Abstract #1528

Discovery and Preclinical Characterization of Fratricide-Resistant TRuC™-T Cells Targeting CD70

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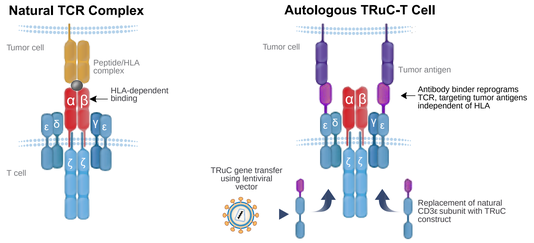
Abstract

Previously we described the design and antitumor activity of T cell receptor fusion constructs (TRuC™) that tether an antibody-derived binder to one of the TCR subunits to achieve redirected T cell killing of tumor cells independent of HLA. Different from CAR-T cells, TRuCs are integrated into the full TCR complex and thus harness its full signaling capacity. The cell surface antigen CD70 represents a promising target for cancer immunotherapy for its selective overexpression in various hematological and solid tumor indications. Because the normal tissue expression of CD70 occurs on activated lymphocytes, including activated T cells, fratricide (self-killing) has been recognized as a significant challenge for CD70-targeted T cell therapies. To address this challenge, we discovered a diverse pool of fully human anti-CD70 scFv binders that were used to make TRuC-T cells and then functionally screened for fratricide-resistance *in vitro*. We successfully identified a CD70-targeted TRuC-T cell candidate that exhibits normal T-cell expansion and an improved memory phenotype, clearly differentiating from fratricide-prone candidates, all while maintaining potent cytotoxicity and cytokine production against tumor cells expressing both low and high levels of CD70. In addition, our CD70-targeted TRuC-T cells showed potent anti-tumor efficacy in multiple xenograft mouse models with no evidence of *in vivo* fratricide. In summary, we have engineered a fratricide-resistant CD70-directed TRuC-T cell therapy that has the potential to treat a wide range of both hematologic and solid cancers.

TRuC platform



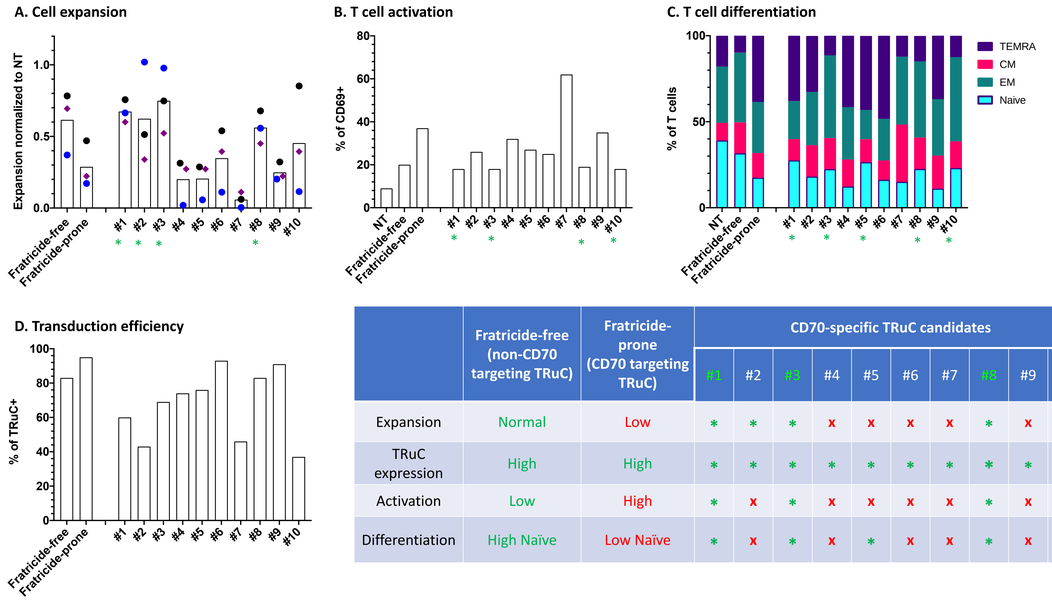
Discovery of CD70-specific binders

- 2 phage libraries were generated and screened for CD70 reactive binders
  - Immunized alpaca VHH library
  - Naïve human scFv library
- Selection of leads was based on binding to recombinant CD70, cell binding, CD27 blocking behavior and sequence diversity.
- VHH candidates identified from the immunized llama VHH library showed low epitopic diversity and TRuC-T cells generated using these binders showed evidence of fratricide (poor expansion, low levels of naïve cells, activated phenotype, limited *in vivo* efficacy).
- A diverse set of 5 scFv binders was selected for testing in two binder orientations, vHvL and vLvH, resulting in a total of 10 TRuC-T cell candidates.

