Engineering CD70-Directed CAR-NK Cells for the Treatment of Hematological and Solid Malignancies

Jae-Woong Chang[1], Joshua Kreuger[1], Joseph G. Skeate[1], Erin M. Stelljes[1], Walker S. Lahr[1], Nicholas J. Slipek[1], Emily Pomeroy[1], Meghan Walsh[2], Jennifer Johnson[2], Charlotte Franco[2], Simarjot Pabla[2], Jared Sewell[2], Eugene Choi[2], Vipin Suri[2], Beau R. Webber[1], and Branden S. Moriarity[1]

[1] *Department of Pediatrics, University of Minnesota, Minneapolis, MN, USA. Masonic Cancer Center, University of Minnesota, Minneapolis, MN, USA. Center for Genome Engineering, University of Minnesota, Minneapolis, MN, USA. Stem Cell Institute, University of Minnesota, Minneapolis, MN, USA.*

[2] *Catamaran Bio, Inc., Cambridge, MA, USA.*

Background

CAR-NK cell therapies are particularly attractive in treating acute myeloid leukemia (**AML**) as the inherent graft-versus-leukemia activity of NK cells can be effectively augmented by a CAR directed to an AML expressed antigen. CD70 is an attractive target for CAR therapy in AML since it is highly expressed on leukemic stem cells and blasts and is not detectable on normal bone marrow hematopoietic stem cells. Additionally, aberrant CD70 expression is associated with several solid tumors and hematological malignancies, including AML, NHL, and renal cell carcinoma (**RCC**) while expression in normal tissue is restricted to immune cells including T, B, DC, and NK cells. Here, we demonstrate that CD70 is not expressed in resting peripheral blood NK cells but is strongly upregulated in response to NK cell activation by engineered feeder cells. As such, integration of a CD70-targeting CAR into activated NK cells leads to substantial reduction of NK cell expansion due to fratricide. Knockout (**KO**) of CD70 by CRISPR/Cas9 editing does not inhibit NK cell expansion nor impair cytotoxicity against various types of tumor cells, therefore a successful engineering strategy with simultaneous CD70 CAR delivery with CD70 knock out via CRISPR/Cas9 would allow for successful propagation of such a CAR-NK therapy. Using the non-viral *TC Buster* transposon system, we were able to deliver transposons polycistronic CD70 CAR while simultaneously knocking out CD70 by CRISPR/Cas9 in primary human peripheral blood NK cells. This single-step process resulted in >75% CD70 CAR integration/expression and >80% knockout of endogenous CD70. The resulting CD70 knockout CD70 CAR-NK cells were resistant to fratricide and expanded comparably to mock-engineered NK cells following feeder cell mediated expansion. Constructs containing soluble IL15 expression enabled enhanced persistence of CAR-NK cells *in vitro* and *in vivo* without exogenous cytokine support.

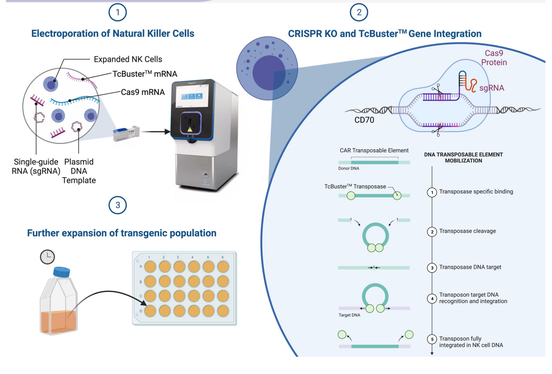
In functional assays, CD70 knockout CAR-NK cells mediated cytotoxicity against multiple CD70-positive tumor cell lines including AML and RCC both *in vitro* and *in vivo*. Overall, the results demonstrate the potential for targeting CD70 with CAR-NK cell therapy for the treatment of AML, RCC, and other CD70-positive malignancies while overcoming fratricide issues by engineering with a non-viral transposon delivery system in combination with CRISPR/Cas9 editing.

