Selective targeting of host CD70⁺ alloreactive cells with a CD70 Dagger™ receptor to prolong allogeneic CAR T cell persistence

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Abstract

Chimeric antigen receptor (CAR) T cell therapy is a cellular immunotherapy approach that takes advantage of unique T cell features for treating cancer. This involves genetically manipulating patient T cells to specifically target tumor antigens. Currently approved treatment approaches employ autogolous T cells, which poses limitations on the widespread use of CAR T cell immunotherapy. Pre-manufactured allogeneic CAR T cells from healthy donors is a promising alternative that may circumvent these issues. However, rejection of allogeneic CAR T cells by the patient's T and natural killer (NK) cells remains a challenge in the allogeneic cell immunotherapy field. We report the development and evaluation of a CAR that prevents rejection of allogeneic CAR T cells by selectively targeting CD70 on patient's NK and T cells. CAR constructs targeting CD70, herein referred to as Dagger receptors, were screened using healthy donor T cells for desired attributes by flow cytometry and function in various *in vitro* rejection models. Allogeneic T cells expressing Dagger receptors selectively eliminated alloreactive T cells and significantly enhanced cell survival compared to control allogeneic T cells. Additionally, T cells expressing first generation CAR-like Dagger receptors lacking an intracellular co-stimulatory domain (ICD) exhibited superior phenotypes compared to variants containing an ICD. Our results demonstrate that arming allogeneic T cells with a CD70 dagger receptor eliminates alloreactive T cells and confers resistance to rejection. This proprietary technology may be applied to improve persistence and long-term efficacy of allogeneic CAR T cells and may potentially allow for the reduction in intensity of lymphodepletion required in patients receiving allogeneic CAR T cell therapy.

CD70 CAR T cells are protected from rejection by host alloreactive T cells

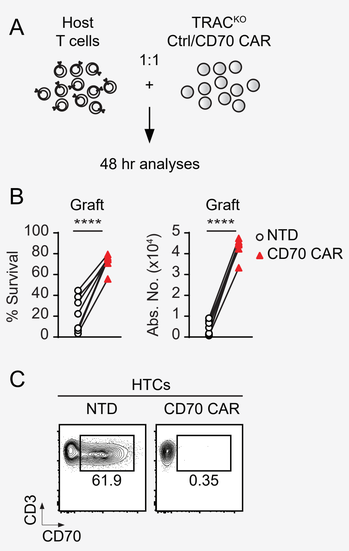

Figure 1. (A) Mixed lymphocyte reactions (MLRs) were performed by co-culturing allogeneic T cells that had been primed for 7 days for increased alloreactivity, with either TRAC^KO control or CD70 CAR T cells. (B) The viability of the surviving control or CAR T cells, referred to as "graft", was determined by flow cytometry. (C) Only host T cells (HTCs) expressing CD70 are eliminated by CD70 CAR T cells. Symbols represent unique graft-host donor pairs (n = 8). Asterisks indicate statistical significance and *p* values denoted as *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001

Generation of CD70 Dagger receptor variants from a CD70 CAR

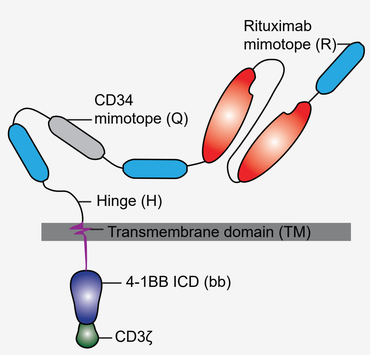

Figure 2. Schematic of CD70 CAR architecture highlighting domains and motifs that were modified for Dagger variant screening in the CD52 locus. KO was performed using TALEN® gene-editing, a technology pioneered and controlled by Cellectis. H: hinge, TM: transmembrane, Q: CD34 mimotope, z: CD3ζ domain, bb: 4-1BB ICD; R: rituximab mimotope

Dagger receptors lacking a costimulatory domain co-express better with a CD19 CAR

