Abstract #841

CRISPR/Cas9 Gene Editing to Produce Multiple Allogeneic CAR-T Cell Candidates Showing Consistently High Potency, Durability, Lack of Alloreactivity, and Ability to Overcome Immune Suppression

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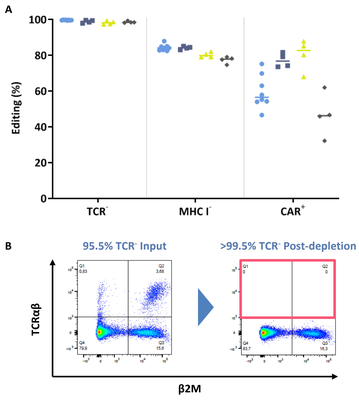
Abstract

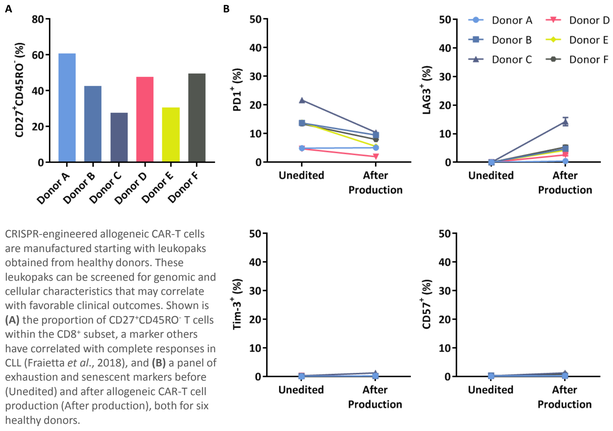
The CRISPR/Cas9 system enables the highly efficient editing of genomes in multiple cell types. The proliferative nature of T cells makes them particularly amendable to CRISPR/Cas9 gene editing for both gene knock-out by non-homologous end joining (NHEJ) and site-specific genetic knock-in by homology-directed repair (HDR). Here we show the consistent production of potent gene-edited allogeneic CAR-T cells targeting multiple tumor antigen targets. Gene knock-out via NHEJ is coupled with HDR to knock-in the CAR construct. The resulting CAR-T cells exhibit the following preclinical properties: (1) highly efficient deletion of the T cell receptor (TCR) to enable allogeneic administration, as supported by lack of graft versus host disease (GvHD) when administered to NSG mice; (2) specific and potent activity against antigen-expressing tumor cells; (3) durability and persistence, as exhibited by multiple tumor cell re-challenges without exhaustion; and (4) resistance to PD-L1-induced immune suppression. These attributes may give the CRISPR/Cas9 gene-edited allogeneic CAR-T cells described here the potential to provide clinical benefit in both hematological and solid tumors.

Figure 1: CRISPR-Engineered Allogeneic CAR-T Cells Show Consistent and High Levels of Gene Editing



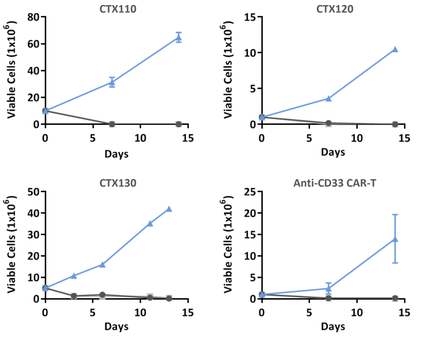
(A) Surface level loss of TCRαβ and MHC I, and expression of CAR resulting from genome editing of either the *TRAC* locus (TCR disruption and CAR insertion) or *B2M* locus (disruption of the invariable subunit of MHC I) is shown across allogeneic CAR-T cells targeting four different tumor antigens: CTX110™ targeting CD19, CTX120™ targeting BCMA, CTX130™ targeting CD70 and anti-CD33 CAR-T cells. Editing rates are consistent across target antigens and CAR-T cell lots produced at either research scale (CTX120, CTX130, anti-CD33 CAR-T cells) or development scale (CTX110). **(B)** TCRαβ purification is performed during the allogeneic CAR-T cell manufacturing process to reduce further the risk of GvHD. Efficient purification of TCR⁻ cells is regularly obtained for lots produced in development, as demonstrated in the representative flow cytometry plots for CTX110 before and after TCRαβ purification shown here.

