CAT-179, an allogeneic NK cell product expressing HER2-CAR, IL-15 and TGFβ dominant negative receptor, durably regresses HER2-expressing xenograft tumors in mice

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Abstract # 2904

ABSTRACT

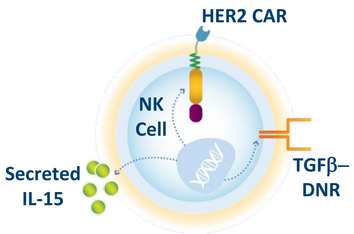
While chimeric antigen receptor (CAR)-engineered immune cell therapies have been at the forefront of cancer immunotherapy for hematological malignancies, patients with solid tumors have yet to benefit from such therapies. Engineered, off-the-shelf, allogeneic natural killer (NK) cells are particularly attractive as cell therapies for solid tumors given their clinical safety, efficacy, and multimodal recognition of tumor cells.

We describe here the pre-clinical pharmacokinetics, efficacy, biodistribution and safety of CAT-179, a novel allogeneic, cryopreserved CAR-NK cell therapy, in naïve animals as well as multiple xenograft models of HER2-amplified ovarian and gastric cancer. CAT-179 cells are engineered to express an optimized HER2-directed CAR to effectively target tumor cells, a transforming growth factor β (TGFβ) dominant negative receptor (DNR) to protect against TGFβ-mediated immunosuppression, and interleukin-15 (IL-15) to enhance NK cell persistence.

A single intravenous (IV) dose of CAT-179 resulted in IL-15-dependent ($p<0.0001$) expansion and persistence of CAT-179 cells for at least 140 days in NOD-scid IL2Rg^{null} (NSG) mice, peaking at approximately 15,000 cells/μL of blood (by day 60). Persisting CAT-179 NK cell levels at day 60 were functionally active and significantly reduced tumor burden when NSG mice were challenged intraperitoneally (IP) with HER2+ SKOV3-luc tumor cells. No significant changes in body weight or condition were observed during this study.

The therapeutic anti-tumor activity of CAT-179 against established tumors was assayed using two different xenograft models. In the first model, NSG mice were given an IP dose of 1 million SKOV3-luc tumor cells (derived from ovarian cancer) on day 0 followed by either 4 million CAT-179 or control NK cells on days 4, 11 and 18. CAT-179 dosed animals showed a rapid and sustained 95% decline in tumor burden ($p<0.0001$) and a significant extension in survival relative to animals dosed with control NK cells ($p<0.0001$). In the second model, 1 million HER2+ N87 cancer cells (derived from gastric cancer) were implanted subcutaneously into the right flank of NSG mice. When tumors reached 70mm^3, a single IV dose of 8M CAT-179 or control NK cells was administered. CAT-179 dosed animals showed a 96% durable tumor regression and significant survival benefit relative to animals dosed with control NK cells ($p<0.0001$). Efficacy strongly correlated with the circulating levels of CAT-179, which significantly infiltrated the tumor xenograft. Our pre-clinical results demonstrate the potential of CAT-179 as a novel, durable, and off-the-shelf cell therapy to overcome the challenges associated with solid tumors and provide quantitative insights into pharmacokinetics, pharmacodynamics and anti-tumor activity of engineered NK cells expressing CAR, TGFβ DNR and IL-15.

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

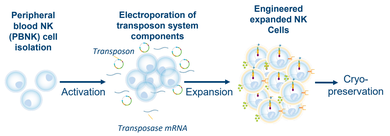


CAT-179 COMPONENTS		
Category	**Specifics**	**Rationale**
Cell Source	• Donor-derived peripheral blood NK cells	• Allogeneic, ready access, safe
Engineered modifications	• HER2-CAR • IL15 • TGFβ-DNR	• Tumor-directed killing • NK cell persistence • Evade TGFβ suppression
Engineering method	• TcBuster™ transposon system	• Highly efficient and consistent across donors
Cell process	• Engineered and expanded	• Highly scalable
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 in the TME.

