P6595

Targeting T cell lymphomas with CRISPR/Cas9-generated anti-CD70 allogeneic CAR-T cells

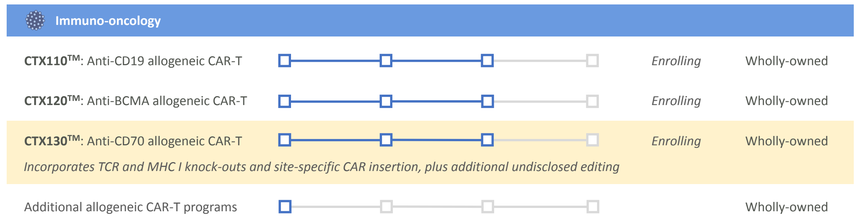
Sushant Karnik, Minh Thu Pham, Pooja Keerthipati, Julie Carson, Zinkal Padalia, Brigid McEwan, Meghna Kuppuraju, Demetrios Kalaitzidis, Tony Ho, Ewelina Morawa, Matthias Will, Jon Terrett, Mary Lee Dequeant

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

Abstract

T cell lymphomas account for 10% to 15% of non-Hodgkin lymphomas and are diverse biologically and clinically. Unlike B-cell lymphomas, T cell lymphomas are rather resistant to conventional therapies, such as chemotherapy and antibody-based therapeutics. This resistance coupled with the diversity of the T cell diseases means that there is a significant unmet need across the T cell lymphoma subtypes. CD70 (CD27 ligand) is a candidate target antigen for T cell lymphomas and has been is the subject of clinical trials using an enhanced ADCC antibody (ARGX-110). Using flow cytometry and immunohistochemistry (IHC) methods, we analyzed the expression of CD70 in cell lines and clinical samples representing T cell lymphomas and found significant expression of CD70 in multiple types of T cell lymphoma, but at highly variable antigen density. CAR-T cells targeting CD70 may thus be a potent new therapy to tackle these diseases. However, the use of autologous CAR-T therapy against T cell lymphomas is complicated by the likelihood of creating lymphoma cells carrying the CAR construct. Thus, we sought to examine the potency of our allogeneic anti-CD70 CAR-T cells (CTX130) against T cell lymphoma cells. CTX130 has previously been shown to be active across a range of CD70 expression levels in other cell types representing other malignancies. Consistent with these prior observations, CTX130 exhibited high potency *in vitro* and *in vivo* against T cell lymphoma cells across a range of CD70 antigen density and representing different types of T cell lymphomas such as Sézary syndrome and cutaneous T cell lymphoma (CTCL). CTX130 may thus be a valid therapeutic to evaluate in T cell lymphoma patients.

Figure 1: CTX130 is CRISPR Therapeutics' first oncology product in clinical phase targeting both solid and liquid tumors



Earlier this year, the U.S. Food and Drug Administration (FDA) accepted CRISPR Therapeutics' Investigational New Drug (IND) application for CTX130, its wholly-owned allogeneic CAR-T cell therapy targeting CD70 for the treatment of both solid tumors, such as renal cell carcinoma (RCC), and T-cell and B-cell hematologic malignancies. Additionally, CRISPR Therapeutics has obtained approval from Health Canada for its Clinical Trial Application (CTA).

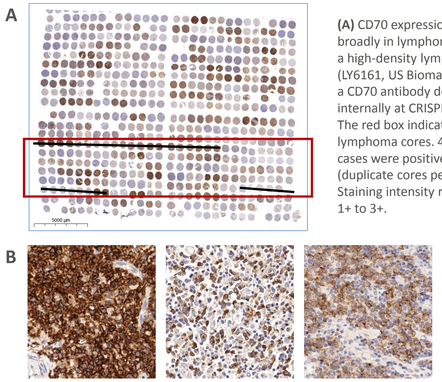
Table 1: CTX130 has demonstrated *in vivo* efficacy in tumor models of a range of CD70-expressing liquid and solid tumors

Indication	CD70⁺ prevalence	CTX130 *in vivo* efficacy in mice**
ccRCC	82%	✓
T cell lymphoma (TCL)	80%*	✓
Pancreatic cancer	25%	✓
Lung cancer	10%	✓
Ovarian cancer	15%	✓

This table shows CD70 expression by IHC from tissue microarray analysis of carcinomas using 1C1 (Br J Cancer. 2010 Aug 24; 103(5): 676–68)

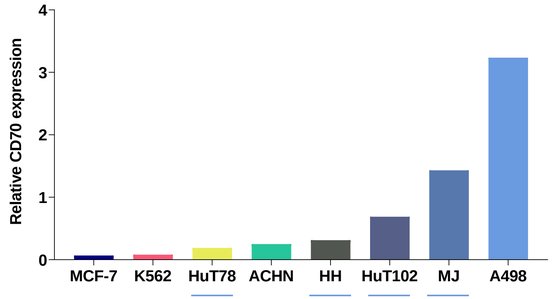
* TCL data were generated internally using a proprietary antibody to CRISPR Therapeutics (See Figure 2A).
**A single injection of CTX130 induced complete tumor regression in xenograft models of clear cell Renal Cell Carcinoma (ccRCC) (A498), TCL (HuT78), lung cancer (H1975), and ovarian cancer (SKOV-3) and nearly complete regression in pancreatic cancer model (Hs766T) (AACR, 2019 and 2020).