Figure 2: Healthy Donor T Cells Can Be Selected For Favorable Phenotypes



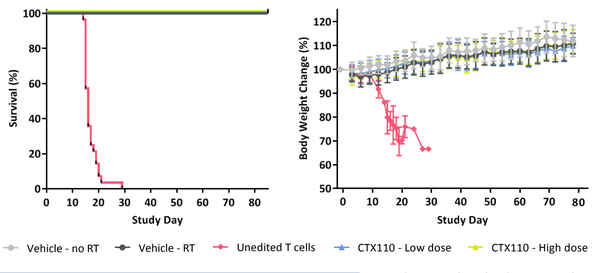
CRISPR-engineered allogeneic CAR-T cells are manufactured starting with leukopaks obtained from healthy donors. These leukopaks can be screened for genomic and cellular characteristics that may correlate with favorable clinical outcomes. Shown is **(A)** the proportion of CD27⁺CD45RO⁻ T cells within the CD8⁺ subset, a marker others have correlated with complete responses in CLL (Fraietta *et al.*, 2018), and **(B)** a panel of exhaustion and senescent markers before (Unedited) and after allogeneic CAR-T cell production (After production), both for six healthy donors.

Figure 3: CRISPR-Engineered Allogeneic CAR-T Cells Do Not Show Any Detectable Cell Outgrowth in the Absence of Cytokines



Approximately two weeks after gene editing, allogeneic CAR-T cells were either kept in full T cell media (Complete Media) or switched to media containing serum but no cytokines (5% Serum, No Cytokines) or neither serum nor cytokines (No Serum, No Cytokines). CTX110, CTX120, CTX130 and anti-CD33 CAR-T cells all remained dependent on serum and cytokines for growth, suggesting that the cells have not transformed following gene editing.

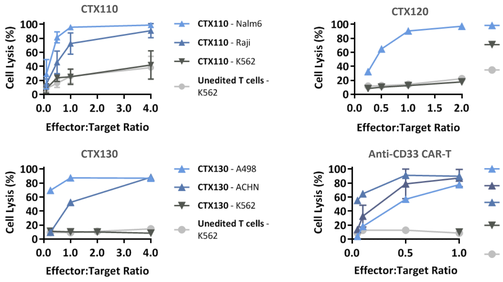
Figure 4: CTX110 Does Not Elicit Xenogeneic Graft Versus Host Disease (GvHD) in an IND-enabling Study



Groups	Dose (Cells/Mouse)	Number of Animals
Vehicle (PBS) – no RT	0	10
Vehicle (PBS) – RT	0	30
Unedited T cells	1x	30
CTX110 – Low dose	2x	30
CTX110 – High dose	4x	30

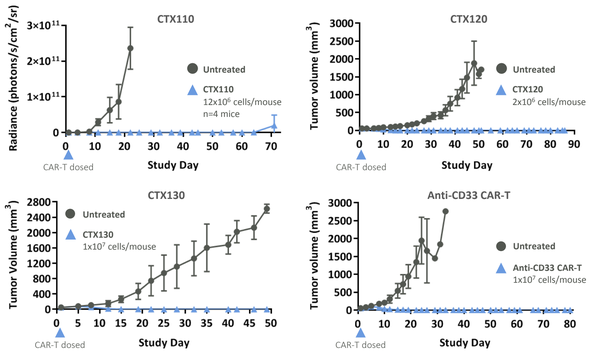
A study was conducted under GLP-compliant conditions to assess whether CTX110 could evoke GvHD in mice. Mice receiving unedited (TCR⁺) T cells rapidly exhibited clinical signs of xenogeneic GvHD, including loss of body weight. These mice all perished within 30 days. The CTX110-treated and control mice exhibited no signs of GvHD and survived throughout the course of the study. All mice received radiation treatment (RT) unless otherwise noted.

Figure 5: Allogeneic CAR-T Cells from Multiple Programs Demonstrate Potent and Specific Cytotoxicity *In Vitro*



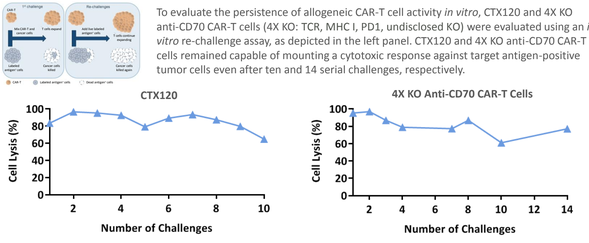
To assess CAR-T cytotoxicity *in vitro*, CAR-T cells were co-cultured with the indicated target antigen-positive or target antigen-negative cells at various effector:target cell ratios. Target cell lysis was quantified after 24 hours, or 4 hours in the case of the CTX120 samples shown here. CTX110, CTX120, CTX130 and anti-CD33 CAR-T cells all exhibited potent lysis of antigen-expressing tumor cells (shown in various shades of blue), but not antigen-negative cells (shown in dark gray).

