CAT-248, an Allogeneic CD70-Directed CAR-NK Cell Therapy, Effectively Controls CD70-Positive Tumor Xenografts

#2898

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ABSTRACT

Engineered, off-the-shelf, allogeneic natural killer (NK) cell therapy is an attractive approach for targeting solid tumors, given their emerging clinical safety, efficacy, and intrinsic anti-tumor recognition and activity. However, improvements to support persistence and maintain durable anti-tumor activity within the tumor microenvironment may be necessary to achieve meaningful clinical efficacy. Here we describe the preclinical activity of CAT-248, a CD70-directed CAR-NK cell therapy, engineered using the TcBuster™ Transposon System (Bio-Techne) multiplexed with CRISPR/Cas9 editing.

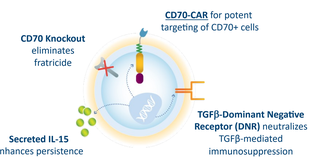
CD70 is highly expressed in many tumor types while normal tissue expression is restricted to a subset of activated immune cells. CAT-248 is an allogeneic, healthy donor peripheral blood-derived NK cell product designed for durable efficacy against CD70 expressing tumors. CAT-248 is engineered to express CD70 CAR, interleukin 15 (IL-15), and transforming growth factor β (TGFβ) dominant negative receptor (DNR). In addition, CAT-248 includes CRISPR/Cas9 knockout of CD70 to mitigate fratricide due to endogenous CD70 expression in activated NK cells. IL-15 enhances persistence of CAT-248 to enable durable efficacy, and TGFβ DNR enables CAT-248 to maintain high activity in TGFβ-enriched and immunosuppressive solid tumor microenvironments. CAT-248 is manufactured using transposon-based engineering which enables stable integration of the three transgenes and CRISPR/Cas9 knockout of CD70 in a single electroporation step, resulting in 40-80% CAR expression and 80-90% knockout of CD70 in CAT-248 NK cells.

CAT-248 activity was characterized across a panel of *in vitro* assays to evaluate the function of CD70 CAR, TGFβ DNR, and IL-15 transgenes. CD70-directed cytotoxicity was assessed against a panel of tumor cell lines with a broad range of CD70 expression. *In vitro*, CAT-248 cells demonstrated both CAR-dependent cytotoxicity and over 2-fold greater secretion of effector cytokines IFNγ and TNFα than control NK cells. TGFβ DNR effectively prevented TGFβ-induced SMAD phosphorylation and TGFβ-induced downregulation of DNAM-1, an NK cell activating receptor. IL-15 secretion enabled *in vitro* NK cell expansion over a 9-day time course without exogenous cytokine support.

To confirm cytolytic activity *in vivo*, CAT-248 cells were administered therapeutically in a 786-O CD70+ renal cell carcinoma xenograft model. CAT-248 cells effectively controlled tumor, demonstrating >98% reduction in tumor burden relative to control NK cells ($p<0.01$). Further, CAT-248 cells demonstrated significant *in vivo* persistence beyond 4 weeks post-dosing in peripheral blood. Overall, the results demonstrate the potential for CAT-248 as a novel off-the-shelf, cryopreserved, allogeneic NK cell therapy for CD70-positive renal cell carcinoma and other solid tumor malignancies.

1 Overview of CAT-248 CAR-NK for CD70-positive solid tumors

CAT-248 is designed to function optimally in solid tumors

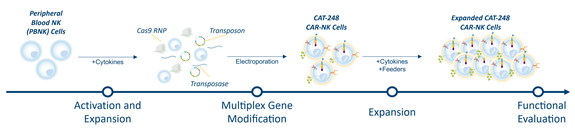


CAT-248 COMPONENTS		
Category	**Specifics**	**Rationale**
Cell Source	Donor-derived peripheral blood NK cells	Allogeneic, ready access, safe
Engineered modifications	CD70-CAR IL-15 TGFβ-DNR CD70 KO	Tumor-directed killing NK cell persistence Evade TGFβ suppression Eliminate fratricide
Engineering method	TcBuster™ transposon system	Highly efficient and consistent across donors
Cell process	Engineered and expanded	High scalable
Formulation	Cryopreserved	Off the shelf

