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

#1691

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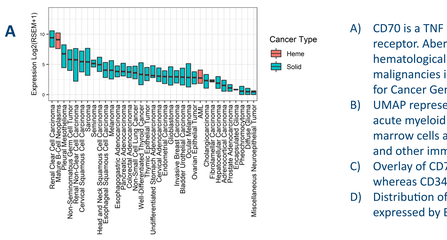
INTRODUCTION

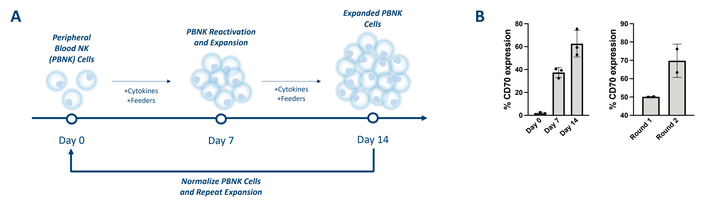
Advances in cellular immunotherapy have led to multiple FDA approvals for autologous CAR-T cell therapies in acute lymphoblastic leukemia (ALL), non-Hodgkin's lymphomas (NHL), and multiple myeloma (MM). While effective, autologous chimeric antigen receptor (CAR)-T cell therapies are limited by safety concerns, lack of scalability for patient derived starting material, and long vein-to-vein timelines. Allogeneic CAR-natural killer (NK) cell therapies have the potential to overcome these limitations by providing an off-the-shelf product capable of delivering clinical benefit without the safety and manufacturing challenges associated with CAR-T therapy. CAR-NK cell therapies are particularly attractive in 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 expression is associated with several malignancies, including AML, NHL, and renal cell carcinoma (RCC), and it is an attractive target for CAR-NK therapy in AML since it is highly expressed on leukemic stem cells and blasts and is absent in normal bone marrow hematopoietic stem cells.[1] While aberrant expression of CD70 is associated with several solid and hematological malignancies, its expression in normal tissue is restricted to certain immune cell types.[2] Here we present preclinical results that highlight the potential for CD70-directed CAR-NK cell therapy using the non-viral TcBuster™ transposon system (Bio-Techne Corp.) in combination with a CRISPR/Cas9 system to engineer human NK cells.

1 CD70 expression across hematological and solid tumor malignancies provides strong rationale for targeting with CAR-NK cell therapy



A) CD70 is a TNF ligand family member and is the sole ligand for the CD27 receptor. Aberrant expression of CD70 is associated with several hematological and solid tumor malignancies. CD70 expression across malignancies is shown from bulk RNAseq data collected from the cBioPortal for Cancer Genomics.[3, 4]
B) UMAP representation of single cell analysis depicting cell composition of acute myeloid leukemia (AML) bone marrow patient samples.[5] AML bone marrow cells are composed of CD34$^+$ blasts, non-AML CD34$^-$/CD38$^+$ cells, and other immune cells.
C) Overlay of CD70 expression on UMAP plot. CD34$^+$ blasts express CD70, whereas CD34$^-$/CD38$^+$ cells lack CD70 expression.
D) Distribution of CD70 expression across various immune cell subsets. CD70 is expressed by B cells, dendritic cells and plasmacytoid dendritic cells.

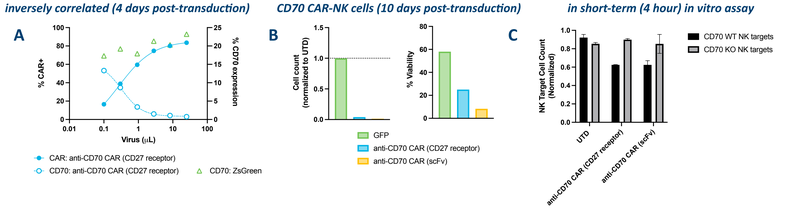
2 CD70 expression is absent in resting peripheral blood NK cells but is upregulated upon activation



A) Peripheral blood NK (PBNK) cells were activated with engineered feeder cells and characterized for CD70 expression by flow cytometry.
B) Prior to activation with engineered feeder cells, CD70 was not detected on PBNK cells. Following feeder cell activation, CD70 expression increased over time and with successive rounds of activation. All data points represent individual donors.

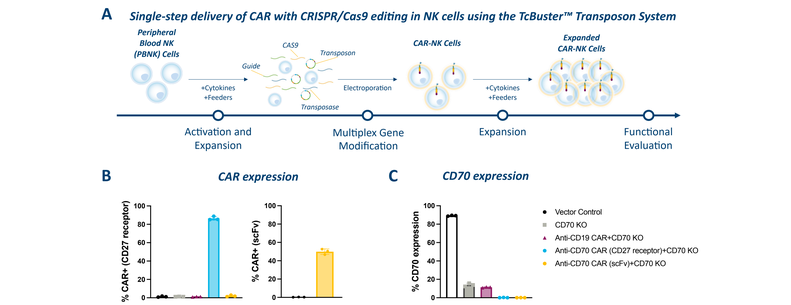
[1] Perna et al. 2017, Cancer Cell. 32:506.
[2] McEarchern et al. 2008, Clin Cancer Res. 14:7763.
[3] Cerami et al. 2012, Cancer Discovery. 2:401.
[4] Gao et al. 2013, Sci. Signal. 6:pl1.
[5] Petti et al. 2019, Nature Comm. 10:3660.

