# Placental Circulating T Cells Expressing CD16 in Combination with Trastuzumab Demonstrate Robust Anti-tumor Antibody-dependent Cellular Cytotoxicity (ADCC) Against Gastric Cancer

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## Introduction

Celularity Inc. is developing PT-CD16VS as a novel platform for use in combination with approved monoclonal antibodies for the potential treatment of multiple cancers. PT-CD16VS is an allogeneic cell therapy product derived from human postpartum placental circulating T (P-T) cells that are genetically modified to express a proprietary CD16 variant with knockout of the endogenous T cell receptor (TCR).
Here we report the characterization of PT-CD16VS and evaluate both the *in vitro* and *in vivo* preclinical efficacy of PT-CD16VS against HER2+ NCI-N87 gastric cancer in combination with Trastuzumab. *In vitro* safety studies assessing the potential for on-target/ off-tumor activity of PT-CD16VS against HER2 low expressing human dermal fibroblasts (HDF) and small airway lung epithelial cells (LEC) are also highlighted.

## Methods

Gene Modification: PT-CD16VS cells were generated through transduction of human placental circulating T cells using a lentiviral vector containing a CD16 construct expressing a high affinity CD16 variant (CD16VS) followed by knock-out of the TCR through transfection.

Phenotypic Characterization: The phenotype of PT-CD16VS cells was determined using flow cytometry. The cells were stained for CD56, CD20, CD5, CD16, and TCR-α/β expression. The viability was assessed using 7AAD staining. For targets, cells were stained for HER2 expression.

Cytotoxicity Assay: The *in vitro* anti-tumor functional activity of PT-CD16VS cells against HER2+ NCI-N87 gastric cancer cells or on-Target/off-Tumor activity against HER2 low expressing Human Dermal Fibroblasts (HDF) and small airway Lung Epithelial Cells (LEC) in combination with Trastuzumab (1μg/mL) or IgG control (1μg/mL) were assessed using the kinetic ACEA –based cytotoxicity assay.

Cytokine Release Assay: The *in vitro* functional activity of PT-CD16VS cells against NCI-N87 or On-Target/Off-Tumor activity against HDF and LEC with Trastuzumab (1μg/mL) or IgG control (1μg/mL) was assessed by co-culturing PT-CD16VS at an E:T ratio of 1:1 for 24 hours and quantifying the levels of proinflammatory cytokines and effector proteins in the supernatant using Meso Scale Discovery (MSD) platform (for NCI-N87) and Luminex platform (for HDF and LEC).

Proliferation Assay: PT-CD16VS proliferation in combination with Trastuzumab or IgG (1μg/mL) against NCI-N87 was assessed by co-culturing PT-CD16VS cells at an E:T ratio of 1:1 over 3 days. Co-cultures were harvested, counted, and analyzed by flow cytometry to enumerate the total number of $CD16^+$ per condition following 2-3 days of co-culture. Fold change was calculated by dividing the number of $CD16^+$ T cells in combination with Trastuzumab by the number of $CD16^+$ T cells detected in combination with the IgG control.

*In vivo* Anti-Tumor Model: A subcutaneous HER2+ gastric cancer (NCI-N87) model was established in NSG mice. Mice were inoculated with 5 x $10^6$ NCI-N87 subcutaneously on Day 0. Ten days post NCI-N87 inoculation, mice were preconditioned with busulfan (30 mg/kg, intraperitoneal injection). On Day 13, mice were randomized by tumor volume into 5 treatment groups of 12 animals per group: Vehicle (Group 1), Trastuzumab 10mg/kg (Group 2), 10 x $10^6$ total PT-CD16VS (Group 3), 10 x $10^6$total PT-CD16VS + 10mg/kg Trastuzumab (Group 4), and Enhertu 10mg/kg (Group 5). On Day 35, the Enhertu group was sacrificed and Groups 2, 3, and 4 received a low dose of Trastuzumab (2.5mg/kg) to evaluate the persistence of PT-CD16VS cells. The remaining mice were sacrificed on Day 45 or Day 49. Tumor volume was evaluated twice weekly.