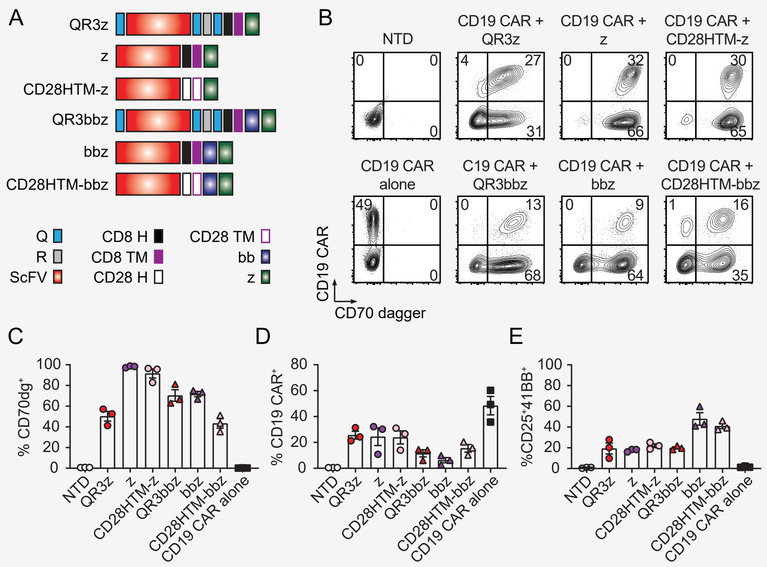

Figure 3. (A) T cells were co-transduced with AAVs encoding a CD19 CAR targeted for integration into the TRAC locus and Dagger variants targeted for integration into the CD52 locus. (B-D) Cells were analyzed by flow cytometry on day 14 or (E) day 9 post-activation. Symbols represent unique donors pairs.

CD19 CAR and Dagger activity are maintained when both receptors are co-expressed on T cells

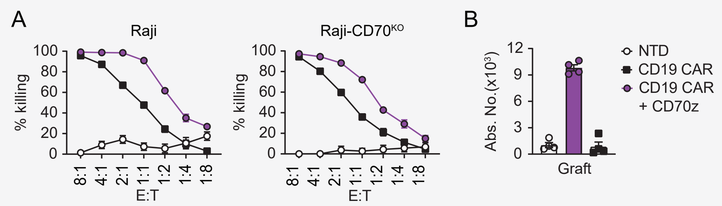

Figure 4. (A). 24 hr short-term killing assay. Raji target cell killing was assessed using bioluminescence. (B) MLRs were performed with TRAC^KO control or Dagger CAR T cells (CD19 CAR + CD70z). Symbols represent unique graft-host donor pairs.

Dagger receptor endows dual specificity and limits antigen escape *in vitro*

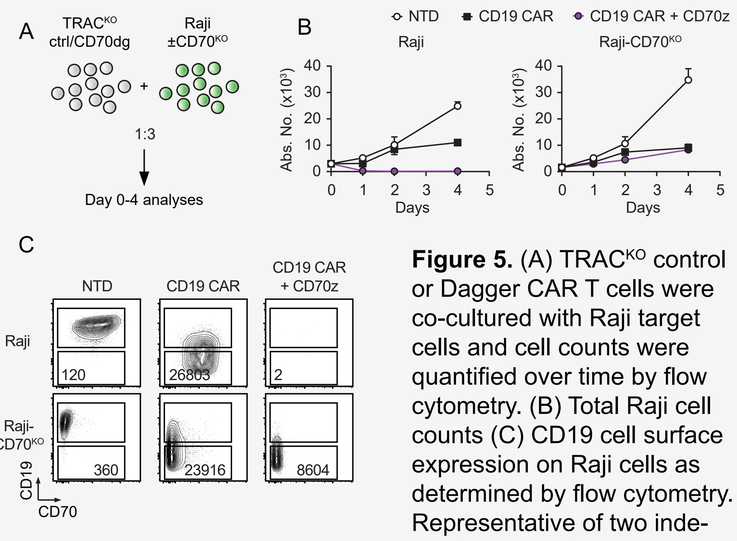

Figure 5. (A) TRAC^KO control or Dagger CAR T cells were co-cultured with Raji target cells and cell counts were quantified over time by flow cytometry. (B) Total Raji cell counts (C) CD19 cell surface expression on Raji cells as determined by flow cytometry. Representative of two independent experiments.

