Chimeric antigen receptor macrophages (CAR-M) elicit a systemic anti-tumor immune response and synergize with PD-1 blockade in immunocompetent mouse models of HER2+ solid tumors

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Introduction

Adoptive cell therapies are effective in patients with hematologic malignancies but exert limited efficacy against solid tumors due to the immunosuppressive tumor microenvironment (TME) and heterogenous antigen expression.

We have previously developed a novel chimeric antigen receptor macrophage (CAR-M) cell therapy platform with potent anti-tumor activity in pre-clinical models[1].

To evaluate the effect of CAR-M in HER2+ immunocompetent solid tumor models alone and in combination with programmed cell death 1 (PD-1) blockade, the objectives of this study were to:

(**1**) Investigate the impact of CAR-M on endogenous anti-tumor immunity

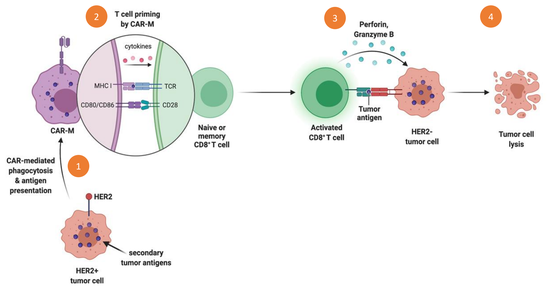
(**2**) Evaluate the impact of CAR-M on the TME

(**3**) Assess CAR-M + PD-1 blockade in a PD1 monotherapy resistant tumor model

Methods

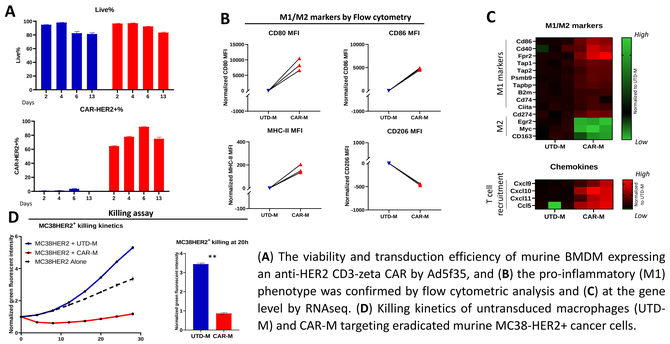
Primary murine bone marrow-derived macrophages were engineered to express an anti-HER2 CAR using the chimeric adenoviral vector Ad5f35. To evaluate the safety and efficacy of CAR-M therapy, immunocompetent mice were engrafted with HER2+ tumors and treated with syngeneic CAR-M monotherapy or in combination with a PD1 blocking antibody. Analysis of the TME, serum biomarkers and organ histology were used to probe the mechanism of action, pharmacokinetics, and pharmacodynamics of CAR-M therapy.

CAR-M Antigen Presentation to CD8$^+$ T cells



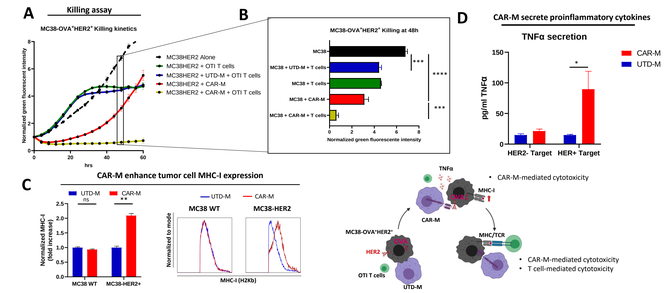
1. CAR-M mediated phagocytosis of target tumor cells leads to capture of secondary tumor antigens.
2. CAR-M process and present secondary tumor antigens to T cells.
3. Primed T cells identify MHC peptide complexes on the surface of tumor cells.
4. CAR-M primed T cells lyse tumor cells and establish immune memory.

