A mesothelin targeting chimeric antigen receptor macrophage (CAR-M) for solid tumor immunotherapy: pre-clinical development of CT-1119

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Abstract 4053

Abstract

While adoptive cell therapies have seen significant success in the treatment of hematological malignancies, solid tumors remain challenging for the field. A significant obstacle is the exclusion of T cells from the tumor microenvironment (TME). In contrast, monocytes/macrophages are naturally recruited to the TME. These cells then have the potential to phagocytose tumor cells, activate the TME, and prime a broad anti-tumor adaptive immune response via T cell recruitment and activation. We have previously developed CT-0508, a chimeric antigen receptor macrophage (CAR-M) targeting HER2 which showed efficacy in a variety of pre-clinical models and is currently in a Phase I clinical trial for patients with HER2+ solid tumors. Mesothelin is overexpressed in a variety of solid tumors, including mesothelioma, lung, pancreatic, and ovarian cancers. To leverage tumor biology with myeloid cells, we engineered primary human macrophages using the chimeric adenoviral vector Ad5f35 to express a CAR containing a human scFv against human mesothelin. We used both *in vitro* cell based assays and *in vivo* xenograft models to assess the activity of CT-1119. 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 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. 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

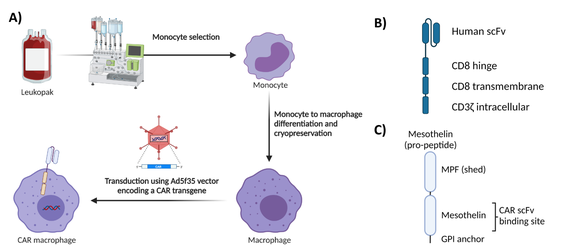

Figure 1 – CT-1119 production and CAR design. **A)** Schematic highlighting the process for the manufacturing of CT-1119 for use in pre-clinical studies. Monocytes isolated from donor leukopaks are differentiated into macrophages via culture with GM-CSF, cryopreserved, thawed, and then transduced with 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. **C)** Diagram of the mesothelin pro-peptide and CAR binding site.

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

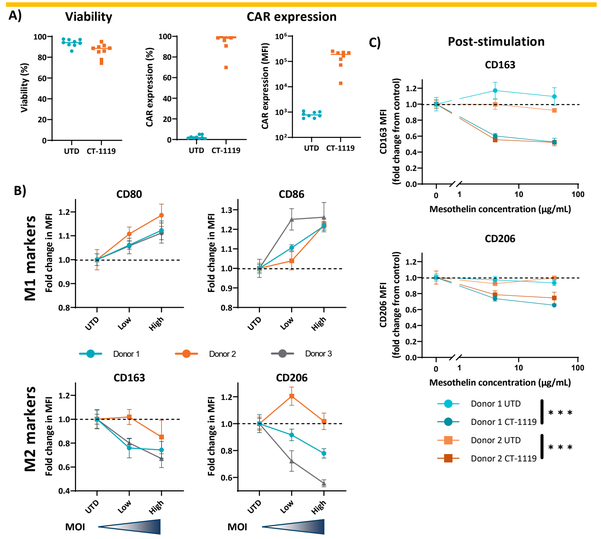

Figure 2 – Flow cytometric characterization of CT-1119. **A)** Plots of viability, the percent of live macrophages expressing the anti-mesothelin CAR, and the MFI of mesothelin CAR expression of all live cells across n=8 independent donors. Line indicates the mean value of all donors. **B)** Comparison 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. **C)** Change in MFI by UTD and CT-1119 following a 24-hour stimulation with recombinant human mesothelin.

CT-1119 has antigen-mediated phagocytosis, killing, and cytokine release

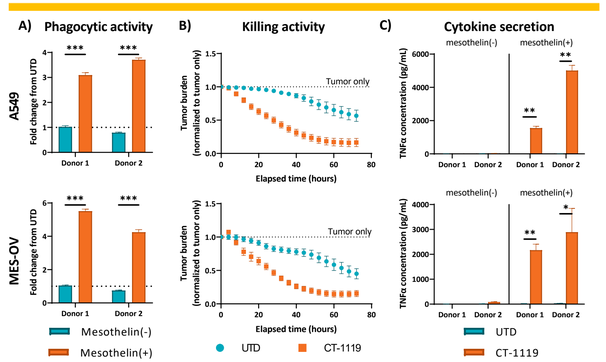

Figure 3 – *In vitro* characterization of the functional activity of CT-1119. **A)** Comparison of the phagocytic ability of UTD-M and CT-1119 macrophages against two target cell lines: lung adenocarcinoma A549 and ovarian cystadenocarcinoma MES-OV. CT-1119 shows a significant increase in the level of phagocytosis compared to UTD only when mesothelin is present on the target cell. **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.

