables killing of DLL3$^+$ tumor cells and depletion of alloreactive host T cells. (B) Representative flow cytometry plots showing DLL3 CAR and CD70 CAR surface expression following integration at the TRAC or SOCS1 locus. (C) Differentiation phenotype of dual CAR T cells at the end of production based on CCR7 and CD45RA expression. (D) Fold expansion of total T cells throughout the production process. (E) *In vitro* cytotoxic activity of dual CAR T cells against NCI-H82 cells assessed using the Incucyte platform at an effector-to-target ratio of 1:9, with images collected every 6 hours. Production data represent results from two unique donors and are presented as mean ± SD.

Figure 6. SOCS1 KI DLL3/CD70 Dual CAR T Cells Show Earlier Expansion and Improved Tumor Control Compared With TRAC KI and TRAC KI/SOCS1 KO Controls



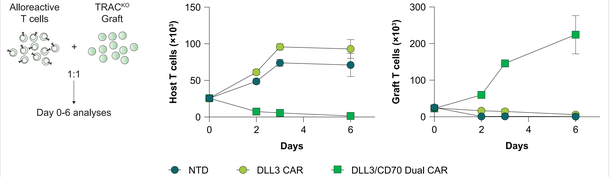
In vivo evaluation of DLL3/CD70 dual CAR T cells generated using different SSI strategies. (A, B) Dual CAR T cells with SSI at TRAC or alternative loci were compared. Antitumor efficacy was evaluated in (A) SHP-77 and (B) NCI-H82 subcutaneous xenograft models. Tumor-bearing NSG mice received a single intravenous dose of 1 × 10^6 and 5 × 10^6 CAR T cells, respectively. (C, D) DLL3/CD70 dual CAR T cells generated by SOCS1 KI were compared with TRAC KI and TRAC KI/SOCS1 KO controls for antitumor efficacy (top) and peripheral expansion (bottom). (C) SHP-77 and (D) NCI-H82 tumor-bearing NSG mice received a single intravenous dose of 5 × 10^5 and 5 × 10^6 CAR T cells, respectively. Data are presented as mean ± SEM (n=3/group for SHP-77 and n=5/group for NCI-H82).

Figure 7. Co-Expression of the CD70 CAR Preserves DLL3-Directed Antitumor Activity



Comparison of the *in vivo* efficacy of SOCS1-integrated DLL3 single CAR and DLL3/CD70 dual CAR T cells in subcutaneous SCLC xenograft models. (A) SHP-77 and (B) NCI-H82 tumor-bearing NSG mice received a single intravenous dose of 3 × 10^5 and 5 × 10^6 CAR T cells, respectively. Data are presented as mean ± SEM (n=3/group for SHP-77 and n=5/group for NCI-H82).

Figure 8. DLL3/CD70 Dual CAR T Cells Show Resistance to Rejection in MLR Assays



(A) Schematic of the primed T-cell MLR assay. Alloreactive host T cells were produced by priming T cells from allogeneic donors against unedited graft T cells. Host T cells were then mixed with graft CAR T cells, and the cell populations were analyzed by flow cytometry at the indicated time points. (B) Representative donor pair showing host T cell (left) and graft T cell (right) counts over time. Data are presented as mean ± SD and representative of multiple independent host/graft donor pairs.

CONCLUSIONS

- Integration of the DLL3 CAR and the DLL3/CD70 dual CAR into the SOCS1 locus was associated with earlier CAR T-cell expansion and improved tumor control compared with TRAC SSI and TRAC SSI plus SOCS1 KO controls
- The enhanced activity of SOCS1 KI relative to SOCS1 KO alone suggests that factors beyond gene disruption contribute to the observed phenotype, supporting the importance of genomic context on CAR expression and function. The underlying mechanism is under investigation
- Co-expression of an anti-rejection CD70 CAR conferred resistance to alloreactive T cells while preserving DLL3 CAR activity
- These findings support the development of allogeneic DLL3/CD70 dual CAR T cells as a differentiated off-the-shelf design that combines optimized CAR function with intrinsic rejection resistance for the treatment of SCLC and neuroendocrine tumors

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Acknowledgments

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Abbreviations

4-1BB, cluster of differentiation 137; AAV, adeno-associated virus; AAVS1, adeno-associated virus integration site 1; alt locus, alternative locus; CD, cluster of differentiation; CD3ζ, cluster of differentiation 3 zeta chain; CD45RA, cluster of differentiation 45, RA isoform; CISH, cytokine-inducible SH2-containing protein; CRISPR, clustered regularly interspaced short palindromic repeats; DLL3, delta-like ligand 3; GSN4, genomic safe harbor locus; HA, homology arm; HPK1, hematopoietic progenitor kinase 1; KI, knock in; KO, knock out; MLR, mixed lymphocyte reaction; NSG, NOD scid gamma; NTD, non-transduced; PD-1, programmed cell death protein 1; PTPN2, protein tyrosine phosphatase non-receptor type 2; RASA2, RAS p21 protein activator 2; scFv, single-chain variable fragment; SCLC, small cell lung cancer; SD, standard deviation; SEM, standard error of the mean; SOCS1, suppressor of cytokine signaling 1; SSI, site-specific integration; T_{CM}, central memory T cell; TCR, T-cell receptor; T_{EFF}, effector T cell; T_{EM}, effector memory T cell; TGFBR2, transforming growth factor beta receptor 2; TRAC, T-cell receptor alpha constant; T_{SCM}, stem cell memory T cell.

Disclosures

All authors are employees of Allogene Therapeutics, Inc.

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