

## TUMOR-SPECIFIC REACTIVITY AND EFFECTOR FUNCTION OF CHIMERIC ANTIGEN RECEPTOR ENGINEERED MACROPHAGES TARGETING MUC1

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**Background** Chimeric antigen receptors (CAR) have demonstrated remarkable efficacy in licensing T cells for antitumor responses against hematopoietic malignancies but have had limited success against solid tumors. Macrophages, both archetypic phagocytes and professional antigen presenting cells, may exert profound effector functions which complement adaptive cellular immunity.<sup>1</sup> Recently, it was shown that human macrophages engineered to express CARs (CAR-Ms) demonstrated antigen-specific phagocytosis, inhibited solid xenograph tumors, and induced an inflammatory tumor microenvironment boosting antitumor T cell responses.<sup>2</sup> Kimura et al. previously completed the first prophylactic cancer vaccine trial based on a non-viral antigen, tumor-associated hypoglycosylated Mucin 1 (MUC1).<sup>3</sup> A panel of fully-human affinity-matured MUC1-specific antibodies raised in healthy subjects following immunization was identified from these patients.<sup>4</sup> Using these MUC1-specific scFv domains for CAR generation, we have now engineered MUC1-targeting CAR-Ms that may potentially possess reduced off-target specificities.

**Methods** Lentiviral CAR expression vectors containing the scFv domains of three unique hypoglycosylated MUC1-specific antibodies or a CD20-specific antibody, the CD3zeta signaling domain, and CD28 and OX40 co-stimulatory domains were constructed. The human monocyte/macrophage U937, SC, and THP-1 lines were stably transduced and flow-sort purified to generate MUC1- or CD20-specific CAR-Ms. CAR-Ms were differentiated into macrophages via 48 hour PMA treatment, and subsequently evaluated for antigen-specific function against MUC1- and/or CD20-expressing K562, ZR-75-1, and Raji cells or cancer cells isolated from solid lung tumors or malignant pleural effusions. CAR-M phenotype was evaluated by flow cytometry following in vitro differentiation and polarization with conventional 'M1' and 'M2' stimuli. Phagocytosis and lysosomal processing of phagocytosed cargo were evaluated by fluorescence microscopy of GFP/CellTrace labeled targets or detection of pH-sensitive pHrodo expression following CAR-M and tumor cell co-culture, respectively. Antigen-specific cytokine production was determined via cytometric bead array following co-culture of CAR-Ms with MUC1- or CD20-expressing tumor cells or 100mer MUC1 peptide.

**Results** Differentiated CAR-Ms possessed an inflammatory phenotype expressing IL-8 and CD86 which was further enhanced by IFN $\gamma$  or LPS treatment and was resistant to 'M2' polarization with conventional stimuli. CAR-Ms exhibited phagocytosis and subsequent lysosomal processing in an antigen-specific manner, with minimal reactivity against tumor cell targets in the absence of the corresponding MUC1 or CD20 antigen. MUC1-specific CAR-Ms stimulated with MUC1 peptide or MUC1+ tumor cells secreted robust levels of pro-inflammatory IL-8, TNF $\alpha$ , and IL-1 $\beta$ , but not immunosuppressive IL-10.

**Conclusions** MUC1-targeting CAR-Ms exert potent tumor-restricted effector function in vitro and may provide a novel treatment strategy either alone or in potential synergistic combination with T cell-mediated immunotherapies.

**Acknowledgements** The authors would like to thank Dr. Olivera J. Finn for generously providing reagents and guidance

and Dr. Michael T. Lotze for his mentorship. This study was supported by funding from the University of Pittsburgh's Department of Cardiothoracic Surgery to ACS and RD.

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**Ethics Approval** The study was approved by the University of Pittsburgh's Institutional Review Board approval number CR19120172-005.

<http://dx.doi.org/10.1136/jitc-2021-SITC2021.112>