Poster #1921

Preclinical Development of CTX120, an Allogeneic CAR-T Cell Product Candidate Targeting BCMA

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Abstract

Autologous CAR-T cells targeting BCMA have induced robust and durable responses in patients with relapsed/refractory multiple myeloma. However, autologous cell therapies face several challenges which will likely limit the number of patients that will have access to these therapies. These limitations include manufacturing failure rates, wait time and supply constraints in addition to other factors such as reimbursement. Allogeneic CAR-T cells can potentially overcome these access challenges and may have several other advantages over autologous therapies. Allogeneic CAR-T cells are derived from robust healthy donor T cells through a batch manufacturing process, which may result in a highly consistent product with greater potency and enable better safety management. Here we show further development and preclinical data for CTX120, an allogeneic "off-the-shelf" CAR-T cell product candidate targeting BCMA. CTX120 is produced using the CRISPR/Cas9 system to eliminate TCR and MHC class I, coupled with specific insertion of the CAR at the *TRAC* locus. CTX120 shows consistent and high percent CAR expression from this controlled insertion and exhibits target-specific cytotoxicity and cytokine secretion in response to BCMA-positive cell lines. CTX120 CAR-T cells retain their cytotoxic capacity over multiple *in vitro* re-challenges, demonstrating durable potency and lack of exhaustion. In mouse models of multiple myeloma, CTX120 showed typical CAR-T persistence and eliminated tumors completely, resulting in long-term survival as compared to untreated animals. These data support the ongoing development of CTX120 for treatment of patients with multiple myeloma and further demonstrate the potential for our CRISPR/Cas9 engineered allogeneic CAR-T platform to generate potent CAR-T cells targeting different tumor antigens.

Figure 1: CRISPR Therapeutics Wholly-Owned Allogeneic CAR-T Pipeline

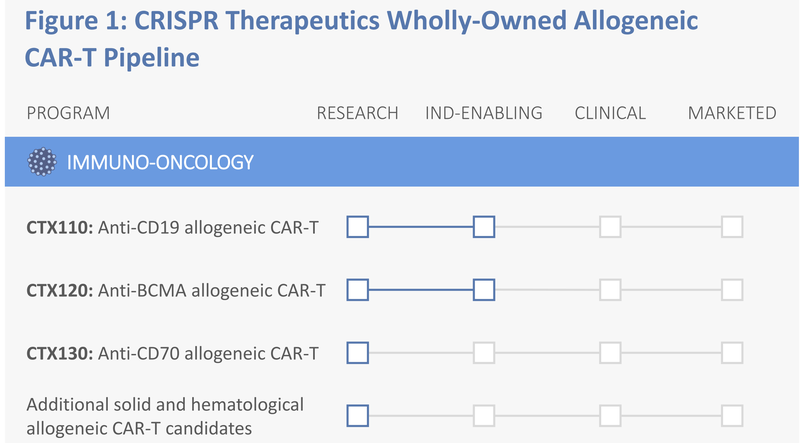
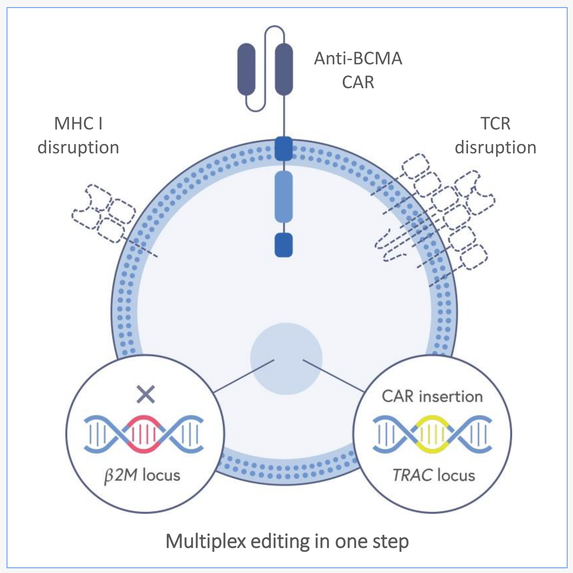
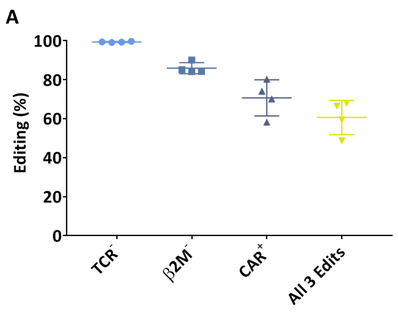