Phenotypic and Functional Analysis of Murine CAR-M



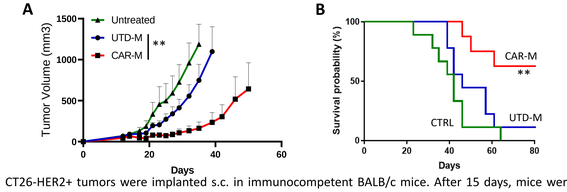
(**A**) The viability and transduction efficiency of murine BMDM expressing an anti-HER2 CD3-zeta CAR by Ad5f35, and (**B**) the pro-inflammatory (M1) phenotype was confirmed by flow cytometric analysis and (**C**) at the gene level by RNAseq. (**D**) Killing kinetics of untransduced macrophages (UTD-M) and CAR-M targeting eradicated murine MC38-HER2+ cancer cells.

CAR-M Promote Immunorecognition of Target by T cells



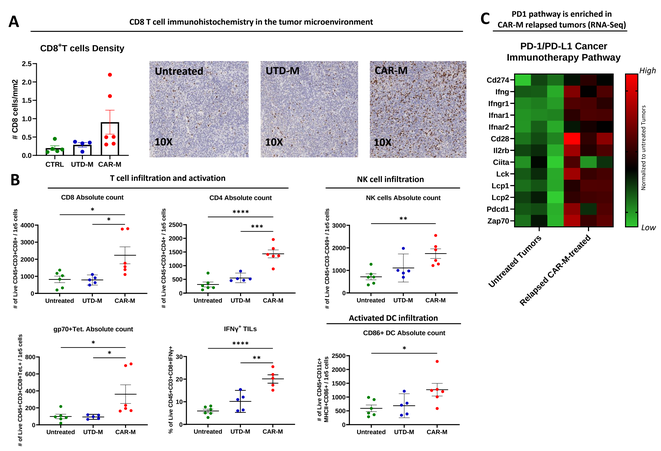
(**A-B**) CAR-M control tumor progression of the MC38-OVA+HER2+ cell line (red line) at a limited E:T ratio in vitro, and enhance the anti-tumor effect of OVA-specific CD8 T cells (OTI T cells; yellow line). The increased cytotoxicity observed was likely due to (**C**) induced MHC-I expression on target cells after exposure to CAR-M to enhance immunorecognition, and (**D**) enhanced TNFα secretion in response to CAR and antigen engagement.

CAR-M Control Tumor Progression and Improve Survival



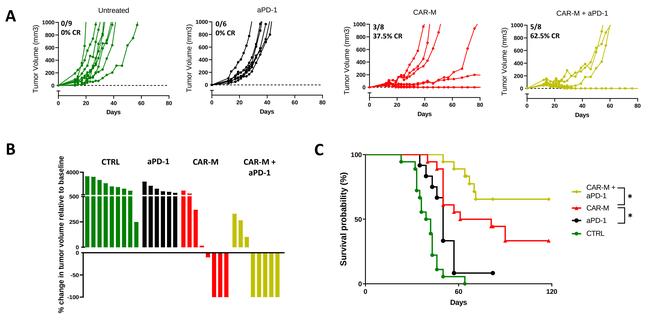
(**A**) CT26-HER2+ tumors were implanted s.c. in immunocompetent BALB/c mice. After 15 days, mice were treated with intratumoral (i.t) injections of CAR-M, UTD-M or left untreated. CAR-M significantly reduced tumor progression and (**B**) increased long term survival compared to control groups. (UTD: untransduced)

CAR-M Reprogram the TME and Prime T cells



(**A**) Immunohistochemistry (IHC) assessment showed that CAR-M treatment increase tumor CD8$^+$ T cell infiltration in the CT26-HER2+ model indicating remodulation of the TME. (**B**) Flow cytometric analysis showed increased infiltration of tumor T cells, natural killer (NK) cells, activated CD86+dendritic cells (DC) and tumor-associated antigen (TAA)-specific CD8 T cells (gp70+) in CAR-M treated mice, suggesting epitope spreading. (**C**) RNAseq analysis performed on relapsed tumors revealed that the PD-1/PD-L1 pathway gene signature was increased after CAR-M treatment in non-responders, indicative of a resistance mechanism.