Dual targeting with Dagger CAR T cells limits antigen escape *in vivo*

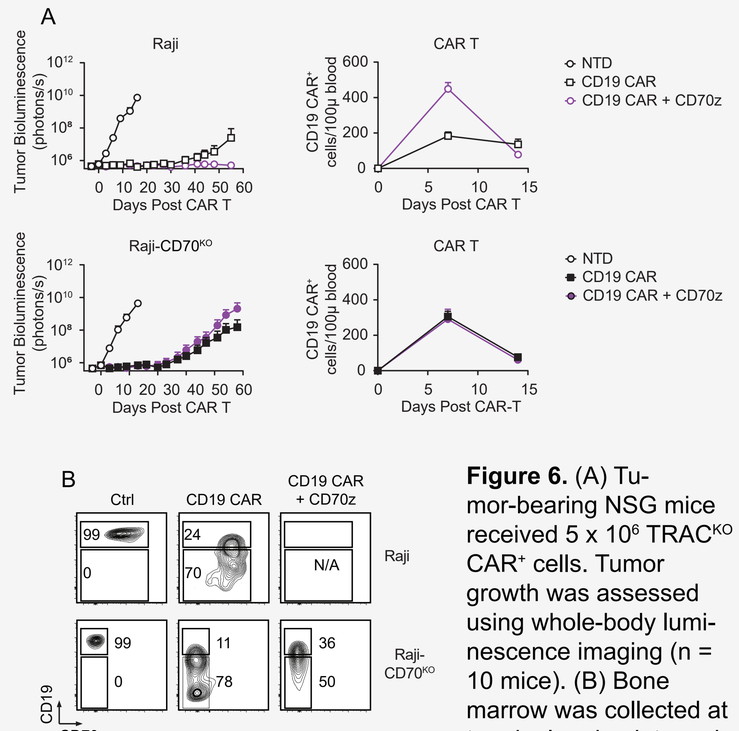

Figure 6. (A) Tumor-bearing NSG mice received 5 x 10⁶ TRAC^KO CAR⁺ cells. Tumor growth was assessed using whole-body luminescence imaging (n = 10 mice). (B) Bone marrow was collected at terminal end points and Raji cells were analyzed *ex vivo* by flow cytometry.

Dagger receptor promotes CAR T cell persistence and anti-tumor activity

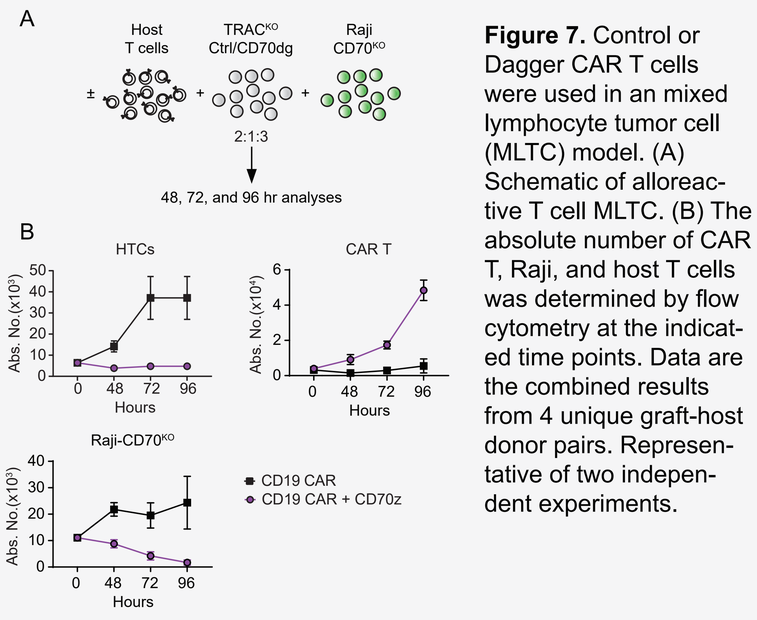

Figure 7. Control or Dagger CAR T cells were used in an mixed lymphocyte tumor cell (MLTC) model. (A) Schematic of alloreactive T cell MLTC. (B) The absolute number of CAR T, Raji, and host T cells was determined by flow cytometry at the indicated time points. Data are the combined results from 4 unique graft-host donor pairs. Representative of two independent experiments.

Conclusion and Future Direction

- CD19 CAR T cells armed with a CD70 Dagger receptor deplete alloreactive host T cells and show higher persistence in MLR assays
- CD70 Dagger receptor endows dual specificity in CD19 CAR T cells and overcomes antigen escape
- Dagger technology may be applied to allogeneic CAR T cells as a dual-purpose receptor to improve anti-tumor efficacy and evade host alloimmune responses