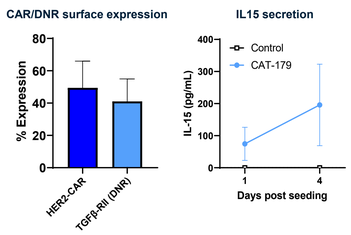
2 High efficiency engineering of CAT-179 NK cells with CAR, DNR, and IL15 is enabled by the TcBuster™ transposon system

A. Engineering process



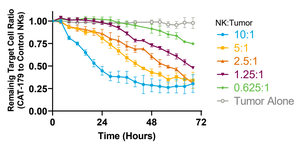
- Consistently high efficiency with multiple donors
- Hundreds of doses from a single leukopak
- Product retains high viability (mean 85%) and activity post-thaw

B. Expression of construct components

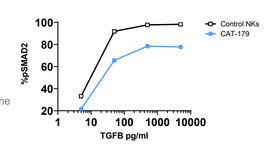


A. NK cell engineering: NK cells were isolated from peripheral blood and activated. A mixture of TcBuster™ (Bio-Techne) transposase-encoding mRNA and transposon plasmid encoding the transposon was then delivered to the NK cells for electroporation. Engineered NK cells were expanded with feeder cells and cryopreserved. Construct schematic of transgene elements showing positions and 2A sequence junctions. TGFβ-DNR structure is described in Wieser et al. 1993.
B. CAT-179 engineered and mock control NK cells were characterized 10 days after electroporation for CAR, TGFβ-DNR, and IL-15 expression. Two donors are shown, AVG±SEM. CAR and DNR expression were determined by flow cytometry. IL-15 secretion by 1E6 NK cells cultured 1 and 4 days was measured by MSD.

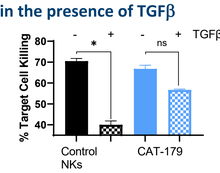
C. Cytotoxicity



D. DNR protects engineered NK cells from TGFβ induced signaling



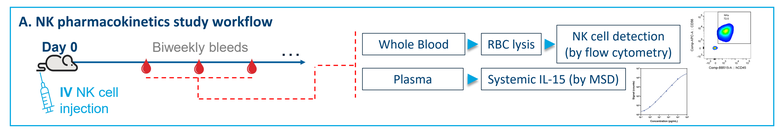
E. DNR restores NK cytotoxicity in the presence of TGFβ



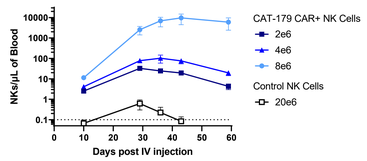
C. HER2+ GFP+ SKOV3 tumor cells were co-cultured with the indicated ratios of either CAT-179 or mock engineered control NK cells. Co-cultures were imaged using an Incucyte S3 to track live GFP+ tumor cells over time. CAR-dependent cytotoxicity was calculated by tumor killing relative to control NK cells. Transposon-engineered CAT-179 cells show CAR-driven cytotoxic activity in vitro.
D. CAT-179 or control engineered NK cells were treated with recombinant TGFβ1 for 1 hour and then fixed, permeabilized, stained for phosphorylated SMAD2 (pSMAD2) and analyzed by flow cytometry. DNR-expressing CAT-197 cells have lower levels of pSMAD2 compared to control.
E. CAT-179 or mock control engineered NK cells were pre-treated with 5 ng/ml TGFβ1 for 6 days then co-cultured for 3 hours with K562-luciferase tumor cells to evaluate innate NK mediated cell. Cytolytic activity for Control NKs is significantly reduced post TGFβ1 treatment. (Kruskal-Wallis with Dunn's multi-comparison correction, *p<0.05)

3 CAT-179 NK cells show dose-dependent in vivo persistence beyond 60 days

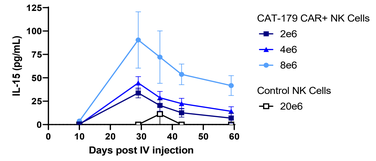
A. NK pharmacokinetics study workflow



B. Peripheral blood NK cell levels



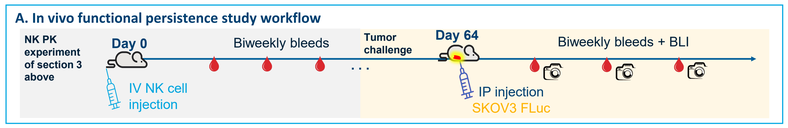
C. Plasma IL15 levels



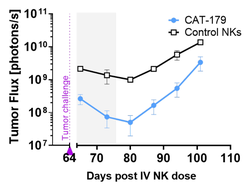
A. Schematic of the NK cell pharmacokinetics (PK) study workflow. A single dose of CAT-179 is administered intravenously (IV) on day 0 (CAR+% = 38) followed by weekly animal body weight check and biweekly blood collection (submandibular vein) to assess NK cell levels in blood (by flow cytometry) and plasma IL15 levels (using MSD)
B. NK cell levels in blood were monitored over time by flow cytometry (RBC lysis followed by staining for hCD45+ and hCD56+ cells). Data shown as AVG±SEM. N=5 mice/arm. CAT-179 cells persist for at least 60 days in vivo with a dose-dependent peak expansion; a significantly higher level than mock engineered cells (Control NKs).
C. MSD-analyzed samples forIL-15 levels. Values over time correlate with CAT-179 expansion levels. Shown as AVG±SEM. N=5 mice/arm

