

CHIMERIC ANTIGEN RECEPTOR MACROPHAGES (CAR-M) SENSITIZE SOLID TUMORS TO ANTI-PD1 IMMUNOTHERAPY

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Background Despite the remarkable efficacy achieved by CAR-T therapy in hematologic malignancies, application in solid tumors has been challenging. 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. A Phase 1 FIH study evaluating the safety and feasibility of CT-0508 (a first in class CAR-M comprised of autologous human monocyte derived macrophages expressing an anti-HER2 CAR) is ongoing.¹

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, multiplexed immunofluorescence, gene expression analysis and TCR sequencing.

Results 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 enhanced epitope spreading. Transcriptomic analysis of post-treatment biopsies collected from patients enrolled in the CT-0508 CAR-M Phase 1 clinical trial demonstrated remodeling of the TME, increased T cell infiltration, T cell activation/proliferation, and increased T cell clonality in the TME. In some patients, increased T cell exhaustion and increased expression of checkpoint receptors was detected post-treatment. Given these results, we evaluated the combination of CAR-M with anti-PD1 in tumors resistant to anti-PD1 monotherapy and found that the combination further reprogrammed the TME, significantly enhanced tumor control, and improved overall survival compared to monotherapy with either agent. Mice that achieved complete responses 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 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 pre-clinical and clinical rationale for the combination of CAR-M with immune checkpoint inhibitors for the treatment of solid tumors.

REFERENCE

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Ethics Approval All animal studies were approved by the IACUC (# 201364) and University Laboratory Animal Resources at the Winstar institute. Mice were treated in accordance with the Winstar institute guidelines.

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