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

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ABSTRACT

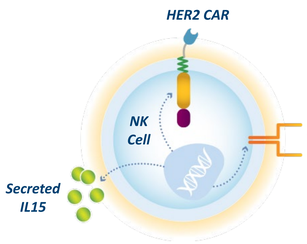
Background: The remarkable clinical responses of chimeric antigen receptor (CAR)-engineered immune cell therapies in hematological malignancies have not been replicated in solid tumors. Engineered, off-the-shelf, allogeneic natural killer (NK) cells are particularly attractive as a chassis for effective cell therapies for solid tumors given their clinical safety, efficacy, and ability to reduce tumor escape through inherent multimodal recognition of tumor cells.
We describe here preclinical efficacy and pharmacodynamics of CAT-179, a novel CAR-NK cell therapy, in multiple models of HER2-amplified ovarian and gastric cancer. CAT-179 cells are engineered to express three transgenes: a HER2-directed CAR to effectively target tumor cells, a transforming growth factor β (TGFβ) dominant negative receptor (DNR) for resistance to TGFβ-mediated immune suppression in the tumor microenvironment, and interleukin-15 (IL15) to enhance NK cell persistence and activity for durable response.

Methods: PBMC-derived NK cells were engineered with a tricistronic construct expressing HER2-directed CAR, TGFβ DNR, and IL15 under the control of a MND promoter using TcBuster™ transposase. CAT-179 activity was assessed *in vitro* by quantifying cytotoxicity and cytokine production upon co-culture with HER2-expressing cell lines. TGFβ DNR activity was assayed by quantifying TGFβ–induced SMAD phosphorylation and DNAM1 receptor expression. *In vivo* persistence and anti-tumor efficacy was evaluated in NSG mice. Anti-tumor efficacy was tested against luciferase-engineered SKOV-3 ovarian cancer cells (SKOV-3-luc) and N87 gastric carcinoma xenografts.

Results: CAT-179 demonstrates both CAR-dependent and innate NK receptor-dependent tumor cell killing *in vitro*, reducing the likelihood of tumor escape through antigen loss. CAT-179 demonstrated high CAR-dependent cytotoxicity as well as TNFα and IFNγ production when co-incubated with multiple HER2-expressing cell lines. Engineered NK cells demonstrated 75% reduction (relative to control NK cells) in TGFβ–induced SMAD2 phosphorylation, prevented TGFβ–induced downregulation of NK cell activating receptors, and restored NK cell cytotoxic activity. Furthermore, TGFβ DNR protected bystander cells from TGFβ–induced phenotypic changes. After a single IV dose, CAT-179 cells persisted for more than two months and retained cytotoxic activity. CAT-179 effectively reduced SKOV-3-luc tumor burden in NSG mice (95% AUC, p<0.0001 for survival).

Conclusions: CAT-179 is a promising demonstration of the Catamaran CAR-NK platform, as a novel off-the-shelf cell therapy to overcome the challenges associated with solid tumors.

1 Overview of CAT-179 for HER2$^+$ tumors

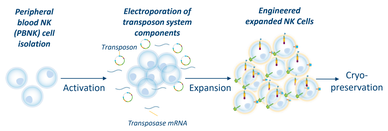


CAT-179 COMPONENTS		
Category	**Specifics**	**Rationale**
Cell Source	Donor-derived peripheral blood NK cells	Allogeneic, ready access
Engineered modifications	HER2-CAR IL15 TGFβ-DNR	Tumor-directed killing NK cell persistence Evade TME suppression
Engineering method	TcBuster™ transposon system	Large 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. HER2+ breast and gastric cancers are associated with TGFβ TME.

