#1537

CD70 knockout: A novel approach to augment CAR-T cell function

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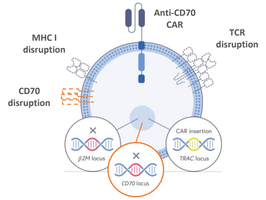
Abstract

CD70 and its ligand, CD27, have been described as both activating and suppressing for different cell types including B and T cells. There has been speculation that the CD70/CD27 axis can act in a checkpoint or co-stimulatory manner in certain immuno-oncology settings. Knockout (KO) of CD70 function scored highly in a CRISPR/Cas9 screen of candidate genes for enhanced T cell activity. Deletion of other checkpoint candidates such as PD1, TIM3, LAG3 and TIGIT scored much lower than CD70, both alone and in combination with each other. T cells with CD70 KO showed resistance to exhaustion upon repeated stimulation in culture. CAR-T cells with CD70 KO similarly showed exhaustion resistance, as well as a reduction in apoptosis, increased proliferation, and improved target cell lysis upon sequential rechallenges. CTX130 is an investigational allogeneic CAR-T therapy currently being studied in patients with CD70-expressing tumors, including clear cell renal cell carcinoma and B and T cell malignancies. CTX130 contains KOs of TRAC to avoid GvHD, B2M to protect the product from patient T cells, and CD70 for enhanced CAR-T performance. Comparing CTX130 with and without CD70 KOs shows that CAR-T cells with CD70 KO have increased potency, enhanced ability to withstand multiple tumor challenges *in vivo*, and increased resistance to overexpression of PD-L1 on target cells. Interestingly, while CD70 surface expression may be extremely low or undetectable after manufacturing of CD70-targeted CAR-T cells without CD70 KO, the beneficial properties outlined here are only achievable when the CD70 gene is genetically knocked out. In summary, KO of CD70 confers benefit to CAR-T cells that far exceeds KO of other checkpoint related genes, and this benefit was present regardless of the antigen being targeted. CD70 KO is included in the CTX130 investigational allogeneic CAR-T therapy currently in clinical trials.

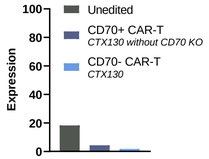
CTX130 is enhanced by CD70 KO

Figure 1: CTX130 – an investigational allogeneic, CRISPR/Cas9 gene-edited, anti-CD70 CAR-T cell therapy CAR-T cell therapy with TRAC, β2M, and CD70 knock-outs



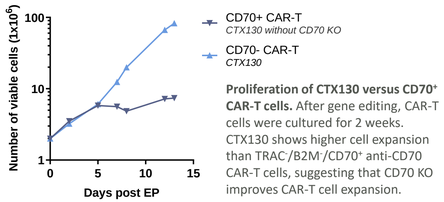
CTX130 cell design. CTX130 includes genetic disruptions of the TRAC, β2M, and CD70 genes. In addition, an anti-CD70 CAR cassette is site-specifically inserted into the TRAC locus by homology-directed repair.

Figure 2: Low CD70 expression can be obtained with or without CD70 KO



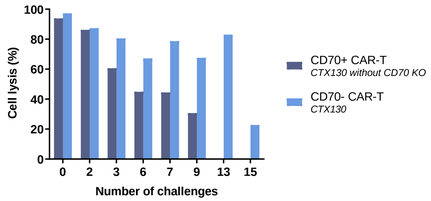
Detection of CD70 by flow cytometry. CD70 was detected on a subset of unedited T cells, consistent with the expected expression pattern of CD70. After manufacturing of anti-CD70 CAR-T cells, CD70 expression was reduced significantly either by fratricide in TRAC$^-$/B2M$^-$/CD70$^+$ CAR-T cells or by KO of the CD70 gene in CTX130.

Figure 3: CAR-T cells with CD70 KO show improved proliferation



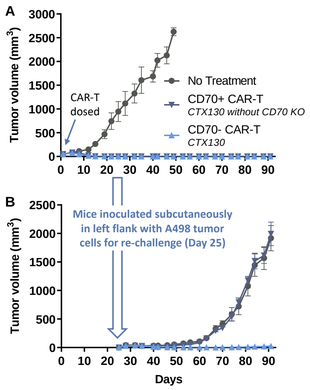
Proliferation of CTX130 versus CD70$^+$ CAR-T cells. After gene editing, CAR-T cells were cultured for 2 weeks. CTX130 shows higher cell expansion than TRAC$^-$/B2M$^-$/CD70$^+$ anti-CD70 CAR-T cells, suggesting that CD70 KO improves CAR-T cell expansion.