Figure 2: CTX120, a CRISPR/Cas9 Gene-Edited Allogeneic Anti-BCMA CAR-T Cell Product Candidate

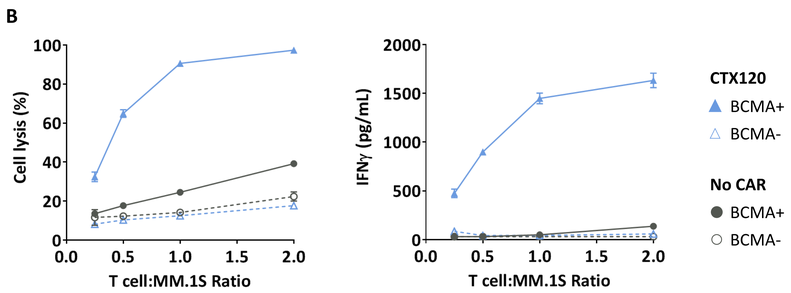


CTX120 is produced from healthy donor T cells using CRISPR/Cas9 gene editing. To prevent GvHD, TCR expression is disrupted by integrating an anti-BCMA CAR construct site-specifically into the *TRAC* locus via homology-directed repair after using CRISPR/Cas9 to introduce a DNA double-strand break. In addition, to improve persistence in the allogeneic setting, MHC I expression is disrupted by knock-out of the *β2M* gene.

Figure 3: High Efficiency CRISPR/Cas9 Gene Editing to Create CTX120, which Displays Potent Cytotoxicity *In Vitro*

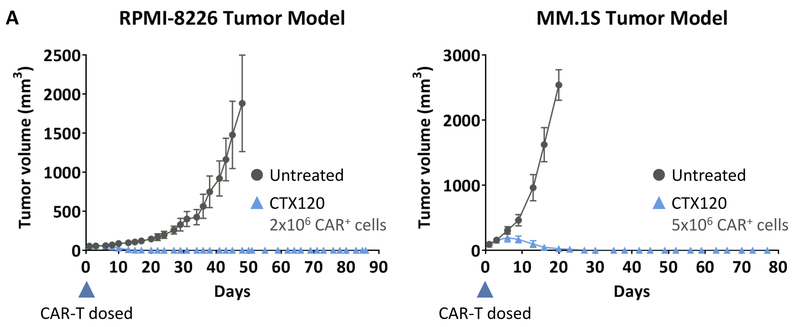


(A) High editing efficiency across multiple donors: High editing rates are achieved across multiple T-cell donors, leading to decreased surface expression of TCR (*TRAC* KO) and MHC I (*β2M* KO), as well as high anti-BCMA CAR expression. More than 60% of cells possess all 3 desired modifications (TCR$^-$/β2M$^-$/CAR$^+$). Each data point represents an individual donor.

