# Pre-clinical Development of CT-1119:
# A Mesothelin-Targeting Chimeric Antigen Receptor Macrophage (CAR-M) for Solid Tumor Immunotherapy

Nicholas R. Anderson[1†], Brinda Shah[1], Alison Worth[1], Rashid Gabbasov[1], Brett Menchel[1], Kerri Ciccaglione[1], Daniel Blumenthal[1], Stefano Pierini[1], Sabrina Delong[1], Sascha Abramson[1], Thomas Condamine[1], Michael Klichinsky[1*]

[1]Carisma Therapeutics, Philadelphia, PA, USA *Corresponding author †Presenting author

## Introduction

### Background

Despite significant success in treating hematological malignancies, adoptive cell therapies have yielded limited efficacy in solid tumors [1]. Macrophages are myeloid cells of the innate immune system and are naturally recruited to solid tumors [2], where they have the potential to phagocytose tumor cells, activate the tumor microenvironment (TME), and prime a broad anti-tumor adaptive immune response via T cell recruitment and activation. We have previously developed chimeric antigen receptor macrophages (CAR-M) targeting HER2 and showed efficacy in a variety of pre-clinical models [3], with a Phase I clinical trial ongoing. Mesothelin is overexpressed in a variety of solid tumors, including mesothelioma, lung, pancreatic, and ovarian cancers [4]. Here, we present preclinical data summarizing the development of CT-1119, a mesothelin targeted CAR-M for solid tumors.

### Methods

Using the chimeric adenoviral vector Ad5f35, we engineered primary human macrophages to express a CAR comprising a human scFv targeted against human mesothelin. To assess the activity of CT-1119, *in vitro* cell based assays and *in vivo* murine xenograft models were utilized. Donor-matched untransduced (UTD) macrophages served as controls.

### Results

Primary human CAR-M engineered with an Ad5f35 vector demonstrated high CAR expression, high viability, upregulated M1 (anti-tumor) macrophage markers, and downregulated M2 (pro-tumor) macrophage markers. CT-1119 demonstrated increased resistance to repolarization by M2 (pro-tumor) polarizing cytokines as compared to donor matched UTD macrophages. CT-1119 specifically bound mesothelin and binding was not impacted by mesothelin shedding. CT-1119 specifically phagocytosed multiple mesothelin expressing tumor cell lines in a CAR-dependent and antigen-dependent manner. CT-1119 demonstrated robust *in vitro* killing of the relevant tumor cell lines A549 and MES-OV expressing mesothelin. CAR engagement also induced the release of pro-inflammatory cytokines such as TNFα following stimulation with mesothelin in both cell-free and cell-based contexts in a dose-dependent manner. *In vivo*, CT-1119 significantly reduced tumor burden in a murine xenograft model of lung cancer. Similarly, human monocytes targeting mesothelin were successfully generated using the same Ad5f35 vector and demonstrated specific activity against mesothelin positive tumor cells.

### Conclusions

The presented results demonstrate that CT-1119, an autologous human anti-mesothelin CAR-M, can cause phagocytosis, tumor cell killing, and pro-inflammatory cytokine release in response to stimulation with mesothelin. These results show that CAR-M is a feasible approach for the treatment of mesothelin expressing sold tumors via the potential for induction of a systemic anti-tumor response.

## Manufacturing process and CAR characteristics

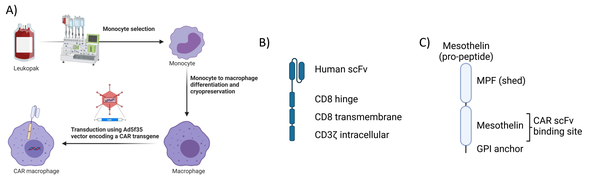



**Figure 1** – CT-1119 production and CAR design. **A)** Schematic highlighting the process for the manufacturing of CT-1119 for use in pre-clinical studies. Monocytes isolated from donor leukopaks are differentiated into macrophages via culture with GM-CSF, cryopreserved, thawed, and then transduced with adenovirus carrying a CAR transgene. CAR-M are ready for use two days post-transduction. **B)** Diagram of the CAR molecule used in CT-1119. The scFv used is fully human and targets the retained portion of mesothelin. The CD3ζ intracellular domain provides a potent pro-phagocytic and pro-cytokine release signal upon CAR engagement. **C)** Diagram of the mesothelin pro-peptide, including the location of the scFv binding site on the cell anchored portion of the protein.

## CT-1119 is an M1-polarized anti-mesothelin CAR-M

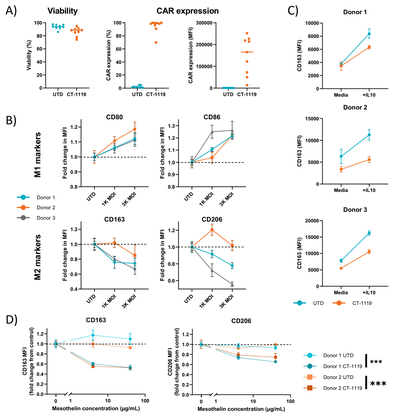



**Figure 2** – Flow cytometric characterization of CT-1119. **A)** Plots of viability, the percent of live macrophages expressing the anti-mesothelin CAR, and the MFI of mesothelin CAR expression of all live cells across nine independent donors following transduction with 3000 MOI of Ad5f35. Line indicates the mean value of all donors. **B)** Comparison of the expression levels of M1 markers CD80 and CD86 and M2 markers CD163 and CD206 on UTD macrophages and CT-1119 generated using two different MOIs. Results of triplicate wells of three independent donors shown. **C)** Change in expression of CD163 in response to treatment for 24 hours with 20 ng/mL IL-10. CT-1119 showed less upregulation of the M2 marker, indicating a resistance to repolarization. **D)** Change in MFI by UTD and CT-1119 following a 24-hour stimulation with recombinant human mesothelin. CT-1119 showed a significant down-regulation of both CD163 and CD206, showing that CT-1119 becomes more M1-like in response to antigen stimulation. Results of triplicate wells of two independent donors shown, two-way ANOVA with Dunnett's multiple comparisons to UTD.

## CT-1119 targeted phagocytosis, killing, & cytokine release

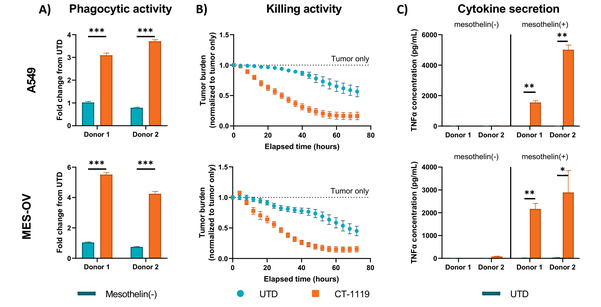



**Figure 3** – *In vitro* characterization of the functional activity of CT-1119. **A)** Comparison of the phagocytic ability of UTD-M and CT-1119 macrophages against two target cell lines: lung adenocarcinoma A549 and ovarian cystadenocarcinoma MES-OV. Cell lines do not endogenously express significant levels of mesothelin and were engineered for over expression. CT-1119 shows a significant increase in the level of phagocytosis (as measured by pHrodo fluorescence) compared to UTD only when mesothelin is present on the target cell. Triplicate wells of two independent donors, two-tailed t test with multiple comparison correction via the Holm-Sidak method. **B)** Comparison of cell line killing by CT-1119 macrophages from three independent donors through 72 hours of co-culture at an initial 1:1 E:T ratio with mesothelin-expressing target cells. The dotted line shows tumor only controls. **C)** Comparison of cytokine release following stimulation of macrophages with the listed target cell in a 1:1 E:T ratio for 24 hours. Some bars may be too short to see on the plot. Triplicate wells of two independent donors, two-tailed t test with multiple comparison correction via the Holm-Sidak method.

## CT-1119 demonstrated durable anti-tumor activity *in vitro*

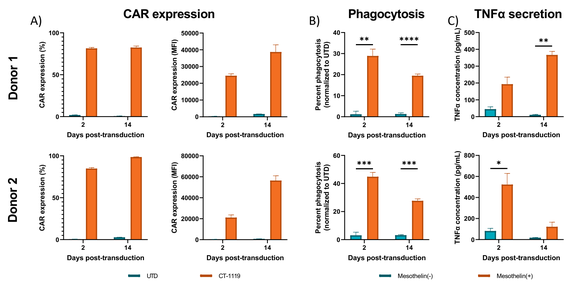



**Figure 4** – CT-1119 expressed CAR and was functional at an early and late timepoint. **A)** Percent of live macrophages expressing anti-mesothelin CAR and CAR expression levels for two timepoints post-transduction (days 2 and 14). **B)** CAR-M show, via flow cytometry, target specific phagocytosis for at least 14 days following transduction with virus. **C)** Secretion of TNFα following stimulation of macrophages at a 1:1 E:T ratio for 24 hours. CT-1119 was able to produce pro-inflammatory cytokines like TNFα at significant levels for up to two weeks following transduction. Longer term culture reduced the level of released cytokines, but CT-1119 was responsive to antigen for at least four weeks (data not shown). All comparisons from triplicate wells of two independent donors, two-tailed t test with multiple comparison correction via the Holm-Sidak method.

