Preclinical evaluation of allogeneic CD19 CAR T cells expressing an anti-rejection CD70 CAR

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Abstract #279

Background: Autologous CAR T cell therapies have revolutionized the treatment of hematologic malignancies but have inherent disadvantages that hinder widespread access, including complex logistics and manufacturing limitations. These challenges may be overcome with off-the-shelf allogeneic CAR T cells derived from healthy donor T cells. Although allogeneic CAR T cells provide immediate availability to patients and scalable manufacturing, they may be susceptible to allorejection and have reduced persistence, limiting clinical responses. To address this challenge, we developed an anti-rejection CD70 CAR capable of selectively depleting activated ($CD70^+$) host lymphocytes. We previously showed that this approach rendered allogeneic CD19 CAR T cells resistant to allorejection while also enhancing antitumor activity by endowing dual targeting in $CD70^+CD19^+$ lymphoma models. Here, we describe an optimized construct for site-specific integration (SSI)-based co-expression of the anti-rejection CD70 CAR and a CD19 CAR from a single locus. The resulting CAR T cell product showed high homogeneity, enrichment of CD19 CAR/CD70 CAR double positive cells, efficacy comparable to CAR T cells expressing only a CD19 CAR, and resistance to allorejection.

Methods: TALEN® gene-editing technology combined with adeno-associated virus (AAV) transduction was employed to knock-in CAR constructs into the T Cell Receptor Alpha Constant (*TRAC*) locus. Constructs encoding a CD19/CD70 tandem CAR (single CAR containing both CD70 and CD19 single-chain variable fragments) or a dual CAR (CD70 CAR and CD19 CAR separated by a self-cleaving peptide) were tested. Cytotoxicity was assessed *in vitro* and *in vivo* using a Raji lymphoma model. Anti-rejection activity of the CD19/CD70 CAR T cells was assessed in mixed lymphocyte reaction (MLR) assays.

Results: SSI of the CD19/CD70 dual CAR transgene in activated T cells was highly efficient and resulted in a high percentage and yield of CD19 CAR/CD70 CAR double positive cells (~80-99%), which showed improved functionality compared to cells expressing tandem CAR constructs. Enrichment and expansion of dual CAR^+ cells was likely enhanced due to CD70-dependent activation during the manufacturing process. Despite this, these cells preserved T cell memory subsets, efficiently eliminated Raji cells *in vitro* and *in vivo*, and resisted allorejection, suggesting that both CARs retain their independent functions.

Design of a CD19/CD70 Dual CAR for integration into the *TRAC* locus

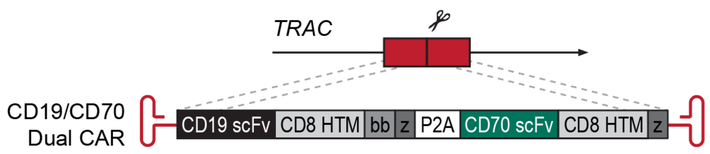

Figure 1. Schematic of the Dual CAR construct for site-specific integration into the *TRAC* locus allowing for co-expression of a CD19 CAR and a CD70 anti-rejection CAR. scFv: single chain variable fragment, H: hinge, TM: transmembrane domain, bb: 4-1BB costimulatory domain, z: CD3ζ activation domain.

High levels of CAR expression achieved with the Dual CAR construct

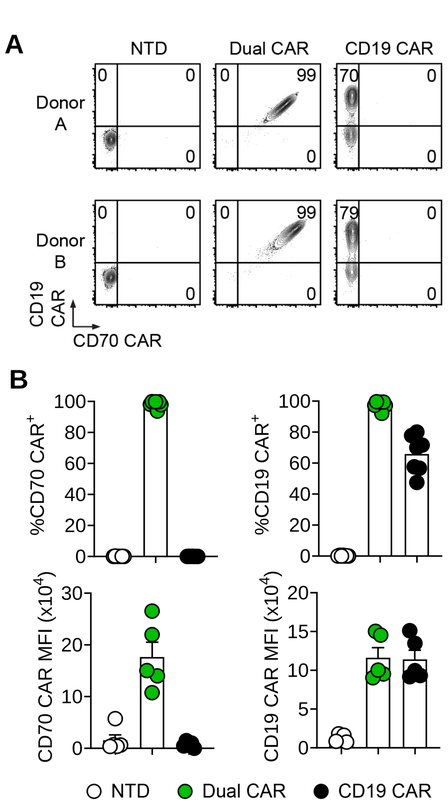

Figure 2. Cells were analyzed by flow cytometry at the end of the production process, day 14 post-activation. (A) Representative flow cytometry plots showing CD19 CAR and CD70 CAR expression from 2 individual donors. (B) Percentage of CAR^+ cells and expression level of the CARs. Symbols represent individual donors. NTD: non-transduced T cells, MFI: mean fluorescence intensity.