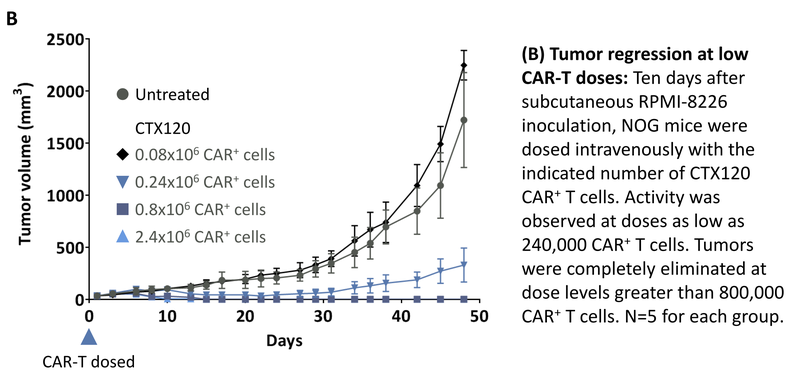


(B) Potent and specific activity *in vitro*: In a 4-hour cell kill assay, CTX120 efficiently kills the BCMA-positive (BCMA+) MM.1S multiple myeloma tumor cell line while sparing the BCMA-negative (BCMA-) K562 cell line. In addition, CTX120 secretes the T cell activation cytokine IFNγ only in response to antigen stimulation. IFNγ values below the limit of detection are shown as the limit of detection.

Figure 4: CTX120 Completely Eliminates Xenograft Tumors, Even at Low CAR-T Cell Doses

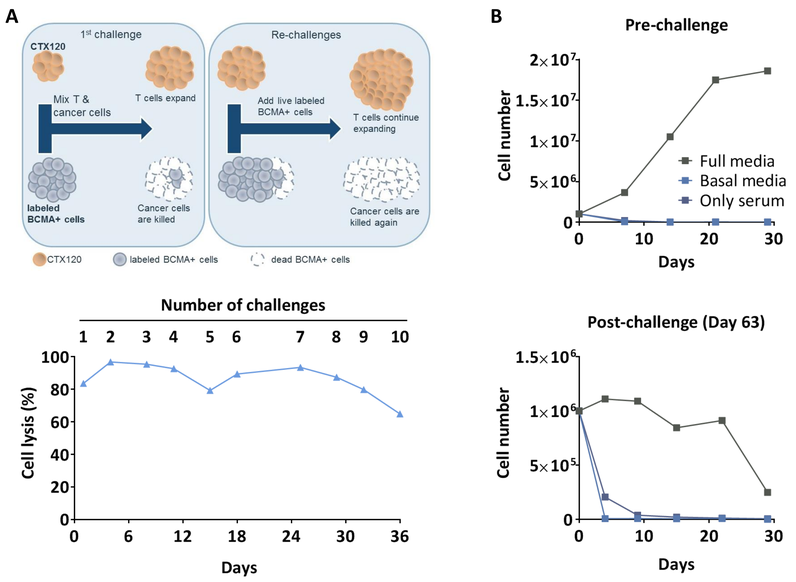


(A) Tumor elimination in multiple subcutaneous xenograft multiple myeloma tumor models: NOG mice inoculated with RPMI-8226 or MM.1S cells were dosed intravenously with the indicated number of CTX120 CAR$^+$ T cells after ten days or upon reaching an average volume of 100 mm^3, respectively. No clinical signs of GvHD were observed in the mice at any timepoint. N=5 for each group.



