A phase 1, first-in-human study of autologous monocytes engineered to express an anti-HER2 chimeric antigen receptor (CAR) in participants with HER2-overexpressing solid tumors

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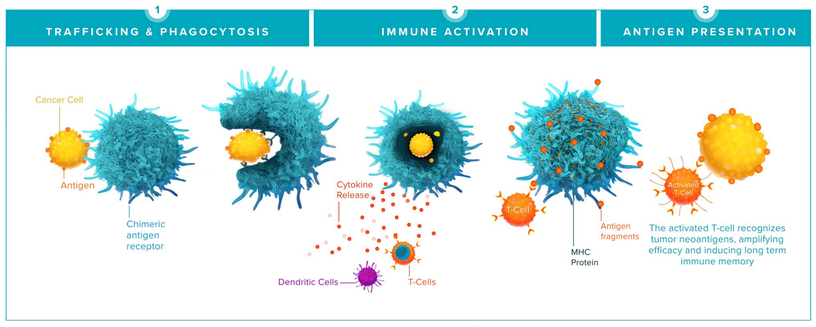
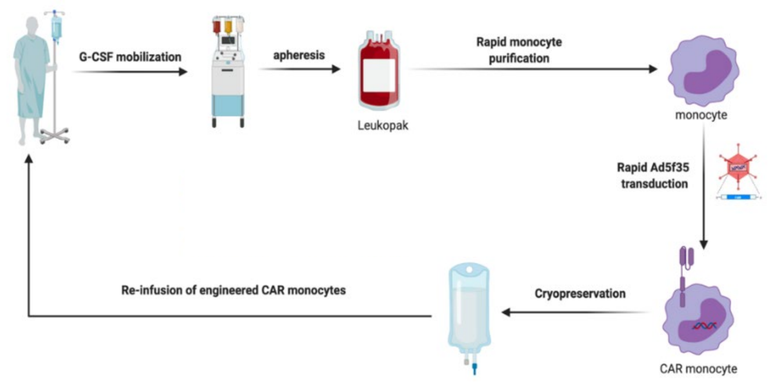
Introduction

Myeloid cells are actively recruited to the solid tumor microenvironment (TME) and have the potential to mediate tumor control via phagocytosis, TME remodeling, and T cell activation. We previously developed human chimeric antigen receptor macrophages and have shown potent anti-tumor activity in pre-clinical solid tumor models[1]. The anti-HER2 CAR-Macrophage cell therapy product, CT-0508, is currently being evaluated in a Phase I trial as a monotherapy and in combination with pembrolizumab. This ongoing first-in-human study evaluates the safety, tolerability, and manufacturing feasibility of CT-0508 along with several customary exploratory secondary endpoints. We have developed a next-generation CAR-Monocyte platform to potentially increase the dose, improve tumor trafficking, enhance persistence, and shorten the manufacturing and vein-to-vein time as compared to CAR-Macrophage therapy. CT-0525 is an autologous anti-HER2 CAR-Monocyte cell therapy based on $CD14^+$ monocytes engineered with an Ad5f35 adenoviral vector to express an anti-HER2 CAR. Pre-clinical studies have demonstrated the feasibility, phenotype, pharmacokinetics, durable CAR expression, cellular fate, antigen specificity, and anti-tumor activity of CT-0525[2]. Pre-clinical studies have shown that CT-0525 differentiated into pro-inflammatory CAR-Macrophages *in vivo* and controlled tumor growth. The CT-0525 manufacturing process takes one day and enables the production of up to 10 billion cells from a single apheresis. CT-0525 is being investigated in a first-in-human, open-label, multi-center, Phase I study in patients with HER2 overexpressing solid tumors.

CT-0525 Mechanism of Action

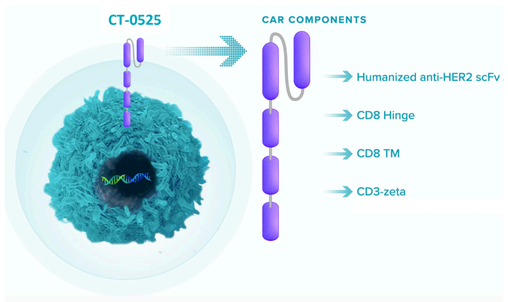


CT-0525 Manufacturing Process



CAR-Monocyte manufacture is a 1-day fully automated process performed on the Miltenyi Prodigy. Vein to vein time is ~2 weeks.