Figure 4: CD70 KO confers superior cytotoxic activity during *in vitro* re-challenge



***In vitro* cytotoxicity re-challenge.** Upon 15 repeated challenges *in vitro* with A498 cells, CTX130 retains cytotoxicity, while TRAC$^-$/B2M$^-$/CD70$^+$ anti-CD70 CAR-T cells lose their ability to expand or kill after 9 challenges.

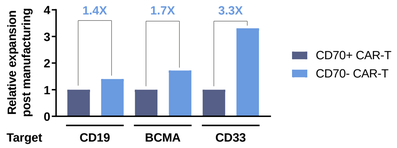
Figure 5: CAR-T cells with and without CD70 KO eliminate an RCC tumor model *in vivo*, but only CTX130 exhibits continued activity following *in vivo* re-challenge



(A) Initial *in vivo* challenge on right hind flank. CTX130 and TRAC$^-$/B2M$^-$/CD70$^+$ anti-CD70 CAR-T cells both eradicate tumors in a subcutaneous A498 xenograft tumor model in NOG mice; n=5 for each group. **(B) *In vivo* re-challenge on left hind flank.** CTX130 cells severely reduce tumor regrowth after *in vivo* re-challenge, while TRAC$^-$/B2M$^-$/CD70$^+$ anti-CD70 CAR-T cells fail to prevent tumor regrowth. No additional CAR-T cells were administered; n=5 for each group.

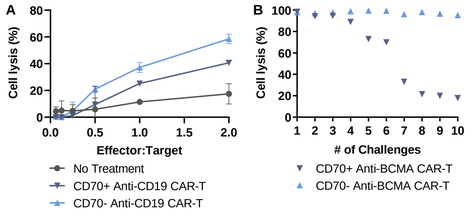
CD70 KO enhances CAR-Ts targeting other antigens

Figure 6: CAR-T cells with CD70 KO show improved proliferation regardless of CAR target

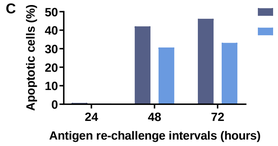


Relative proliferation of CD70$^-$ and CD70$^+$ CAR-T cells targeting additional antigens. CD70 KO demonstrates advantages beyond the specific context of a CD70-targeted CAR-T cell.

Figure 7: CD70 KO improves *in vitro* cytotoxic activity of CAR-T cells targeting CD19 and BCMA



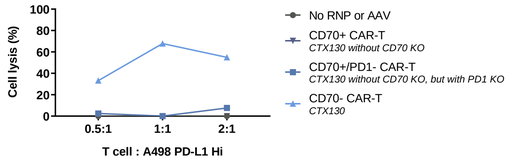
***In vitro* cytotoxicity co-culture and re-challenge assays. (A)** Anti-CD19 CAR-T cells with CD70 KO show greater potency than anti-CD19 CAR-T cells with the CD70 gene intact and **(B)** anti-BCMA CAR-T cells with CD70 KO show an increased ability to serially lyse BCMA$^+$ target cells as compared to anti-BCMA CAR-T cells with CD70 intact, demonstrating the advantages of CD70 KO CAR-T cells regardless of the target antigen.



(C) Apoptosis assay. Anti-BCMA CAR-T cells with CD70 KO showed decreased apoptosis upon exposure to antigen rechallenge.

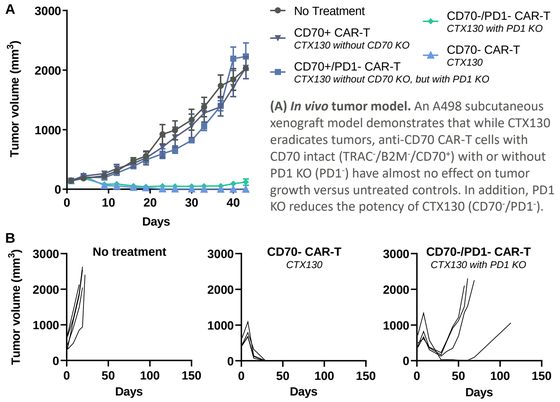
KO of certain checkpoint genes is detrimental to CAR-T function

Figure 8: CD70 KO confers superior cytotoxic activity than PD1 KO against an RCC tumor cell line overexpressing PD-L1



***In vitro* cytotoxicity co-culture assay.** A498 cells engineered to over express PD-L1 (A498 PD-L1 Hi) were used as target cells. CTX130 shows a potent dose response against the A498 PD-L1 Hi cells, whereas anti-CD70 CAR-T cells with CD70 intact (TRAC$^-$/B2M$^-$/CD70$^+$) show almost no cytolytic activity towards the A498 PD-L1 Hi cells, even when PD1 is also knocked out (PD1$^-$).