## Mesothelin-targeting CAR-Monocytes can be produced in a single day process

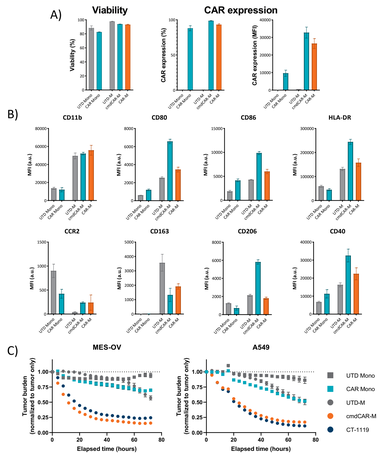



**Figure 5** – Production of anti-mesothelin CAR monocytes. In comparison to CAR-M, manufacturing of CAR monocytes is a single day process immediately following isolation from the leukopak. Differentiation of CAR monocytes *in vitro* leads to CAR monocyte-derived CAR macrophages (cmdCAR-M) following a week of culture. CAR-M were manufactured from donor-matched monocytes following differentiation with GM-CSF for five days. **A)** Flow cytometric comparison of UTD monocytes, CAR monocytes, UTD macrophages, CAR macrophages, and cmdCAR-M from two independent donors. Transduction with CAR did not significantly impact viability of the monocytes or the resulting macrophages, while causing robust expression of CAR. **B)** Expression of macrophage phenotype markers. Transduction with Ad5f35 containing CAR did not impact the differentiation of monocytes in macrophages. The resulting CAR monocyte derived macrophages displayed an M1-like phenotype, along with upregulated antigen presentation machinery. Triplicate wells of two representative donors. **C)** Comparison of the ability of monocytes and macrophages to selectively killing antigen positive tumor cells at a 2:1 E:T ratio over 72 hours from two independent donors. CAR monocytes were not effective against the tumor lines. However, anti-meso cmdCAR-M was similarly effective as CT-1119 in terms of killing.

## CT-1119 reduced tumor burden in a lung cancer model

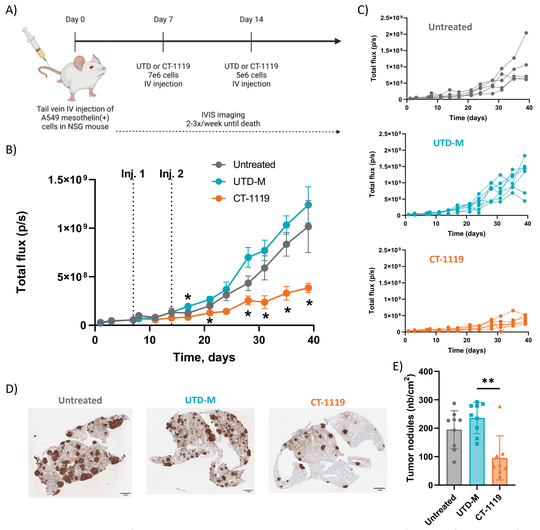



**Figure 6** – *In vivo* assessment of CT-1119 therapy activity in a metastatic lung cancer xenograft model. **A)** Schematic of the in vivo experiment design. NSG mice were injected with A549 mesothelin/CBG expressing cells and the tumor allowed to establish. On days 7 and 14 post-tumor injection, donor matched UTD and CT-1119 macrophages were IV dosed and tumor growth monitored via IVIS. **B)** Average luminesce for treatment groups as measured by IVIS. Two-way ANOVA with Tukey's multiple comparisons showed significance between UTD and CT-1119 groups. **C)** Individual tumor plots for treatment groups. **D)** IHC staining of human mesothelin in mouse lungs from listed treatment groups showing tumor nodules. Scale bar 1 mm. **E)** Quantification of number of tumor nodules per cm². Kruskal-Wallis test with Dunn's multiple comparisons showed significance between UTD and CT-1119 groups.

## Key Takeaways

- **CT-1119 CAR-M demonstrated high viability, high CAR expression, and M1 polarization with relative resistance to M2 conversion**
- **CT-1119 displayed targeted phagocytosis, killing, and cytokine release in response to multiple mesothelin-positive target cell lines**
- **CT-1119 showed durable CAR expression and activity *in vitro***
- **Anti-Mesothelin CAR-Monocytes could be produced and differentiated into functional M1-polarized CAR-M**
- **CT-1119 reduced tumor burden in a mesothelin overexpressing metastatic lung cancer xenograft model**

**References**

1. Hou A, Chen L, Chen Y, Navigating CAR-T cells through the solid-tumour microenvironment, Nat Rev Drug Discov. 2021; 20:531-550.
2. Biswas S, Allavena P, Mantovani A, Tumor associated macrophages: functional diversity, clinical significance, and open questions, Semin Immunopathol. 2013; 35:585-600.
3. Klichinsky M, *et al*, Human chimeric antigen receptor macrophages for cancer immunotherapy, Nat Biotechnol. 2020: 1-7.
4. Lv J, Li P, Mesothelin as a biomarker for targeted therapy, Biomarker Res. 2019; 7: 1-18.

Graphics were created with Biorender.

carisma THERAPEUTICS

# Macrophages Engineered to Express Synthetic Cytokine Switch Receptors Act as Living Microenvironment Converters

Chris Sloas[1], Yuhao Huangfu[1], Thomas Condamine[1], Michael Klichinsky[1], Yumi Ohtani[1]

[1]Carisma Therapeutics, Philadelphia, PA

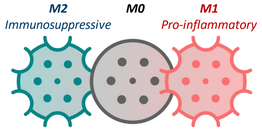
## Introduction

### Balancing pro- and anti-inflammatory cytokines in disease

- Cytokines in tissue microenvironments regulate pro- and anti-inflammatory signals
- Dysregulated cytokines can cause pathogenic immunosuppression or inflammation
- Rebalancing inflammation or immunosuppression offers a generalizable approach to treating many diseases, but systemic cytokine blockade carries risks such as increased risk of infection

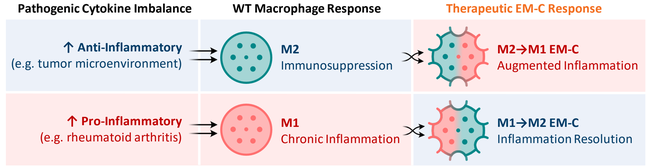
### Macrophage cell therapies for rebalancing inflammation

- Cell therapies offer a localized solution to rebalance inflammation
- Macrophages are proficient at both initiating and resolving inflammation
- Engineered macrophages have demonstrated promising ability to target tumor cells using CARs [1-2]




## Objectives

**Goal: Develop Engineered Microenvironment Converters (EM-C) as a platform technology to regulate inflammation in disease**




## Materials and Methods

- EM-C are generated by expressing Switch Receptors (SR) in primary human macrophages or monocytes, or primary murine BMDMs
- SR are delivered using VPX-Lentiviral particles
- SR are generated to target IL10 or TGFβ for M2→M1 signal conversion, or to target IFNγ for M1→M2 conversion
- All data shown are representative of at least three independent experiments
- Measurements are reported as mean ± SD for experimental replicates or, when stated, for donor replicates

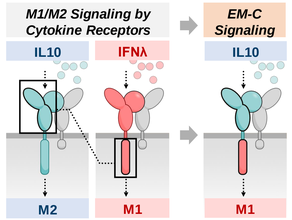



**Figure 1. Conceptual design of Switch Receptors for EM-C signaling.** Switch Receptors (SR) are chimeric proteins consisting of the ligand binding domain from one cytokine receptor, paired with a compatible cytosolic domain from a second receptor. Successful SR design enables EM-C to convert anti-inflammatory M2 cytokines into M1 signals, or *vice versa*.

## IL10 EM-C exhibit a pro-inflammatory response to IL-10

**IL10 EM-C express a SR that converts IL10, a common immunosuppressive factor in solid tumors, into a pro-inflammatory (M1) signal. Primary human and murine EM-C stimulated with IL10 activate STAT1/2 signaling and have an augmented M1 phenotype.**

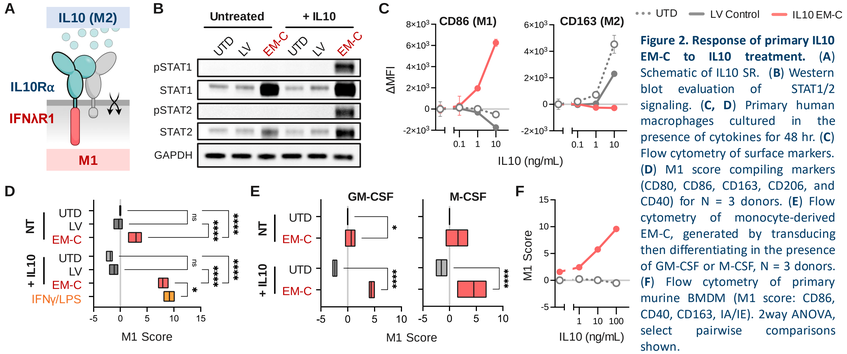



**Figure 2. Response of primary IL10 EM-C to IL10 treatment.** (**A**) Schematic of IL10 SR. (**B**) Western blot evaluation of STAT1/2 signaling. (**C, D**) Primary human macrophages cultured in the presence of cytokines for 48 hr. (**C**) Flow cytometry of surface markers. (**D**) M1 score compiling markers (CD80, CD86, CD163, CD206, and CD40) for N = 3 donors. (**E**) Flow cytometry of monocyte-derived EM-C, generated by transducing then differentiating in the presence of GM-CSF or M-CSF, N = 3 donors. (**F**) Flow cytometry of primary murine BMDM (M1 score: CD86, CD40, CD163, IA/IE). 2way ANOVA, select pairwise comparisons shown.