Conclusions

- A CD70-targeted scFv discovery campaign using a naïve human scFv library identified a sequence-diverse pool of αCD70 scFv candidates.
- Candidate construct #1 generates fratricide-resistant anti-CD70 TRuC-T cells that exhibit a similar fold-expansion and phenotype to fratricide-free TRuC-T cells
- Fratricide-resistant candidate #1 shows robust cytotoxicity and cytokine responses against human RCC and AML tumor cell lines with high and low CD70 expression.
- Candidate #1 displays potent efficacy in various xenograft models, including models with clinically relevant levels of CD70 expression (MOLM-13, ACHN) with functional persistence observed in an RCC model (786-O).
- We have identified a fratricide-resistant anti-CD70 TRuC-T cell lead candidate that holds promise as a novel cell therapy for the treatment of CD70-expressing liquid and solid tumors.

Fratricide-resistant CD70-targeted TRuC-T cell candidates were selected based on T-cell expansion and phenotype



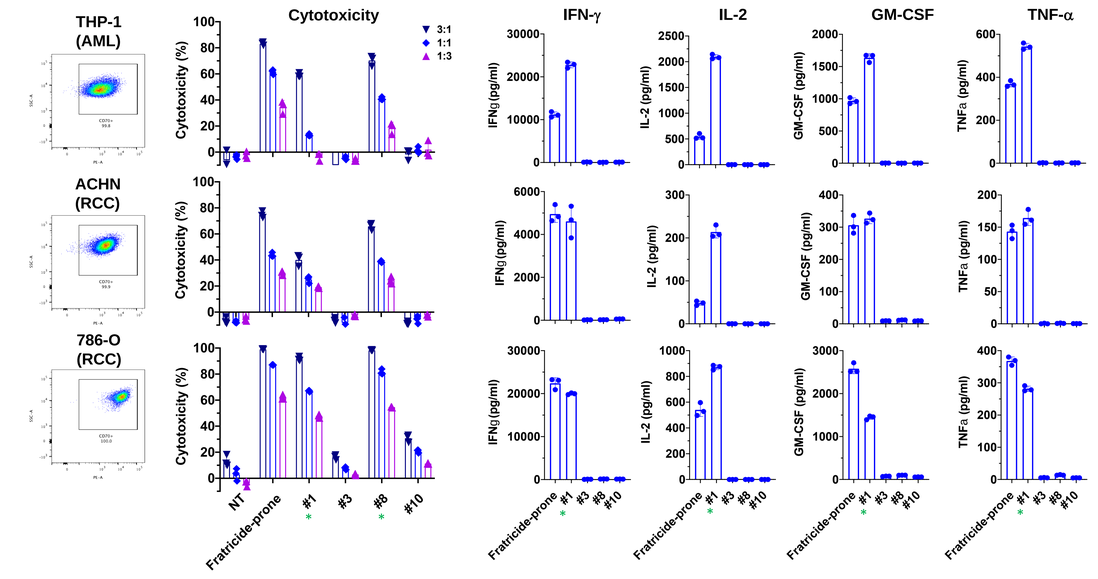
	Fratricide-free (non-CD70 targeting TRuC)	Fratricide-prone (CD70 targeting TRuC)	CD70-specific TRuC candidates									
			#1	#2	#3	#4	#5	#6	#7	#8	#9	#10
Expansion	Normal	Low	*	*	*	x	x	x	x	*	x	x
TRuC expression	High	High	*	*	*	*	*	*	*	*	*	*
Activation	Low	High	*	x	*	x	x	x	x	*	x	*
Differentiation	High Naïve	Low Naïve	*	x	*	x	*	x	x	*	x	*

T cells were activated and expanded with Human T cell TransAct (Miltenyi Biotech), with recombinant human IL-7 and IL-15, for 10 days.