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

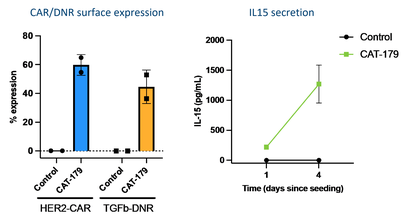


- Consistently high efficiency w/multiple donors (40-80%)
- Hundreds of doses from a single leukopak
- Product retains high viability (range 74%-97%; mean 85%) and activity post-thaw

B. Construct Schematic

Transposon Construct

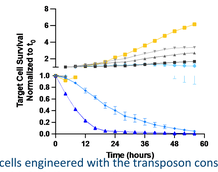
C. Expression of Construct Components



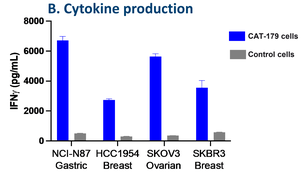
A) NK cell engineering: NK cells were isolated from peripheral blood and activated. A mixture of TcBuster™ (Bio-Techne) transposase-encoding mRNA and transposon plasmid encoding the transposon construct was then added to the activated NK cells for electroporation. Engineered NK cells were then expanded 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 10 days after electroporation. Control cells were mock engineered without a construct, CAT-179 cells were engineered with the construct shown in panel B. 2 donors are shown, AVG+/-SEM. CAR and DNR expression were determined by flow cytometry. IL15 secretion was quantified by Meso Scale Discovery (MSD) ELISA by plating 1E6 NK cells with IL2 and recovering supernatants after 1 day or 4 days in culture.

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

A. Cytotoxicity



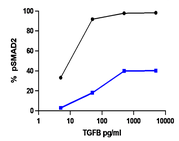
B. Cytokine production



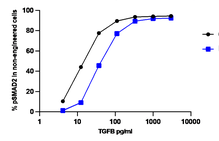
A. CAT-179 cells engineered with the transposon construct show CAR-driven cytotoxic activity. Cells were tested for *in vitro* cytotoxicity activity by co-culturing with SKOV3 tumor cells (engineered to express GFP) for time shown at the indicated effector:target (E:T) ratios. Conditions were tested in technical triplicates. Viable tumor cells were imaged every four hours in an Incucyte S3 instrument, quantified, and normalized to the start of the assay (t=0). NCI-N87, HCC1954, and SKBR3 cell lines were run in similar studies not shown.
B. CAT-179 cells produce interferon gamma (IFNγ) in response to target cells. Supernatant from the 24-hour timepoint of the cytotoxicity assay (in panel A) was collected and IFNγ levels were determined using the V-plex Proinflammatory Panel 1 Human Kit from MSD. Data shown is from an E:T ratio of 2.5:1

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

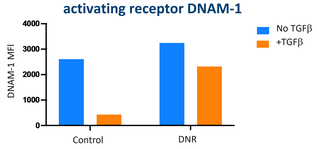
A. DNR protects engineered NK cells from TGFβ induced signaling



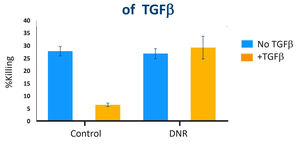
B. DNR protects neighboring non-engineered NK cells from TGFβ induced signaling (trap)



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



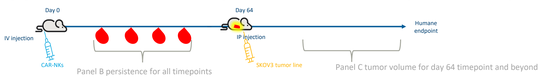
D. DNR restores NK cytotoxicity in the presence of TGFβ



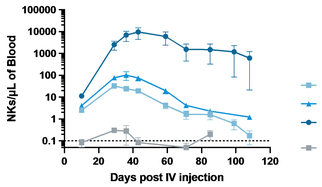
A. DNR engineered or mock control engineered NK cells were treated with recombinant TGFβ1 for 1hr and then fixed, permeabilized, stained for phosphorylated SMAD2 (pSMAD) and analyzed by flow cytometry. % of pSMAD2 positive cells was determined by gating on engineered NK cells.
B. DNR engineered or mock 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 persistence and cytotoxicity *in vivo*

