Poster #367

CRISPR Edited Allogeneic Anti-CD83 CAR-T Cells Show Potent Activity in GvHD and AML Tumor Models

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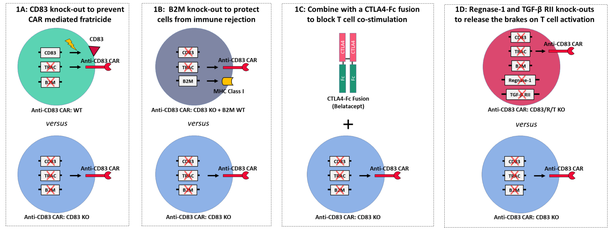
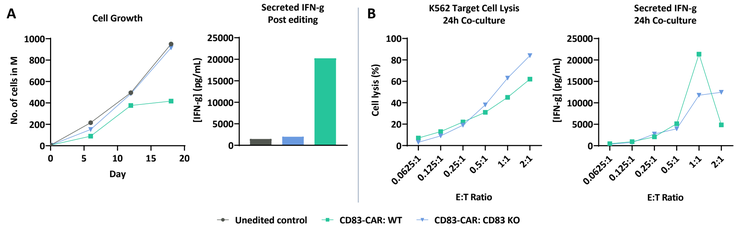
Abstract CD83 is expressed on acute myeloid leukemia (AML) blasts and allo-activated immune cells, making it an attractive target for preventing relapse and graft versus host disease (GvHD) in AML patients who undergo allogeneic hematopoietic cell transplantation. A recent publication showed that autologous anti-CD83 CAR-T cells are active in both AML tumor and GvHD animal models[1]. In this study, CRISPR/Cas9 editing was used to make allogeneic anti-CD83 CAR-T cells and test if knock-outs could improve CAR-T activity *in vitro* and *in vivo*. Knockout of CD83 expression improved activity by preventing CAR mediated fratricide while knockout of β2M expression protected allogeneic cells from immune rejection. Anti-CD83 CAR-T cell activity was further enhanced by knocking out genes that function as 'brakes' on T cell activation or combining CAR-T cells with a CTLA4-Fc fusion protein that blocks co-stimulation of T cells. Collectively, these data support the clinical evaluation of gene-edited, potency-enhanced, allogeneic anti-CD83 CAR-T cells in relapsed/refractory AML patients.

Figure 1: Strategies to enhance the potency of anti-CD83 CAR-T Cells



All CAR-T cells included TRAC knock-out to prevent GvHD and anti-CD83 CAR transgene insertion into the TRAC locus. Additional knock-outs are described in panels 1A-1D.

Figure 2: CD83 knock-out improves anti-CD83 CAR-T cell expansion with minimal impact on effector function *in vitro*



(A) Anti-CD83 CAR-T cells (CD83-CAR) with and without CD83 gene disruption (CD83 KO vs. WT) were edited on Day 0 and cultured in parallel with an unedited control. CD83-expressing WT CAR-T cells appeared to undergo CAR-mediated fratricide and knocking out CD83 reduced IFN-γ secretion and improved cell expansion. **(B)** Effector function was assessed by co-culturing a fixed number of CAR-T cells with increasing numbers of CD83+ K562 target cells. Both CD83 KO and WT CAR-T cells showed robust target cell killing and cytokine secretion *in vitro*.

Figure 3: CD83 knock-out enhanced the activity of anti-CD83 CAR-T cells in a murine xenograft tumor model

Both WT and CD83 KO anti-CD83 CAR-T cells substantially delayed tumor growth in a THP1 xenograft tumor model. Treatment with CD83 KO CAR-T cells showed even higher activity and led to durable complete responses (CRs) in 3/5 mice.

