CHIMERIC ANTIGEN RECEPTOR MACROPHAGES (CAR-M) ELICIT A SYSTEMIC ANTI-TUMOR IMMUNE RESPONSE AND SYNERGIZE WITH PD1 BLOCKADE IN IMMUNOCOMPETENT MOUSE MODELS OF HER2+ SOLID TUMORS

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Background Despite the remarkable efficacy achieved by CAR-T therapy in hematologic malignancies, application in solid tumors has been challenging. We previously developed human CAR-M and demonstrated that adoptive transfer of CAR-M into xenograft models of human cancer controls tumor progression and improves overall survival.¹ Given that CAR-M are M1-polarized macrophages with the potential to remodel the tumor microenvironment (TME) and act as professional antigen presenting cells, we developed an immunocompetent animal model to evaluate the interaction of CAR-M with the TME and the adaptive immune system.

Methods Murine bone marrow-derived macrophages were engineered to express an anti-HER2 CAR using the chimeric adenoviral vector Ad5f35. To evaluate the safety and efficacy of CAR-M therapy, immunocompetent mice were engrafted with HER2+ tumors and treated with syngeneic CAR-M monotherapy or in combination with a PD1 blocking antibody. Tumors were collected at various time points and dynamic changes in the TME were assessed using flow cytometry, immunohistochemistry, and gene expression analysis.

Results In addition to efficient gene delivery, Ad5f35 transduction promoted a pro-inflammatory (M1) phenotype in murine macrophages. CAR-M, but not control macrophages, phagocytosed and killed HER2-overexpressing tumor cell lines. CAR-M induced MHC-I expression on tumor cells and enhanced the cytotoxicity of CD8+ T cells. In vivo, CAR-M led to significant tumor regression and improved overall survival in multiple syngeneic models. Analysis of the TME showed that CAR-M led to increased infiltration of intratumoral CD4+ and CD8+ T, NK, and dendritic cells – as well as an increase in T cell responsiveness to tumor-associated antigens, indicating enhanced epitope spreading. Given the impact of CAR-M on the endogenous adaptive immune system, we evaluated the combination of CAR-M with anti-PD1 in the CT26-HER2 model, which is resistant to anti-PD1 monotherapy, and found that the combination further reprogrammed the TME, enhanced tumor control, and improved overall survival compared to monotherapy with either agent. Mice that achieved complete responses (CRs) after CAR-M therapy were protected against antigen-negative relapse, indicating long-term anti-tumor immunity. Finally, the combination of CAR-M with anti-PD1 did not trigger sustained elevations of any serum analyte associated with cytokine release syndrome (CRS) and was well tolerated across numerous safety assessments.

Conclusions These results demonstrate that CAR-M reprogram the TME, induce epitope spreading, and orchestrate a systemic immune response against solid tumors. Moreover, our findings provide rationale for the combination of CAR-M with immune checkpoint inhibitors for the treatment of solid tumors.

REFERENCE

1. Klichinsky M, Ruella M, Shestova O, et al. Human chimeric antigen receptor macrophages for cancer immunotherapy. *Nat Biotechnol* 2020;**38**(8):947–953