## IL10 EM-C augment the surrounding microenvironment

**IL10 EM-C release a repertoire of soluble pro-inflammatory factors in response to IL10. Furthermore, TAM-like M2 macrophages cultured with EM-C are skewed towards an M1 phenotype, demonstrating that EM-C can repolarize surrounding immune cells.**

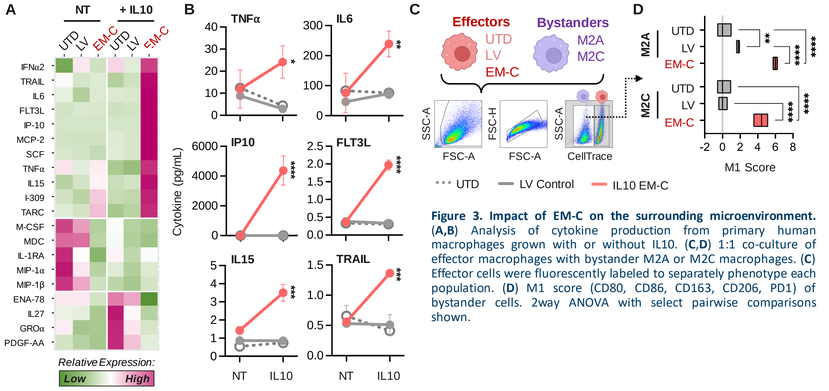



**Figure 3. Impact of EM-C on the surrounding microenvironment.** (**A,B**) Analysis of cytokine production from primary human macrophages grown with or without IL10. (**C,D**) 1:1 co-culture of effector macrophages with bystander M2A or M2C macrophages. (**C**) Effector cells were fluorescently labeled to separately phenotype each population. (**D**) M1 score (CD80, CD86, CD163, CD206, PD1) of bystander cells. 2way ANOVA with select pairwise comparisons shown.

## Development of EM-C targeting TGFβ (M2 → M1)

**The EM-C platform is broadened to target TGFβ, an additional immunosuppressive factor prevalent in solid tumors. TGFβ EM-C adopt a pro-inflammatory phenotype in response to TGFβ, and additionally sequester TGFβ from the supernatant.**

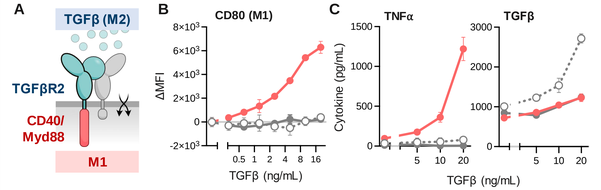



**Figure 4. Development of primary EM-C expressing a TGFβ SR.** (**A**) Schematic of TGFβ SR. (**B, C**) Primary human were cultured in the presence of TGFβ for 48hr. (**B**) Flow cytometry analysis of the M1 marker CD80. (**C**) Cytokine analysis quantifies TNFα production and TGFβ sequestering.

## Expanding the EM-C platform for inflammatory diseases (M1 → M2)

**EM-C are modular and can instead target inflammatory cytokines, such as IFNγ, a canonical M1 cytokine. Here, stimulated IFNγ EM-C exhibit an M2 phenotype, diminish inflammatory cytokine production, and sequester of IFNγ from the supernatant.**

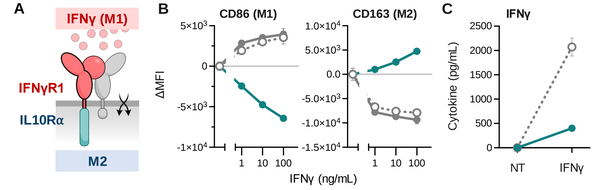



**Figure 5. Development of anti-inflammatory EM-C targeting IFNγ.** (**A**) Schematic of IFNγ SR. (**B,C**) Primary human macrophages were cultured in the presence of IFNγ for 24 hr. (**B**) Flow cytometry analyses of the M1 marker CD86 and M2 marker CD163. (**C**) Cytokine analysis quantifies IFNγ sequestering.

## Key Takeaways

**The EM-C platform represents a novel immunotherapy that harnesses macrophages as 'living converters' to locally regulate inflammation**

- Robust control of inflammatory phenotypes in engineered and bystander cells
- Modular approach that accommodates diverse cytokine targets
- Applicable for both oncology (M2→M1) and inflammatory (M1→M2) indications

[1] Klichinsky, M. *et al.* Human chimeric antigen receptor macrophages for cancer immunotherapy. *Nat Biotechnol* 1–7 (2020) doi:10.1038/s41587-020-0462-y.
[2] Anderson, N. R., Minutolo, N. G., Gill, S. & Klichinsky, M. Macrophage-Based Approaches for Cancer Immunotherapy. *Cancer Res* **81**, 1201–1208 (2021).

**BMDM**: Bone Marrow-Derived Macrophage
**DN** TGFβR2: Dominant Negative TGFβR2
**EM-C**: Engineered Microenvironment Converter
**LV**: Lentivirus
**MFI**: Median Fluorescence Intensity
**NT**: Nontreated/untreated
**SR**: Switch Receptor
**UTD**: Untransduced

Join us!

carisma THERAPEUTICS

# Chimeric antigen receptor macrophages (CAR-M) sensitize solid tumors to anti-PD1 immunotherapy

Stefano Pierini[1], Rashid Gabbasov[1], Oliveira-Nunes Maria Cecilia[1], Alison Worth[1], Yumi Ohtani[1], Sascha Abramson[1], Olga Shestova[2], Saar Gill[2], Jonathan Stuart Serody[3], Michael Ball[1], Rehman Qureshi[1], Thomas Condamine[1], Michael Klichinsky[1]

[1] Carisma Therapeutics, Philadelphia, PA, USA; [2] University of Pennsylvania Abramson Cancer Center, Philadelphia, PA; [3] UNC Lineberger Comprehensive Cancer Center, Chapel Hill, NC.

## Introduction

Adoptive cell therapies are effective in patients with hematologic malignancies but exert limited efficacy against solid tumors due to the immunosuppressive tumor microenvironment (TME) and heterogenous antigen expression.

We have previously developed a novel chimeric antigen receptor macrophage (CAR-M) cell therapy platform with potent anti-tumor activity in pre-clinical models[1].

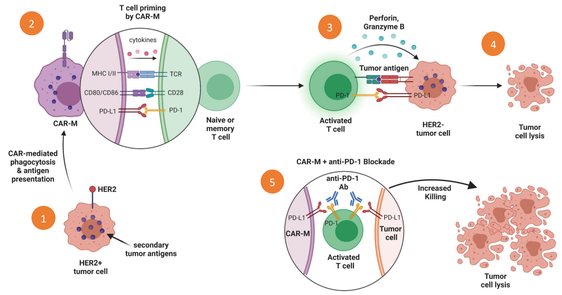
A Phase 1 FIH study evaluating the safety and feasibility of CT-0508 (a first in class CAR-M comprised of autologous human monocyte derived macrophages expressing an anti-HER2 CAR) is ongoing[2].

To evaluate the effect of CAR-M in HER2+ immunocompetent solid tumor models alone and in combination with programmed cell death 1 (PD-1) blockade, the objectives of this study were to:

1. **Investigate the impact of CAR-M on endogenous anti-tumor immunity**
2. **Evaluate the impact of CAR-M on the TME**
3. **Assess CAR-M + PD-1 blockade in a PD1 monotherapy resistant tumor models**

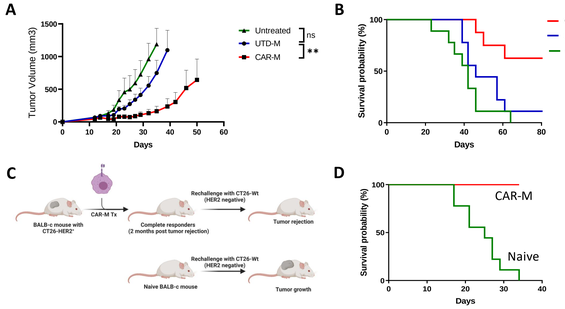
## Methods

Primary murine bone marrow-derived macrophages were engineered to express an anti-HER2 CAR using the chimeric adenoviral vector Ad5f35. To evaluate the safety and efficacy of CAR-M therapy, immunocompetent mice were engrafted with HER2+ tumors and treated with syngeneic CAR-M monotherapy or in combination with a PD1 blocking antibody. Analysis of the TME, TCR sequencing, and organ histology were used to probe the mechanism of action, pharmacokinetics, and pharmacodynamics of CAR-M therapy.




1. **CAR-M mediated phagocytosis of target tumor cells leads to capture of secondary tumor antigens.**
2. **CAR-M process and present secondary tumor antigens to T cells.**
3. **Primed T cells identify MHC peptide complexes on the surface of tumor cells.**
4. **CAR-M primed T cells lyse tumor cells and establish immune memory.**
5. **Anti-PD-1 blockade enhances CAR-M primed T cells cytotoxic effect.**

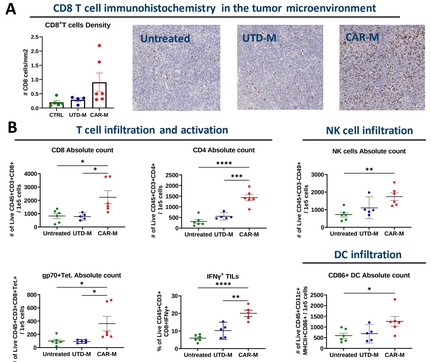
## CAR-M controlled tumor growth, improved survival and induced long-term protection against antigen-negative relapse



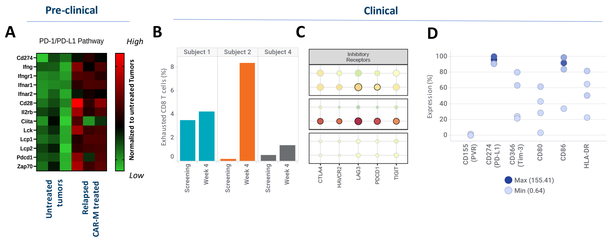

(**A**) CT26-HER2+ tumors were implanted s.c. in immunocompetent BALB/c mice. After 15 days, mice were treated with intratumoral (IT) injections of CAR-M, UTD-M or left untreated. CAR-M significantly reduced tumor progression and (**B**) increased long term survival compared to control groups. (**C**) Mice achieving complete responses (CR) post CAR-M therapy were re-challenged with HER2-negative CT26-Wt tumors to model antigen negative relapse. (**D**) Naive mice succumbed to disease within 35 days, while 100% of the mice from the CAR-M treatment group survived, indicating long-term tumor protection against antigen-negative relapse.