Figure 2: CD70 expression on a mixed TCL array shows approximately 80% prevalence by IHC



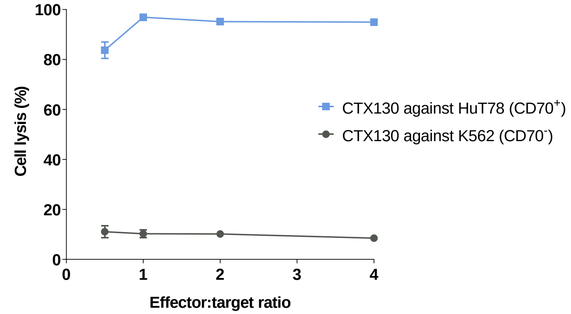
(A) CD70 expression is observed broadly in lymphoma samples on a high-density lymphoma array (LY6161, US Biomax) stained with a CD70 antibody developed internally at CRISPR Therapeutics. The red box indicates mixed T cell lymphoma cores. 43 out of 53 cases were positive for CD70 (duplicate cores per case). **(B)** Staining intensity ranged from 1+ to 3+.

Figure 3: TCL cancer cell lines display varying levels of CD70 protein expression



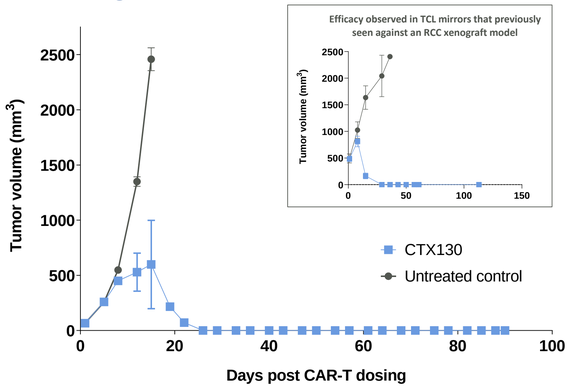
CD70 expression by flow cytometry in TCL and RCC cancer cell lines. Consistent with the IHC data, TCL cell lines HuT78, HH, HuT102 and MJ (indicated by the blue lines) show a range of CD70 expression from low/medium to high. RCC cell lines A498 and ACHN show high and low CD70 expression, respectively, and were both previously shown to be effectively eliminated by our allogeneic anti-CD70 CAR-T cells (AACR, 2018). MCF-7 and K562 are CD70-negative cells lines shown as negative controls for background staining.

Figure 4: CTX130 shows potent and specific cytotoxicity against a low-expressing CD70-positive TCL cell line



***In vitro* cytotoxicity assay to evaluate CTX130 activity against the CD70-positive HuT78 cancer cell line and CD70-negative K562 cell line.** CTX130 was cocultured with HuT78 or K562 cells for 24 hours at T cell:tumor cell ratios ranging from 0.5:1 to 4:1. Tumor cell viability was assayed using flow cytometry. The mean (± standard deviation) cell lysis is shown from three technical replicates. The high activity seen against a low-expressing TCL cell line suggests CTX130 could potentially prove effective against even tumors with low CD70 expression.

Figure 5: CTX130 shows potent activity against a low-expressing CD70-positive TCL tumor xenograft model



***In vivo* efficacy against an established HuT78 xenograft tumor model of Sézary Syndrome.** $3x10^6$ HuT78 cells were injected subcutaneously into the right flank of NSG (NOD.Cg-Prkdcscid Il2rgtm1Wjl/SzJ) mice. When mean tumor size reached an average size of approximately 66 mm^3, mice were either left untreated or injected intravenously (N=5 per group) with $8.6x10^6$ CAR⁺ CTX130 cells per mouse. Tumor volumes were measured twice weekly for the duration of the study. Each point represents the mean tumor volume ± standard error. Four of the five mice treated with CTX130 remain tumor free at the end of study, with durable responses observed out to at least until 90 days**. Insert: CTX130 completely eliminated a subcutaneous A498 RCC model of large tumor size.** A498 cells were injected subcutaneously into NSG mice. When mean tumor size reached an average size of approximately 500 mm^3, mice were injected intravenously (N=4 group) with $10x10^6$ CAR⁺ CTX130 cells per mouse.

Conclusions from preclinical studies

- CTX130 is an allogeneic CAR-T cell therapy targeting CD70 for the treatment of both solid tumors, such as renal cell carcinoma, and T-cell and B-cell hematologic malignancies
- Given that CD70 shows high (~80%) prevalence in TCL, as shown by IHC, we explored the ability of CTX130 as a treatment for these cancers
- CTX130 shows potential for the treatment of TCL, including tumors with low CD70 expression, as demonstrated by *in vitro* and *in vivo* studies:
  - CTX130 shows potent and specific activity *in vitro* against a low-expressing CD70⁺ TCL line
  - CTX130 shows potent efficacy *in vivo* against a low-expressing CD70⁺ TCL xenograft tumor model
- CTX130 has entered clinical phase and CRISPR Therapeutics expects to begin treating patients with CTX130 in the second half of 2020