Figure 6: Allogeneic CAR-T Cells from Multiple Programs Demonstrate Potent *In Vivo* Anti-Tumor Activity in Xenograft Models



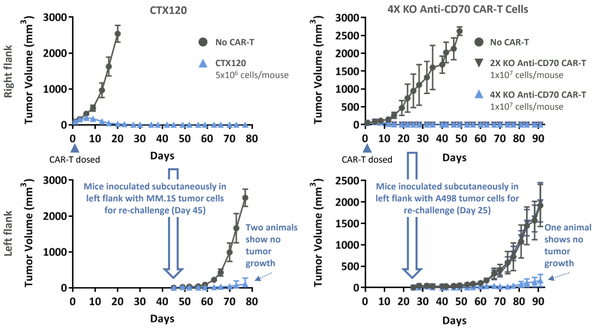
CTX110 prolonged the survival of mice bearing a disseminated Nalm6-luciferase B-cell acute lymphoblastic leukemia model and prevented tumor growth in all animals for ~60 days before tumors regrew (top left panel). CTX120, CTX130 and anti-CD33 CAR-T cells all completely eliminated subcutaneous xenograft tumors of RPMI-8226 (multiple myeloma), A498 (renal cell carcinoma) and THP-1 (acute myeloid leukemia), respectively, with no relapse observed under the observation period. Total body luminescence was used to measure tumor burden for the disseminated model, while tumor volumes on the flanks of mice were directly measured for the subcutaneous models. N=5 mice for all groups unless otherwise noted.

Figure 7: Allogeneic CAR-T Cells Maintain the Ability to Lyse Cancer Cells Following Numerous Re-Challenges *In Vitro*



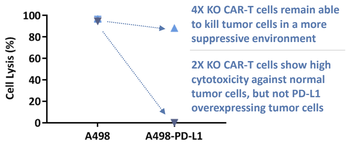
To evaluate the persistence of allogeneic CAR-T cell activity *in vitro*, CTX120 and 4X KO anti-CD70 CAR-T cells (4X KO: TCR, MHC I, PD1, undisclosed KO) were evaluated using an *in vitro* re-challenge assay, as depicted in the left panel. CTX120 and 4X KO anti-CD70 CAR-T cells remained capable of mounting a cytotoxic response against target antigen-positive tumor cells even after ten and 14 serial challenges, respectively.

Figure 8: Allogeneic CAR-T Cells Maintain Anti-Tumor Activity Following Xenograft Tumor Re-Challenge *In Vivo*



Mice that received a single inoculation of either the MM.1S multiple myeloma cells (left panel) or A498 cells (right panel) achieved complete tumor regression after a single dose of CTX120 (left panel) or anti-CD70 CAR-T cells with (4X KO) or without (2X KO: TCR, MHC I) additional gene edits (right panel). Mice from these cohorts were then subjected to a second challenge of tumor cells in their left hind flanks. Mice that had received a previous single dose of either CTX120 or 4X KO anti-CD70 CAR-T cells showed control of tumor growth in the left flank. N=5 mice for all groups.

Figure 9: Additional Editing Can Overcome Suppression by PD-L1 *In Vitro*



2X KO or 4X KO anti-CD70 CAR-T cells efficiently lysed A498 cells in a 24-hour cell kill assay, shown here at a 2:1 effector:target ratio. Against A498 cells overexpressing PD-L1, however, the 2X KO CAR-T cells could no longer mount a potent response while 4X KO CAR-T cells overcame PD-L1 suppression and displayed potent cytotoxic activity *in vitro*.

▼ 2X KO ▲ 4X KO

Conclusions from Preclinical Studies

- Allogeneic CAR-T cells targeting CD19⁺ (CTX110), BCMA⁺ (CTX120), CD70⁺ (CTX130) or CD33⁺ malignancies are produced with high and consistent editing rates
- CRISPR Tx CAR-T cells do not evoke xenogeneic GvHD or display cytokine-independent growth
- CRISPR Tx CAR-T cells show potent anti-tumor activity *in vitro* and *in vivo*
- CRISPR Tx CAR-T cells show persistent activity, maintaining function after multiple tumor cell challenges *in vitro* and *in vivo*
- CRISPR Tx CAR-T cells can be further engineered to resist immunosuppressive tumor microenvironments