Poster #1428

Allogeneic CRISPR/Cas9 Gene-edited CAR-T Cells Targeting CD33 Show Potent Preclinical Activity Against AML Cells

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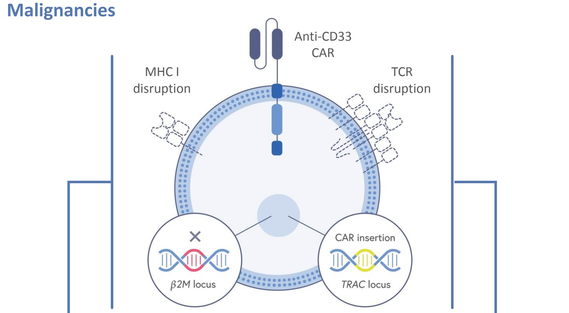
Abstract

Acute myelogenous leukemia (AML) has a high mortality rate and remains difficult to treat, making new treatment approaches critical. Chimeric antigen receptor T (CAR-T) cell therapies have shown impressive clinical responses in B-cell neoplasia. However, comparable successes have not been reported to date in myeloid malignancies, potentially due to difficulty in manufacturing efficacious CAR-T cells from AML patients and lack of a suitable tumor antigen for AML. To address these issues, we have developed allogeneic CAR-T cells from healthy donors targeting the CD33/Siglec-3 antigen, a protein expressed on most AML cells and subpopulations in the majority of AML patients at presentation and relapse. Allogeneic anti-CD33 CAR-T cells were produced from healthy donor-derived T cells using CRISPR/Cas9 gene editing. In these cells, the *TRAC* locus was disrupted to reduce the risk of graft versus host disease (GvHD). At the same time, a CAR construct targeting CD33 was inserted site-specifically into the *TRAC* locus. In addition, the beta-2-macroglobulin locus was disrupted to prevent clearance of the allogeneic CAR-T cells by the host immune system. These allogeneic anti-CD33 CAR-T cells showed potent effector activity *in vitro* against human AML-derived cell lines, as measured by both tumor cell lysis and effector cytokine secretion. The allogeneic anti-CD33 CAR-T cells also potently reduced AML tumors *in vivo* in xenograft mouse models.

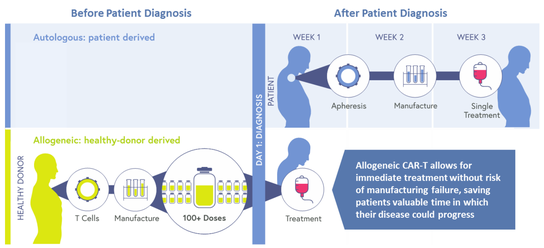
Figure 1: CRISPR-Edited Allogeneic CAR-T Cells for CD33⁺ Malignancies



- **Improve persistence in the allogeneic setting** with β2M knock-out to eliminate MHC I expression
- **Prevent GvHD** via TCR disruption
- **Improve safety and potency** by precise insertion of CAR construct into *TRAC* locus

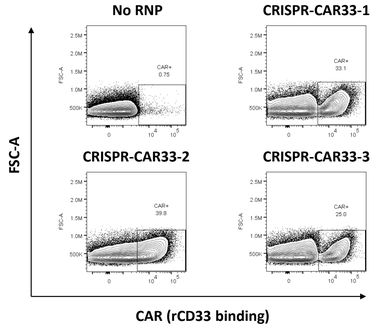
CRISPR-edited allogeneic CAR-T cells are produced by targeted disruption of the *TRAC* locus with concomitant insertion of an anti-CD33 CAR cassette into the *TRAC* locus by homology-directed repair. *TRAC* gene disruption leads to loss of surface expressed TCRαβ complex and reduces the risk of graft versus host disease (GvHD). Targeted disruption of the β2M gene leads to loss of surface expressed MHC I and the reduced risk of rejection of allogeneic CAR-T cells by the host immune system.

Figure 2: Healthy Donor-derived CAR-T Cells Offer Benefits Over Autologous Approaches



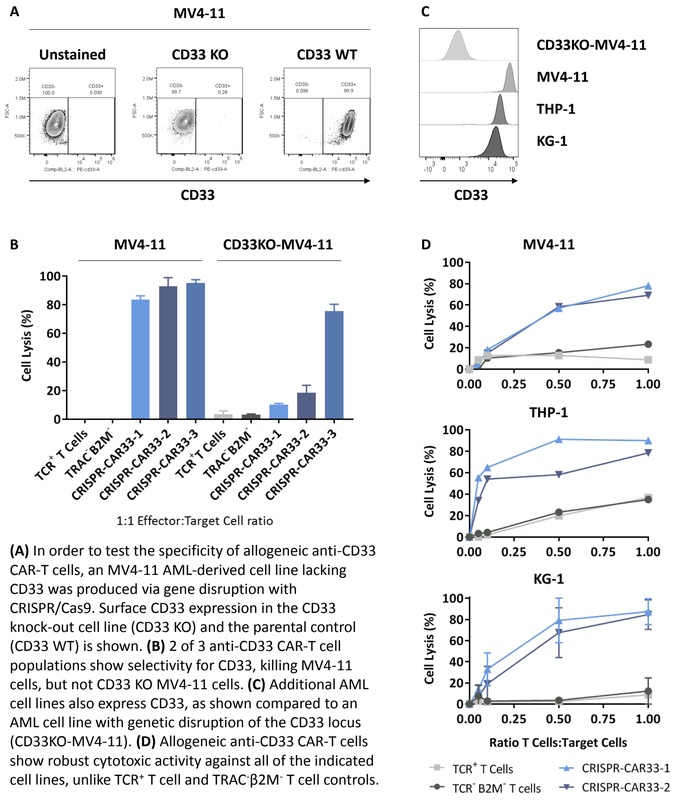
The function of autologous CAR-T cells depends on the health of the donor and quality of the T cells, which are often compromised due to disease and prior lines of therapy. An "off-the-shelf" allogeneic CAR-T cell approach allows for the controlled manufacturing of product from healthy donor T cell populations. Benefits of the allogeneic approach include off-the-shelf administration, more potent starting material, a more consistent product, broader access, and flexible dosing (e.g., re-dosing).