4 CAT-179 NK cells retain cytolytic activity after >2 months in vivo

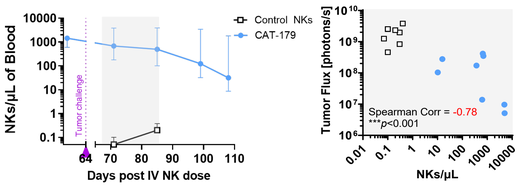
A. In vivo functional persistence study workflow



B. Persistent CAT-179 cells retain potential for anti-tumor efficacy



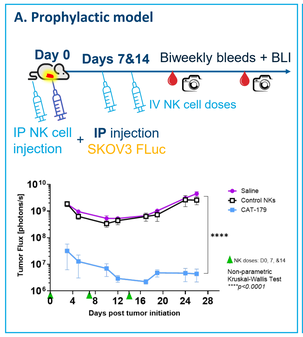
C. Systemic NK cell levels inversely correlate with tumor burden during the initial weeks post tumor challenge



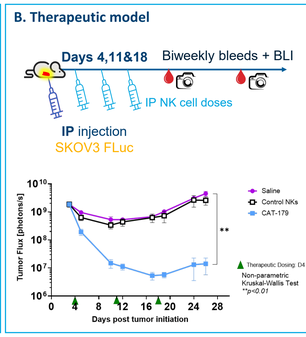
A. Following the PK study of section (3) above, CAT-179 mice with blood NK cell levels >10/μL on Day 60, along with all the control group mice, were challenged with 1x10^6 of HER2+ ovarian cancer cells (SKOV3) intraperitoneally (IP) on Day 64 followed by weekly assessment of tumor burden by IVIS Lumina™.
B. SKOV3-fluc mice were imaged at the indicated time points to quantify tumor burden. Luciferase signal is reported as flux (photons/second). Data shown as AVG±SEM. Persistent engineered NK cells retained cytotoxicity with lower tumor burden detected from animals treated with CAT-179 cells.
C. (**Left**) NK cell levels in blood at the indicated timepoints. (**Right**) To assess the role of the NK cells on the observed efficacy of panel B, initial systemic NK cell levels of each mouse from the two groups (shaded region of Fig. 4B) were plotted against their initial tumor burden (flux, shaded region in Fig. 4C-left). Higher systemic NK cell levels significantly correlated with lower tumor burden (Spearman correlation, two-tailed)

5 CAT-179 delivers prophylactic and therapeutic efficacy in IP tumor models

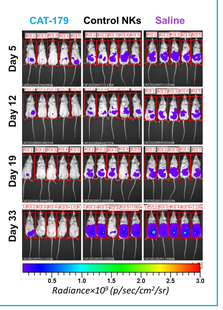
A. Prophylactic model



B. Therapeutic model



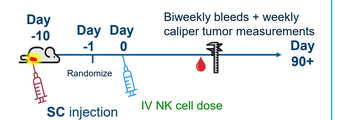
C. Bioluminescent imaging (BLI)



A. (**Top**) Schematic demonstrating the prophylactic intraperitoneal (IP) efficacy study workflow. 1x10^6 ovarian cancer cells expressing high levels of HER2 (SKOV3) were co-injected IP with 4E6 CAR+ CAT-179 cells (CAR+% = 61). Second and third 4E6 CAR+ doses were administered on days 7 and 14. Tumor burden (flux) was quantified twice a week using an IVIS Lumina imaging system and systemic NK cell levels were quantified using flow cytometry. (**Bottom**). Data shown as *AVG±SEM, Kruskal-Wallis, Dunn's multiple comparison correction.* CAT-179 resulted in a significantly lower tumor burden (blue) compared to control NKs and saline (purple and black).
B. (**Top**) Schematic demonstrating the therapeutic IP efficacy study workflow. Unlike the prophylactic study, on days 4, 11, and 18 post-tumor injection, NKs were administered IP and tumor burden (flux) was assessed longitudinally by IVIS imaging. (**Bottom**) Tumor flux data over time as measured by an IVIS Lumina. Data shown as *AVG±SEM, Kruskal-Wallis non-parametric test with Dunn's multiple comparison correctio.* CAT-179 resulted in a significantly lower tumor burden compared to control NKs and saline.
C. Therapeutic study IVIS BL images demonstrating the lower flux from tumors treated with CAT-179 relative to the control groups

