# CAT-248 (Engineered NK cells expressing CD70 CAR, IL15, and TGFβ DNR) demonstrates in vivo expansion, tumor infiltration, and durable regression of multiple CD70-expressing xenograft tumors

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## ABSTRACT

Durable and effective therapies are needed for solid tumors despite recent advances with targeted therapies and immunotherapies as many patients relapse or are refractory to these agents. Engineered immune cell therapies can be effective options given their potency, specificity, and durability. However, solid tumors present unique challenges for cell therapies due to heterogenous antigen expression, poor infiltration of immune cells, and an immunosuppressive tumor microenvironment.

CAT-248 is an allogeneic, engineered peripheral blood derived natural killer (NK) cell product with four modifications: (1) a chimeric antigen receptor (CAR) targeting CD70, an antigen frequently overexpressed in many solid tumors as well as hematological malignancies, (2) Interleukin 15 (IL15) to enhance expansion and persistence of engineered NK cells, (3) TGFβ dominant negative receptor (TGFβ DNR) to maintain activity in TGFβ rich tumor microenvironment of solid tumors, and (4) knockout of endogenous CD70 using CRISPR/Cas9 to prevent fratricide by CD70 CAR.

We evaluated *in vitro* and *in vivo* efficacy of CAT-248 in renal cell carcinoma (RCC) and EGFR inhibitor resistant non-small cell lung cancer (NSCLC) models as both these malignancies are associated with high CD70 expression. We tested CAT-248 in the context CD70 high (A498 and 786-O), CD70 medium (Caki-2), and CD70 low NCI-H1975 NSCLC xenografts. CAT-248 was dosed intravenously (IV) to established subcutaneous xenografts in NOD/SCID/IL2Rγ$^{null}$ (NSG) mice, and tumor volumes were quantified over time until control groups reached humane end points.

CAT-248 demonstrated high *in vitro* cytotoxicity (>90% at 10:1 effector: target ratio after 24 hrs, $p < 0.05$) upon co-culture with RCC and NSCLC cell lines expressing CD70 at various levels. Cytotoxicity was dependent on the CD70 CAR expression on CAT-248 NK cells and CD70 (ligand) expression on target tumor cells. Following IV administration in mice with or without implanted tumors, CAT-248 cells rapidly expanded and persisted for over 100 days. Expanding CAT-248 cells led to durable, rapid regression of established A498, NCI-H1975 and Caki-2 xenografts and substantial reduction (>75%, *p<0.05*) in 786-O xenograft volumes relative to control groups dosed with either mock NK cells or saline. Immunohistochemistry of 786-O xenografts two weeks post start of treatment revealed extensive infiltration of CAT-248 NK cells into the tumor and significant reduction of CD70-expressing tumor cells.

CAT-248 infiltrates and durably regresses tumor xenografts expressing CD70 at high, medium or low antigen densities. CAT-248 has the potential to be a potent and durably effective allogeneic NK cell therapy for CD70 expressing solid tumors.