CT-0525 – HER2 Targeted CAR-Monocytes

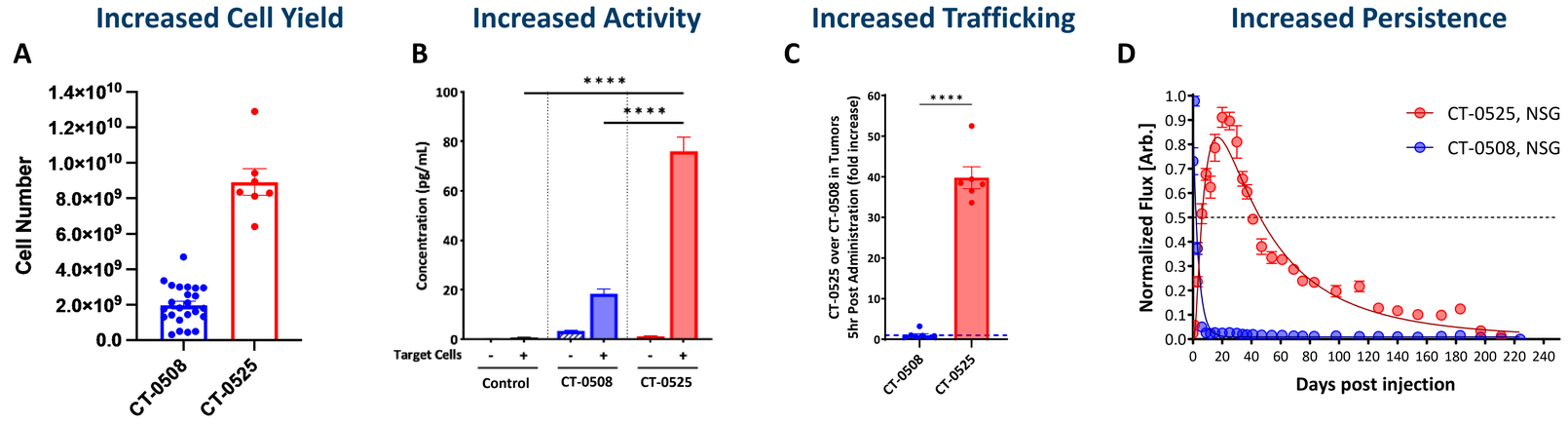


CT-0525 Product	
Cells	Autologous monocytes
Vector	Ad5f35
Phenotype	M1
CAR	1st Generation

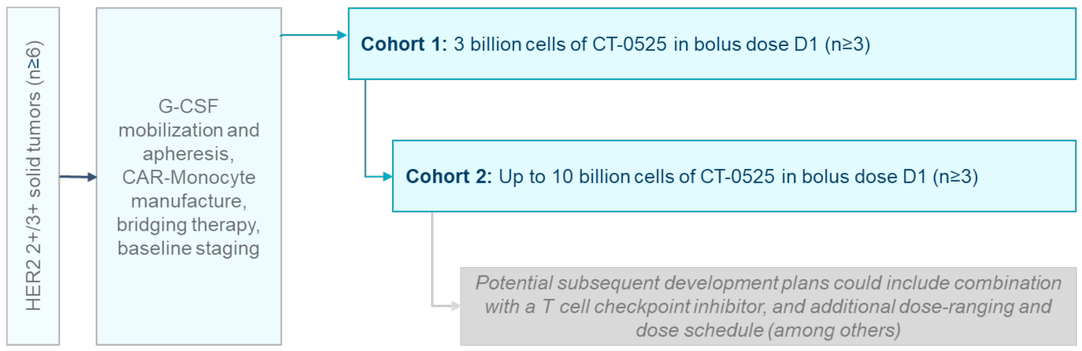
CT-0525 is comprised of autologous monocytes engineered using a chimeric adenoviral vector Ad5f35 that delivers a first generation anti-HER2 CAR and simultaneously induces an M1 phenotype.