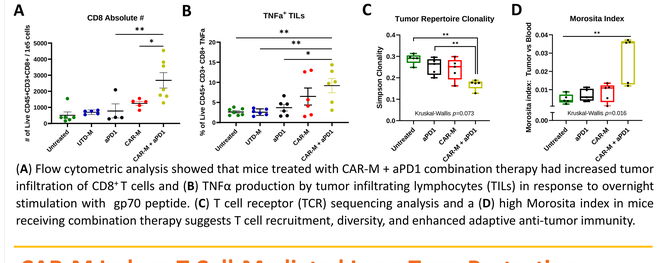
CAR-M and Anti PD-1 Combination Therapy Improves Tumor Control and Survival in the CT26-HER2 Model



To evaluate the combination therapy of CAR-M with PD-1 blockade in the CT26-HER2+ model, aPD-1 was administered four times, at 3-day intervals, starting 14 days post tumor inoculation (CAR-M therapy initiated on day 15). (**A-B**) aPD-1 and CAR-M combination therapy improved tumor control and (**C**) significantly improved survival probability (38.9% CR CAR-M vs 66.7% CR CAR-M + aPD-1).

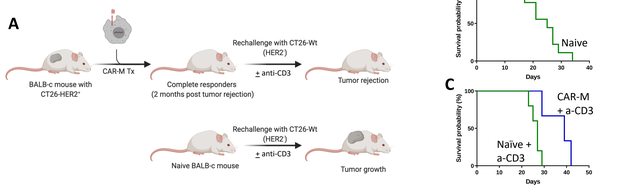
aPD-1 = anti-PD-1 mAb; **CR**= complete response

CAR-M and Anti PD-1 Combination Therapy Modulates the TME and TCR Repertoire



(**A**) Flow cytometric analysis showed that mice treated with CAR-M + aPD1 combination therapy had increased tumor infiltration of CD8$^+$ T cells and (**B**) TNFα production by tumor infiltrating lymphocytes (TILs) in response to overnight stimulation with gp70 peptide. (**C**) T cell receptor (TCR) sequencing analysis and a (**D**) high Morosita index in mice receiving combination therapy suggests T cell recruitment, diversity, and enhanced adaptive anti-tumor immunity.

CAR-M Induce T Cell-Mediated Long-Term Protection Against Antigen-Negative Relapse



(**A**) Mice achieving complete responses (CR) post CAR-M therapy were re-challenged with HER2-negative CT26-Wt tumors to model antigen negative relapse. (**B**) Naïve mice succumbed to disease within 35 days compared to the CAR-M treatment group indicating long-term tumor protection against antigen-negative relapse. (**C**) Depletion of CD3$^+$ T cells in complete responders negated tumor protection, highlighting the ability of CAR-M to induce long term T-cell memory. *=P<0.05; **=P<0.01; ***=P<0.001

Conclusion

- In vitro, CAR-M enhanced T cell-mediated recognition of target tumor cells.
- In vivo, CAR-M reduced tumor burden and prolonged overall survival in syngeneic models of HER2+ tumors.
- CAR-M reprogrammed the TME and primed T cells against secondary antigens, indicating epitope spreading.
- The PD-1/PD-L1 pathway gene signature was increased after CAR-M treatment in non-responders, indicative of a potential resistance mechanism.
- CAR-M and aPD-1 therapy improved tumor control, prolonged survival and synergistically modulated the TME and TCR repertoire diversity in a HER2+ solid tumor model.
- Complete responders to CAR-M therapy were protected against antigen negative relapse, indicating epitope spreading and long-term anti-tumor immunity.

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

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