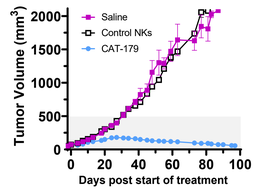
6 CAT-179 cells deliver prolonged protective efficacy versus HER2$^+$ SC tumor

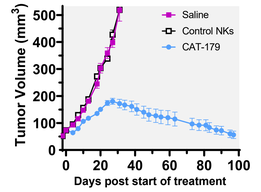
A. Single-dose SC tumor, IV NK cell efficacy



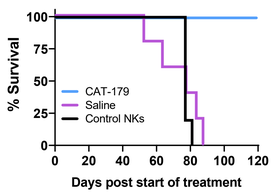
A. Schematic of single-dose efficacy model. NSG mice (N=10) were implanted SC with 1e6 HER2$^+$ NK87 gastric tumor cells then treated IV with 8e6 40% CAR+ CAT-179 or 2e7 mock engineered control NK cells, or saline. Tumor growth and mouse survival were tracked >90 days.
B. CAT-179 NK cells caused durable tumor regression vs controls (AUC, $p<0.01$, Kruskal-Wallis with Dunn's.(**Right**) Scale of tumor volume adjusted to show 30 days of tumor growth followed by >60 days sustained regression.
C. CAT-179 NK cells prevented death of all treated tumor-bearing mice. Survival benefit was not reached (>76 days and ongoing) versus median survival of 77 days for saline and control NK cell groups.

B. Single CAT-179 dose regresses established N87 gastric SC tumors



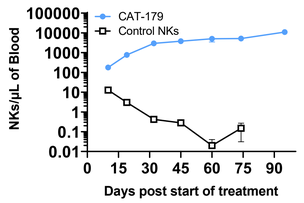


C. CAT-179-treated mice survive SC tumor

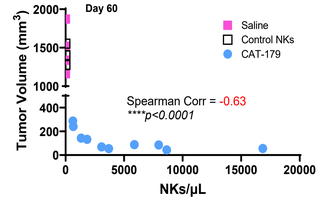


7 Stabilizing expansion of CAT-179 NK cells associates with tumor infiltration and control

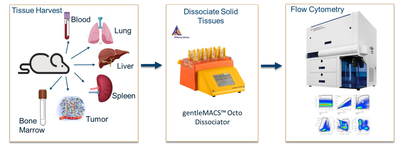
A. NK cell expansion and stabilization



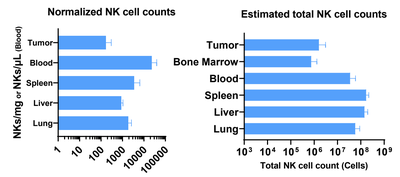
B. Sustained NK levels inversely correlates with tumor burden



C. Biodistribution assessment



D. CAT-179 biodistribution in tumor-bearing NSG mice



A. Blood samples were analyzed over time to track circulating populations of NK cells by flow cytometry. Control NK cells steadily diminished from before day 7 to day 60. CAT-179 NK cells increased with expansion changing from rapid to near homeostatic after day 30.
B. Blood NK cell counts inversely correlate with tumor burden 60 days after NK cell transfer ($p<0.0001$, two-tailed Spearman -0.63)
C. To assess biodistribution, 90 days after CAT-179 IV injection, major organs and remaining tumor xenografts were harvested, dissociated, and quantitatively analyzed by flow cytometry
D. Persistence and protective efficacy of engineered NK cells are associated with high biodistribution of NK cells in tumor-bearing mice. Normalized (left) and total estimated (right) CAT-179 counts are shown

SUMMARY AND CONCLUSIONS

- We describe here the preclinical evaluation of CAT-179, a novel engineered NK cell therapy expressing HER2-CAR, TGFβ-DNR, and IL15
- We demonstrate 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
- 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; CAT-179 cells will be protected from TGFβ-mediated immune suppression in the TME and can protect neighboring cells
- Following a single dose of 8E6 CAR+ cells, the engineering of IL15 in CAT-179 significantly enhances the persistence and expansion *in vivo* up to 140 days in NSG mice without adverse effects
- In HER2+ SKOV3 (Ovarian) and N87 (Gastric) tumor xenograft IP and SC models, CAT-179 cells show potent persistence, expansion, biodistribution and anti-tumor activity, leading to a significant survival benefit in treated NSG mice relevant to control groups
- Peripheral blood CAT-179 cell levels inversely correlate with tumor burden

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