Allogeneic CRISPR Engineered Anti-CD70 CAR-T Cells Demonstrate Potent Preclinical Activity Against Both Solid and Hematological Cancer Cells

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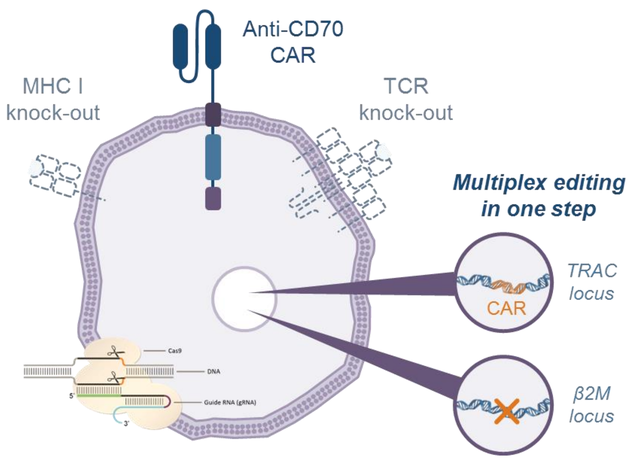
Abstract

Autologous CAR-T therapeutics have recently been approved for use in B-cell malignancies. While responses have been impressive using CD19-directed CAR-T cells, there has been a lack of comparable success for CAR-T cells directed at solid tumor antigens. In an effort to address the need for effective and durable off-the-shelf therapies for both hematologic and solid tumors, we have developed allogeneic CAR-T cells targeting the CD70 antigen. CD70 is expressed in both hematologic malignancies as well as in solid cancers such as renal cell carcinoma (RCC), while its expression in normal tissues is restricted to a subset of lymphoid cell types. CAR-T cells expressing a CD70-targeting CAR were generated by CRISPR/Cas9 genome editing. T cells from healthy donors were edited to express a CD70 CAR by knocking this construct into the concurrently knocked out TCR alpha constant region (*TRAC*). Loss of the TCR reduces the risk of graft versus host disease enabling an allogeneic therapeutic. CD70 CAR-T cells displayed potent cell killing function *in vitro* against CD70-expressing lymphoid and renal cancer derived cell lines across a broad range of antigen expression levels. CD70 CAR-T cells also secreted IFNγ, released Granzyme B, and proliferated in a CD70-specific manner. Furthermore, the CD70-targeting CAR-T cells were able to eliminate established ccRCC tumor xenografts in mice.

Figure 1: CRISPR Therapeutics Allogeneic CAR-T Pipeline

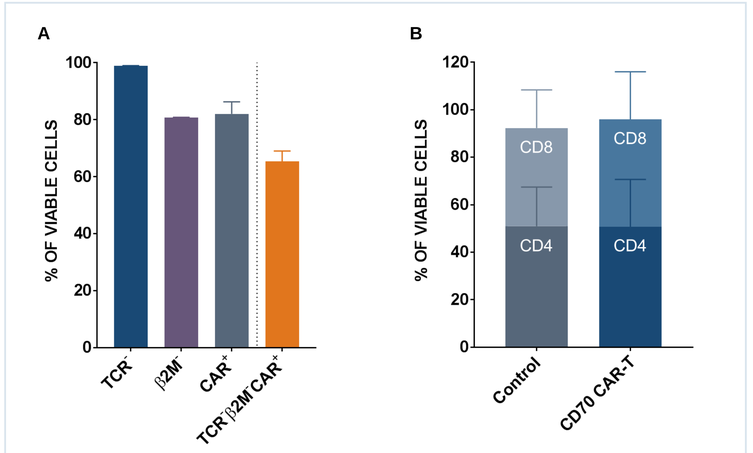
Program	Editing approach	Research	IND-enabling	Ph I/II	Partner
CTX101: Anti-CD19 allogeneic CAR-T	Disruption & Insertion			*IND filing Q4 2018*	Wholly-owned
CTX102: Anti-BCMA allogeneic CAR-T	Disruption & Insertion				Wholly-owned
CTX103: Anti-CD70 allogeneic CAR-T	Disruption & Insertion				Wholly-owned
Multiple solid tumor allogeneic CAR-T	Disruption & Insertion				Wholly-owned

Figure 2: CRISPR/Cas9 Gene-Edited Allogeneic Anti-CD70 CAR-T Cells



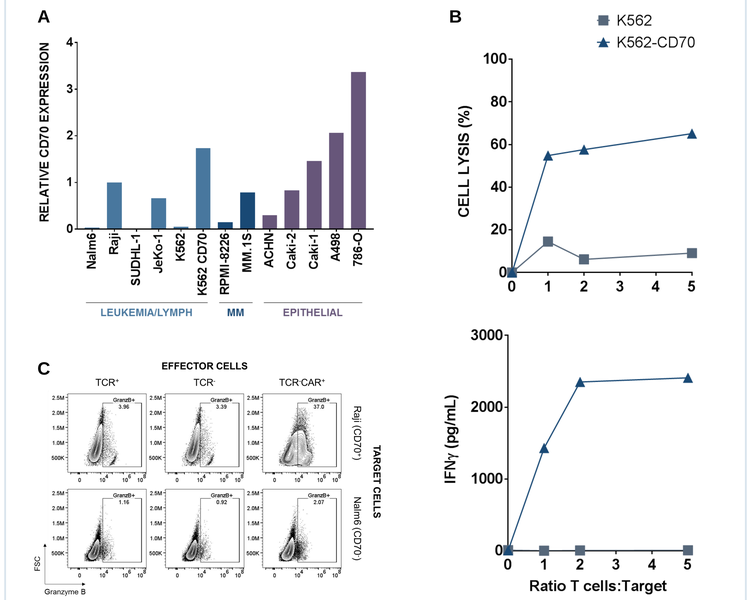
CRISPR/Cas9 gene editing of T cells from healthy donors is used to produce allogeneic CAR-T cells. To prevent GvHD, TCR expression is ablated by integrating an anti-CD70 CAR construct into the *TRAC* locus by homology-directed repair after using CRISPR/Cas9 to introduce a site-specific double strand break. To enhance persistence of allogeneic cells, MHC-I expression is eliminated by disrupting the *β2M* gene.