Comparable phenotype and expansion between Dual and single CAR T cells

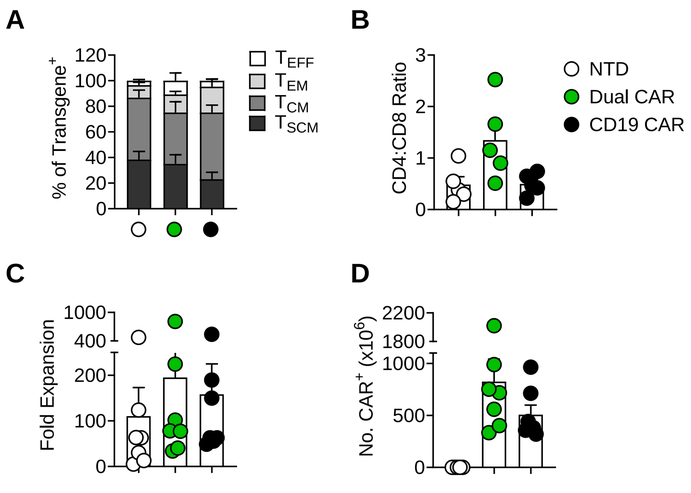

Figure 3. Cells were analyzed by flow cytometry at the end of the production process, day 14 post-activation. (A) Analysis of T cell subsets, (B) CD4/CD8 ratios, (C) total fold expansion, and (D) total CD19 CAR^+ cell yields at the end of the production process. Symbols represent individual donors.

CD19/CD70 Dual CAR T cells show no evidence of aberrant growth *in vitro*

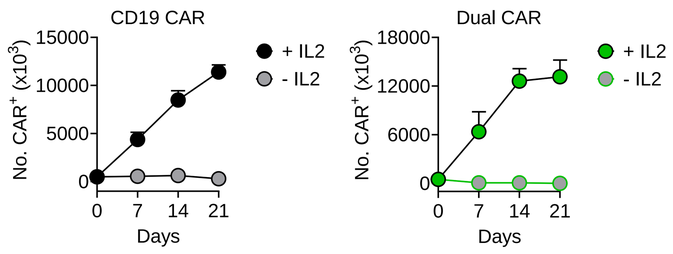

Figure 4. CD19 CAR T cells and CD19/CD70 Dual CAR T cells were cultured with or without IL-2 and cell growth was monitored over time by flow cytometry. Data are the combined results from 5 individual donors

CD19/CD70 Dual CAR T cells resist rejection by alloreactive host T cells

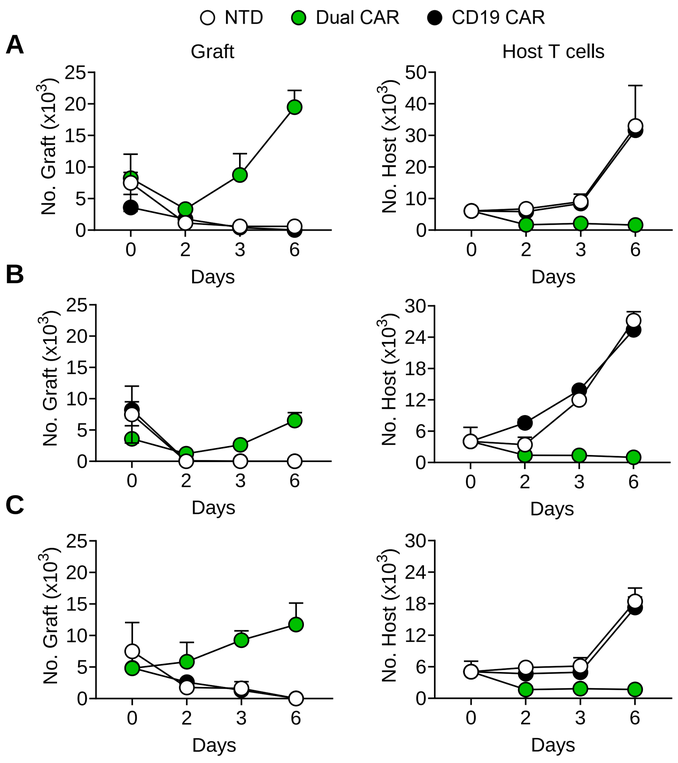

Figure 5. MLR assays were performed by co-culturing allogeneic T cells that had been primed for 7 days for increased alloreactivity, with either $TRAC^{KO}$ control T cells, Dual CAR T cells, or CD19 CAR T cells. Viability of the surviving control or CAR T cells, referred to as "graft", and host T cells (HTCs) was determined by flow cytometry. Absolute number of graft (left) and HTCs (right) are shown over time. Data are representative of 17 unique graft:host donor pairs tested. (A) Graft C paired with Host A. (B) Graft C paired with Host B. (C) Graft D paired with Host A.

