## 194 PRE-CLINICAL DEVELOPMENT OF CT-1119, A MESOTHELIN TARGETING CHIMERIC ANTIGEN RECEPTOR MACROPHAGE (CAR-M), FOR SOLID TUMOR IMMUNOTHERAPY

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**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.

### REFERENCES

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**Ethics Approval** All studies involving animals were approved by the IACUC of the Wistar Institute (protocol 201364).

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