(A) The fold-expansion at the end of the manufacturing process for each tested construct was normalized to the expansion of NT T cells (n=3 donors). (B) T-cell activation was evaluated by surface CD69 expression. (C) T-cell differentiation was determined by surface expression of CD45RA and CCR7 (Naïve, CD45RA⁺CCR7⁺; CM, CD45RA⁻CCR7⁺; EM, CD45RA⁻CCR7⁻; TEMRA, CD45RA⁺CCR7⁻). (D) Transduction efficiency was assessed by flow cytometric detection of anti-CD70 binder surface expression using Fc-CD70 protein. Data from representative donors are shown for (B-D).

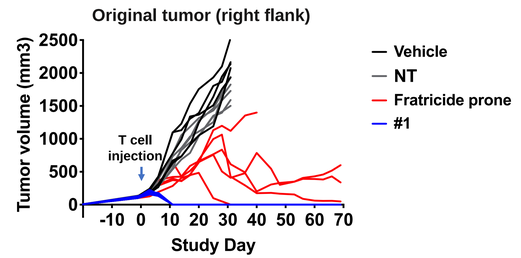
NT, non-transduced T cells. "Fratricide-free" is a non-CD70 targeting TRuC that shows a robust proportion of naïve cells and typical TRuC fold-expansion. "Fratricide-prone" comparator is a CD70-targeted TRuC with diminished expansion and a reduced naïve subset.

Fratricide-resistant CD70-targeted TRuC-T cell candidate #1 exhibits potent cytotoxicity and cytokine production against RCC and AML cell lines with CD70^{high} and CD70^{low} expression

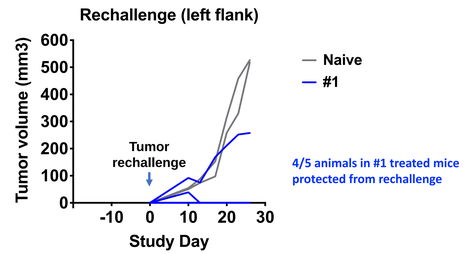


CD70 expression in tumor cell lines was detected using αCD70 (BioLegend clone 113-16). TRuC-T cells were co-cultured with tumor cell lines at indicated effector-to-target ratios. Cytokine levels were measured by U-Plex MSD kits. For cytokines, data from 3:1 ratio are shown.

Fratricide-resistant CD70-targeted TRuC-T cell candidate #1 exhibits potent and persistent *in vivo* efficacy

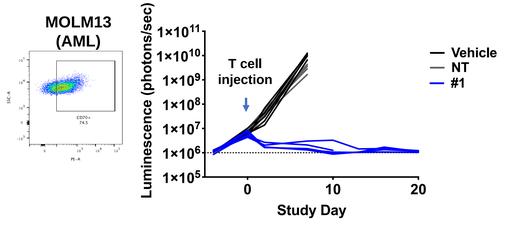


NSG mice were injected s.c. with 3x10⁶ of 786-O cells. Eighteen days later, on study day 0, tumor-bearing mice were treated with PBS (vehicle), non-transduced T cells (NT), fratricide prone CD70 TRuC-T cells or fratricide-resistant candidate #1 TRuC-T cells. Individual animals were administered 3x10⁶ TRuC-T cells.

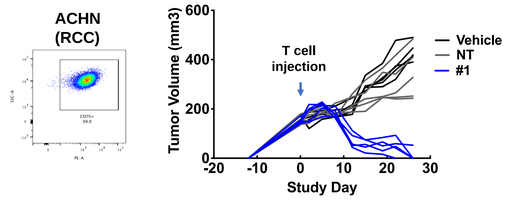


5 tumor-free mice treated with #1 TRuC-T cells were rechallenged on study day 43 with 3x10⁶ of 786-O cells (s.c.) per animal. Naïve mice that received no treatment were inoculated with tumor cells as a control.

Fratricide-resistant candidate #1 exhibits potent efficacy against tumor models with low to moderate CD70 expression



NSG mice were injected i.v. with 5x10⁴ MOLM-13-Luc cells. Four days later, on study day 0, tumor-bearing mice received single infusions of the indicated treatments. Individual animals were administered 1x10⁷ TRuC-T cells. N=5 mice/group.



NSG mice were injected s.c. with 2x10⁶ ACHN cells. Twelve days later, on study day 0, tumor-bearing mice were normalized and received single infusions of the indicated treatments. Individual animals were administered 5x10⁶ TRuC-T cells. N=5 mice/group.