Objectives

- Generate CD70 CAR NK cells using the non-viral *TC-Buster* transposon system
- Validate anti-tumor activity of CD70 CAR NK cells against AML and RCC *in vitro* and *in vivo*
- Generate and test armored CD70 CAR NK Cells

Experimental Approach

Generating CAR NK Cells
with TcBuster™ and Cas9



Results

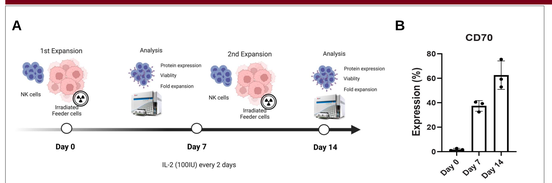


Figure 1. CD70 expression is absent in resting peripheral blood NK cells but is highly upregulated upon activation. (A) Timeline for production of NK cells. Primary human peripheral blood NK cells (n=3 human donors) are activated with mbIL21- and 41BBL-expressing K562 feeder cells at a 2:1 (feeder: NK) ratio for 7 days with exogenous IL-2 (100 IU) every 2 days. NK cells are expanded again for one week at a 1:1(feeder: NK) ratio with exogenous IL-2 (100 IU) every 2 days. (B) CD70 expression was measured by flow cytometry at each time point.

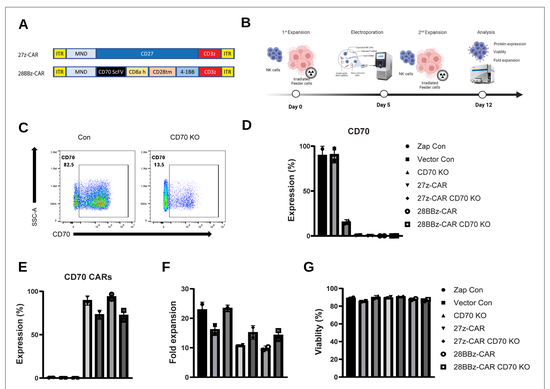


Figure 2. CD70 knockout enhances CD70 CARs NK cells expansion. (A) CD70 CAR constructs. The transposon flanked by *TcBuster* ITRs, containing an MND promoter, CD70 ligand-based CAR (27z-CAR) or CD70 scFv-based CAR (28BBz-CAR). Elements are separated by 2A ribosomal skip sequences. (B) Timeline for production of CD70 CARs NK cells. (C) FACS analysis of CD70 expression in Control (left) or CD70 KO NK cells using CRISPR/Cas9 (Right). (D-G) CD70, CD70 CARs expression were measured by flow cytometry (D and E), fold expansion was calculated (F). Viability was measured by Trypan Blue exclusion assay (G).

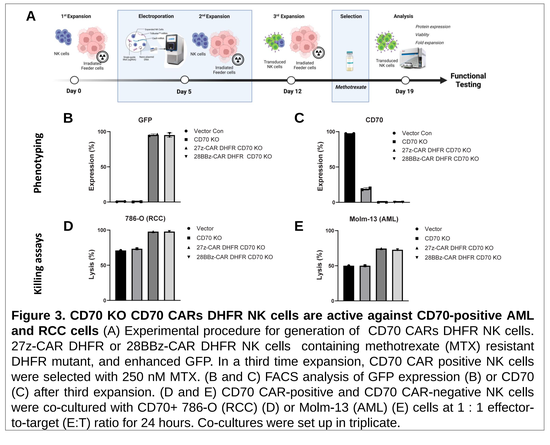


Figure 3. CD70 KO CD70 CARs DHFR NK cells are active against CD70-positive AML and RCC cells (A) Experimental procedure for generation of CD70 CARs DHFR NK cells. 27z-CAR DHFR or 28BBz-CAR DHFR NK cells containing methotrexate (MTX) resistant DHFR mutant, and enhanced GFP. In a third time expansion, CD70 CAR positive NK cells were selected with 250 nM MTX. (B and C) FACS analysis of GFP expression (B) or CD70 (C) after third expansion. (D and E) CD70 CAR-positive and CD70 CAR-negative NK cells were co-cultured with CD70+ 786-O (RCC) (D) or Molm-13 (AML) (E) cells at 1 : 1 effector-to-target (E:T) ratio for 24 hours. Co-cultures were set up in triplicate.

