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

Henia Dar, Ph.D., Daniel Henderson, B.S., Zinkal Padalia, M.S., Ashley Porras, B.A., Dakai Mu, M.S., Kelly Maeng, Ph.D., Seshidhar Police, Ph.D., Demetrios Kalaitzidis, Ph.D., Jonathan Terrett, Ph.D., Jason Sagert, Ph.D.

CRISPR Therapeutics, 610 Main Street, Cambridge, MA, USA 02139

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 TCR locus via homology for each group. 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

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 locus) and MHC I (β2M locus), 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.15 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.15 cells were dosed intravenously with the indicated number of CTX120 CAR+ T cells after ten days or upon reaching an average volume of 100 mm³, 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

Conclusions

- Using CRISPR/Cas9 gene editing, we have generated CTX120, an allogeneic CAR-T 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