Allogeneic natural killer cells engineered to express HER2 CAR, interleukin 15, and TGF-beta dominant negative receptor effectively control HER2⁺ tumors

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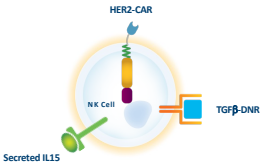
INTRODUCTION

Despite the success of HER2-targeted therapies in HER2+ breast and gastric cancer, additional therapies are needed to address treatment-resistant metastatic disease. Adoptive immune cell therapy is a promising therapeutic modality given the remarkable clinical responses seen with autologous chimeric antigen receptor (CAR) T cells in hematological malignancies. However, success of cell therapy in solid tumors has been more limited. Three major impediments to the success of adoptive cell therapies in solid tumors are the heterogeneity of antigen expression, the immune suppressive tumor microenvironment (TME), and the inherent challenges of manufacturing autologous cells and consequent variability of these cell products. Engineered, off-the-shelf, allogeneic Natural Killer (NK) cells provide a solution to these challenges. We describe here CAT-179, a novel transposon engineered CAR-NK cell therapy for HER2+ solid tumors. CAT-179 cells express three transgenes: a HER2-directed CAR to effectively eliminate tumor cells, a transforming growth factor β (TGFβ) dominant negative receptor (DNR) for resistance to TGFβ-mediated immune suppression in the TME, and interleukin 15 (IL15) cytokine to enhance NK cell persistence and activity for durable response. CAT-179 addresses key hurdles to allogeneic cell therapy for solid tumors and is a promising new therapeutic approach for HER2 expressing breast, gastric and other tumors.

1 Overview of CAT-179 for HER2⁺ breast and gastric cancer



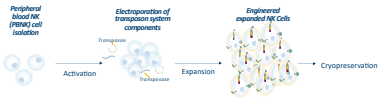
CAT-179 COMPONENTS		
Category	**Specifics**	**Rationale**
Cell Source	Donor-derived peripheral blood NK cells	Allogeneic, ready access
Engineered modifications	HER2-CAR IL15 TGFβ-DNR	Tumor-directed killing NK cell persistence Evade TME suppression
Engineering method	TcBuster™ transposon system	Large cargo compatible
Cell process	Engineered and expanded	High yield
Formulation	Cryopreserved	Off the shelf

CAT-179 is a cryopreserved off-the-shelf allogeneic engineered NK cell. It is derived from peripheral blood NK cells that are engineered using the TcBuster™ transposon system (Bio-Techne). CAT-179 is engineered to express a HER2-targeting CAR for specific targeting to HER2 positive tumors, a secreted IL15 to enable NK cell persistence, and a TGFβ-dominant negative receptor (DNR) to provide resistance to the suppressive effects of TGFβ in the tumor microenvironment.

2 High efficiency engineering of CAT-179 NK cells with CAR, DNR, and IL15 is enabled by the non-viral TcBuster™ transposon system

A. Engineering process

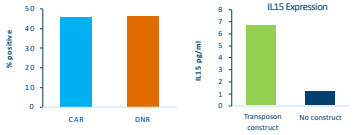
- Consistently high efficiency w/multiple donors (40-80%)
- Hundreds of doses from a single leukopak
- Product retains high viability (range 74%-97%; mean 85%) and activity post-thaw



B. Construct Schematic

Transposon Construct

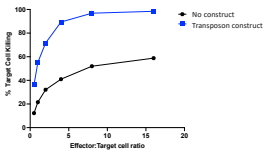
C. Expression of Construct Components



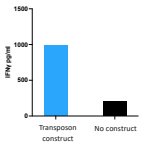
A) NK cell engineering: NK cells were isolated from peripheral blood and activated. A mixture of TcBuster™ (Bio-Techne) transposase-encoding mRNA and transposon plasmid encoding the transposon construct was then added to the activated NK cells for electroporation. Engineered NK cells were then expanded with feeder cells and cryopreserved.
B) Schematic of the transgene elements, separated by 2A sequences, and their relative positions within the transposon plasmid. TGFβ-DNR structure is described in Wieser et al. 1993.
C) Engineered expanded NK cells were characterized for CAR, TGFβ-DNR, and IL15 expression 14 days after electroporation. CAR and DNR expression were determined by flow cytometry. IL15 expression was quantified by Meso Scale Discovery (MSD) ELISA by plating 160,000 NK cells with SKOV3 tumor cells and recovering supernatants after 24 hours in culture.