## CAR-M activated the TME and primed T cells

(**A**) Immunohistochemistry (IHC) assessment showed that CAR-M treatment increases tumor $CD8^+$ T cell infiltration in the CT26-HER2+ model indicating modulation of the TME. (**B**) Flow cytometric analysis showed increased infiltration of tumor CD4 and CD8 T cells, natural killer (NK) cells, activated CD86+ dendritic cells (DC) and tumor-associated antigen (TAA)-specific CD8 T cells (gp70+) in CAR-M treated mice. Ex vivo restimulation of tumor-infiltrating lymphocytes (TILs) with the $gp70_{423-431}$ peptide revealed that TILs from CAR-M-treated mice produced more IFN gamma in response to the antigen, indicating enhanced epitope spreading in the CAR-M treated group.

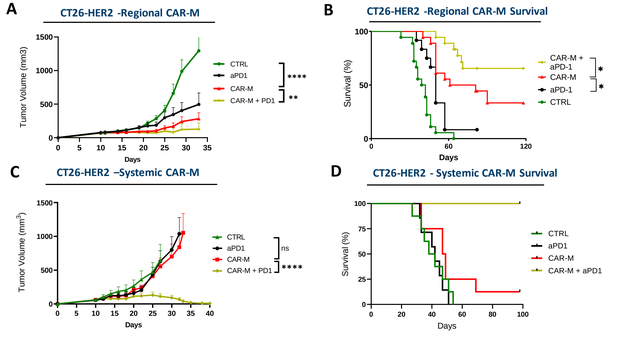



## CT-0508 remodeled the tumor immune landscape (CT-0508 Phase 1 FIH Study)



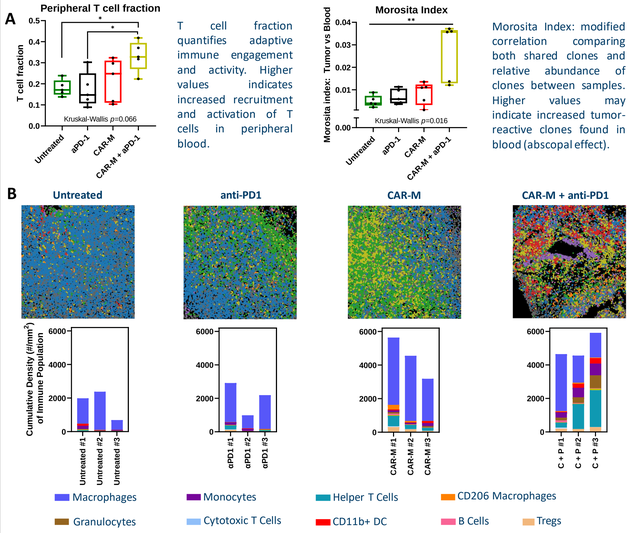

(A) RNAseq analysis performed on CT26-HER2 relapsed tumors reveled that the PD-1/PD-L1 pathway gene signature is increased after CAR-M treatment in non-responders. (B) ScRNA seq analysis performed on fresh tumor biopsy of participants from the CT-0508 Phase 1 FIH study in HER2 overexpressing tumors, showed increased frequency of exhausted T cells in all three participants 4 weeks after CT-0508 infusion. (C) Differential gene expression demonstrated increased expression of genes associated with T cells exhaustion. (D) Flow cytometry analysis on CT-0508 pre-infusion showed high expression level of PD-L1 protein as well as elevation of M1 markers. Taken together, preclinical and clinical results provide the rational for combining CAR-M with PD-1 blockade to enhance potency of therapy.

## CAR-M and anti-PD1 combination therapy improved tumor control and survival in CT26-HER2 model




To evaluate efficacy of CAR-M + anti-PD-1 combination therapy in anti-PD1-resistant tumor models, CT26-HER2 colorectal tumors were implanted subcutaneously in the lower back of BALB/c mice. (A) Combination of regional (IT) CAR-M and systemic (IP) anti-PD-1 lowered CT26-HER2 progression and (B) increased mice survival compared to controls (cumulative data from 3 experiments). (C and D) Combination of systemic (IV) CAR-M + systemic (IP) anti-PD-1 reduced tumor progression and improved survival rate of CT26-HER2-bearing mice compared to monotherapies.

## CAR-M + anti-PD1 combination therapy modulated the TME and TCR Repertoire




T cell fraction quantifies adaptive immune engagement and activity. Higher values indicates increased recruitment and activation of T cells in peripheral blood.

Morosita Index: modified correlation comparing both shared clones and relative abundance of clones between samples. Higher values may indicate increased tumor-reactive clones found in blood (abscopal effect).

(A) T cell receptor (TCR) sequencing analysis in mice receiving combination therapy suggests T cell recruitment, diversity, and enhanced adaptive anti-tumor immunity. (B) Multiplexed IHC showed that the tumor/fibroblast population decreased by half in the CAR-M and the combo groups, whereas immune cell densities more than double in these same CAR-M and combo groups. T cells were significantly higher in both the CAR-M and CAR-M + anti-PD-1 samples compared to control. Macrophages tended to be greatest in the CAR-M sample while helper T cells, DC, neutrophils and monocytes were greatest in the combo group.

## Conclusions

- **In vivo, CAR-M reduced tumor burden, prolonged overall survival in CT26-HER2+ tumor and induced long-term anti-tumor immune response against antigen-negative relapses.**
- **CAR-M reprogrammed the TME and primed T cells against secondary antigens.**
- **In a Phase 1 FIH study, CT-0508 increased intratumoral infiltration of T cells which exhibited an exhausted phenotype.**
- **CAR-M + anti-PD-1 combo improved tumor control, prolonged survival and synergistically modulated the TME in HER2+ solid tumor models.**

1. Klichinsky M, et al. Human chimeric antigen receptor macrophages for cancer immunotherapy. Nat Biotechnol. 2020;38(8):947-95

2. Reiss K.A. et al. A phase 1, first-in-human (FIH) study of the anti-HER2 CAR macrophage CT-0508 in subjects with HER2 overexpressing solid tumors. ASCO (2022) #2533

Graphics were created using BioRender (BioRender.com)

carisma THERAPEUTICS

# A Phase 1, First in Human (FIH) Study of Autologous Macrophages Containing an Anti-HER2 Chimeric Antigen Receptor (CAR) in Participants with HER2 Overexpressing Solid Tumors

Kim A. Reiss,[1] Yuan Yuan,[2] Naoto T. Ueno,[3] Melissa L. Johnson,[4] E. Claire Dees,[5] Mathew Angelos,[1] Joseph Chao,[2] Saar I. Gill,[1] Olga Shestova[1], Jonathan S. Serody, Saul J. Priceman, Amy Ronczka[6], Rehman Qureshi,[6] Poonam Sonawane,[6] Daniel Cushing,[6] Debora Barton,[6]* Michael Klichinsky,[6] Thomas Condamine,[6] Ramona F. Swaby,[6] Yara Abdou[5]

1. University of Pennsylvania Abramson Cancer Center, 2. City of Hope Cancer Center, 3. The University of Texas M.D. Anderson Cancer Center, 4. Sarah Cancer Research Institute, 5. University of North Carolina Lineberger Comprehensive Cancer Center, 6. Carisma Therapeutics.

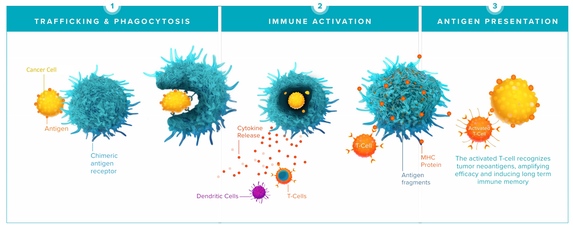
## Introduction

Macrophages are phenotypically plastic cells that are abundant in the solid tumor microenvironment (sTME) and can promote tumor growth (M2) or enhance anti-tumor immunity (M1). Macrophage function can be redirected by CAR expression to selectively target and phagocytose antigen overexpressing cancer cells. CAR macrophages can reprogram the sTME and present neoantigens to T cells, leading to epitope spreading and anti-tumor immune memory.

CT-0508 is comprised of autologous monocyte-derived pro-inflammatory macrophages expressing an anti-HER2 CAR. Pre-clinical studies showed that CT-0508 induced targeted cancer cell phagocytosis while sparing normal cells, decreased tumor burden, prolonged survival, and was safe and effective in humanized xenograft mouse models of human HER2 overexpressing ovarian cancer. In immunocompetent mouse models of HER2 overexpressing solid tumors, syngeneic anti-HER2 CAR-M mediated tumor control and improved overall survival as compared to untreated or control untransduced macrophage (UTD) treated mice. Notably, anti-HER2 CAR-M treatment led to significant activation of the sTME, with notable infiltration of CD8+ and CD4+T cells, NK cells, dendritic cells, and an increase in activated CD8+tumor infiltrating lymphocytes.

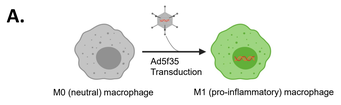
Anti-HER2 CAR-M were evaluated in a PD1 blockade resistant syngeneic model – mice that received both therapies had improved tumor control, overall survival, and sTME activation as compared to either treatment alone, indicating synergy and the capacity for CAR-M to sensitize solid tumors to checkpoint blockade.

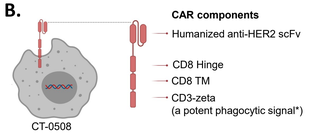
## CT-0508 Mechanism of Action

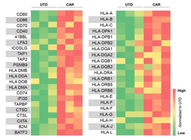



## CT-0508 is an M1-Polarized Anti-HER2 CAR-M

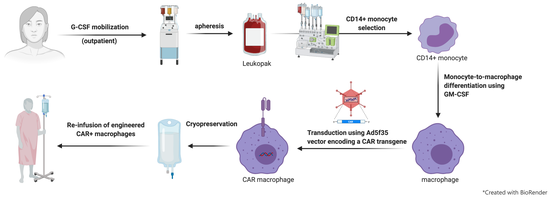
The chimeric adenoviral vector Ad5f35 is a pro-inflammatory, high-efficiency vector (A), that delivers the CAR (B) and simultaneously induces an M1 phenotype (C).