*In vivo* PT-CD16VS Tumor Infiltration: Using the subcutaneous NCI-N87 model described above, tumors were grown until approximate volume of 210-225 $mm^3$ and treated with vehicle, 10 x $10^6$total PT-CD16VS, or 10 x $10^6$ total PT-CD16VS + 10mg/kg Trastuzumab. Six days post treatment mice were sacrificed, and tumors were harvested and fixed for IHC. Sections were stained using Opal Multiplex IHC Confocal system for the following markers: CD45, CD16, HER2, and Ki67. Quantitative data was derived from 3 tumor samples with 5 or 6 independent paraffin sections per sample.

## Summary

- PT-CD16VS cells exhibited high viability ($7AAD^-$), T cell purity ($CD56^-$/ $CD20^-$/ $CD5^+$), and CD16 transduction efficiency across eleven P-T donors. PT-CD16VS expressed no TCR-α/β following manufacture.
- *In vitro*, PT-CD16VS cells in combination with Trastuzumab demonstrated robust cytotoxicity against HER2+ NCI-N87 gastric cancer cells at low E:T ratio of 0.625:1 and 12 hours of co-culture.
- PT-CD16VS cells proliferated in response to co-culture with NCI-N87 in combination with Trastuzumab following 48 and 72 hours of incubation.
- PT-CD16VS cells secreted pro-inflammatory cytokines and effector proteins when co-cultured with NCI-N87 in combination with Trastuzumab for 24 hours.
- As compared to CYNK-101 (placental CD34-derived NK cells), PT-CD16VS showed greater cytotoxicity in combination with Trastuzumab against NCI-N87 with equivalent or lower cytokine secretion following 24 hours of co-culture.
- PT-CD16VS in combination with Trastuzumab did not elicit on-target/ off-tumor cytotoxicity or cytokine release in response to low HER2 expression on normal Human Dermal Fibroblasts (HDF) and small airway Lung Epithelial Cells (LEC) .
- *In vivo*, PT-CD16VS cells in combination with Trastuzumab were well tolerated and significantly reduced tumor burden compared to vehicle control, Trastuzumab alone, PT-CD16VS cells alone, and Enhertu on Day 35. The combination of PT-CD16VS and Trastuzumab resulted in an 8.3% complete response and a 58.3% partial response of greater than 85% reduction in tumor volume compared to a 0% and 8.3% in Enhertu treated mice for complete response and greater than 85% partial reduction, respectively. At termination, the combination treatment group demonstrated a complete response rate of 75%.
- After a low dose (2.5mg/kg) administration of Trastuzumab on Day 35 to Groups 2, 3, and 4, the continued reduction of tumor volume in the PT-CD16VS+Trastuzumab group suggested persistence of PT-CD16VS as continued tumor growth was observed in the PT-CD16VS alone group.
- IHC analysis demonstrated PT-CD16VS infiltration into the NCI-N87 tumor was Trastuzumab dependent at 6 days post-cell administration. Infiltrating cells were close to 100% $CD16^+$ and greater than 75% were proliferating ($Ki67^+$).

## Conclusions

- Celularity Inc. developed allogeneic cell therapy product PT-CD16VS has robust *in vitro* and *in vivo* preclinical efficacy against HER2+ NCI-N87 gastric cancer cells in combination with Trastuzumab. Furthermore, *in vitro*, there was no observed on-target/off-tumor activity of PT-CD16VS against non-cancerous cells expressing low levels of HER2.
- PT-CD16VS can be combined with various monoclonal antibodies to engage in anti-tumor antibody-dependent cellular cytotoxicity against diverse cancer types using a "universal receptor" approach.

## Results

### Figure 1. CD16 Construct and Phenotype of PT-CD16VS Cells

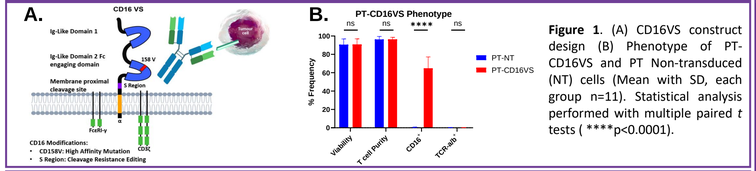



**Figure 1**. (A) CD16VS construct design (B) Phenotype of PT-CD16VS and PT Non-transduced (NT) cells (Mean with SD, each group n=11). Statistical analysis performed with multiple paired *t* tests ( ****p<0.0001).

