Allogeneic Chimeric Antigen Receptor T Cells Targeting B Cell Maturation Antigen

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Abstract # 1540

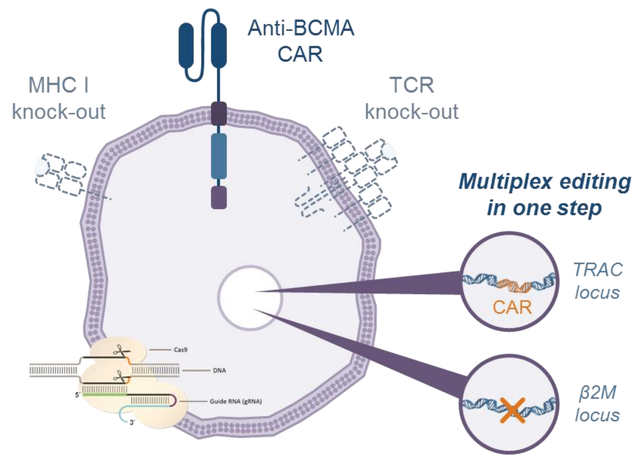
Abstract

B cell maturation antigen (BCMA) is a tumor necrosis factor family cell surface receptor that binds B cell activating factor (BAFF) and a proliferation inducing ligand (APRIL) and is involved in the long-term survival of B cells. BCMA has been shown to be expressed broadly on monoclonal gammopathy of undetermined significance (MGUS) and multiple myeloma (MM) cells and for this reason has been pursued as a potential antigen for the treatment of MM. Indeed, clinical studies evaluating autologous chimeric antigen receptor T cells (CAR-T cells) targeting BCMA for the treatment of MM have shown promising efficacy with >80% response rates. However, logistical challenges will potentially limit the number of patients that have access to autologous therapies. Using the CRISPR/Cas9 system, we generated allogeneic CAR-T cells targeting BCMA by disrupting the beta-2-microglobulin (*β2M*) and T cell receptor alpha constant (*TRAC*) genes and inserting an anti-BCMA CAR into the *TRAC* locus. This results in allogeneic CAR-T cells lacking the major histocompatibility complex (MHC-I) and endogenous T cell receptor (TCR) expression while being potently cytotoxic towards cells expressing BCMA. These data show proof-of-concept for an "off-the-shelf" CAR-T therapy targeting BCMA.

Figure 1: CRISPR Therapeutics Allogeneic CAR-T Pipeline

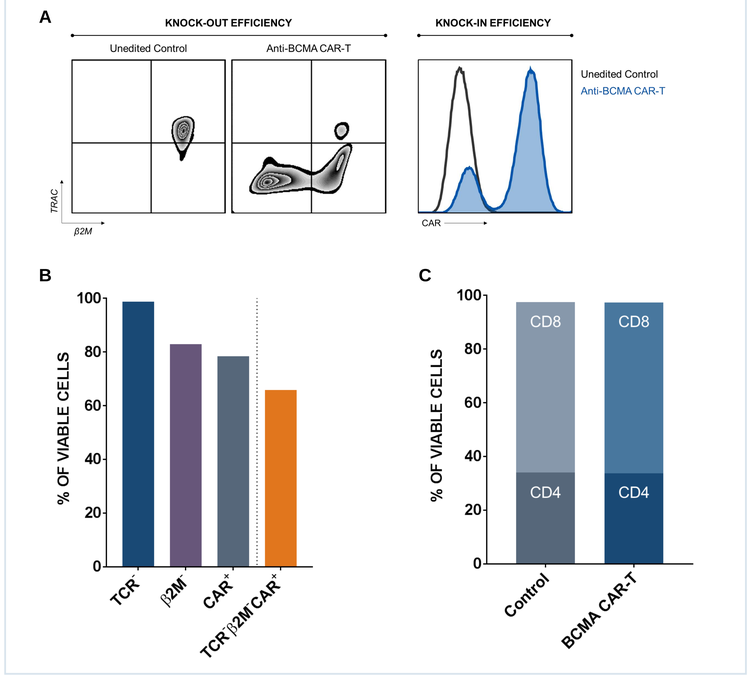
Program	Editing approach	Research	IND-enabling	Ph I/II	Partner
CTX101: Anti-CD19 allogeneic CAR-T	Disruption & Insertion			*IND filing Q4 2018*	Wholly-owned
CTX102: Anti-BCMA allogeneic CAR-T	Disruption & Insertion				Wholly-owned
CTX103: Anti-CD70 allogeneic CAR-T	Disruption & Insertion				Wholly-owned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

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



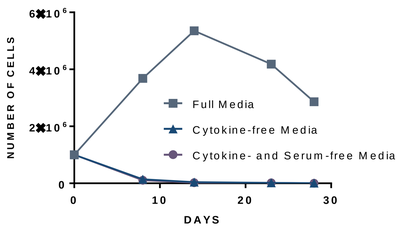
CRISPR/Cas9 gene editing of T cells from healthy donors is used to produce allogeneic CAR-T cells. To prevent GvHD, TCR expression is ablated by integrating an anti-BCMA CAR construct into the *TRAC* locus by homology-directed repair after using CRISPR/Cas9 to introduce a site-specific double strand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