CAT-248 is an off-the-shelf CAR-NK cell therapy engineered for optimal CD70 targeting, protection from TGFβ-mediated immunosuppression, and enhanced NK cell persistence. Peripheral blood NK cells are engineered in a single-step process that enables simultaneous non-viral delivery of a multiplex CAR construct with the TcBuster™ Transposon System (Bio-Techne) and CRISPR/Cas9 editing to knockout CD70 and prevent fratricide.

2 Single-step process enables simultaneous, non-viral delivery of multiplex CAR construct and CRISPR/Cas9 editing of NK cells

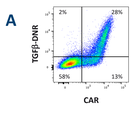
Single-step delivery of multiplex CAR construct with CRISPR/Cas9 editing in NK cells using the TcBuster™ Transposon System



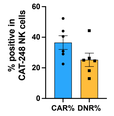
CAT-248 is a peripheral blood NK cell therapy product, engineered in a single-step process for multiplex CAR construct delivery and CRISPR/Cas9 editing. PBNK cells are activated with a mixture of human cytokines prior to genetic modification. A mixture of TcBuster transposase-encoding mRNA, transposon plasmid, Cas9 RNP, and CD70 sgRNA are then added to the activated NK cells for electroporation. The process results in simultaneous delivery of four components, a transposon encoding anti-CD70 CAR, TGFβ-DNR, and IL-15 and CRISPR/Cas9 editing to knockout CD70 and prevent fratricide. Engineered NK cells are expanded with feeder cells to generate the final CAR-NK cell product for functional evaluation.

3 CAT-248 is engineered for CD70 CAR targeting and protection from TGFβ-mediated immunosuppression

CAR and DNR expression

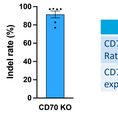


Efficient engineering of CAR and DNR



	Average	SD
CAR % positive	36.5%	11.0%
DNR % positive	25.3%	10.7%

Efficient knockout of CD70

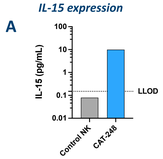


	Average	SD
CD70 Indel Rate (%)	91.5%	9.2%
CD70 protein expression (%)	0.06%	0.02%

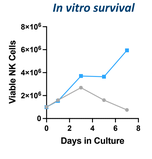
A. Representative flow cytometry profile of CD70 CAR and TGFβ-DNR expression in CAT-248 NK cells. CD70 CAR was detected with recombinant CD70 protein, and TGFβ DNR was detected with an anti-TGFβRII antibody.
B. Efficient expression of CD70 CAR and TGFβ-DNR. CAR expression averaged 36.5% of total NK cells, and TGFβ-DNR expression averaged 25.3% of total NK cells across a panel of 6 independent donors.
C. Efficient knockout of CD70 to prevent NK cell fratricide. Across a panel of 6 independent donors, genomic analysis indicated an average indel rate for CD70 knockout of 91.5%. After NK cell expansion, the percentage of CD70$^+$ cells was reduced to 0.06% of total NK cells by flow cytometry.

4 CAT-248 expresses IL-15 to enable NK cell expansion and persistence

IL-15 expression



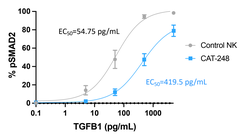
In vitro survival



A. CAT-248 demonstrates enhanced IL-15 production. Cell culture supernatants were collected from CAT-248 or control NK cells cultured for 24-hours, and IL-15 levels were measured by ELISA. IL-15 production was confirmed from CAT-248 cells while only background levels of IL-15 were detected from CD70 KO only control NK cells.
B. CAT-248 cells expand and persist without exogenous cytokine support *in vitro*. Over a 7-day culture without any exogenous cytokines, CAT-248 cells expanded while control NK cell counts decreased.