Figure 3: Development of CRISPR-Edited Allogeneic Anti-CD33 CAR-T Cells



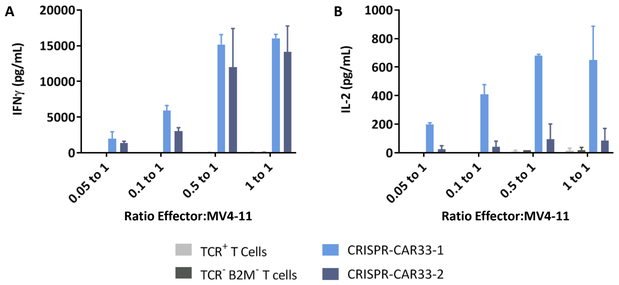
Multiple anti-CD33 CAR constructs were screened in human primary T cells to identify ones with high surface staining. CAR surface expression is shown for three constructs selected for further evaluation: CRISPR-CAR33-1, CRISPR-CAR33-2, and CRISPR-CAR33-3. No RNP represents T cells mock transfected without Cas9:sgRNA ribonucleoprotein (RNP).

Figure 4: CRISPR-Edited Anti-CD33 CAR-T Cells Exhibit Specific and Potent Cell Killing of AML Cells *In Vitro*



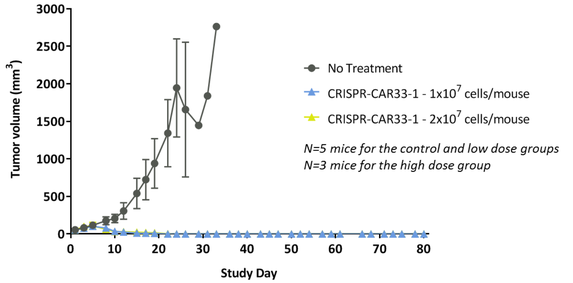
(A) In order to test the specificity of allogeneic anti-CD33 CAR-T cells, an MV4-11 AML-derived cell line lacking CD33 was produced via gene disruption with CRISPR/Cas9. Surface CD33 expression in the CD33 knock-out cell line (CD33 KO) and the parental control (CD33 WT) is shown. **(B)** 2 of 3 anti-CD33 CAR-T cell populations show selectivity for CD33, killing MV4-11 cells, but not CD33 KO MV4-11 cells. **(C)** Additional AML cell lines also express CD33, as shown compared to an AML cell line with genetic disruption of the CD33 locus (CD33KO-MV4-11). **(D)** Allogeneic anti-CD33 CAR-T cells show robust cytotoxic activity against all of the indicated cell lines, unlike TCR⁺ T cell and TRAC⁻β2M⁻ T cell controls.

Figure 5: CRISPR-Edited Anti-CD33 CAR-T Cells Secrete Effector Cytokines in the Presence of AML Cells *In Vitro*



Effector cytokine secretion in response to target cell engagement was also examined. **(A)** CRISPR-CAR33-1 and CRISPR-CAR33-2 both secrete high levels of IFNγ when exposed to target cells. **(B)** However, CRISPR-CAR33-1 secretes higher levels of IL-2 than CRISPR-CAR33-2.

Figure 6: Allogeneic Anti-CD33 CAR-T Cells Eliminate AML Cells *In Vivo*



CRISPR-CAR33-1 was then tested against a THP-1 tumor xenograft model in NOG mice. Subcutaneous THP-1 tumors were allowed to grow to 50 mm³, at which point allogeneic anti-CD33 CAR-T cells were injected intravenously into the mice. Tumor volumes were measured over the indicated observation period. CRISPR-CAR33-1 eliminated all THP-1 tumors. No relapse was observed.

Conclusions

- CRISPR-edited allogeneic anti-CD33 CAR-positive T cells can be generated from healthy donor T cells
- Allogeneic anti-CD33 CAR-T cells can potently and specifically kill CD33-expressing AML cells *in vitro*
- CRISPR-CAR33-1 CAR-T cells displayed robust IFNγ and IL-2 secretion when exposed to target cells
- CRISPR-CAR33-1 CAR-T cells eliminate AML cells in a xenograft mouse tumor model
- Allogeneic CAR-T cells targeted towards CD33 hold promise for the treatment of AML, a disease with substantial need of new treatment approaches