## 1. CAT-248 is an engineered NK cell product optimized for durable efficacy in CD70 expressing solid tumors

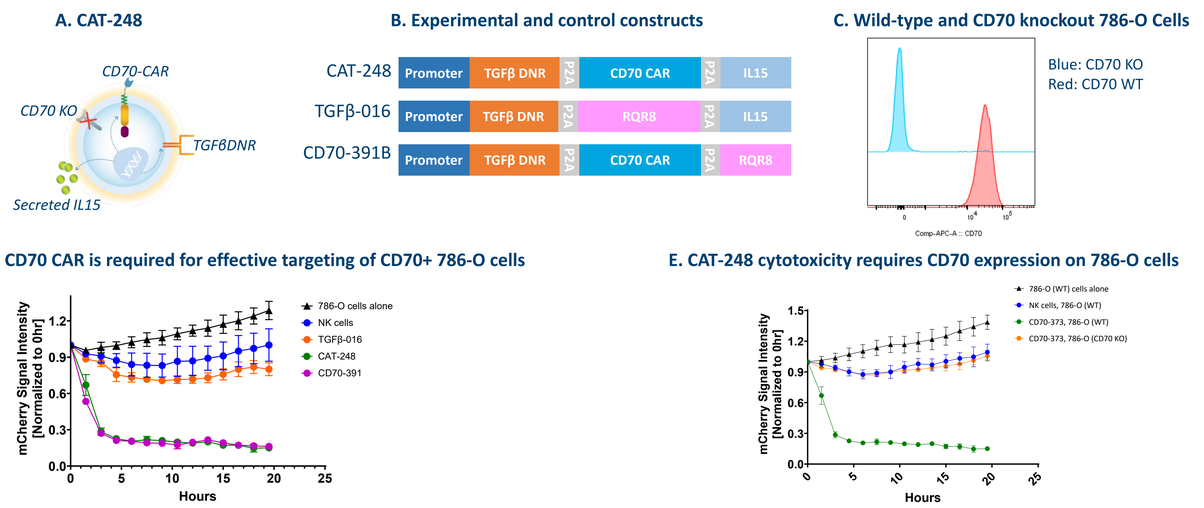



Fig 1. (A) CAT-248 is a donor derived peripheral blood natural killer cell product engineered to express CD70 CAR, TGFβ DNR and IL15 using TcBuster Transposon system with CD70 locus knocked out using Cas9. (B) CAT-248 construct consists of TGFβ DNR, CD70-CAR and IL15 expressed as a single transcript under the control of MND promoter with P2A ribosomal skipping sequences. TGFβ-016 control construct expresses TGFβ DNR and IL15 while CD70-391B control construct expresses TGFβ DNR and CD70-CAR. (C) CD70 locus in 786-O cell line was knocked out using Cas9 and the knockout cells (blue) express significantly less CD70 that wild-type 786-O cells (Red) on cell surface. CD70 was quantified using FACS. (D) CD70 CAR is highly cytotoxic towards 786-O cells. CAT-248 or control NK cells were incubated with mCherry expressing-786-O cells at 10:1 ratio. Incucyte was used to quantify mCherry signal and the signal at times indicated was normalized to signal at the start of the incubation period. (E) CAT-248 is significantly less cytotoxic towards 786-O cells knocked out for CD70. Control NK cells or CAT-248 cells were incubated with CD70 knockout 786-O cells at 10:1 ratio and cytotoxicity was quantified as described in (D)