5 CAT-248 NK cells are protected from TGFβ-induced dysfunction

TGFβ-DNR function: pSMAD2 signaling

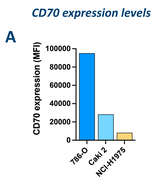


TGFβ1 (pg/mL)	% Reduction in pSMAD2 signal with CAT-248
500	46.9%
50	35.8%
5	12.0%

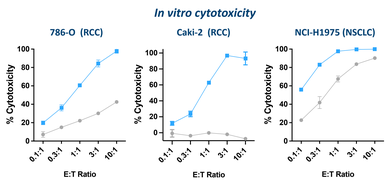
CAT-248 NK cells are protected from TGFβ-induced dysfunction. In results averaged across two independent donors, DNR-engineered CAT-248 cells show reduced levels of phospho-SMAD2, a key downstream mediator of TGFβ suppressive signaling. Engineering efficiency differed across donors (55% CAR$^+$DNR$^+$ for Donor 1, 71% CAR$^+$DNR$^+$ for Donor 2). Despite the presence of unengineered NK cells, the total NK cell population was protected from TGFβ-mediated effects with a 7.7-fold decrease in the pSMAD2 EC_{50} in CAT-248 relative to control NK cells. In the overall population of engineered and non-engineered cells in CAT-248, the percentage of pSMAD2$^+$ cells decreased by 46.9% in the presence of 500 pg/mL TGFβ1 relative to control NK cells.

6 Potent lysis of CD70-positive target cells and release inflammatory cytokines with CAT-248

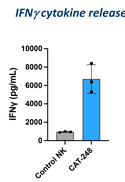
CD70 expression levels



In vitro cytotoxicity



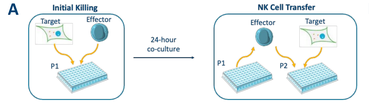
IFNγ cytokine release



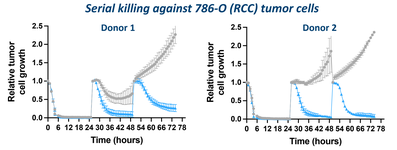
A. Broad range of CD70 expression across tumor cell lines. CD70 expression was evaluated by flow cytometry for a panel of solid tumor cell lines. CD70 expression was highest for 786-O (renal cell carcinoma), intermediate for Caki-2 (renal cell carcinoma), and lowest for NCI-H1975 (non-small cell lung carcinoma).
B. CAR-mediated cytotoxicity against a range of CD70-expressing cell lines. CAT-248 demonstrated potent cytotoxicity against tumor cell lines with varying levels of CD70 expression. Tumor cell lines expressing firefly luciferase were co-cultured with either CAT-248 CAR-NK cells or CD70 KO only control NK cells. Co-cultures were treated with a mixture of neutralizing antibodies against NKG2D, 2B4, DNAM-1, NKp30, and NKp46 to reduce background levels of NK cell cytotoxicity. Luciferase signal was measured after a 24-hour co-culture to measure cytotoxicity.
C. CAR-mediated cytokine release against 786-O target cells. CAT-248 CAR-NK cells or CD70 KO only control NK cells were co-cultured with 786-O tumor cells at an effector:target (E:T) ratio of 3:1 for 24-hours (without any neutralizing antibodies). Cell culture supernatants were collected and analyzed for IFNγ cytokine release by ELISA. Substantially greater levels of IFNγ were detected from CAT-248 CAR-NK samples than control NK cells.