CT-1119 exhibited durable anti-tumor activity in vitro

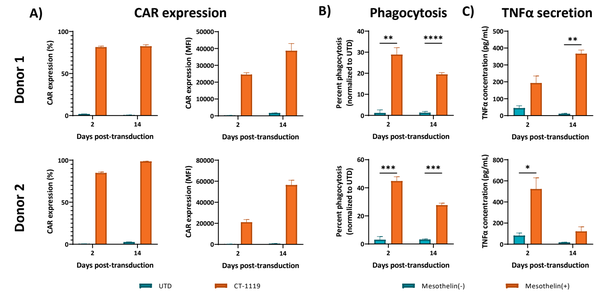

Figure 4 – CT-1119 expressed CAR and was functional at an early and late timepoints. **A)** Percent of live macrophages expressing anti-mesothelin CAR and CAR expression levels for two timepoints post-transduction (days 2 and 14). **B)** CAR-M show target specific phagocytosis for at least 14 days following 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. CT-1119 was responsive to antigen for at least four weeks (data not shown).

CT-1119 reduced tumor burden in a lung cancer model

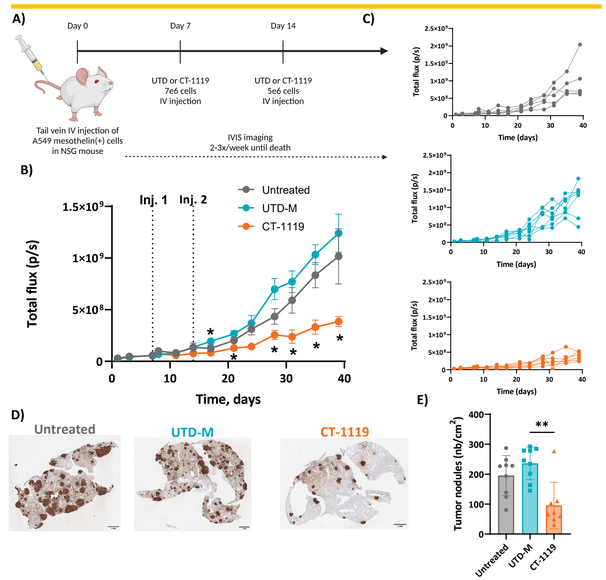

Figure 5 – *In vivo* assessment of CT-1119 therapy activity in a metastatic lung cancer xenograft model. **A)** Schematic of the in vivo experiment design. NSG mice were injected with A549 mesothelin/CBG expressing cells and the tumor allowed to establish. On days 7 and 14 post-tumor injection, donor 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. **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.

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

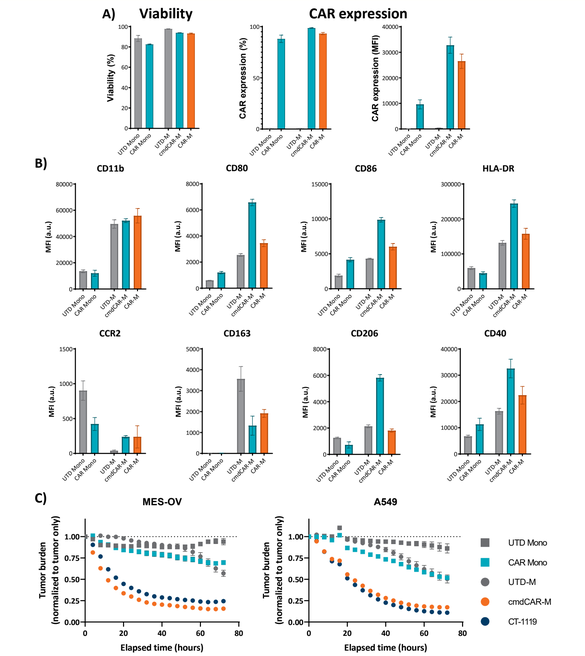

Figure 6 – Production of anti-mesothelin CAR monocytes. Manufacturing of CAR monocytes is a single day process immediately following isolation from the leukopak. Differentiation of CAR monocytes in vitro leads to CAR monocyte-derived CAR macrophages (cmdCAR-M) following a week of culture. CAR-M were 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. **B)** Expression of macrophage phenotype markers. Transduction with Ad5f35 containing CAR did not impact the differentiation of monocytes in macrophages. **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.

Key results

- **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**
- **CT-1119 reduced tumor burden in a mesothelin overexpressing metastatic lung cancer xenograft model**
- **Anti-mesothelin CAR-Monocytes could be produced and differentiated into functional M1-polarized CAR-M**

Figures 1A, 1B, 1C, and 5A created with Biorender

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