## 2. CAT-248 effectively targets tumor cells with low, medium or high CD70 expression

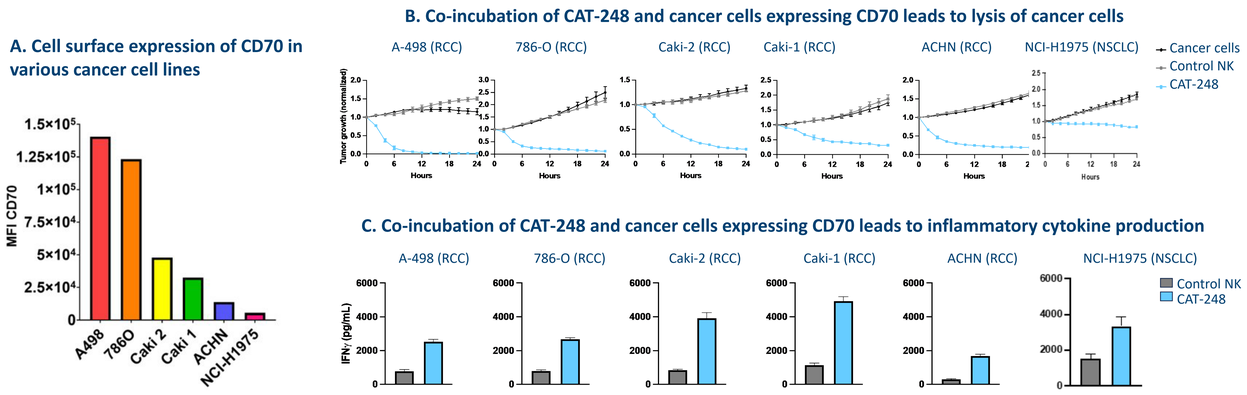



Fig 2. (A) Expression of CD70 on various renal cell carcinoma (RCC) or Non-small cell lung cancer (NSCLC) cell lines was quantified using flow cytometry. (B) CAT-248 or control NK cells were co-cultured with various cancer cell lines expressing a range of CD70 levels at an E:T ratio of 10:1 (NK cells:Target cells). Cytotoxicity was quantified using IncuCyte as described in Fig. 1D. (C) CAT-248 (blue) or Control NK (grey) cells were co-cultured with various CD70 expressing cell lines for 24 hours. Following the co-culture, IFNγ in supernatant was quantified using MesoScaleDiscovery (MSD) assay and cytokine levels were determined from standard curve.

## 3. CAT-248 expands and persists in mice and persisting cells continue to demonstrate anti-tumor activity

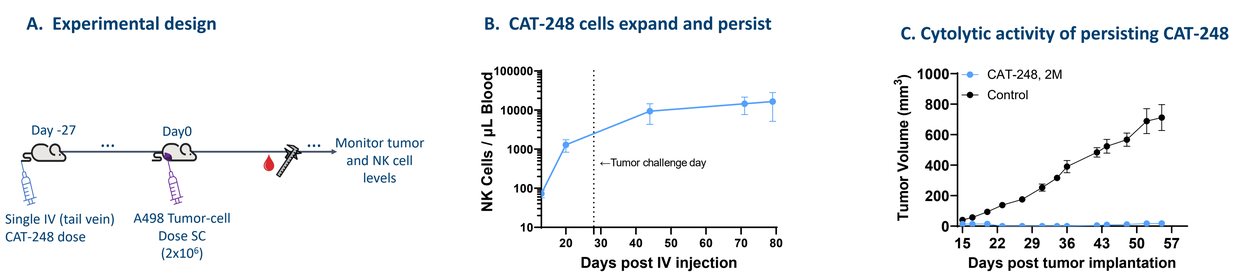



Fig 3. (A) A single intravenous dose of 2M CAT-248 cells was administered to NSG mice. 27 days later, 2M A498 cancer cells were injected into left flank of female NSG mice. Tumor volumes and circulating NK cells were quantified at the times indicated. (B) CAT-248 NK cells expand and persist in NSG mice. Circulating NK cells were quantified at the indicated times by measuring live human CD45+ CD56+ cells using flow cytometry. (B) Persisting NK cells continue to be functional and maintain their cytolytic function by prevent the outgrowth of the subcutaneously injected A498 xenograft tumors