3 CD70 expression drives CD70 CAR-NK-mediated fratricide *in vitro*



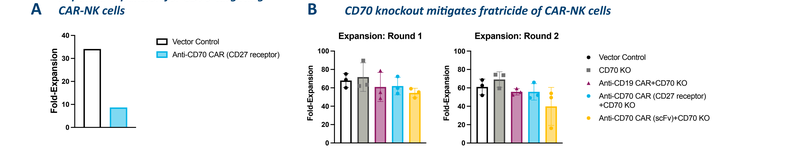
A) PBNK cells were transduced with varying amounts of virus to express either anti-CD70 CAR (CD27 receptor) or zsGreen control. CAR and CD70 expression were evaluated 4 days post-transduction. Increasing expression of CAR correlated with decreasing levels of CD70 for anti-CD70 CAR but not the ZsGreen control.
B) PBNK cells were transduced to express GFP control, anti-CD70 CAR (CD27 receptor), or anti-CD70 CAR (scFv). Viability and cell counts (normalized to untransduced control) were measured 10 days post-transduction. Cell viability and yield were lower for both anti-CD70 CAR-NK cells relative to the GFP control.
C) Wild-type (WT) or CD70 knockout (CD70 KO) PBNK cells were transduced to express anti-CD70 CAR (CD27 receptor), anti-CD70 CAR (scFv), or not transduced (UTD). Untransduced cells were labeled with CellTrace™ Violet dye (Thermo Fisher Scientific). WT or CD70 KO CAR-NK cells (effectors) were co-cultured with either CD70$^+$ or CD70$^-$ UTD NK cells (targets) at a 2:1 E:T ratio. Target cell counts were measured after 4 hours and normalized to no effector control. CD70 CAR-NK cells depleted CD70 WT NK cells but not CD70 KO NK cells.

4 Single-step process enables simultaneous non-viral CAR delivery and CRISPR/Cas9 editing of NK cells



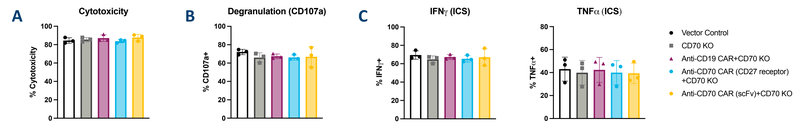
A) PBNK cells were co-cultured with engineered feeder cells and recombinant human IL-2. A mixture of TcBuster transposase-encoding mRNA, transposon plasmid encoding anti-CD70 CAR or control CAR, Cas9-encoding mRNA, and CD70 sgRNA was then added to the feeder cell-activated NK cells for electroporation. Engineered NK cells were then re-activated with feeder cells for two consecutive rounds to expand NK cells. After expansion, NK cells were characterized for CAR and CD70 expression, NK fold-expansion, and *in vitro* functionality.
B) CAR expression was characterized for engineered NK cells following expansion with either anti-CD27 antibody (CD27 receptor detection) or recombinant CD70 protein (scFv detection). Controls included vector control (plasmid without transposase), CD70 KO only, and anti-CD19 CAR with CD70 KO. Efficient delivery of CAR plasmid was confirmed for both anti-CD70 CAR (CD27 receptor) (86%) and anti-CD70 CAR (scFv) (50%) in CD70 KO NK cells. All data points represent individual donors.
C) CD70 expression was characterized in the engineered CD70 KO NK cells. The percentage of CD70$^+$ cells was reduced from 90% in vector control NK cells to less than 15% in CD70 KO and anti-CD19 CAR+CD70 KO controls. CD70 was expressed in less than 1% of anti-CD70 CAR (CD27 receptor) and anti-CD70 CAR (scFv) cells following CD70 KO, and the lower levels of CD70 expression are likely due to residual fratricide. All data points represent individual donors.