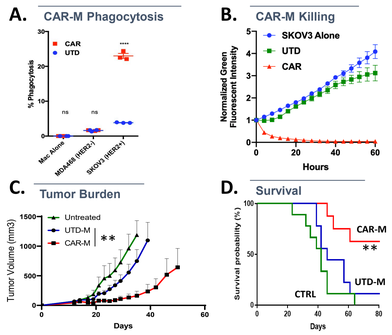

## Manufacturing Process



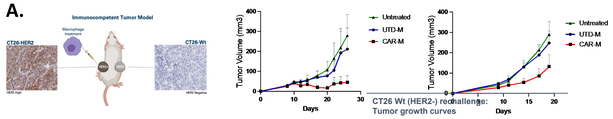

## CT-0508 is Effective in Preclinical Models

Insertion of a CAR confers on the macrophages the ability to selectively recognize, phagocytose (A) and kill HER2 overexpressing SKOV3 cancer cells, leading to targeted tumor cell eradication (B) and cytokine production (not shown).

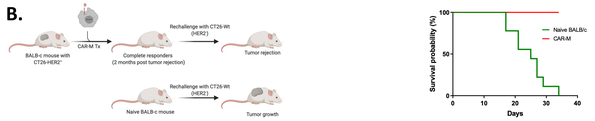
In a CT26-HER2 immunocompetent mouse model, CAR-M control tumor growth (C) and improve overall survival (D).




## Epitope Spreading Prevents Antigen Negative Relapse

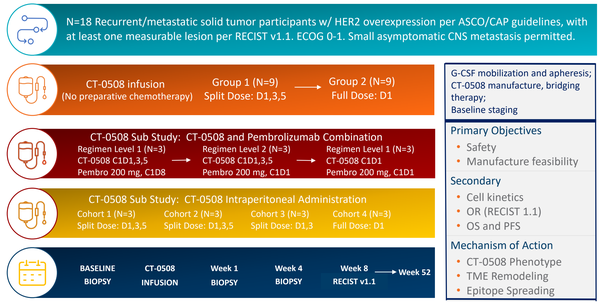



Abscopal effect is observed on HER2 negative tumor after intratumoral injection on HER2 positive tumor (A). CAR-M epitope spread led to rejection of antigen negative tumor rechallenge (B).




## CT-0508 Clinical Trial Design (NCT04660929)

This is an ongoing open label, first-in-human Phase 1 study to evaluate the safety, tolerability, cell manufacturing feasibility, trafficking, and preliminary evidence of efficacy of the investigational cell product CT-0508 in 18 subjects with advanced solid tumors overexpressing HER2.




G-CSF mobilization and apheresis; CT-0508 manufacture, bridging therapy; Baseline staging

**Primary Objectives**
- Safety
- Manufacture feasibility

**Secondary**
- Cell kinetics
- OR (RECIST 1.1)
- OS and PFS

**Mechanism of Action**
- CT-0508 Phenotype
- TME Remodeling
- Epitope Spreading

- Filgrastim (G-CSF), is being used to mobilize autologous monocytes into the peripheral blood for collection by apheresis.
- The CT-0508 cell product is then prepared, cryopreserved and released.
- The first 3 subjects in the study will be hospitalized for 8 days after the first infusion of CT-0508 (Day 1 to Day 8). There is no preparative chemotherapy prior to the cell product infusion.
- The first 9 subjects (Group 1) enrollment has been completed.
- Approximately 9 subjects in Group 2 will receive up to $5 \times 10^9$ of total manufactured CT-0508 cells on Day 1.
- AE reporting begins at the start of mobilization and continues until any toxicities resolve or are deemed irreversible. Subjects are continually reassessed for evidence of acute and/or cumulative toxicity.

## Objectives

**Primary**
- Assess the safety and tolerability of CT-0508 in subjects with HER2 overexpressing solid tumors.
- Assess the feasibility of manufacturing CT-0508.

**Secondary**
- Characterize the in vivo cellular kinetics profile (levels, persistence, trafficking) of CT-0508 transgene into peripheral blood and target tissues.
- Estimate the objective response rate (ORR), according to RECIST v1.1, of at least 1 dose of CT-0508 among subjects with HER2 overexpressing solid tumors.
- Estimate overall survival (OS).
- Estimate progression-free survival (PFS).
- Estimate duration of response (DOR).
- Estimate rates of 6-month and 12-month survival.

Tertiary/Exploratory
- Estimate iORR, iPFS, and iDOR.

## Main Eligibility Criteria

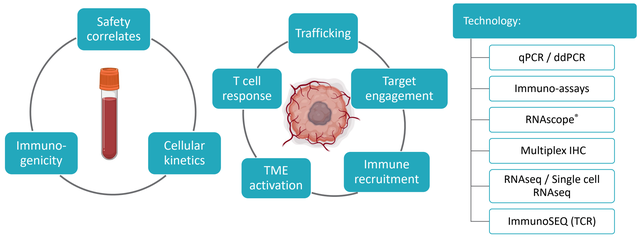
- Subjects with HER2-positive tumors after most recent therapy, by immunohistochemistry (IHC) using standard local assay resulting 3+, or 2+ with confirmation by In Situ Hybridization (ISH)
  - IHC and ISH assays and interpretation must follow the most recent ASCO/CAP guidelines and performed in an accredited laboratory. Other tumor types (non-breast, non-gastroesophageal) will be tested according to the breast cancer ASCO/CAP guidelines
- Female or male, at least 18 years of age
- Recurrent or metastatic solid tumor for which there are no available curative treatment options, AND after failure of, or ineligibility to receive the approved HER2 targeted agents, when available.
- Willingness to undergo serial biopsies
- At least one measurable lesion per RECIST criteria
- ECOG 0-1
- No concurrent infections or use of chronic steroids
- Satisfactory organ function

## Safety Observations and Assessments

- Adverse events of special interest have been selected according to other cell therapies and HER2 targeted agent experience and will be closely monitored. They include fever, cytokine release syndrome, hypersensitivity reactions, cardiovascular toxicity, ICANS and others. Cytokine release syndrome will be graded and treated following ASTCT Guidelines.
- Dose limiting toxicities will be observed for a period of 4 weeks, and reviewed by an independent Safety Review Committee.

## Correlative Plan

- Tumor tissue samples: subjects enrolled in Study 101 undergo one pre-treatment and 2 on-treatment biopsies to assess trafficking, target antigen engagement, TME reprogramming, epitope spreading, and other PK/PD assessments.
- Blood samples: collected over a period of 52 weeks for biomarker evaluation.




## Acknowledgements

**We are indebted to our patients, as well as the Clinical Trial Sites and Apheresis Unit staff of University of Pennsylvania, University of North Carolina, City of Hope, Sarah Cannon Research Institute Cancer Center and The University of Texas MD Anderson Cancer Centers.**

1. Klichinsky M, Ruella M, Shestova O, et al. Human chimeric antigen receptor macrophages for cancer immunotherapy. Nat Biotechnol. 2020;38(8):947-953.

www.HER2MacrophageTrial.com

# Pre-clinical Development of a CAR Monocyte Platform for Cancer Immunotherapy

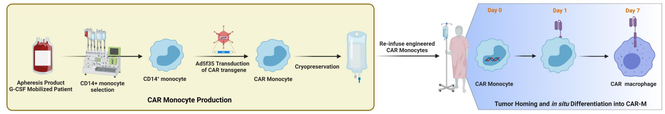
Linara Gabitova[1], Brett Menchel[1], Silvia Beghi[1], Larissa Ishikawa[1], Rehman Qureshi[1], Andrew Best[1], Sabrina Ceeraz DeLong[1], Sascha Abramson[1], Thomas Condamine[1], Daniel Blumenthal[1], Michael Klichinsky[1]

[1]Carisma Therapeutics, Philadelphia, PA

## Introduction

Engineered cell therapies have demonstrated significant clinical activity against hematologic malignancies, but responses against solid tumors remain rare. Our previously developed human chimeric antigen receptor macrophage (CAR-M) platform has shown potent anti-tumor activity in pre-clinical solid tumor models[1], and an anti-HER2 CAR-M product (CT-0508) is currently being evaluated in a Phase I trial. Use of myeloid cells for immunotherapy has the potential to overcome the main challenges presented by solid tumors – tumor infiltration, immunosuppression within the tumor microenvironment (TME), lymphocyte exclusion, and target antigen heterogeneity. Currently, CAR-M are generated in a week-long ex-vivo process in which peripheral blood monocytes are differentiated into macrophages prior to genetic manipulation. Here, we demonstrated the feasibility, phenotype, pharmacokinetics, durable CAR expression, cellular fate, specificity, and anti-tumor activity of human CD14+ CAR monocytes.

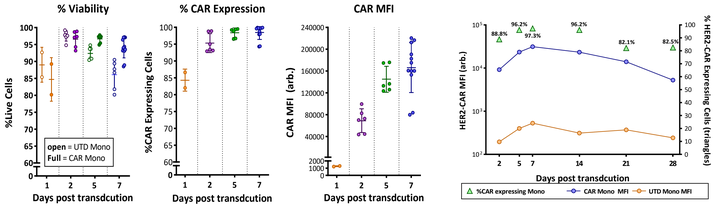
## Single day CAR Monocyte production process





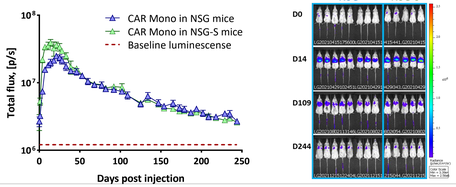


## Ad5f35 efficiently transduced human monocytes




Viability, %CAR expressing cells and CAR MFI of HER2-CAR transduced monocytes at different time points post transduction. (N=6 donors, day 1 N=2 donors) CAR expression persisted during at least 28 days of *in vitro* culture.