### Figure 2. PT-CD16VS Cells Exhibit Potent Functional Activity vs. HER2+ NCI-N87 Gastric Tumor Cells

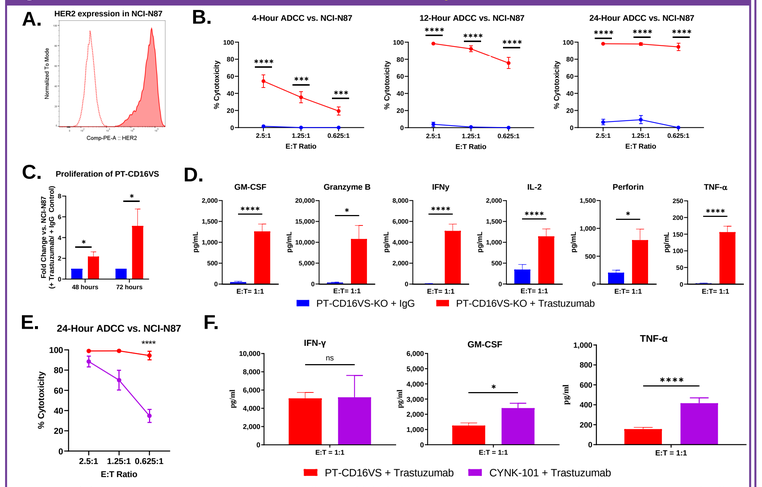



**Figure 2**. (A) HER2 expression in NCI-N87 tumor cells in red labeled with PE anti-human HER2 antibody. Isotype- stained cells are shown in dotted line. (B) ACEA kinetic cytotoxicity assay against NCI-N87 target cells in combination with Trastuzumab (Mean with SEM, each E:T Ratio n=8-11). (C) Proliferation fold change of PT-CD16VS with Trastuzumab against NCI-N87 target cells (Mean with SEM, each timepoint n=5-7). (D) The secretion of proinflammatory cytokines and cytolytic proteins by PT-CD16VS in combination with Trastuzumab against NCI-N87 tumor cells. (Mean with SEM, each group n=9). (E) ACEA kinetic cytotoxicity of PT-CD16VS-KO (Mean with SEM, n=8) vs. CYNK-101 (Mean with SEM, n=4). (F) Cytokine release of PT-CD16VS (Mean with SEM, n=9) vs. CYNK-101 (Mean with SEM, n=5). Statistical analyses for B, C, E performed with multiple *t* tests, and for D, F with paired *t* test (*p<0.05, **p<0.01, ***p<0.005, and ****p<0.0001).

### Figure 3. PT-CD16VS Cells Do Not Elicit On-Target/Off-Tumor Activity vs. HER2 Low Normal Cells

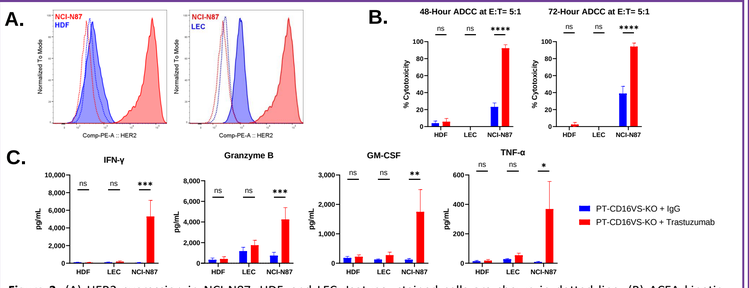
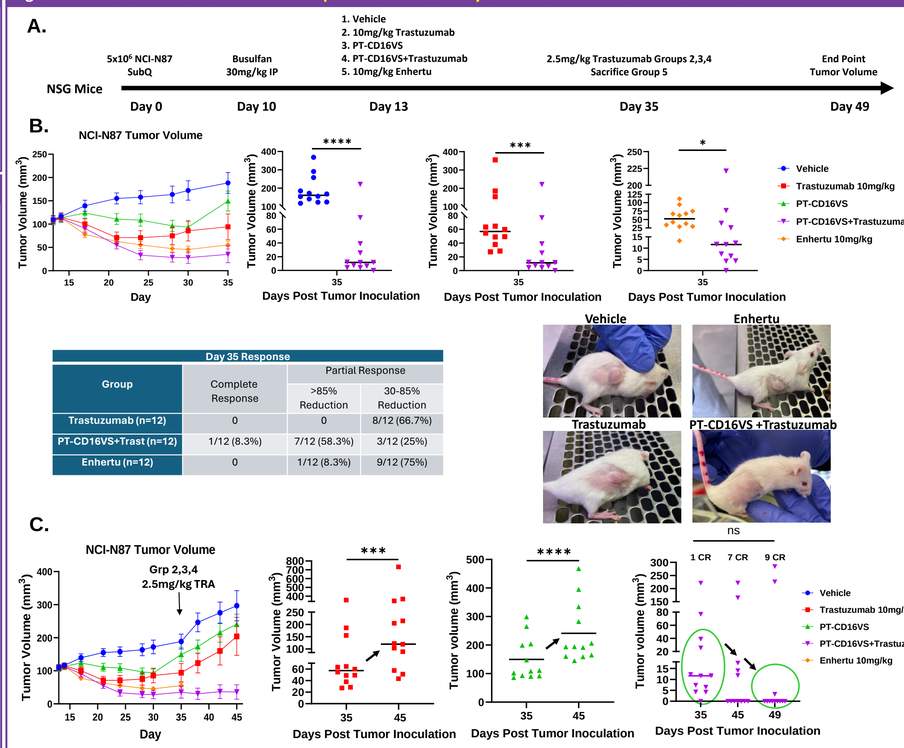



