CD83 is expressed on acute myeloid leukemia (AML) cells and activated immune cells, making it an attractive target for CAR-T cell therapy. However, CD83 expression is present on both myeloid and T cells, limiting the specificity and safety of anti-CD83 CAR-T cells. A recent publication showed that knockouts and CD83+ CAR-T cells are active in both AML and CAR-T cell therapy, suggesting that using KO CAR-T cells could improve CAR-T activity. However, knockout of CD83 expression impaired activity by preventing CAR-T cells from identifying CD83+ myeloid-derived suppressor cells (MDSC) and tumor-associated macrophages. 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, enhanced-allogeneic anti-CD83 CAR-T cells in idiopathic refractory AML patients.

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

- **A** Fixed number of CAR-T cells were co-cultured with increasing numbers of CD83+ K562 target cells. Both CD83 KO and WT CAR-T cells showed higher activity and led to durable complete remission with CD83 KO CAR-T cells showing even better tumor and GvHD animal models. In this study, CRISPR/Cas9 editing was used to make allogeneic anti-CD83 CAR-T cells and test additional potency edits, these allogeneic CAR-T cells can match the survival seen with autologous cells (Figure 9).

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

- **A** Anti-CD83 CAR-T cells (CD83-CAR) with and without CD83 gene disruption (CD83 KO vs. WT) were edited on Day 0 and co-cultured with 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-anti CD83 CAR-T cells co-cultured with autologous AML tumor in a THP1-xenograft tumor model. Treatment with CD83 KO allo CAR-T cells showed increased tumor growth suppression, higher activity and led to durable complete responses (PFS) in 2/5 mice.

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

- A fixed number of CAR-T cells were co-cultured with CD83+ A498 target cells on Day 0 and challenged with increasing number of target cells on Days 2, 5, and 7. AML CAR-T KO CAR-T cells showed enhanced target cell killing and proliferation upon no challenge with target cells, indicating that disrupting the Regnase-1 and TGFβRII genes can further increase anti-CD83 CAR-T cell potency.

### Figure 5: B2M knock-out improves potency of allogeneic anti-CD83 CAR-T cells in P B M C humanized N G mice

- **A** To test if B2M knock-out (B2M KO) extends anti-CD83 T cell activity, three groups of CAR-T cells were made: autologous cells from the B2M KO mice, and allogeneic CAR-T cells from the same donors with and without B2M KO. CAR-T cells were then cultured with autologous PBMC and humanized N G mice.

### Figure 6: Combining CD83 and B2M knock-out further enhances GvHD protection

- **A** A fixed number of CAR-T cells were co-cultured with CD83+ A498 target cells on Day 0 and challenged with increasing number of target cells on Days 2, 5, and 7. AML CAR-T KO CAR-T cells showed enhanced target cell killing and proliferation upon no challenge with target cells, indicating that disrupting the Regnase-1 and TGFβRII genes can further increase anti-CD83 CAR-T cell potency.

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

- A fixed number of CAR-T cells were co-cultured with CD83+ A498 target cells on Day 0 and challenged with increasing number of target cells on Days 2, 5, and 7. AML CAR-T KO CAR-T cells showed enhanced target cell killing and proliferation upon no challenge with target cells, indicating that disrupting the Regnase-1 and TGFβRII genes can further increase anti-CD83 CAR-T cell potency.

### Figure 8: Addition of the Regnase-1 and TGFβRII 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 (PFS) were observed in all mice treated with anti-CD83 CAR-T cells (R/T/CD83 KO) in contrast, with half (6/3) CR was observed in mice treated with CD83 KO anti-CD83 CAR-T cells.