CT-0525 CAR-Monocytes demonstrate improved function, trafficking and persistence over CT-0508 CAR-Macrophages

CT-0525 manufacturing yields ~5x more cells than CT-0508 **(A).** Pre-clinical studies demonstrated that CT-0525 CAR-Monocytes have improved function in vitro as demonstrated by their improved ability to kill (not shown) and secrete pro-inflammatory cytokines **(B)** upon co-culture with HER2 overexpressing tumor cell lines. CT-0525 also demonstrated improved trafficking potential into solid tumors **(C)** and *in vivo* persistence **(D)** in immuno-compromised mouse models, relative to CT-0508.



CT-0525 Clinical Trial Design (NCT06254807)



Filgrastim (G-CSF), is being used to mobilize autologous monocytes into the peripheral blood for collection by apheresis. The CT-0525 cell product is then prepared, cryopreserved and released. There is no preparative chemotherapy prior to the cell product infusion. Approximately 6 participants will be enrolled in 2 cohorts exploring two different dose regimens ($3x10^9$ CAR^+ cells and up to $10x10^9$ CAR^+ cells). AE reporting begins at the start of mobilization and continues until any toxicities resolve or are deemed irreversible. Participants are continually reassessed for evidence of acute and/or cumulative toxicity.

Study Objectives

Primary

- Assess the safety and tolerability of CT-0525 in participants with HER2 overexpressing solid tumors.
- Assess the feasibility of manufacturing CT-0525.

Secondary

- Characterize the *in vivo* cellular kinetics profile (levels, persistence, trafficking) of CT-0525 transgene into peripheral blood and target tissues.
- Estimate the objective response rate (ORR), according to RECIST v1.1, of CT-0525 by assessing the proportion of participants with a confirmed objective response.
- Estimate duration of response (DOR).

Tertiary/Exploratory

- Estimate progression-free survival (PFS).
- Estimate overall survival (OS).

Main Inclusion Criteria

- Participant must be ≥ 18 years of age
- Tumor tissue that is HER2-positive, following most recent therapy, by IHC using standard local assay resulting in 3+, or 2+ with confirmation by ISH testing.
  - ✓ IHC, ISH assays and interpretation must follow the most recent ASCO/CAP guidelines and be performed in an accredited laboratory. Other tumor types (non-breast, non-gastroesophageal) will be tested according to the breast cancer ASCO/CAP guidelines
- Histologically confirmed recurrent or metastatic solid HER2-positive tumor for which there are no available curative treatment options, and after failure of approved systemic therapies used for the treatment of recurrent (unresectable) and/or metastatic disease
- Willingness to undergo serial biopsies
- At least one measurable lesion per RECIST v1.1 criteria
- ECOG 0-1 at screening
- No concurrent infections
- Good organ function

Safety Observations and Assessments

- Adverse events of special interest have been selected according to experience from other cell therapies and HER2 targeted agents and will be closely monitored. They include fever, cytokine release syndrome, hypersensitivity reactions, cardiovascular toxicity, Immune effector Cell Associated Neurotoxicity Syndrome (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 Studies

- **Peripheral blood:** Samples are collected over a period of 52 weeks for biomarker evaluation.
- **Tumor Biopsy:** Participants enrolled in Study 102 undergo one pre-treatment and 2 on-treatment biopsies (7 days and 4 weeks post infusion).

Peripheral blood	**Safety Correlates**	• Serum cytokines • Immunogenicity
	Cellular Kinetics	• Blood persistence • Trafficking
Tumor biopsy	**Mechanism of action**	• Target engagement • TME activation • Immune cell recruitment • Adaptive immune response

Current Status

The study is currently enrolling participants in the USA and the following sites are supporting the study (* denotes initiated sites):

- UNC Lineberger Comprehensive Cancer Center*
- MD Anderson Cancer Center*
- OHSU Knight Cancer Institute
- Mayo Clinic Comprehensive Cancer Center
- Cedars-Sinai, Los Angeles, CA
- Roswell Park Comprehensive Cancer Center
- University of Pennsylvania Abramson Cancer Center
- Cleveland Clinic Cancer Center
- University of Cincinnati Cancer Center*

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- 1. Klichinsky M, et al. Human chimeric antigen receptor macrophages for cancer immunotherapy. Nature Biotechnology. 2020; 38: 947-953.
- 2. Gabitova L, et al. 318 - Pre-clinical development of a CAR monocyte platform for cancer immunotherapy. Journal for ImmunoTherapy of Cancer 2022;10: doi: 10.1136/jitc-2022-SITC2022.0318.

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