7 Durable activity in a serial killing challenge assay with CAT-248

Design of serial killing challenge assay



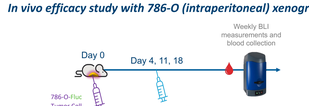
Serial killing against 786-O (RCC) tumor cells



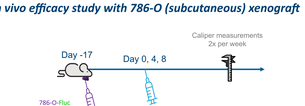
A. Schematic of *in vitro* serial killing challenge assay. For the initial round of cell killing, GFP$^+$ 786-O tumor cells (targets) and NK cells (effectors) were co-cultured at a 5:1 effector:target (E:T) ratio for 24 hours. For each additional round of challenge, effector cells were transferred from the initial assay plate (P1) to an assay plate seeded with fresh tumor cells (P2). A total of three rounds of challenge were performed over a 72-hour time course, imaged every hour in an Incucyte S3 instrument. Relative tumor cell growth measured by GFP signal intensity (CU x mm2) and normalized at the start of each round of target cell challenge (t=0, 24, and 48 hr).
B. CAT-248 CAR-NK cells demonstrated durable activity in a serial killing cytotoxicity assay. In the absence of any NK neutralizing antibodies, both CAT-248 and control NK cells (CD70 KO only) lyse 786-O tumor cells after a single round of challenge at an E:T ratio of 5:1. However, upon subsequent rounds of tumor cell challenge, only CAT-248 cells were able to repeatedly lyse 786-O tumor cells while control NK cells were unable to control tumor cell growth.

8 CAT-248 effectively controls CD70-positive RCC tumor xenografts

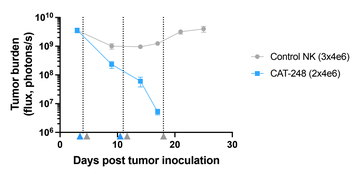
In vivo efficacy study with 786-O (intraperitoneal) xenograft



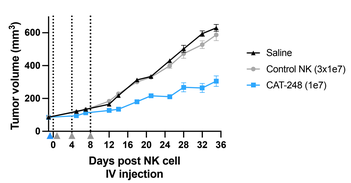
In vivo efficacy study with 786-O (subcutaneous) xenograft



Therapeutic administration of CAT-248 reduces tumor burden of 786-O (IP) xenografts



Therapeutic administration of CAT-248 suppresses growth of 786-O (SC) xenografts



A. 786-O (IP/IP) *in vivo* efficacy model. NSG mice (n=8/group) were intraperitoneally (IP) implanted with 1e6 786-O-Fluc CD70$^+$ renal cell carcinoma tumor cells. Groups were dosed intraperitoneally with either CAT-248 CAR$^+$ NK cells (CD70-373 lead) or control NK cells on Days 4, 11, and 18 after tumor inoculation. Tumor bioluminescence intensity (BLI) signal was recorded weekly to quantify tumor burden. Treatment with CAT-248 (2x 4e6 CAR$^+$ NK cells) resulted in 98% reduction in tumor burden relative to mock engineered NK cells ($p<0.05$, non-parametric t-test).
B. 786-O (SC/IV) *in vivo* efficacy model. NSG mice (n=10/group) were subcutaneously (SC) implanted with 2e6 786-O CD70$^+$ renal cell carcinoma tumor cells. Groups were dosed intravenously with either CAT-248 CAR$^+$ NK cells, control NK cells, or saline on Day 17 after tumor inoculation when tumor volumes had reached 70-100 mm^3. CAT-248 was administered as a single-dose treatment while control NK cells were administered three times, every 4 days. Tumor volumes were recorded twice per week with caliper measurements to quantify tumor burden. Single-dose treatment of CAT-248 significantly suppressed tumor growth ($p<0.05$, non-parametric t-test) compared to both control NK and saline groups.

SUMMARY

CAT-248 is a highly differentiated CAR-NK cell therapy, currently in development for the treatment of CD70-positive solid tumors.

- Demonstration that CAT-248 significantly reduces growth of 786-O renal cell carcinoma xenografts in both intraperitoneal and subcutaneous *in vivo* models
- Use of a novel, single-step engineering solution for simultaneous, non-viral delivery of a CAR, TGFβ dominant-negative receptor (DNR), and secreted IL-15 in combination with CRISPR/Cas9 knockout of CD70 in primary human peripheral blood NK cells to prevent fratricide
- Evidence that the incorporation of a TGFβ DNR provides protection from TGFβ-mediated immunosuppression
- The use of secreted IL-15 leads to enhanced NK cell persistence