318 PRE-CLINICAL DEVELOPMENT OF A CAR MONOCYTE PLATFORM FOR CANCER IMMUNOTHERAPY

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Background 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 demonstrate the feasibility, phenotype, pharmacokinetics, durable CAR expression, cellular fate, specificity, and anti-tumor activity of human CD14+ CAR monocytes.

Methods Using the chimeric adenoviral vector Ad5f35, we engineered primary human CD14+ monocytes to durably express an anti-HER2 CAR (CAR-mono). Using a partially automated approach, we established a process that allowed for same day manufacturing (from Leukopak to cryopreserved CAR-mono cell product).

Results CAR-mono showed high CAR expression and viability (>90%), and efficiently differentiated into CAR-expressing macrophages. The production process was designed to precondition CAR-mono to differentiate into M1-like CAR macrophages with strong pro-inflammatory effector functions. CAR-mono derived CAR-M (cmdCAR-M) demonstrated potent anti-tumor activity regardless of exposure to GM-CSF or M-CSF, and were protected against M2 switching by immunosuppressive factors. Treating CAR-mono with GM-CSF and IL-4 resulted in their differentiation to monocyte-derived CAR-DCs with an activated phenotype, indicating that these cells retained their myeloid differentiation potential. *In vivo*, intravenous administrated CAR-mono demonstrated the ability to traffic to tumors and showed remarkable long-term CAR expression and persistence (>180 days) in both NSG and NSG-S mouse models, demonstrating lasting persistence and CAR expression irrespective of human cytokine support. CAR-mono differentiated into strong pro-inflammatory CAR-M even when injected directly into well-established tumors. Finally, CAR-mono induced anti-tumor activity in various HER2+ solid tumor xenograft models.

Conclusions The CAR-mono platform allows for a rapid, same-day manufacturing process while maintaining the key characteristics of CAR-M therapy. Ad5f35 engineered human CAR monocytes are primed toward M1 macrophage differentiation, demonstrate durable CAR expression and persistence, and produce a cell population highly similar to our established CT-0508 product. These data provide strong pre-clinical support to advance the CAR-mono platform into clinical testing.

REFERENCE

1. Klichinsky M, *et al*. Human chimeric antigen receptor macrophages for cancer immunotherapy. *Nature Biotechnology*. 2020; **38**: 947-953.