104 DEVELOPMENT AND CHARACTERIZATION OF HUMAN CHIMERIC ANTIGEN RECEPTOR MONOCYTES (CAR-MONO), A NOVEL CELL THERAPY PLATFORM

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Background Engineered cell therapies have demonstrated significant clinical activity against hematologic malignancies, but solid tumors remain an intractable challenge. We have previously developed a human chimeric antigen receptor macrophage (CAR-M) platform for adoptive cell therapy and shown potent anti-tumor activity in pre-clinical solid tumor models.[1] CAR-M overcome critical solid tumor challenges such as tumor infiltration, immunosuppression within the tumor microenvironment, 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 production feasibility, phenotype, pharmacokinetics, 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 express a CAR targeted against human epidermal growth factor receptor 2 (HER2) (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 expression and cell viability exceeded 90%, and cells efficiently differentiated into CAR-expressing macrophages. The adenoviral based gene modification method led to pre-conditioning of CAR-mono cells resulting in a strong M1 phenotype upon differentiation, and potent anti-tumor activity regardless of exposure to GM-CSF, M-CSF, or immunosuppressive factors. Treating CAR-mono cells with GM-CSF and IL-4 resulted in their differentiation to monocyte-derived CAR-DCs, indicating that these cells retain their myeloid differentiation potential. In vivo, CAR-mono treatment induced anti-tumor activity in various HER2+ solid tumor xenograft models. Following intravenous administration, CAR-mono demonstrated the ability to traffic to both GM-CSF < sup >high</sup > and GM-CSF< sup >low</sup >expressing tumors. Notably, CAR-mono showed long-term CAR expression and persistence (>100 days) in both NSG and NSG-S mouse models, demonstrating lasting persistence irrespective of human cytokine support.

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 monocytes are primed toward M1 macrophage differentiation and produce a cell population highly similar to our established CAR-M platform. Collectively, these findings 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* March 2020.

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