5 CD70 knockout restores expansion of CD70 CAR-NK cells



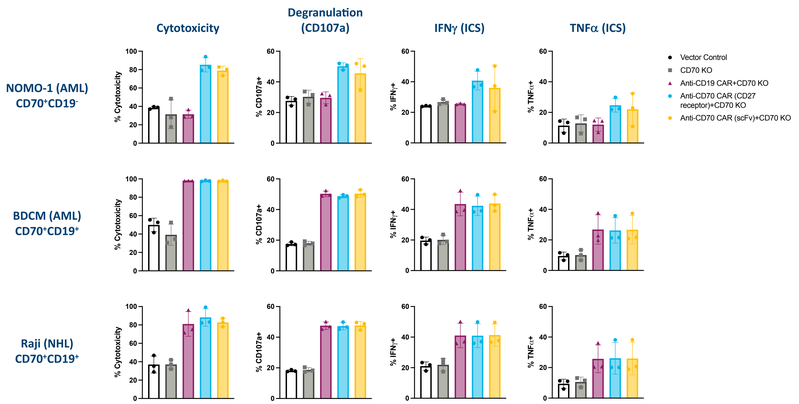
A) NK cells engineered to express anti-CD70 CAR (CD27 receptor) showed reduced fold-expansion levels relative to vector and CD70 KO controls.
B) Both anti-CD70 CARs show comparable fold-expansion levels to vector, CD70 KO, and anti-CD19 CAR+CD70 KO controls. NK cells were normalized to 1x10^6 cells per sample prior to each round of expansion, and fold-expansion was calculated by dividing the cell count following engineered feeder cell expansion by 1x10^6. All data points represent individual donors, and error bars represent standard deviation.

6 CD70 KO CAR-NK cells maintain innate cytotoxicity



Engineered CD70 KO PBNK cells were characterized for endogenous *in vitro* activity against K562 target cells.
A) NK cells were co-cultured with luciferase-expressing K562 target cells at a 1:2 E:T ratio. CD70 KO NK cells were engineered to express either anti-CD70 CAR (CD27 receptor) or anti-CD70 CAR (scFv). Controls included vector control (plasmid without transposase), CD70 KO only, and anti-CD19 CAR with CD70 KO. Target cells without NK effector cells served as a negative control (not shown). Cells were incubated at 37°C for 24 hours, then treated with D-luciferin substrate to measure luminescence signal. Cytotoxicity was calculated relative to the target cell only negative control.
B) Engineered NK cells were co-cultured with luciferase-expressing K562 target cells at a 1:1 E:T ratio, then stained with an anti-CD107a antibody to characterize NK cell degranulation by flow cytometry.
C) For intracellular staining, cells were treated for an additional 4 hours with brefeldin A and monensin, following CD107a staining. Cells were stained with a fixable viability dye and antibodies against NK cell markers. Finally, cells were fixed and permeabilized before staining for intracellular IFNγ and TNFα.
Compared to CD70 WT vector control cells, CD70 KO cells (with or without CAR co-expression) showed similar levels of cytotoxicity and expression of CD107a, IFNγ, and TNFα in response to K562 target cells. All data points represent individual donors, and error bars represent standard deviation.

7 PBNK cells engineered with CD70 CAR and CRISPR/Cas9 KO of CD70 are active against CD70-positive AML and NHL tumor lines



Engineered NK cells were characterized for CAR-mediated *in vitro* activity against CD70-positive AML and non-Hodgkin's lymphoma (NHL) target cells. NK cells were co-cultured with luciferase-expressing NOMO-1 (AML), BDCM (AML), or Raji (Burkitts NHL) target cells. Cytotoxicity, degranulation, and cytokine expression were characterized as described above. Background activity levels were similar for vector control and CD70 KO NK cells across all functional readouts. Anti-CD19 CAR+CD70 KO NK cells maintain specific activity against CD19-positive BDCM and Raji tumor lines but not the CD19-negative NOMO-1 tumor line. Both anti-CD70 CAR+CD70 CARs demonstrated functional *in vitro* activity against CD70-positive tumor lines (NOMO-1, BDCM, and Raji). All data points represent individual donors, and error bars represent standard deviation.

CONCLUSIONS

CD70-targeted cell therapy is strongly supported by its expression across several hematological and solid tumor malignancies and its limited expression in normal tissue.

- Scalable production of CD70-targeted cell therapy is hindered by CD70 expression in a subset of immune cells, including activated NK cells. Introduction of CD70-targeting CAR leads to substantial reduction of NK cell expansion due to fratricide.
- Novel single-step engineering solution for simultaneous, non-viral CAR delivery in combination with CRISPR/Cas9 editing of primary human peripheral blood NK cells resulted in CAR-NK cells resistant to fratricide with *in vitro* CAR-specific functionality against CD70-positive AML and NHL tumor cell lines.
- CAR-NK activity can be further extended using this non-viral transposon system to enable delivery of larger genetic payloads, including elements to improve fitness in the immunosuppressive tumor microenvironment or cytokines such as IL-15 to aid NK cell persistence. Furthermore, the transposon system provides a more streamlined manufacturing process over viral delivery methods.

Overall, these results demonstrate a path to enable CD70-targeted CAR-NK cell therapy for the treatment of AML, RCC, and other CD70-positive malignancies.