CRISPR/CAS9 GENE-EDITED, ALLOGENEIC ANTI-CD83 CAR-T CELLS DEMONSTRATE POTENT ACTIVITY IN GVHD AND AML TUMOR MODELS

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**Background**
CD83 is expressed on blood cancer cells, including acute myeloid leukemia (AML) blasts, and on allo-activated immune cells that are implicated in graft-versus-host disease (GvHD).\(^1\) Targeting CD83 thus has the potential to improve outcomes in AML, as well as to reduce the risk of GvHD, which is a significant cause of mortality and morbidity in patients who undergo allogeneic hematopoietic cell transplant. The potential benefit of targeting CD83 is supported by literature showing that depletion of CD83+ cells in preclinical models of GvHD and AML is an effective therapeutic strategy.\(^1,2\)

**Methods**
We assessed the effectiveness of CRISPR/Cas9 gene-edited, allogeneic anti-CD83 CAR-T cells against animal models of GvHD and AML, and whether additional gene edits could enhance CAR-T potency in these settings. Anti-CD83 CAR-T cells were made with TRAC disruption to reduce the risk of GvHD, B2M disruption to reduce allogeneic host rejection, and insertion of an anti-CD83 CAR construct into the TRAC locus. To assess whether CD83 expression on activated T cells causes CAR-mediated fratricide, the CD83 gene was disrupted. To increase potency further, two additional gene disruptions were introduced: ZC3H12A (which encodes Regnase-1) and TGFBR2 (which encodes TGFBR2).

**Results**
We found that CD83 knockout (KO) improved the *in vitro* expansion of anti-CD83 CAR-T cells and enhanced *in vivo* activity. In a THP-1 tumor model, treatment with CD83 KO anti-CD83 CAR-T cells improved median survival when compared with wild-type cells (not reached vs. 59 days). In a xenogeneic GvHD model, CD83 KO cells were more effective at delaying GvHD at a dose of 1e6 CAR+ cells (median survival 71.5 vs. 51 days) and prevented GvHD entirely at a dose of 3e6 CAR+ cells. Activity could also be enhanced by combining CD83 KO cells with belatacept, a CTLA4-Fc fusion protein that blocks co-stimulation of T cells.

Anti-CD83 CAR-T cells with KO of Regnase-1, TGFBR2, and CD83 (R/T/83 KO cells) maintained robust expansion *in vitro*, demonstrated increased target cell killing *in vitro*, and showed enhanced *in vivo* activity. In a THP-1 tumor model, durable complete responses were observed in all animals (5/5) treated with R/T/83 KO cells and in 60% of animals (3/5) treated with CD83 KO cells. In a xenogeneic GvHD model, a single dose of 1e6 CAR+ R/T/83 KO cells prevented GvHD while CD83 KO cells delayed GvHD onset.

**Conclusions**
Collectively, these data support the clinical evaluation of gene-edited, potency-enhanced, allogeneic anti-CD83 CAR-T cells in relapsed/refractory AML patients.

**REFERENCES**


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