Chimeric antigen receptor macrophages (CAR-M) sensitize solid tumors to anti-PD1 immunotherapy

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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

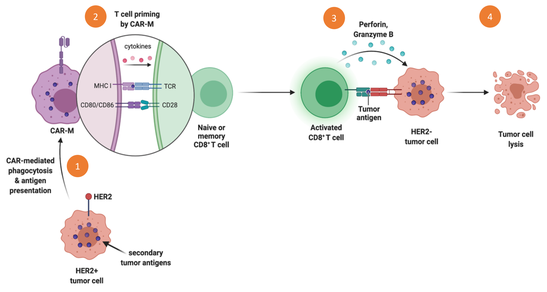
(2) Evaluate the impact of CAR-M on the TME

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

Methods

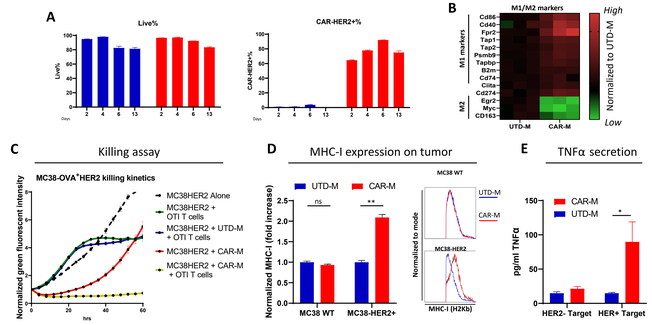
Primary murine bone marrow-derived macrophages were engineered to express an anti-HER2 CAR using the chimeric adenoviral vector Ad5f35. To evaluate the safety and efficacy of CAR-M therapy, immunocompetent mice were engrafted with HER2+ tumors and treated with syngeneic CAR-M monotherapy or in combination 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



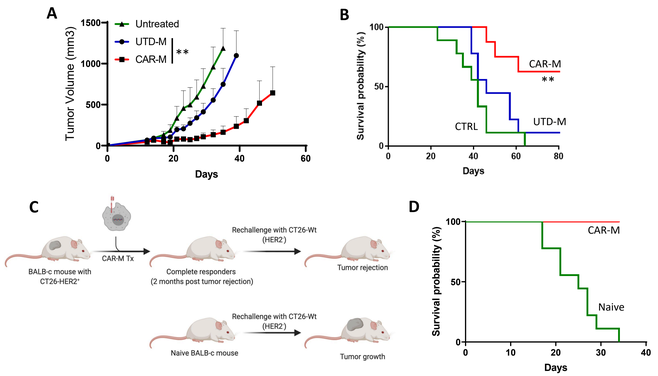
1. **CAR-M mediated phagocytosis of target tumor cells leads to capture of secondary tumor antigens.**
2. **CAR-M process and present secondary tumor antigens to T cells.**
3. **Primed T cells identify MHC peptide complexes on the surface of tumor cells.**
4. **CAR-M primed T cells lyse tumor cells and 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 was confirmed by Flow cytometry, and (**B**) the pro-inflammatory (M1) phenotype was confirmed at the gene level by RNAseq. (**C**) 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 (**D**) induced MHC-I expression on target cells after exposure to CAR-M to enhance immunorecognition, and (**E**) enhanced TNFα secretion in response to CAR - antigen engagement.

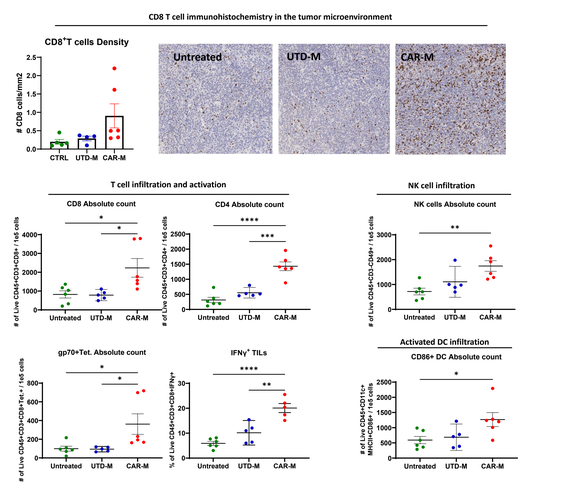
CAR-M Control Tumor Progression, Improve Survival and Induce Lon-Term Protection against Antigen-negative Relapse