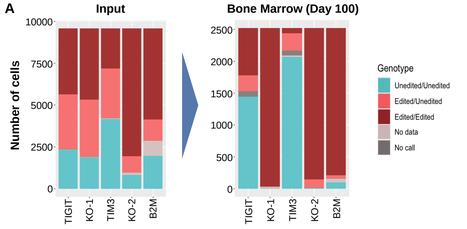
Figure 9: PD1 KO alone does not improve CAR-T activity and PD1 KO in the context of CD70 KO reduces CAR-T activity in multiple *in vivo* xenograft studies



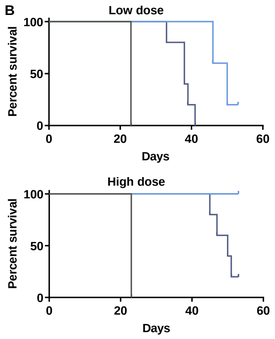
(A) *In vivo* tumor model. An A498 subcutaneous xenograft model demonstrates that while CTX130 eradicates tumors, anti-CD70 CAR-T cells with CD70 intact (TRAC$^-$/B2M$^-$/CD70$^+$) with or without PD1 KO (PD1$^-$) have almost no effect on tumor growth versus untreated controls. In addition, PD1 KO reduces the potency of CTX130 (CD70$^-$/PD1$^-$).

(B) *In vivo* "large" tumor model. CTX130 completely eliminated "large" subcutaneous A498 xenograft tumors without relapse. In contrast, TRAC$^-$/B2M$^-$/CD70$^+$/PD1$^-$ anti-CD70 CAR-T cells were unable to achieve complete elimination of the tumor or maintain a prolonged response.

Figure 10: KO of multiple checkpoint genes impairs CAR-T cell function



(A) Single-cell DNA sequencing *in vivo* CRISPR screen. CAR-T cells with multiple genes edited were used to treat Nalm6 leukemia-bearing mice. After ~100 days, cells from mouse bone marrow were isolated and DNA from single cells was sequenced. The number of single human cells and allelic editing at the indicated genes is shown (representative data from one mouse). The results demonstrated positive selection of disrupted alleles for two un-named genes and negative selection of disrupted alleles of the checkpoint genes TIGIT and TIM3, suggesting that disruptive edits of these genes impairs CAR-T function.



(B) *In vivo* leukemia model with checkpoint knockout CAR-T cells. TRAC$^-$/B2M$^-$ anti-CD19 CAR-T cells were produced with and without additional disruptions in multiple checkpoint genes (PD1, TIGIT, TIM3, LAG3). The edited CAR-T cells were used to treat Nalm6 bearing NOG mice at 4x10^6 CAR$^+$ T cells (top) and 8x10^6 CAR$^+$ T cells (bottom). Disruption of these four checkpoint genes reduces the potency of CD19-targeted CAR-T cells. We have previously shown the generation of CAR-T cells with up to 9 different genes knocked out, which showed enhanced potency in an *in vivo* model [McEwan, et al. (2020) at AACR].

Conclusions from Preclinical Studies

- We applied CRISPR/Cas9 editing to examine the effects of knocking out the gene function of multiple checkpoint-related genes in CAR-T cells, including both "obvious" choices such as PD1 and LAG3 where antagonism with antibodies has shown anti-cancer properties in humans and mice, as well as less obvious candidates such as CD70
- Surprisingly, not only did CD70 KO perform better than any of the more obvious checkpoint genes, CD70 KO also provided advantages for CAR-T cells targeting multiple antigens other than CD70
- In contrast, CAR-T cells with classical checkpoint genes knocked out showed no improved properties. In fact, these knock-outs often proved detrimental to CAR-T function
- We have applied the CD70 KO strategy to our CTX130 program currently in clinical trials for solid and heme malignancies