Figure 3: High Efficiency CRISPR/Cas9 Gene Editing to Produce Allogeneic Anti-CD70 CAR-T Cells



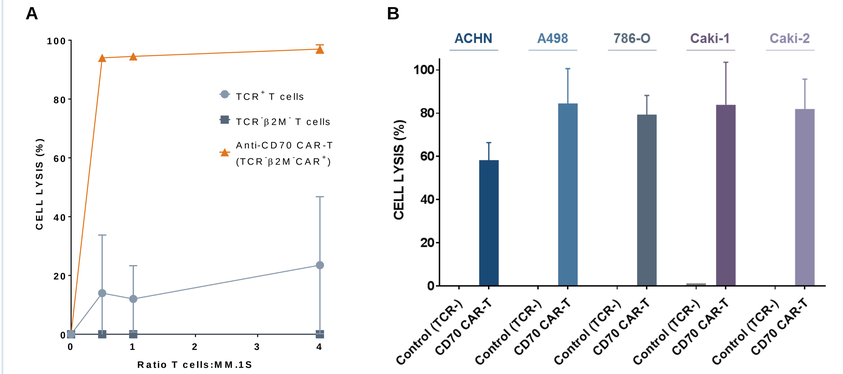
(**A**) High editing rates are achieved at the *TRAC* and *β2M* loci resulting in decreased surface expression of TCR and MHC-I. Highly efficient site-specific integration and expression of the CAR from the *TRAC* locus is also detected. Data are from 3 healthy donors. (**B**) Production of allogeneic anti-CD70 CAR-T cells (TCR-β2M-CAR+) preserves CD4 and CD8 proportions.

Figure 4: Anti-CD70 CAR-T Cells Display Effector Functions in a CD70-Specific Manner



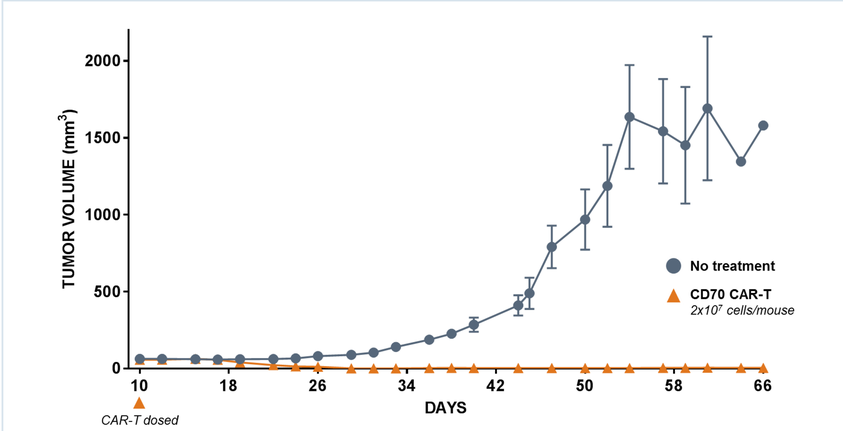
(**A**) High levels of surface CD70 are expressed in cell lines derived from multiple cancers. RCC cell lines show particularly high levels of CD70 expression. (**B**) Allogeneic anti-CD70 CAR-T cells (TCR-β2M-CAR+) kill CD70-expressing cells (top panel) and secrete IFNγ (bottom panel) in a CD70-specific manner. (**C**) TCR-deficient anti-CD70 CAR-T cells (TCR-CAR+) also secrete Granzyme B in a CD70-specific manner.

Figure 5: Anti-CD70 CAR-T Cells Kill Multiple Myeloma and Renal Cell Carcinoma Cells



(**A**) Allogeneic anti-CD70 CAR-T cells (TCR-β2M-CAR+) show potent cytotoxicity against the CD70+ MM.1S multiple myeloma cell line. (**B**) TCR-deficient anti-CD70 CAR-T cells (TCR-CAR+) display cell killing activity against a panel of RCC cell lines with varying CD70 expression.

Figure 6: Anti-CD70 CAR-T Cells Display Robust Anti-Tumor Activity in a Renal Cell Carcinoma Mouse Model



TCR-deficient anti-CD70 CAR-T (TCR-CAR+) cells eradicate tumors in a subcutaneous A498 RCC tumor xenograft model. 5x10[6] A498 cells were injected subcutaneously into NOG mice, followed by CAR-T cells intravenously 10 days after inoculation. No clinical signs of GvHD were observed in the mice at any timepoint. n=4 for both groups.

Summary and Conclusion

- Using CRISPR/Cas9 gene editing, we have generated an allogeneic anti-CD70 CAR-T product at high efficiency, with over 60% of the cells harboring all 3 desired edits
- The CAR-T cells selectively kill CD70+ cells and secrete the cytokine IFNγ following encounter with CD70-expressing cells.
- The CAR-T cells kill CD70+ cell lines derived from both solid and hematopoietic cancers
- The CAR-T cells eradicate RCC cells in a subcutaneous A498 tumor xenograft model, confirming potent activity *in vivo*