3 CAT-179 CAR-NK cells kill HER2⁺ SKOV3 cells *in vitro* and produce interferon gamma

A. Cytotoxicity



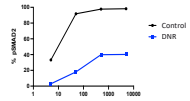
B. Cytokine production



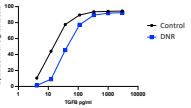
A) CAT-179 cells engineered with the transposon construct show CAR-driven cytotoxic activity. Cells were tested for *in vitro* cytotoxicity activity by co-culturing with SKOV3-fluc tumor cells (SKOV3 cells engineered to express firefly luciferase) for 24 hours at the indicated E:T ratios in the presence of antibodies that block the endogenous NK activating receptors (2B4, NKp30, NKp46, NKG2D, and DNAM-1) in order to determine the degree of CAR-specific killing without background from innate cell killing.
B) CAT-179 cells produce interferon gamma (IFNγ) in response to target cells. Supernatant from the SKOV3-fluc cytotoxicity assay (in panel A) was collected and IFNγ levels were determined using a human cytokine MSD ECL assay kit. Data shown is from an E:T ratio of 8:1.

4 TGFβ-DNR in CAT-179 protects NK cells from TGFβ-mediated immuno-suppression by dual DNR and trap mechanisms

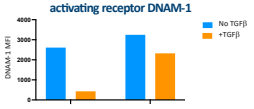
A. DNR protects engineered NK cells from TGFβ induced signaling



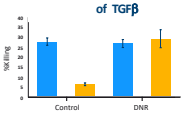
B. DNR protects neighboring non-engineered NK cells from TGFβ induced signaling (trap)



C. DNR protects against downregulation of NK activating receptor DNAM-1



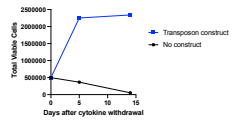
D. DNR restores NK cytotoxicity in the presence of TGFβ



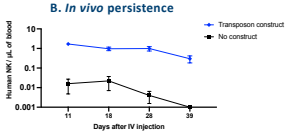
A) DNR engineered or mock control engineered NK cells were treated with recombinant TGFβ1 for 1hr and then fixed, permeabilized, stained for phosphorylated SMAD2 (pSMAD) and analyzed by flow cytometry. % of pSMAD2 positive cells was determined by gating on engineered NK cells.
B) DNR engineered or mock control engineered NK cells were treated with recombinant TGFβ1 for 1hr and then fixed, permeabilized, and stained for phosphorylated SMAD2 (pSMAD) and analyzed by flow cytometry. % of pSMAD2 positive cells was determined by gating on non-engineered NK cells.
C) DNR engineered or mock control engineered were treated with or without 10 ng/ml TGFβ for 5 days. Cells were then stained for DNAM-1 and analyzed by flow cytometry by gating on the engineered population. MFI= mean fluorescence intensity.
D) DNR engineered or mock control engineered NK cells were pre-treated with 5 ng/ml TGFβ for 5 days then co-cultured for 3 hours with K562-luciferase-tagged tumor cells to evaluate innate NK mediated cell killing either in the presence of TGFβ (orange) or the absence of TGFβ (blue).

