Development of CTX112: A Next Generation Allogeneic Multiplexed CRISPR-edited CAR T Cell Therapy with Disruptions of the TGFBR2 and Regnase-1 Genes for Improved Manufacturing and Potency

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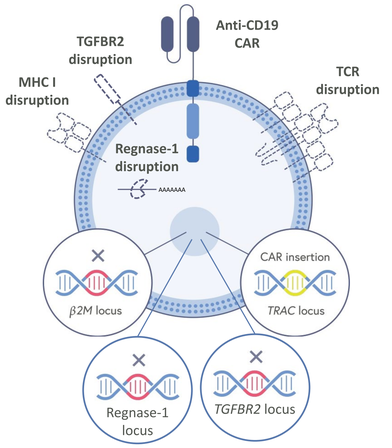
Introduction

- CTX110, a CD19-directed CRISPR-edited CAR T cell therapeutic candidate for B-cell lymphoma, has induced clinical responses and durable remissions beyond two years in some patients (CARBON trial, NCT04035434)
- CTX112, a next-generation CRISPR-edited CAR T cell therapeutic candidate, contains the edits used to produce CTX110 along with additional edits to the TGFBR2 and Regnase-1 genes
- TGFBR2 KO aims to avoid immune suppression of CAR T cell activity by cells of the tumor environment, and Regnase-1 KO aims to increase functional persistence
- CTX112 controls CD19+ disease in mice at one-tenth the dose of CTX110 and shows additional manufacturing advantages

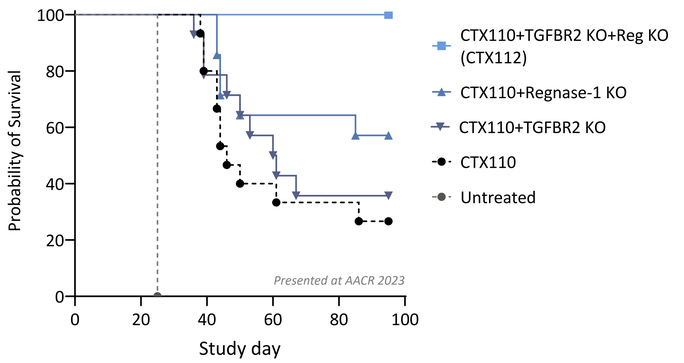
Figure 1: Design of CTX112 – CRISPR/Cas9 gene-edited allogeneic CAR T cells

- The manufacturing process begins with activation of T cells from a healthy donor, followed by electroporation of Cas9 ribonucleoprotein to produce the following modifications:



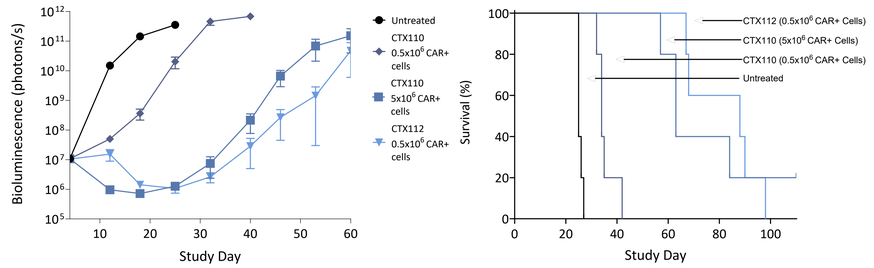
- **TCR KO** to minimizes the risk of GvHD
- **β2M KO** to eliminate MHC class I expression and mitigate host T cell-mediated clearance of CAR T cells
- **CAR KI** via precise insertion of CAR transgene into the TRAC locus using an AAV template
- **Regnase-1** to remove an intrinsic "brake" on T cell function
- **TGFBR2 KO** to remove a key extrinsic "brake" on T cell anti-tumor activity

Figure 2: The additional edits in CTX112 extend survival in Nalm6-Luc mice



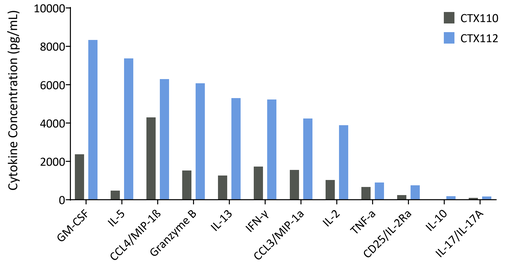
- Mice grafted with Nalm6-Luc leukemia cells were dosed on Day 4 with 4e6 CAR+ cells per mouse per group (N=15 per treated group; N=5 for control group)

Figure 3: CTX112 controls disease at one-tenth the dose of CTX110



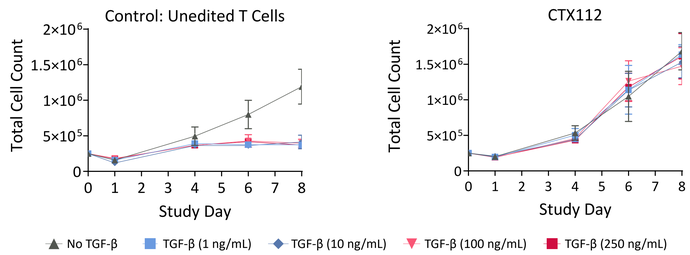
- A 10x lower dose of CTX112 vs. CTX110 (0.5e6 vs. 5e6 CAR+ T cells) shows equivalent efficacy in controlling leukemia burden in Nalm6-Luc mice (n=5 per group dosed at Day 4) (left), resulting in significantly longer survival (right). For these experiments, CTX110 and CTX112 were produced using the same T cell donor