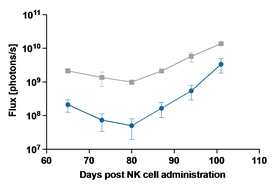
A. Study design



B. *In vivo* persistence



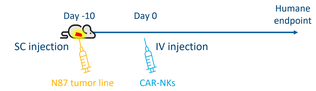
C. *In vivo* efficacy of long-lasting engineered NK cells



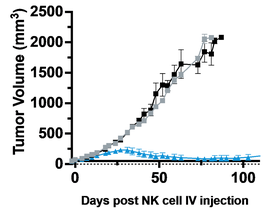
A. Schematic of long-term functional assessment of engineered NK cells for persistence and efficacy.
B. To evaluate the impact of engineered IL15 on persistence of NK cells *in vivo*, 2E6, 4E6 or 8E6 CAR$^+$ (6.5E6, 13E6, or 26E6 total cells respectively) cells engineered with the transposon construct or mock engineered cells (EP only) 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). AVG+/-SEM
C. Persistent engineered NK cells retained cytotoxicity, as shown by challenging the animals in the persistence study (panel B) with SKOV3-fluc tumor cells intraperitoneally on day 64. No cells were detected in the EP only group, similar to vehicle control. 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). AVG+/-SEM

6 CAT-179 cells are efficacious *in vivo* against HER2$^+$ tumor xenografts and prolong survival of xenografted mice

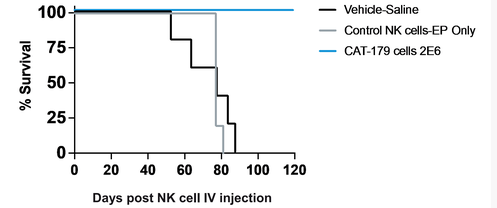
A. Study design



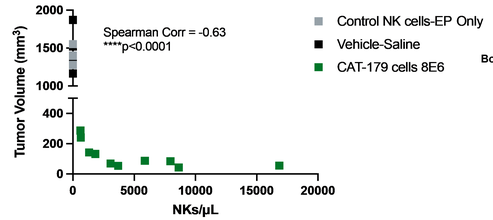
B. Tumor signal quantitation



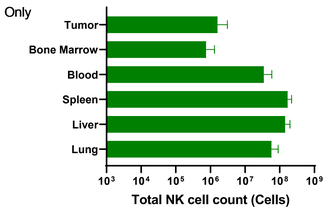
C. Mouse survival



D. Correlation of NK cells and tumor volume



E. NK cell biodistribution



A. Schematic of *in vivo* efficacy study design. NSG mice (n=10) were subcutaneously (SC) injected with 1x10^6 N87 HER2$^+$ gastric cancer tumor cells. 2-8 million CAR$^+$ NK cells engineered with the transposon construct, 20 million mock engineered cells, or saline were dosed intravenously after tumor cell injection on day 10.
B. Tumor was palpated at the indicated time points to quantify tumor burden. CAT-179 cells (at 2E6 dose) showed significantly lower tumor burden (p<0.0001, non-parametric t-test) compared to either control.
C. Kaplan-Meyer analysis demonstrates significant (p<0.0001) prolonged survival of mice dosed with CAT-179 cells in the *in vivo* efficacy study relative to control arms.
D. Blood analysis from a different arm of the same study at day 60, NK cells circulating in peripheral blood were enumerated using flow cytometry and tumor volume was measured by caliper.
E. For a separate study arm, animals were sacrificed at 90 days after start of NK cell administration. Dissected organs were dissociated and tested by flow cytometry for the presence of human CD56+ NK cells.

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 (~4 Kb) cargo containing CAR, IL15, and DNR in CAT-179 using the non-viral TcBuster™ transposon system. Transposon engineering of CAT-179 results in stable expression of CAR (45% CAR at day 10-14 post electroporation) 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+ N87 gastric cancer cell line and lead to a significant survival benefit in tumor bearing mice.

CAT-179 is a demonstration of the power of the Catamaran CAR-NK platform to deliver promising off-the-shelf cell therapies to overcome the challenges associated with solid tumors.