5 Engineered IL15 supports CAT-179 cell survival *in vitro* and persistence *in vivo*

A. *In vitro* survival



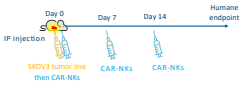
B. *In vivo* persistence



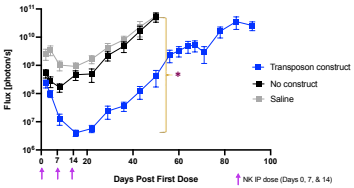
A) To evaluate the function of engineered IL15, NK cells engineered with the transposon construct were compared with mock engineered cells (no construct) for their ability to survive *in vitro* in the absence of exogenous cytokine support. Cells were cultured for 14 days in the absence of exogenous cytokine and NK cells were counted over time.
B) To evaluate the impact of engineered IL15 on persistence of NK cells *in vivo*, 4 million CAR⁺ cells (12 million total cells) engineered with the transposon construct or mock engineered cells (no construct) were injected intravenously (IV) in NSG mice (n=5) and NK cells counts were monitored over time in blood samples by flow cytometry (staining for hCD45⁺, hCD56⁺ cells).

6 CAT-179 cells are efficacious *in vivo* against HER2⁺ tumor and enable prolonged survival of mice

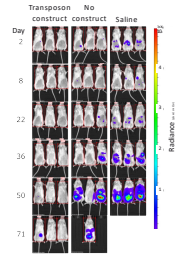
A. Study design



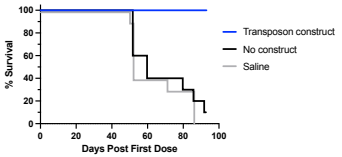
B. Tumor signal quantitation



C. Tumor images



D. Mouse survival



A) Schematic of *in vivo* efficacy study design. NSG mice (n=10) were intraperitoneally (IP) injected with 1x10⁶ SKOV3-luciferase tagged (SKOV3-fluc) HER2⁺ ovarian cancer tumor cells. 4 million CAR⁺ NK cells engineered with the transposon construct (12 million total NK cells), 12 million mock engineered cells, or saline were dosed IP after tumor cell injection on day 0 and again on days 7 and 14.
B) SKOV3-fluc mice were imaged (using an IVIS *in vivo* imaging system) at the indicated time points to quantify tumor burden. Luciferase signal is reported as flux (photons/second). *p<0.05 using non-parametric t-test at individual time points between transposon construct and both controls. Flux signal in control arms were not included in the analysis after >/=40% of the mice in the group died or were euthanized due to tumor burden.
C) Representative bioluminescence images of tumor in mice
D) Kaplan-Meyer analysis demonstrates significant (p<0.0001) prolonged survival of mice dosed with transposon construct engineered NK cells in the *in vivo* efficacy study relative to control arms.

SUMMARY AND CONCLUSIONS

We describe here the evaluation of CAT-179, a novel engineered NK cell therapy expressing HER2-CAR, TGFβ-DNR, and IL15.

- We have demonstrated high efficiency engineering of the large (~4Kb) cargo containing CAR, IL15, and DNR in CAT-179 using the non-viral TcBuster™ transposon system (Bio-Techne). Transposon engineering of CAT-179 results in stable expression of CAR (45% CAR at day 14 post gene delivery) without the need for post-engineering selection.
- CAT-179 demonstrates HER2-CAR-driven interferon gamma production and tumor cell killing *in vitro* when co-cultured with HER2⁺ tumor cells.
- The TGFβ-DNR in CAT-179 demonstrates resistance to TGFβ mediated immunosuppression, as evidenced by reduction in TGFβ-induced phosphorylation of SMAD2 in both engineered cells and non-engineered NK cells, as well as prevention of TGFβ-induced downregulation of NK cell activating receptor DNAM-1 and restoration of NK cell cytotoxic activity. These data suggest CAT-179 cells will be protected from TGFβ-mediated immune suppression in the TME and can protect neighboring cells.
- The engineering of IL15 in CAT-179 significantly enhances survival *in vitro* without the need for exogenous cytokines. Moreover, CAT-179 cells show enhanced persistence *in vivo* over non-engineered cells up to 40 days in NSG mice.
- Finally, CAT-179 show potent anti-tumor activity *in vivo* against the xenografted HER2+ SKOV3-fluc ovarian cancer cell line and lead to a significant survival benefit in tumor bearing mice.

CAT-179 addresses key hurdles to allogeneic cell therapy for solid tumors and is a promising new therapeutic approach for HER2-expressing breast, gastric and other tumors.