## Long-term *in vivo* persistence of human CAR Monocytes

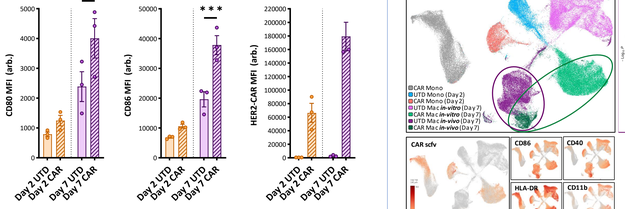






Ad5f35 engineered CAR Monocytes showed long term *in vivo* persistence in both NSG and NSG-S mice independent of human cytokine support.

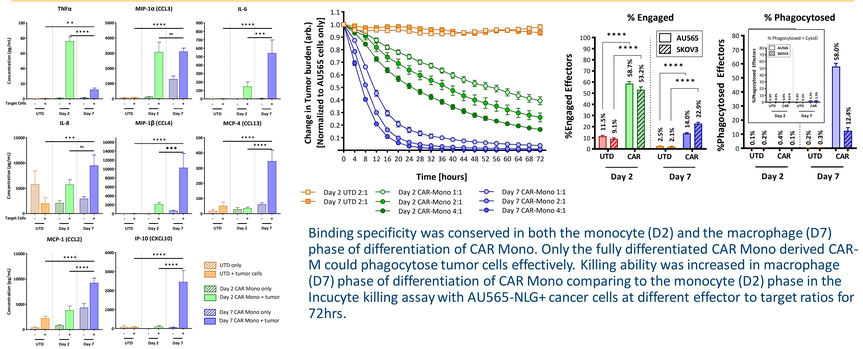
## CAR Monocytes differentiated into M1 CAR Macrophages *in vitro* and *in vivo*




CAR Mono showed a progressively increasing M1 phenotype. CAR Mono derived CAR-M showed morphology and phenotype similar to our current CAR-M product.

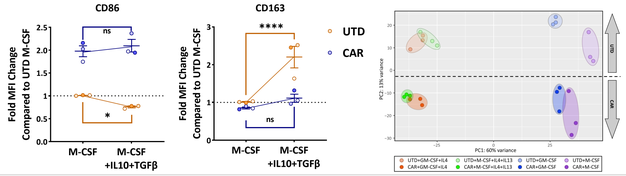
CAR Mono-derived CAR-M showed strong pro-inflammatory profile enhanced by intratumoral differentiation in the presence of HER2 expressing cancer cells. (scRNA-Seq, N=3 donors)

## CAR Monocyte anti-tumor activity increased with differentiation



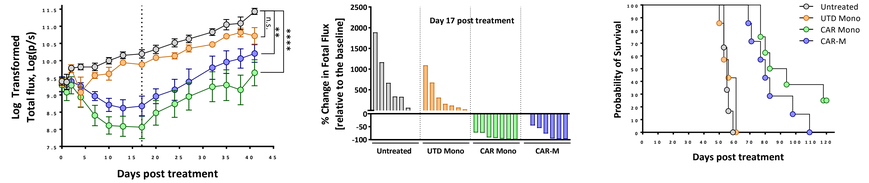

Binding specificity was conserved in both the monocyte (D2) and the macrophage (D7) phase of differentiation of CAR Mono. Only the fully differentiated CAR Mono derived CAR-M could phagocytose tumor cells effectively. Killing ability was increased in macrophage (D7) phase of differentiation of CAR Mono comparing to the monocyte (D2) phase in the Incucyte killing assay with AU565-NLG+ cancer cells at different effector to target ratios for 72hrs.

## CAR Monocytes were protected against M2 skewing

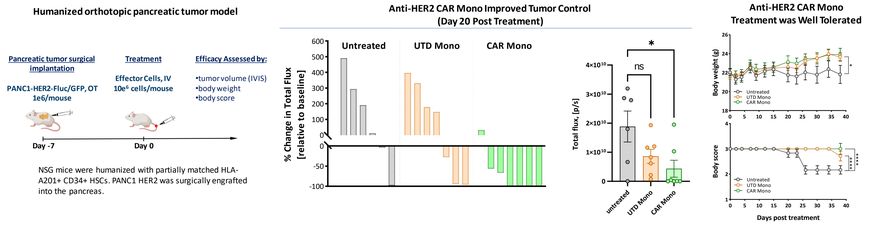



Impact of M2c skewing on expression of M1/M2 markers MFI normalized to UTD with M-CSF only. Bulk RNA sequencing showed M2a and MoDC skewed cells clustered together irrespective of UTD or CAR transduced state (N=3 donors).

## CAR Monocytes suppressed tumor growth *in vivo*




Human anti-HER2 CAR Mono suppressed the growth of HER2-expressing ovarian cancer SKOV3 following single intraperitoneal administration in a xenograft IP/IP mouse model. Mice treated with CAR Mono or CAR-M showed significant decrease of tumor burden as well as improved body score and body weight (not shown) and overall survival. Median Survival: Untreated/UTD = 55 days, CAR Mono = 83 days.




Human anti-HER2 CAR Mono suppressed the growth of pancreatic cancer PANC1-HER2 following single intravenous administration in the orthotopic tumor model in CD34+-humanized NSG mice. Mice treated with CAR Mono showed significantly lower signal at day 20 (P<0.05). Body weight and body score were significantly improved by the CAR Mono treatment.

## Conclusions:

1. Primary human CAR Monocytes were successfully generated with high efficiency and viability in a single day manufacturing process using Ad5f35
2. CAR Monocytes differentiated into M1 polarized CAR macrophages and resisted M2 subversion
3. CAR Monocytes showed targeted anti-tumor activity that increased with differentiation
4. CAR Monocytes suppressed tumor growth in xenograft and CD34+humanized mouse models with both regional and systemic administration *in vivo*

## References

1. Klichinsky M, et al. Human chimeric antigen receptor macrophages for cancer immunotherapy. Nature Biotechnology. 2020

Some Figures were created with BioRender.com

# A Phase 1, First-in-Human (FIH) Clinical Trial of the Anti-HER2 CAR Macrophage CT-0508 in Participants with HER2 Overexpressing Solid Tumors.

Kim A. Reiss[1], Yuan Yuan[2], Naoto T. Ueno[3], Melissa L. Johnson[4], E. Claire Dees[5], Mathew Angelos[1], Joseph Chao[2], Saar I. Gill[1], Olga Shestova[1], Jonathan S. Serody[5], Saul J. Priceman[2], Amy Ronczka[6], Rehman Qureshi[6], Poonam Sonawane[6], Daniel Cushing[6], Debora Barton[6], Michael Klichinsky[6], Thomas Condamine[6], Ramona F. Swaby[6], Yara Abdou[5]

1. University of Pennsylvania Abramson Cancer Center, 2. City of Hope Cancer Center, 3. The University of Texas M.D. Anderson Cancer Center, 4. Sarah Cancer Research Institute, 5. University of North Carolina Lineberger Comprehensive Cancer Center, 5. Carisma Therapeutics.

## Introduction

- In pre-clinical studies, CAR macrophages (CAR-M) phagocytose tumor cells, activate the tumor micro-environment (TME), recruit T-cells, and induce anti-tumor T cell immunity.
- CT-0508 is a first in class CAR-M, comprised of autologous monocyte derived macrophages expressing an anti-HER2 CAR.
- Here we present preliminary clinical results from the CT-0508 Phase 1 FIH study in participants with advanced HER2 overexpressing solid tumors.

## CT-0508 is an M1-Polarized Anti-HER2 CAR-M

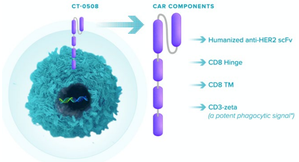
**Cells**: Autologous monocyte derived macrophages

**Vector**: Ad5f35

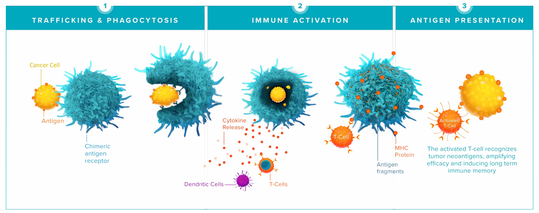
**Phenotype**: M1-polarized

**Target**: HER2

**FDA Fast Track Designation Granted Sept 2021**




## CT-0508 Mechanism of Action




## Phase 1 Clinical Trial Design

N=18 Recurrent/metastatic solid tumor participants w/ HER2 overexpression per ASCO/CAP guidelines, with at least one measurable lesion per RECIST v1.1. ECOG 0-1. Small asymptomatic CNS metastasis permitted.