**Figure 3**. (A) HER2 expression in NCI-N87, HDF, and LEC. Isotype- stained cells are shown in dotted line. (B) ACEA kinetic cytotoxicity assay against HDF, LEC, and NCI-N87 in combination with Trastuzumab (Mean with SEM, each group n=4). (C) The cytokine release by PT-CD16VS in combination with Trastuzumab against HDF, LEC, and NCI-N87 (Mean with SEM, each group n=4). Statistical analyses performed with multiple *t* tests (*p<0.05, **p<0.01, ***p<0.005, and ****p<0.0001).

### Figure 4. PT-CD16VS Cells Demonstrate Superior *In-vivo* Efficacy in NCI-N87 Subcutaneous Model in NSG Mice




| Day 35 Response | | | |
|---|---|---|---|
| Group | Complete Response | Partial Response | |
| | | >85% Reduction | 30-85% Reduction |
| Trastuzumab (n=12) | 0 | 0 | 8/12 (66.7%) |
| PT-CD16VS+Trast (n=12) | 1/12 (8.3%) | 7/12 (58.3%) | 3/12 (25%) |
| Enhertu (n=12) | 0 | 1/12 (8.3%) | 9/12 (75%) |

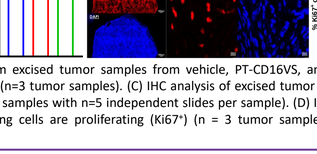



**Figure 4**. (A) Schema of NCI-N87 tumor model. (B) Tumor volumes on Day 35 demonstrating superior tumor reduction in the PT-CD16VS + Trastuzumab group (Mean with SEM, each group n=12, Statistical analyses performed using Wilcoxon Rank Sum test), and objective response rates. (C) Tumor volumes after second, low dose administration of Trastuzumab suggesting PT-CD16VS persistence is Trastuzumab dependent (Mean with SEM, each group n=12). Statistical analyses performed with paired *t* test (*p<0.05, ***p<0.005, and ****p<0.0001).

### Figure 5. PT-CD16VS Infiltration and Proliferation in NCI-N87 Subcutaneous Tumor is Trastuzumab Dependent




**Figure 5**. (A) Schema for NCI-N87 tumor model for IHC analysis. (B) IHC analysis from excised tumor samples from vehicle, PT-CD16VS, and PT-CD16VS +Trastuzumab demonstrates tumor infiltration by PT-CD16VS is Trastuzumab dependent (n=3 tumor samples). (C) IHC analysis of excised tumor samples from PT-CD16VS + Trastuzumab shows close to 100% of infiltrating cells are $CD16^+$ (n=3 tumor samples with n=5 independent slides per sample). (D) IHC analysis of excised tumor samples from PT-CD16VS+Trastuzumab demonstrates >75% of infiltrating cells are proliferating ($Ki67^+$) (n = 3 tumor samples with n = 5 or 6 independent sections per sample).