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

**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 heterogeneous 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 hematologic 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 NSCLC in a relevant non-small cell lung cancer (NSCLC) xenograft models as both these malignancies are associated with high CD70 expression. We tested CAT-248 in the context of RCC (A498 and 786-O), C70 medium (Caki-2), and CD70 low NCI-H1975 NSCLC xenografts. CAT-248 was dosed intravenously (i.v.) to establish subcutaneous xenografts in NSG/NSGCD2F1m<sup>−/−</sup> (NSG) mice, and tumor volumes were quantified over time until control groups reached humane endpoints.

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 (lgand) 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 regeneration 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 tumor expressing expressing tumor microenvironment.

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 durable 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**

   **A. CAT-248**

   **B. Experimental and control constructs**

   **C. Wild-type and CD70 knockout 786-O Cells**

   **D. CD70 CAR is required for effective targeting of 786-O cells**

   **E. CAT-248 cytotoxicity requires CD70 expression on 786-O cells**

2. **CAT-248 effectively targets tumors with low, medium or high CD70 expression**

   **A. Cell surface expression of CD70 in various cancer cell lines**

   **B. Co-incubation of CAT-248 and cancer cells expressing CD70 leads to lysis of cancer cells**

   **C. Co-incubation of CAT-248 and cancer cells expressing CD70 leads to inflammatory cytokine production**

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

   **A. Experimental design**

   **B. CAT-248 cells expand and persist**

   **C. Cytolytic activity of persisting CAT-248**

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

   **A. A498 (RCC, CD70 high)**

   **B. NCI-H1975 (NSCLC, CD70 low)**

   **C. Caki-2 (RCC, CD70 medium)**

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

   **A. CAT-248 infiltrates A498 xenografts**

   **B. Expression of phenotypic markers on tumor associated and spleen associated CAT-248 NK cells (by flow cytometry)**

6. **Clinical scale manufacturing of CAT-248**

   **A. NK Cell Isolation and Activation**

   **B. Transposon Cell Modification**

   **C. CAR-NK Cell Expansion**

   **D. Fill/Finish/Cryopreservation**

7. **SUMMARY AND CONCLUSIONS**

   1. CAT-248 is a healthy donor derived peripheral blood natural killer cell product engineered to express a 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 MNDR promoter with 3’A ribosomal slipping sequences. TGFβ-3’A control construct expresses TGFβ DNR and IL15 while CD70-391B control constructs express 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 than 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 incubated with CD70 knocking-out-786-O cells at 10:1 ratio and cytotoxicity was quantified as described in (D).

   2. The experimental and control constructs were transduced into K562 cells using the TcBuster Transposon system. K562 cells were sorted on a FACS and the purified population was cultured for 15 days in cytokine rich expansion media before cryopreservation. The CAR-NK cell line was generated by transducing TcBuster Transposon engineered K562 cells with the CAR construct. The CAR construct was comprised of a P2A promoter, a hCD16β subunit, and a hCD152β subunit. CAR transductions were performed using P2A promoter engineered K562 cells. The CAR transduction efficiency was >90% as measured by flow cytometry. The CAR-NK cell line was expanded using cytokine-rich expansion media before cryopreservation. The CAR-NK cell line was expanded using cytokine-rich expansion media before cryopreservation. The CAR-NK cell line was expanded using cytokine-rich expansion media before cryopreservation.

   3. Purity: 92.8±5.1% (CD56+), Fold-expansion: 1116±798, Viability: 94-98%

   4. The CAR-NK cell line was assayed for transduction efficiency, expression of CD16 and CD70 by flow cytometry. The CAR-NK cell line was cryopreserved.

   5. The CAR-NK cell line was assayed for transduction efficiency, expression of CD16 and CD70 by flow cytometry. The CAR-NK cell line was cryopreserved.

   6. The CAR-NK cell line was assayed for transduction efficiency, expression of CD16 and CD70 by flow cytometry. The CAR-NK cell line was cryopreserved.

   7. The CAR-NK cell line was assayed for transduction efficiency, expression of CD16 and CD70 by flow cytometry. The CAR-NK cell line was cryopreserved.

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