Figure 4: CTX112 secretes higher levels of a broader array of cytokines compared to CTX110



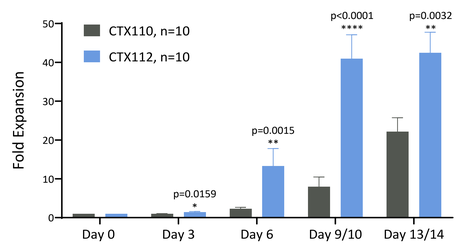
- CTX112 and CTX110 both upregulate multiple cytokines when co-cultured with CD19+ Nalm6 leukemia cells at 1:1 ratio for approximately 24 hours, with CTX112 showing even greater upregulation than CTX110

Figure 5: CTX112 is insensitive to TGF-β-mediated inhibition



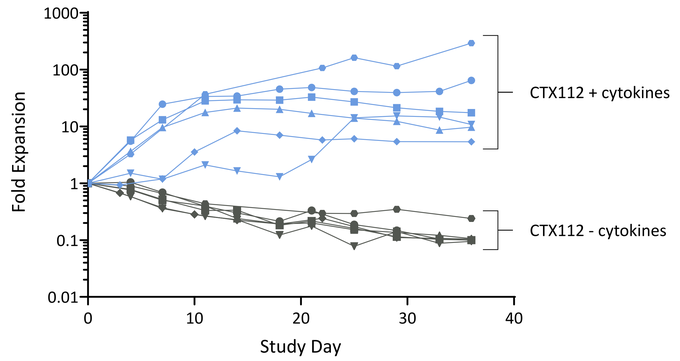
- Knockout of TGFBR2 makes CTX112 resistant to the inhibitory effect of TGF-β, an inhibitory cytokine often expressed in the tumor microenvironment

Figure 6: CTX112 shows even greater expansion *in vitro* than CTX110



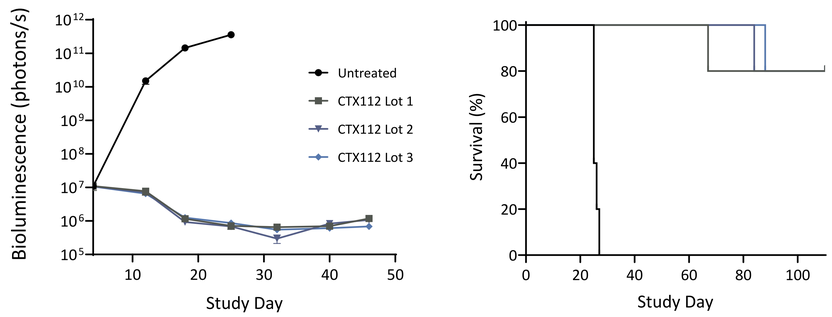
- CTX112 expands significantly more than CTX110 *in vitro* in the presence of 100U/mL human IL-2 and IL-7. The graph depicts the fold expansion of CTX110 and CTX112 manufactured from 10 different donors

Figure 7: CTX112 remains dependent on cytokines for growth



- CTX112 retains expansion properties after a freeze-thaw cycle when cultured with 100U/mL of human IL-2 and IL-7 cytokines, but does not show proliferation or expansion in the absence of human IL-2 and IL-7 cytokines

Figure 8: CTX112 lots produced at our internal manufacturing facility show robust activity *in vivo* at very low cell doses



- CTX112 cells made at our manufacturing facility were administered to mice grafted with Nalm6-Luc leukemia cells (n=5 per group dosed at Day 4). At a dose of 0.5e6 CAR+ T cells, mice in all lot groups demonstrated lower tumor burden (left) and higher survival (right) than mice in the control group, as well as compared to mice administered research-grade CTX112 dosed at the same cell dose in Figure 3

Conclusions

- CTX112, a potency-enhanced CRISPR-edited allogeneic CAR T cell candidate for the treatment of CD19+ malignancies, incorporates disruption of the TGFBR2 and Regnase-1 genes to increase cytokine secretion and sensitivity, as well as functional persistence, with the aim of improving effector function on tumors
- CTX112 demonstrates even better performance than CTX110 across numerous preclinical assessments, including:
  - Secretion of a broader array of cytokines at higher levels *in vitro*
  - Greater sensitivity to cytokine and antigen stimulation, while maintaining cytokine dependence
  - Greater efficacy in cancer models *in vivo* at lower doses
- CRISPR Therapeutics' manufacturing site has produced CTX112 lots that have performed even better than research lots in murine models *in vivo*
- CTX112 is being evaluated in a clinical trial for B cell malignancies (NCT05643742)