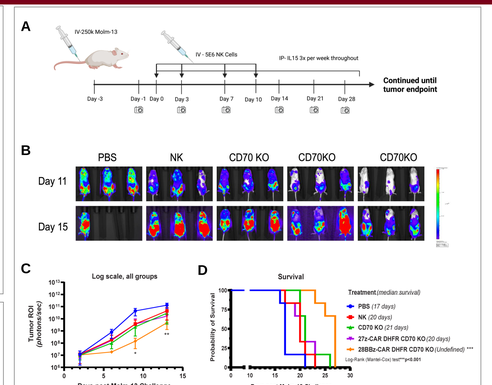


Figure 4. CD70 KO CD70 CAR NK cells enhance *in vivo* efficacy in a xenograft model of AML. (A) Experimental procedure of AML xenograft model with Molm-13 cells. A total of 2.5 × 10^5 luciferase-expressing Molm-13 cells were delivered i.v. to NSG mice. Three days after tumor injection, PBS or 5 × 10^6 Pulse control (NK), CD70 KO, 27z-CAR DHFR CD70 KO or 28BBz-CAR DHFR CD70 KO NK cells were delivered i.v. two times weekly for 14 days. NK cells were supported with a 1 μg dose of IL-15 three times weekly for 4 weeks. Tumor burden was assessed with bioluminescence imaging weekly (n = 6 mice per group) (B) Representative images showing AML progression in individual mice at day 11 and 15. (C) Kinetics of AML progression in individual mice that received PBS control or CD70KO or CD70 CARs DHFR NK cells. (D) Kaplan-Meier curves showing survival of mice in each experimental group

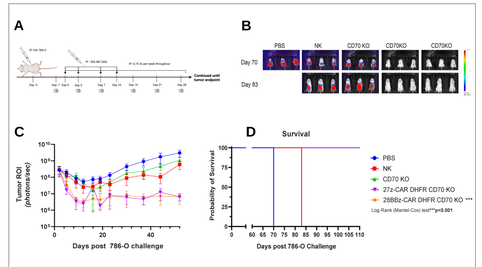


Figure 5. CD70 KO CD70 CARs NK cells enhance *in vivo* efficacy in a xenograft model of RCC. (A) Experimental procedure of RCC xenograft model with 786-O cells. A total of 1 × 10^6 luciferase-expressing 786-O cells were delivered i.p. to NSG mice. Three days after tumor injection, PBS, 5 × 10^6 Pulse control (NK), CD70 KO, 27z-CAR DHFR CD70 KO or 28BBz-CAR DHFR CD70 KO NK cells were delivered i.p. two times weekly for 14 days. NK cells were supported with a 1 μg dose of IL-15 three times weekly for 4 weeks. Tumor burden was assessed with bioluminescence imaging weekly (n = 3 mice per group) (B) Representative images showing RCC progression in individual mice at day 70 and 83. (C) Kinetics of RCC progression in individual mice that received PBS control, CD70 KO NK or CD70 CARs NK cells. (D) Kaplan-Meier curves showing survival of mice in each experimental group.(E) Persistence of NK cells in mice blood (Left) and Intraperitoneal fluid (Right) upon necropsy. Number of mCD45-CD56+ cells was measured by flow cytometry.