## 4. CAT-248 regresses tumor xenografts expressing high, medium or low levels of CD70

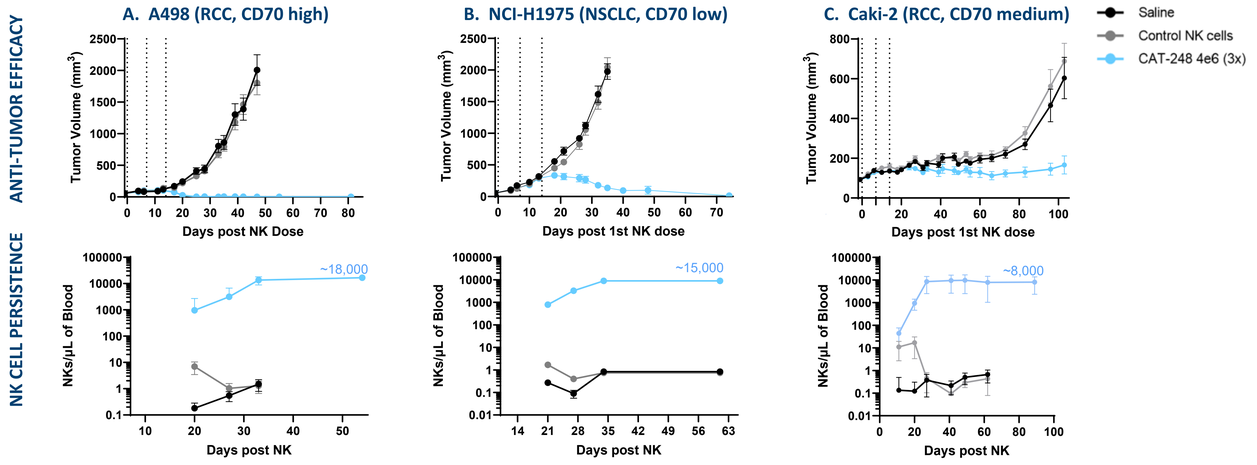



Fig 4. In vivo efficacy of CAT-248 was tested in three RCC or NSCLC xenograft models expressing different levels of CD70. (A) CAT-248 durably regresses A498 RCC subcutaneous xenografts. A498 (2M) cells were implanted subcutaneously into left flank of female NSG mice (n=10/group). 14 days post implantation, average tumor volume reached ~70-100 mm$^3$ and mice were administered an intravenous dose of saline, 4M CAT-248 or control NK cells. Mice were given two additional intravenous doses of NK cells 7 and 14 days after the initial dose. Tumor volumes were measured on days indicated (top panel). Mouse blood was sampled for circulating NK cells on days indicated (bottom panel). (B) CAT-248 durably regresses NCI-H1975 NSCLC subcutaneous xenografts. Experimental design was identical to that described in Fig. 3(A) except NCI-H1975 cells were implanted. (C) CAT-248 controls Caki-2 RCC subcutaneous xenografts. Experimental design was identical to that described in Fig. 3(A) except Caki-2 cells were implanted in the right flank.

## 5. CAT-248 infiltrates tumor xenografts and tumor associated NK cells are phenotypically distinct

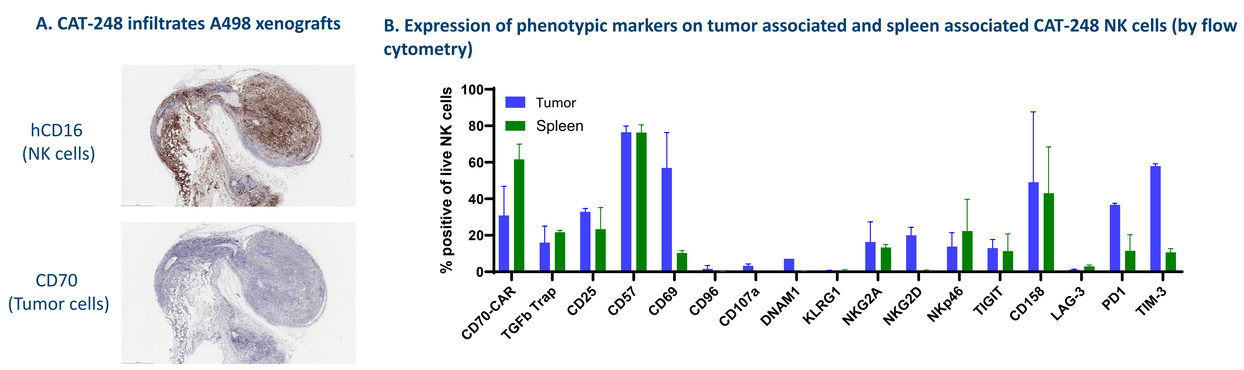
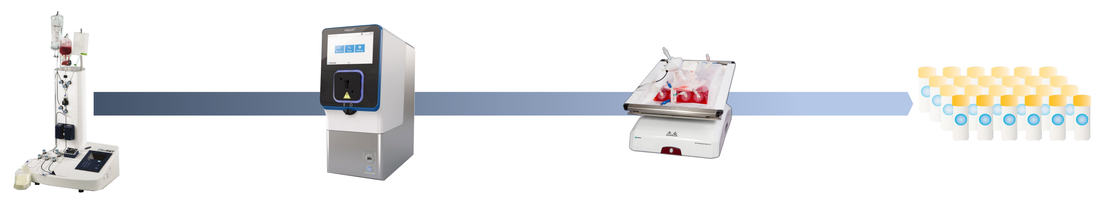