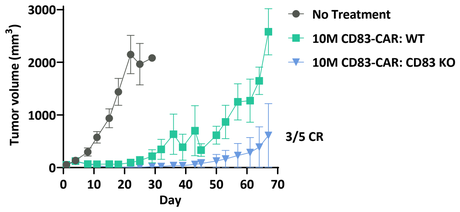
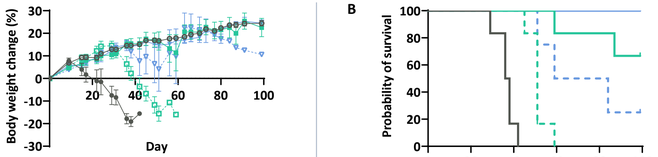
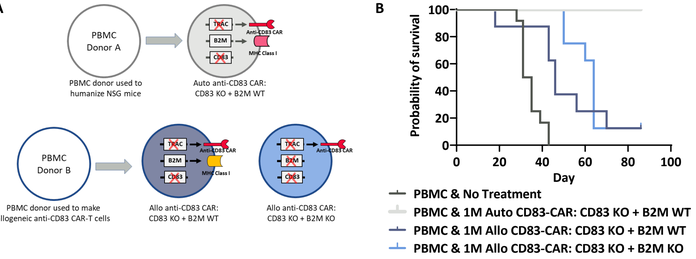


Figure 4: CD83 knock-out enhanced the activity of anti-CD83 CAR-T cells in a preventative GvHD model



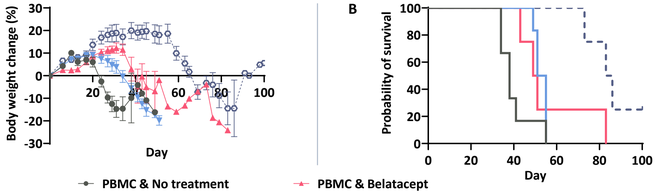
Mice engrafted with human PBMCs experienced severe weight loss (**A**) and died by Day 42 (**B**). CD83 KO anti-CD83 CAR-T cells delayed or eliminated weight loss (**A**) and improved survival **(B)** better than WT anti-CD83 CAR-T cells, including complete prevention of GvHD at the 3M CAR+ T cell dose. The CAR-T cells used in this study were allogeneic to the PBMC donor.

Figure 5: B2M knock-out improves potency of allogeneic anti-CD83 CAR-T cells in PBMC humanized NSG mice



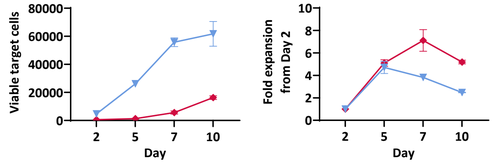
(A) To test if PBMC-derived T cells drive both GvHD and allogeneic CAR-T cell rejection, three types of anti-CD83 CAR-T cells were made: autologous CAR-T cells from the PBMC donor used for humanization, and allogeneic CAR-T cells from an unrelated donor with and without B2M knock-out (to assess the contribution of MHC class I mediated immune rejection). **(B)** Allogenic rejection drives reduced potency at lower CAR-T doses, and B2M knock-out helps protect from allogeneic rejection. With additional potency edits, these allogeneic CAR-T cells can match the survival seen with autologous cells (Figure 9).

Figure 6: Combining CD83 KO anti-CD83 CAR-T cells with belatacept delays GvHD more effectively than either treatment alone



A single dose of allogeneic CD83 KO anti-CD83 CAR-T cells, multiple doses of the CTLA-4 Fc fusion protein belatacept (12 x 100 µg 3 times per week), or a combination of the two were assessed in a preventative GvHD model. The combination delayed body weight loss and improved survival more effectively than either treatment alone. These data show that belatacept does not impact CAR-T effector function *in vivo* and support this combination strategy for GvHD prophylaxis.

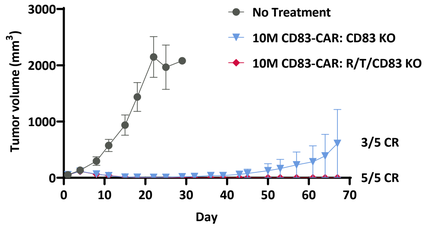
Figure 7: Addition of the Regnase-1 and TGFβ-RII knock-outs increases CD83 KO anti-CD83 CAR-T proliferation and target cell killing *in vitro*



Co-culture conditions	Day 0	Day 2	Day 5	Day 7
A498 target cells	30,000	60,000	120,000	150,000
Anti-CD83 CAR-T cells	7,500	-	-	-