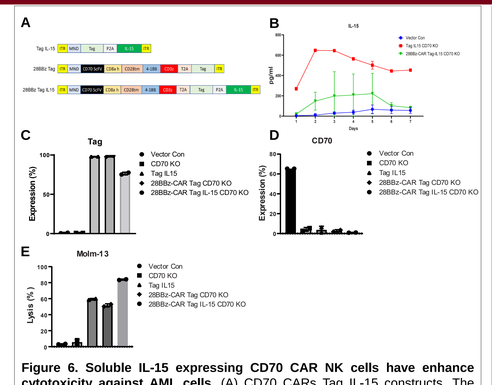


Figure 6. Soluble IL-15 expressing CD70 CAR NK cells have enhance cytotoxicity against AML cells. (A) CD70 CARs Tag IL-15 constructs. The transposon flanked by *TcBuster* ITRs, containing an MND promoter, Tag, IL-15 (Tag IL-15) or 28BBz-CAR containing Tag (28BBz-CAR Tag) or Tag IL-15 (28BBz-CAR Tag IL-15). Elements are separated by 2A ribosomal skip sequences. (B) Detection of secreted IL-15 by Tag IL-15 or 28BBz-CAR Tag IL-15 NK cells. NK cells were electroporated with transposition reagent and subsequently supernatant was collected from each experimental samples at the indicated time points. Secreted IL-15 was measured by ELISA assay. (C and D) Tag and CD70 expression of each group of NK cells were measured by flow cytometry. (E) CD70 CAR-positive and CD70 CAR-negative NK cells were co-cultured with CD70+ at 1 : 1 effector-to-target (E:T) ratio for 24 hours. Co-cultures were set up in triplicate.

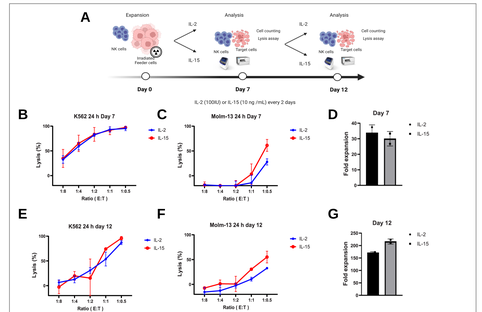


Figure 7. Continuous IL-15 stimulation does not inhibit NK cell cytotoxicity and expansion. (A) Timeline for production of NK cells. Primary human peripheral blood NK cells (n=2 human donors) are activated with mbIL21- and 41BBL-expressing K562 feeder cells at a 2:1 (feeder: NK) ratio for 12 days with IL-2 (100 IU) or IL-15 (10 ng / mL). (B-G) NK cells were co-cultured with K562 (B and E) or Molm-13 (C and F) cells at the indicated effector-to-target (E:T) ratios for 24 hours and fold expansion was calculated at day7 (D) or Day 12 (G). Co-cultures were set up in triplicate.

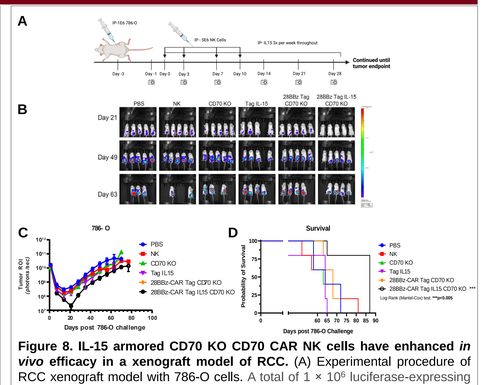


Figure 8. IL-15 armored CD70 KO CD70 CAR NK cells have enhanced *in vivo* efficacy in a xenograft model of RCC. (A) Experimental procedure of RCC xenograft model with 786-O cells. A total of 1 × 10^6 luciferase-expressing 786-O cells were delivered i.p. to NSG mice. Three days after tumor injection, PBS or 5 × 10^6 Pulse control (NK), CD70 KO, Tag IL-15, 28BBz-CAR Tag CD70 KO, or 28BBz-CAR Tag IL-15 CD70 KO NK cells were delivered i.p. at day 0. Tumor burden was assessed with bioluminescence imaging weekly (n = 5 mice per group). (B) Representative images showing RCC progression in individual mice at day 21,49, and 63. (C) Kinetics of RCC progression in individual treated mice. (D) Kaplan-Meier curves showing survival of mice in each experimental group.

Conclusions

- CD70 is upregulated in NK cells upon activation and expansion and results in fratricide in CD70 CAR-NK cell cultures
- Simultaneous CRISPR/Cas9 knockout with transposition enhances expansion of CD70 CAR-NK cells
- CD70 CAR NK cells demonstrate enhanced killing of CD70+ cancer cells *in vitro and in vivo*
- IL-15 armored CD70 CAR NK cells show enhanced killing without exogenous cytokine *in vitro* and *in vivo*

References

- Perna et al. 2017, Cancer Cell. 32:506-519.
- McEarchern et al. 2008, Clin Cancer Res. 14(23):7763-7772.

Questions or Comments ?

Contact information : jwchang@umn.edu