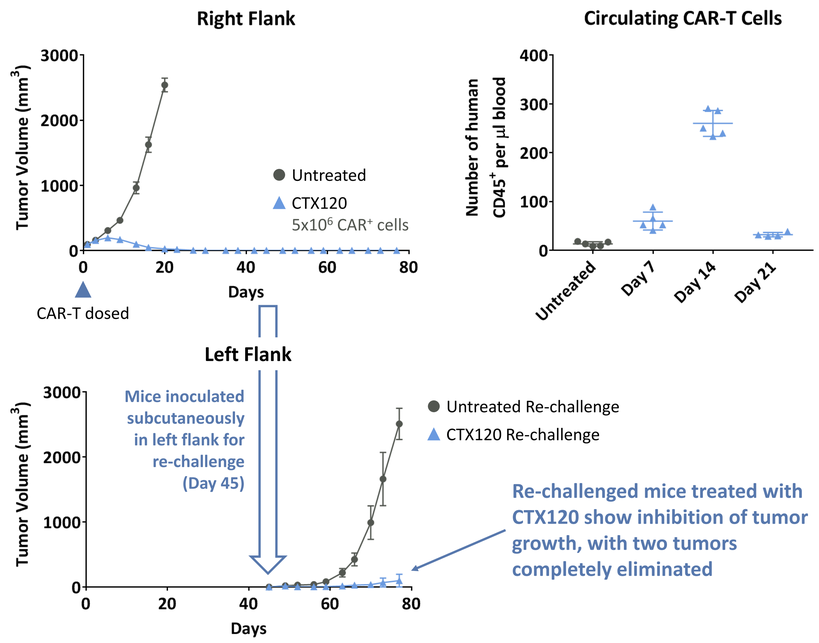
(B) Tumor regression at low CAR-T doses: Ten days after subcutaneous RPMI-8226 inoculation, NOG mice were dosed intravenously with the indicated number of CTX120 CAR$^+$ T cells. Activity was observed at doses as low as 240,000 CAR$^+$ T cells. Tumors were completely eliminated at dose levels greater than 800,000 CAR$^+$ T cells. N=5 for each group.

Figure 5: CTX120 Clears Multiple Myeloma Cells Upon Re-Challenge *In Vitro*



(**A**) **Serial cell killing *in vitro*:** In this *in vitro* re-challenge assay, CTX120 is mixed with BCMA+ target cells at a ratio of 1 CAR-T cell:2 target cells. After 3-4 days, the viability of BCMA+ target cells is analyzed via FACS and CTX120 is re-challenged with new target cells. The assay is serially repeated every 3-4 days with cytotoxicity evaluated at the end of each cycle. In this assay, CTX120 retains the ability to mount a cytotoxic response against BCMA+ cells even after ten serial re-challenges.
(**B**) **Cytokine dependency maintained after serial challenges:** To confirm that the persistent cytotoxicity of CTX120 is not due to editing-induced transformation, a cytokine-free growth assay was performed with input CAR-T cells and CAR-T cells from the final re-challenge. CTX120 required cytokines for growth both prior and subsequent to the re-challenge assay.

Figure 6: CTX120 Clears Multiple Myeloma Cells Upon Re-Challenge *In Vivo*



Tumor clearance upon *in vivo* re-challenge: To evaluate the persistence of CTX120 activity *in vivo*, a tumor re-challenge was performed in a xenograft tumor model. MM.1S tumors were inoculated subcutaneously in the right hind flank of NOG mice and allowed to grow to an average volume of 100 mm^3 prior to treatment with the indicated number of CTX120 CAR$^+$ T cells. 45 days following CTX120 injection, MM.1S cells were inoculated subcutaneously in the left hind flank of the treatment group and a new control group. In contrast to control mice, which all developed tumors, mice previously treated by CTX120 showed markedly slower progression or complete inhibition of left flank tumors. Examination of circulating CTX120 showed an expansion of the T cells following initial dosing. Upon re-challenge, CTX120-treated animals showed inhibited or eliminated tumor growth without subsequent expansion of circulating CAR-T cells. N=5 for each group.

Conclusions

- Using CRISPR/Cas9 gene editing, we have generated CTX120, an allogeneic CAR-T cell product candidate targeting BCMA, at high efficiency across multiple donors. In most donors, over 60% of the cells harbor all three desired edits
- CTX120 shows high potency and specificity *in vitro*, as exhibited by cytotoxicity against a BCMA+ cell line and secretion of T cell activation cytokines
- CTX120 eradicates multiple myeloma cells in multiple xenograft mouse models, confirming potent preclinical activity *in vivo*, even at low doses
- CTX120 exhibits persistent activity both *in vitro* and *in vivo* xenograft re-challenge models, demonstrating the ability to regress BCMA+ tumor growth as late as 45 days after CAR-T administration