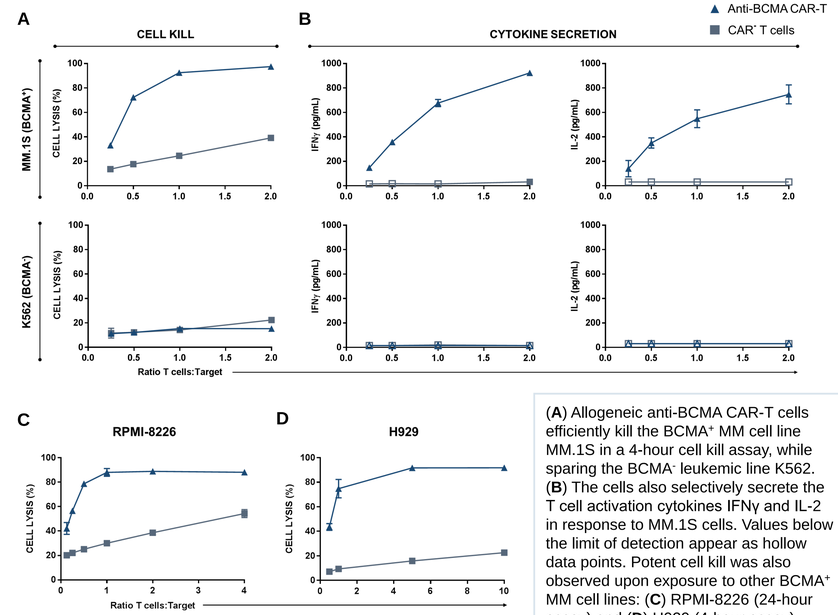
(**A**) Representative FACS plots of *TRAC* and *β2M* expression (left panel) and CAR expression following site-specific knock-in to the *TRAC* locus (right panel) 1 week following gene editing. (**B**) Editing results in decreased surface expression of TCR and MHC-I, as well as high CAR expression. More than 60% cells possess all 3 desired modifications (TCR$^-$/β2M$^-$/CAR$^+$). (**C**) Production of allogeneic anti-BCMA CAR-T cells preserves CD4 and CD8 proportions.

Figure 4: Allogeneic CAR-T Cells Remain Dependent on Cytokines for Growth Following Editing



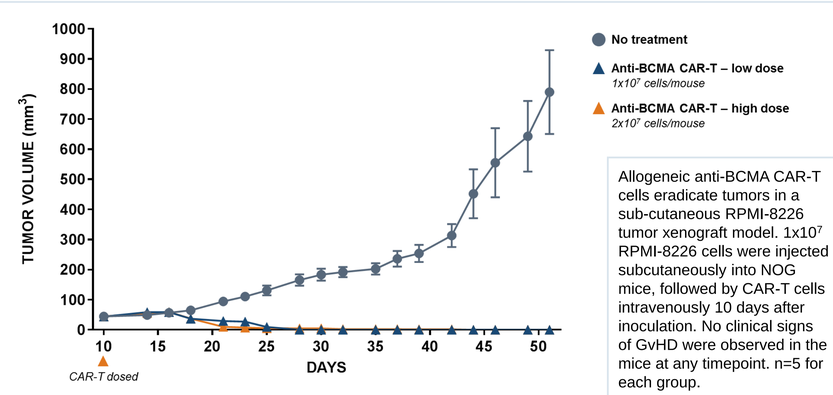
To examine whether editing results in transformations that confer cytokine-independent growth to T cells, cytokines and/or serum were removed from the growth media 2 weeks following editing and CAR knock-in. No further proliferation of T cells was observed in the absence of cytokines, indicating that the T cells remained dependent on cytokines for growth.

Figure 5: Allogeneic Anti-BCMA CAR-T Cells Show Potent Yet Specific Activity *In Vitro*



(**A**) Allogeneic anti-BCMA CAR-T cells efficiently kill the BCMA$^+$ MM cell line MM.1S in a 4-hour cell kill assay, while sparing the BCMA$^-$ leukemic line K562. (**B**) The cells also selectively secrete the T cell activation cytokines IFNγ and IL-2 in response to MM.1S cells. Values below the limit of detection appear as hollow data points. Potent cell kill was also observed upon exposure to other BCMA$^+$ MM cell lines: (**C**) RPMI-8226 (24-hour assay) and (**D**) H929 (4-hour assay).

Figure 6: Allogeneic Anti-BCMA CAR-T Cells Display Robust Anti-Tumor Activity in a Mouse Model



Allogeneic anti-BCMA CAR-T cells eradicate tumors in a sub-cutaneous RPMI-8226 tumor xenograft model. 1x10^7 RPMI-8226 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=5 for each group.

Summary and Conclusion

- Using CRISPR/Cas9 gene editing, we have generated an allogeneic anti-BCMA CAR-T product at high efficiency, with over 60% of the cells harboring all 3 desired edits
- The CAR-T cells maintain a similar CD4/CD8 ratio compared to controls, as well as characteristic cytokine dependency, suggesting neither abnormal tonic signaling from CAR insertion nor transformation due to the editing process have occurred
- The CAR-T cells selectively kill BCMA$^+$ cells and secrete T cell activation cytokines following encounter with BCMA-expressing cells
- The CAR-T cells eradicate MM cells in a subcutaneous RPMI-8226 tumor xenograft model, confirming potent activity *in vivo*