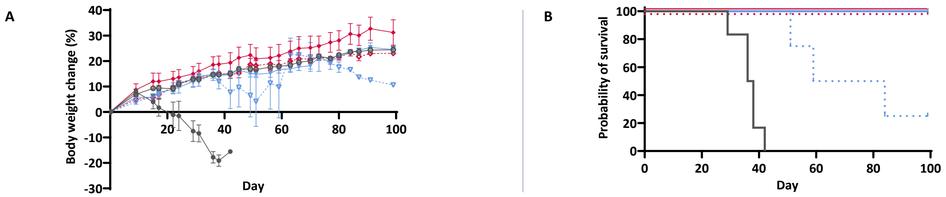
A fixed number of CAR-T cells were co-cultured with CD83+ A498 target cells on Day 0 and challenged with increasing numbers of target cells on Days 2, 5, and 7. R/T/CD83 KO CAR-T cells exhibited enhanced target cell killing and proliferation upon re-challenge with target cells, indicating that disrupting the Regnase-1 and TGFBRII genes can further increase anti-CD83 CAR-T cell potency.

Figure 8: Addition of the Regnase-1 and TGFβ-RII knock-outs enhances the activity of CD83 KO anti-CD83 CAR-T cells in a murine xenograft tumor model



In an established THP1 xenograft tumor model, durable complete responses (CRs) were observed in all mice treated with anti-CD83 CAR R/T/CD83 KO cells. In contrast, a 60% (3/5) CR rate was observed in mice treated with CD83 KO anti-CD83 CAR-T cells.

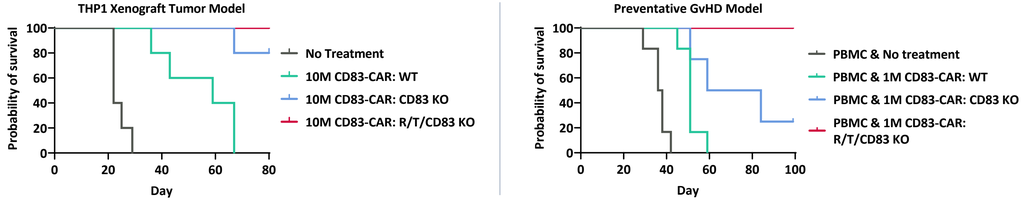
Figure 9: Addition of the Regnase-1 and TGFβ-RII knock-outs enhances the activity of CD83 KO anti-CD83 CAR-T cells in a preventative GvHD model



Allogeneic R/T/CD83 KO anti-CD83 CAR-T cells prevented GvHD at both the 1M and 3M CAR+ T cell dose levels. In contrast, allogeneic CD83 KO anti-CD83 CAR-T cells prevented GvHD only at the 3M CAR+ T cells dose level and delayed GvHD at the 1M CAR+ T cells dose level.

Conclusions — Potency enhanced allogeneic anti-CD83 CAR-T cells have the potential to prevent relapse and GvHD in AML patients

- CD83 is a promising CAR-T target for the treatment of acute myeloid leukemia and prevention of graft vs. host disease
- While anti-CD83 CAR-T cells show encouraging activity alone, that activity can be improved through a variety of means, including knock out of CD83 to prevent CAR-mediated fratricide, knock out of B2M to reduce allogeneic rejection, and combination with belatacept
- CRISPR/Cas9-mediated disruption of Regnase-1 and TGF-β RII expression further improves potency and survival in both AML and GvHD models *in vivo* (see below)
- We plan to assess the safety and efficacy of both autologous anti-CD83 CAR-T cells and gene-edited, potency-enhanced, allogeneic anti-CD83 CAR-T cells in R/R AML patients



References: 1. Shrestha B, Walton K, Reff J, Sagatys EM, Tu N, Boucher J, Li G, Ghafoor T, Felices M, Miller JS, Pidala J, Blazar BR, Anasetti C, Betts BC, Davila ML. Human CD83-targeted chimeric antigen receptor T cells prevent and treat graft-versus-host disease. J Clin Invest. 2020 Sep 1;130(9):4652-4662.