Fig 5. (A) CAT-248 infiltrates A498 xenografts and eliminates CD70 expressing tumor cells. NSG mice bearing A498 xenografts were treated with intravenous dose of 10M CAR$^+$ NK cells. Tumors were harvested 17 days post NK cell administration, sectioned and immuno-stained for human CD16 (top panel) or CD70 (bottom panel). Extensive infiltration of tumor and surrounding stroma with CD16+ NK cells is observed (brown staining) while CD70 signal from tumor cells is not detectable above background. (B) Tumor- and spleen-associated CAT-248 cells are phenotypically distinct when profiled by flow cytometry from tissues harvested ~30 days post a single IV dose of 1 million CAR+ NK cells (AVG±SD)

## 6. Clinical scale manufacturing of CAT-248



| NK Cell Isolation and Activation | Transposon Cell Modification | CAR-NK Cell Expansion | Fill/Finish/Cryopreservation |
|---|---|---|---|
| **Day 0-5** | **Day 5** | **Day 5-Day 15** | **Day 15** |
| NK cells/Leukopak: 5±2.3E8<br>Purity: 92.8±5.1% (CD56$^+$) | CAR$^+$: 50% +/- 17%<br>CD70$^+$ <0.6% | Fold-expansion: 1116±798<br>Projected Yield/Leukopak: 5.6E11 | Viability: 94-98%<br>Purity (CD56$^+$) > 95%<br>CD3$^+$ < 0.07% |

Fig 6. Manufacturing process for CAT-248 is based on non-viral engineering with TcBuster transposon system and expansion using engineered K562 (eK562) feeder cell line. Peripheral blood Natural Killer cells from healthy donor leukopaks are isolated by CD56 positive selection and CD3 depletion using CliniMACS system. NK cells are then activated with a cytokine cocktail and electroporated using Maxcyte ExPERT system. Electroporated cells are then mixed with irradiated eK562 feeder cells and expanded for 10 days in Xuri bioreactor system. Expanded NK cells are harvested and cryopreserved.

## SUMMARY AND CONCLUSIONS

1. CAT-248 is a healthy donor derived peripheral blood NK cell product engineered to express a CD70 CAR, Interleukin 15, and TGFβ dominant negative receptor, and knocked out at CD70 locus.
2. In vitro, CAT-248 is cytotoxic towards various cancer cell lines expressing low, medium or high levels of CD70 cell surface. CAT-248 activity requires CD70 CAR as well as expression of CD70 on cancer cells.
3. Intravenous dosed CAT-248 expands and persists in NSG mice and persisting CAT-248 cells continue to demonstrate anti-tumor activity.
4. Intravenous dosed CAT-248 durably regresses subcutaneous xenografts of CD70 low, medium or high RCC or NSCLC cell lines
5. CAT-248 effectively infiltrates subcutaneous xenografts and eliminates CD70 expressing cancer cells. Tumor associated CAT-248 is phenotypically distinct from spleen associated CAT-248.
6. CAT-248 is engineered using non-viral TcBuster Transposon technology and a clinical scale 15-day manufacturing process generates hundreds of doses of active cryopreserved drug product
7. CAT-248 will be clinically evaluated in CD70 expressing solid tumors such as RCC and NSCLC

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