CT-0508 infusion (No preparative chemotherapy) — Group 1 (N=9) Split Dose: D1,3,5 → Group 2 (N=9) Full Dose: D1

CT-0508 Sub Study: CT-0508 and Pembrolizumab Combination
Regimen Level 1 (N=3): CT-0508 C1D1,3,5 + Pembro 200 mg, C1D8
Regimen Level 2 (N=3): CT-0508 C1D1,3,5 + Pembro 200 mg, C1D1
Regimen Level 1 (N=3): CT-0508 C1D1 + Pembro 200 mg, C1D1

CT-0508 Sub Study: CT-0508 Intraperitoneal Administration
Cohort 1 (N=3) Split Dose: D1,3,5
Cohort 2 (N=3) Split Dose: D1,3,5
Cohort 3 (N=3) Split Dose: D1,3
Cohort 4 (N=3) Full Dose: D1

BIOPSY — CT-0508 — Week 1 BIOPSY — Week 4 BIOPSY — Week 8 RECIST v1.1 — Week 52

G-CSF mobilization and apheresis; CT-0508 manufacture, bridging therapy; Baseline staging

**Primary Objectives**
- Safety
- Manufacture feasibility

**Secondary**
- Cell kinetics
- OR (RECIST 1.1)
- OS and PFS

**Mechanism of Action**
- CT-0508 Phenotype
- TME Remodeling
- Epitope Spreading

## CT-0508 Manufacturing Process Overview

**Source**: Autologous mobilized peripheral blood

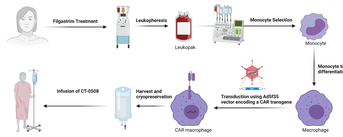
**Manufacturing time**: 1 week

**Vein to vein**: ~3 weeks

**Vector**: Ad5f35

**Process**: Automated

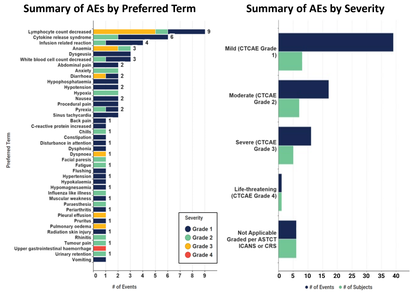
**Fill format**: Cryopreserved




## Summary of Treated Participants

| Summary of Participant and Tumor Characteristics | | | |
|---|---|---|---|
| **Characteristic** | **N = 9** | **Characteristic** | **N = 9** |
| Median age (range), years | 58 (45, 73) | Tumor Type, n (%) | |
| | | Breast Cancer | 4 (44.4) |
| | | Esophageal Cancer | 2 (22.2) |
| | | Cholangiocarcinoma | 1 (11.1) |
| | | Ovarian Cancer | 1 (11.1) |
| | | Salivary Carcinoma | 1 (11.1) |
| Gender, n (%) | | | |
| Male | 3 (33.3) | | |
| Race, n (%) | | Median Number of Prior Cancer Therapies, n (range) | |
| White | 9 (100) | | 3 (2, 11) |
| ECOG PS, n (%) | | | |
| 0 | 6 (66.7) | Median Number of Prior Anti-HER2 Therapies, n (range) | |
| 1 | 3 (33.3) | | 2 (0, 9) |
| HER2 Overexpression, n (%) | | | |
| IHC 3+ | 7(77.8) | Prior Radiotherapy, n (%) | |
| IHC2+/FISH+ | 2(22.2) | Yes | 5 (55.6) |

## CT-0508 was Well Tolerated with No Dose-Limiting Toxicities



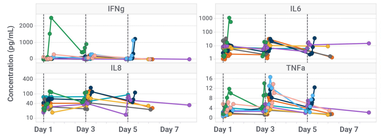

CT-0508 was well tolerated with most AEs being Grade 1-2. 5 SAEs were reported: 3 related (2 CRS & 1 infusion reaction) and 2 unrelated (1 GI hemorrhage and 1 worsening dyspnea both related to PD). No AEs leading to CT-0508 dose modification or discontinuation and no major organ toxicity were observed.

| Adverse Events of Special Interest | |
|---|---|
| **Category** | **N=9 (%)** |
| Infusion Reaction | |
| Grade 1 | 3 (33.3) |
| Grade 2 | 1 (11.1) |
| CRS (cytokine release syndrome) | |
| Grade 1 | 4 (44.4) |
| Grade 2 | 2 (22.2) |
| Grade 3-4 | 0 (0.0) |
| ICANS | 0 (0.0) |

## Overall Response

| Evaluated Participants | N = 9 |
|---|---|
| **Best Overall Response (RECIST 1.1)** | Stable Disease: 5 (55.6%) |

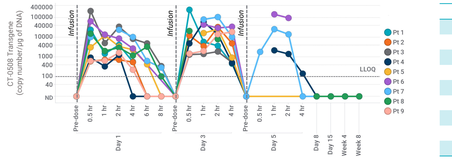
## CT-0508 Led to Transient Elevations of Pro-Inflammatory Cytokines




In concordance with clinical observations, minor and transient increases in serum IFNγ, IL-6, IL-8 and TNFα were observed. These increases were observed within an hour following infusion (dotted line) and resolved within 48 hours.

## CT-0508 Rapidly Migrated Out of the Blood and was Detected Within the TME

**Peripheral blood CT-0508 pharmacokinetics:**

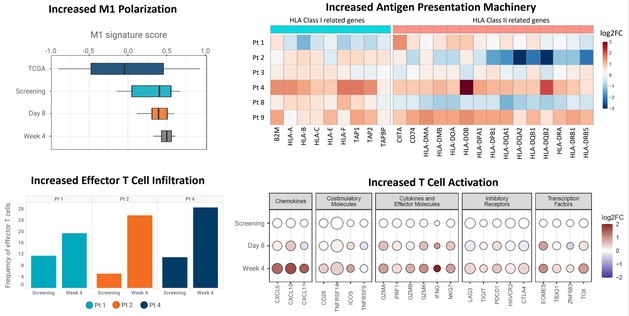



**CT-0508 detection in the TME:**

| Pt | Day 8 | Week 4 |
|---|---|---|
| 1 | - | + |
| 2 | + | + |
| 3 | + | - |
| 4 | - | + |
| 5 | + | N/A |
| 6 | + | - |
| 7 | + | - |
| 8 | + | - |
| 9 | - | - |

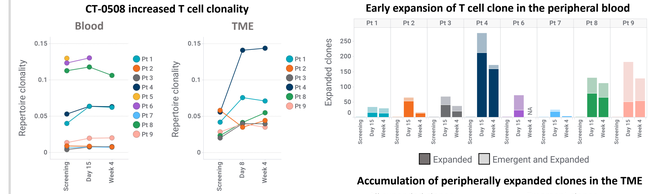
A similar pharmacokinetic profile was observed for all 9 participants enrolled in group 1, with CT-0508 detectable in blood on infusion days for 4-8 hours post-infusion, consistent with rapid migration of CAR-M from the blood to tissues following infusion. CT-0508 was detected within the TME of 8 of the 9 participants in group 1 using RNAscope™ technology.

## CT-0508 Activated the Tumor Microenvironment

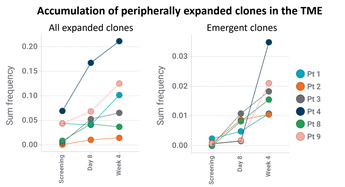



Transcriptomic analysis of biopsies collected pre and post treatment (Screening, Day 8 and week 4 post-infusion, n=6) demonstrated increase in M1 polarization (using a published M1 gene signature[2]), increase in antigen presentation machinery related genes (Fold change at week 4 compared to screening is depicted), increase in frequency of effector T cells (assessed in 3 patients with matched biopsies analyzed by scRNAseq) and increase in T cells activation (the plots are colored based on fold change compared to screening and size is based on the level of expression of each genes).

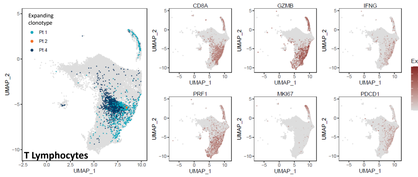
## CT-0508 Induced Increased T Cell Clonality and Led to the Accumulation of Newly Emergent T Cell Clones in the TME




TCR repertoire analysis revealed a significant increase in clonality as early as 15 days post infusion and expansion of T cell clones in the blood of participants post CT-0508 infusion. These changes are indicative of initiation of an adaptive immune response. These observations were associated with a similar increase in clonality in the TME and peripherally expanded and peripherally emergent clones were found to accumulate in the TME over time.




## Expanded T Cell Clones Adopted a Cytotoxic Phenotype in the TME




Single cell transcriptomic analysis demonstrated that expanding T cell clonotypes from patient 1, 2 and 4 at week 4 post-infusion clustered with cells expressing high levels of CD8α, perforin, granzyme B, Ki67, IFNγ, and PD1 demonstrating their activated and/or proliferating CD8 cytotoxic T lymphocyte phenotype.

## Conclusions

**CT-0508 Demonstrated Safety and Feasibility**
- CT-0508 was successfully manufactured
- No dose limiting toxicities observed
- No severe CRS, no ICANS, and no major organ toxicity observed
- Best Overall Response of Stable Disease (per RECIST 1.1) achieved in 5 of 9 participants

**Clinical Validation of CAR-M MOA**
- CT-0508 tumor infiltration was detected in 8 of 9 evaluated participants
- CT-0508 activated and remodeled the TME
- CT-0508 led to increased T cell clonality and significant expansion of newly emergent T cell clones within the TME with concomitant CD8 T cell activation, demonstrating induction of anti-tumor immunity

## Acknowledgements

**We are indebted to our patients, Clinical Trial Sites and Apheresis Unit staff, and our investigators.**

1. Klichinsky M, Ruella M, Shestova O, et al. Human chimeric antigen receptor macrophages for cancer immunotherapy. Nat Biotechnol. 2020;38(8):947-953.