Co-expressing the anti-CD70 CAR does not affect the antitumor activity of the CD19 CAR

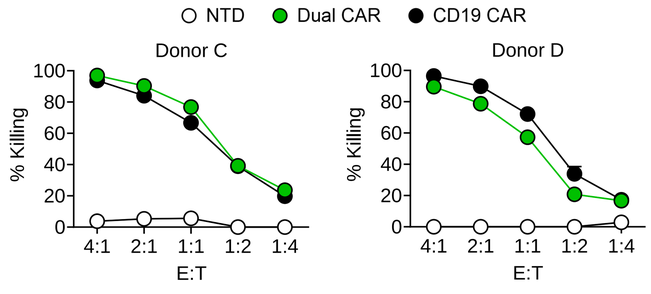

Figure 6. (A) 24-hour killing assay. Cytotoxic activity against luciferase (luc)-labeled $CD70^{KO}$ Raji cells was assessed using bioluminescence. Data are representative of 5 unique donors tested.

CD19/CD70 Dual CAR T cells eradicate antigenically heterogeneous tumors

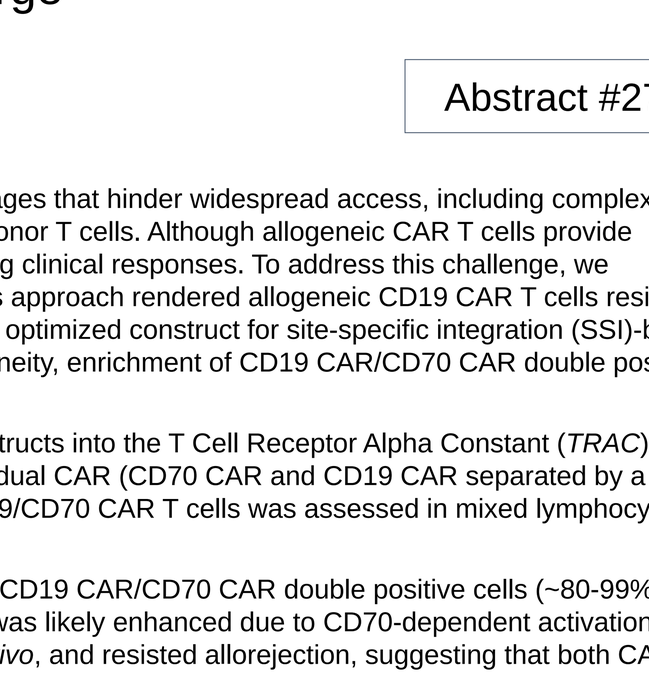

Figure 7. To mimic a tumor with heterogenous target expression and to model antigen escape, $CD19^{KO}$ and parental ($CD19^{WT}$) Raji cells were mixed at a ratio of 1:1 prior to co-culturing with CAR T cells at an E:T ratio of 1:3. (A) Flow cytometry plots of CD19 expression on Raji tumor cells before and after co-culture with effector T cells. Absolute number of $CD19^{neg}$ Raji population indicated. (B) Total $CD19^{neg}$ Raji cell counts.

CD19/CD70 Dual CAR T cells are efficacious *in vivo*

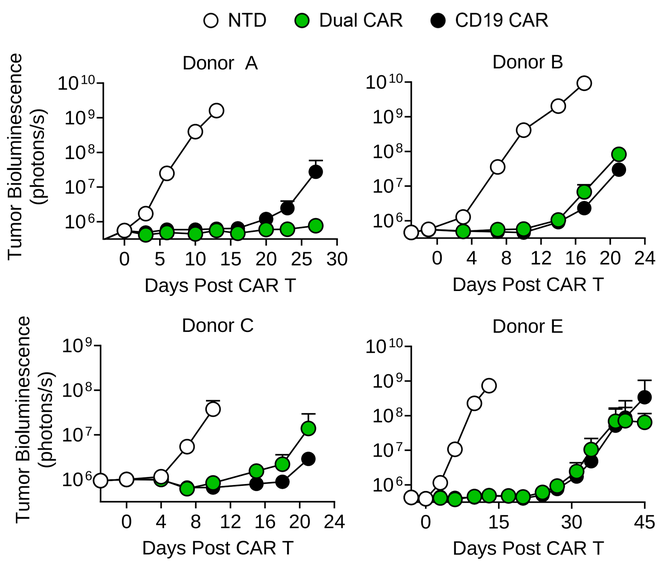

Figure 8. NSG mice engrafted with luc-labeled $CD70^{KO}$ Raji tumors received a dose of $5x10^6$ TCRα-deficient CAR^+ cells and tumor growth was monitored using whole-body luminescence imaging (n = 8-10 mice). Representative of 5 individual donors tested.

Conclusions

- CD19/CD70 Dual CAR T cells produced via SSI are efficacious *in vitro* and *in vivo* and can overcome antigen escape
- Co-expressing a CD70 CAR with a CD19 CAR allows allogeneic CD19 CAR T cells to resist rejection.
- The anti-rejection CD70 CAR technology is designed to enhance engraftment and expansion of AlloCAR T™ product candidates