University of Pennsylvania Abramson Cancer Center · City of Hope · The University of Texas MD Anderson Cancer Center · Making Cancer History® · Sarah Cannon Fighting Cancer Together™ · UNC Lineberger Comprehensive Cancer Center · carisma THERAPEUTICS

# Characterization of CT-0508, an Anti-HER2 Chimeric Antigen Receptor Macrophage (CAR-M), Manufactured from Patients Enrolled in the Phase 1, First in Human, Clinical Trial of CT-0508

Michael Ball[1], Madison Kremp[1], Rehman Qureshi[1], Poonam Sonawane[1], Maggie Schmierer[1], Josh VanDuzer[1], Michael Lynch[1], Adam Pfendt[1], Ken Locke[1], Ramona Swaby[1], Daniel Cushing[1], Michael Klichinsky[1], Thomas Condamine[1]

[1]Carisma Therapeutics, Philadelphia, PA, USA

## Introduction

- Macrophages are actively recruited and abundantly present in the solid tumor microenvironment (sTME). Tumor associated macrophages are predominantly immunosuppressive and support tumor growth (M2), while a subset of proinflammatory macrophages enhance anti-tumor immunity (M1).
- Pre-clinical studies have shown that chimeric antigen receptor macrophages (CAR-M) infiltrate tumors, phagocytose tumor cells, activate the sTME, recruit T cells, and present tumor antigens to T cells leading to epitope spreading and robust anti-tumor immunity.[1,2]
- CT-0508 is a cell product comprised of autologous monocyte-derived proinflammatory macrophages expressing an anti-HER2 CAR and is being investigated in a currently ongoing first in human clinical trial (NCT04660929).
- Here we show that functional CT-0508 was successfully manufactured with an M1 phenotype and that CAR-antigen interaction amplified the activation of CT-0508 CAR-M. These data confirm that M1 polarized, polyfunctional autologous CAR-M can be successfully manufactured from heavily pre-treated patients with advanced cancer.

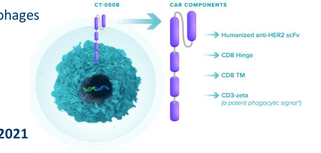
## CT-0508 is an M1-polarized anti-HER2 CAR-M

**Cells**: Autologous monocyte derived macrophages

**Vector**: Ad5f35

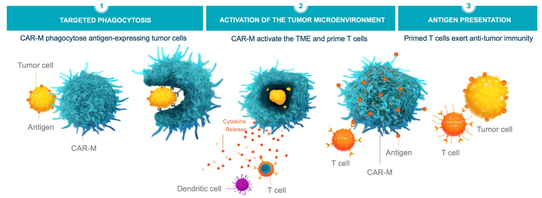
**Phenotype**: M1-polarized

**Target**: HER2




**FDA Fast Track Designation Granted Sept 2021**

## CT-0508 mechanism of action




## CT-0508 manufacturing process

**Source**: Autologous mobilized peripheral blood

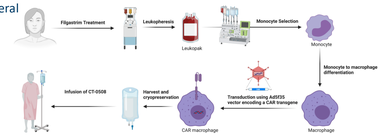
**Manufacturing time**: 1 week

**Vein to vein**: ~3 weeks

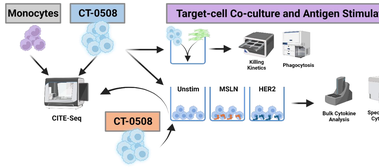
**Vector**: Ad5f35

**Process**: Automated

**Fill format**: Cryopreserved

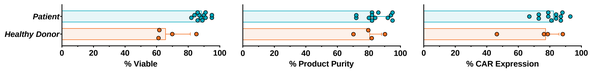



## Methods overview



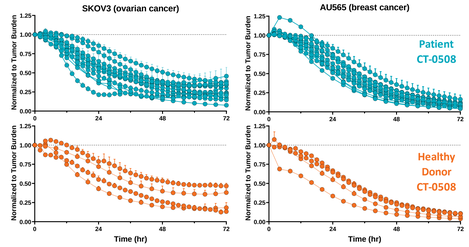

- CITE-Seq of **patient-derived CT-0508** and associated precursor monocytes.
- CT-0508 co-culture with target cells.
- HER2 stimulation of CT-0508.
- CITE-Seq of antigen-stimulated **healthy donor-derived CT-0508**.

## CT-0508 was successfully produced for Group-1 Patients



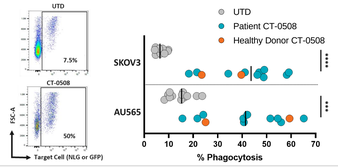

**Patient derived CT-0508** was successfully manufactured with high viability (left-panel), purity (middle-panel), and CAR expression (right-panel), in both patient derived product (n=12), and **healthy donor-derived CT-0508** (n=4).

## CT-0508 efficiently killed HER2$^+$ tumor cells *in vitro*



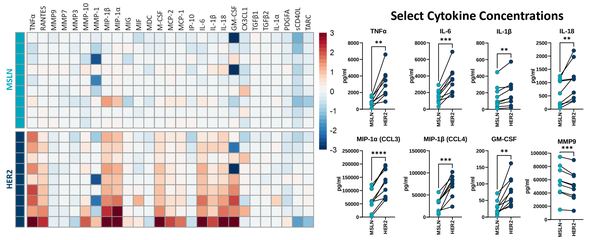

**Patient-derived CT-0508** (n=12) and **healthy donor-derived CT-0508** (n=4) can kill multiple different HER2+ tumor cells *in vitro*. Each line represents the killing of a single donor over the course of 72 hours normalized to the growth of the tumor cell line (dotted line).

## CT-0508 phagocytosed HER2$^+$ tumor cells *in vitro*



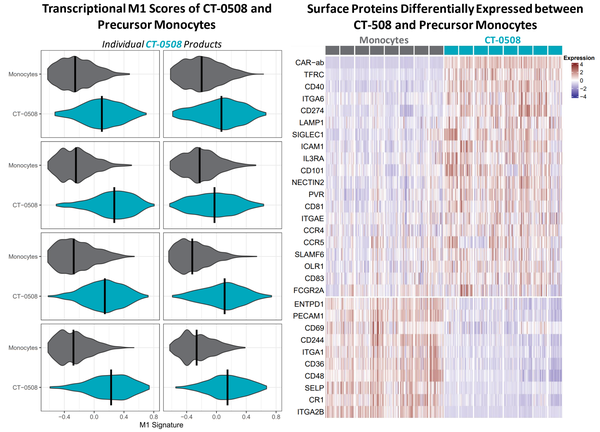

**Patient-derived CT-0508** (n=11) and **healthy donor-derived CT-0508** (n=2) can efficiently phagocytose multiple HER2+ cell lines after 4-hour co-incubation compared to **UTD**. Phagocytosis is defined as percentage of GFP/NLG+ cells within the macrophage population.

## Patient CT-0508 secreted pro-inflammatory cytokines in response to HER2 antigen stimulation



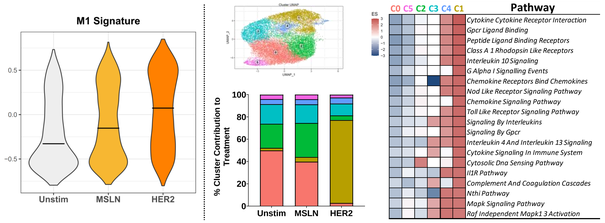

**Patient-derived CT-0508** (n = 9 )was stimulated for 4-hours in the presence of plate-bound HER2 protein, irrelevant protein control (plate-bound MSLN), or without protein (Unstim). Secreted factors were measured by multiplex MSD and changes over Unstim were observed for several cytokines (left panel).

## Single cell transcriptomic analysis demonstrates differentiation of CT-0508 to M1 macrophages



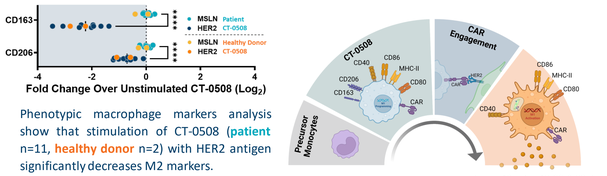

Manufacturing of **CT-0508** from isolated patient **monocytes** yields differentiated macrophages with an increased M1 gene signature[3] (n = 8, left panel). Changes in surface protein expression as captured by CITE-Seq (right panel) reflect this differentiation and polarization (ex. ↑TFRC, CD40, CD274, ICAM1, CD81)

## CAR engagement further activates the pro-inflammatory transcriptional profile of CT-0508




**Healthy donor-derived CT-0508** was stimulated for 4-hours in the presence of plate-bound HER2 protein, irrelevant protein control (plate-bound MSLN), or without protein (Unstim). scRNA-Sequencing revealed a unique transcriptional profile of HER2 antigen-stimulated CT-0508, potentiating an M1 phenotype and up-regulating several activation and immune-stimulatory pathways within the product.

## Target-specific activation of CT-0508 drives further decrease in M2 phenotypic markers




Phenotypic macrophage markers analysis show that stimulation of CT-0508 (**patient** n=11, **healthy donor** n=2) with HER2 antigen significantly decreases M2 markers.

## Key Takeaways

- CT-0508 was successfully manufactured for all patients in group 1 of the first in human Phase 1 clinical trial.
- CT-0508 specifically and efficiently killed and phagocytosed HER2$^+$ target tumor cells.
- Antigen-specific stimulation of patient derived CT-0508 induces expression of key pro-inflammatory factors, including TNFα, IL-1β, and IL-18, in addition to several chemotactic and growth factors.
- Single-cell transcriptomic and surface marker expression analysis confirms that CT-0508 is an M1 polarized CAR-macrophage product. In addition, CAR-mediated signaling further potentiates this phenotype by increasing the M1 gene signature and upregulating the expression of numerous genes involved in macrophage innate signaling and immune-stimulatory pathways.

## Acknowledgements

**We are indebted to our patients, as well as the Clinical Trial Sites and Apheresis Unit staff of University of Pennsylvania, University of North Carolina, City of Hope, Sarah Cannon Research Institute Cancer Center, and The University of Texas MD Anderson Cancer Center**

1. Klichinsky M, et al. Human chimeric antigen receptor macrophages for cancer immunotherapy. Nat Biotechnol. 2020;38(8):947-953.
2. Pierini S, et al. Chimeric Antigen Receptor Macrophages (CAR-M) Induce Anti-Tumor Immunity and Synergize With T Cell Checkpoint Inhibitors in Pre-Clinical Solid Tumor Models. AACR (2021) #63.
3. Sijin Cheng, et al. A pan-cancer single-cell transcriptional atlas of tumor infiltrating myeloid cells. Cell. 2021;184(3):792-809.
4. Graphics created with BioRender.com

carisma THERAPEUTICS