(**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. (**C**) Mice achieving complete responses (CR) post CAR-M therapy were re-challenged with HER2-negative CT26-Wt tumors to model antigen negative relapse. (**D**) Naïve mice succumbed to disease within 35 days, while 100% of the mice from the CAR-M treatment group survived, indicating long-term tumor protection against antigen-negative relapse.

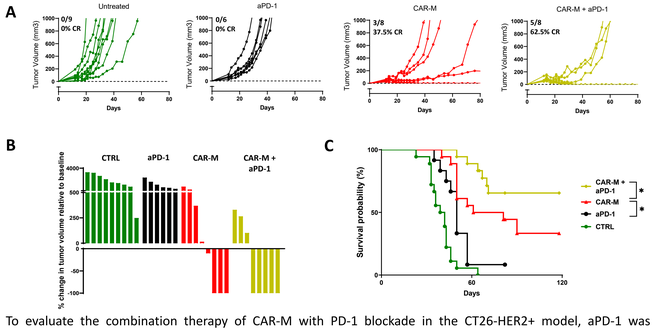
UTD = untransduced

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.

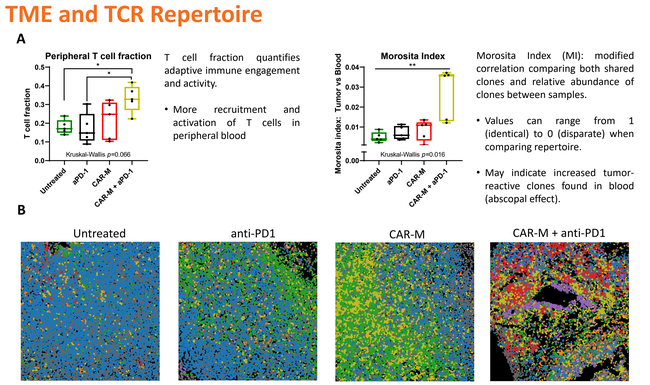
CAR-M and Anti-PD-1 Combination Therapy Improves Tumor Control and Survival in the CT26-HER2 Model



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 (Cumulative survival; 0% CR CTRL, 8.3% CR anti-PD-1, 38.9% CR CAR-M and 66.7% CR CAR-M + anti-PD-1).

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

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



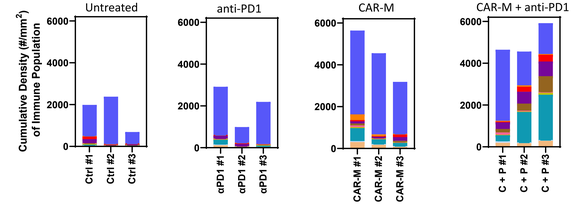
T cell fraction quantifies adaptive immune engagement and activity.

- More recruitment and activation of T cells in peripheral blood

Morosita Index (MI): modified correlation comparing both shared clones and relative abundance of clones between samples.

- Values can range from 1 (identical) to 0 (disparate) when comparing repertoire.
- May indicate increased tumor-reactive clones found in blood (abscopal effect).

Quantification of immune cell populations: Cumulative density



(**A**) T cell receptor (TCR) sequencing analysis in mice receiving combination therapy suggests T cell recruitment, diversity, and enhanced adaptive anti-tumor immunity. (**B**) Multiplexed IHC showed that the tumor/fibroblast population decreased by half in the CAR-M and the combo groups, whereas immune cell densities more than double in these same CAR-M and combo groups. T cells were significantly higher in both the CAR-M and CAR-M + anti-PD-1 samples compared to control. Macrophages tended to be greatest in the CAR-M sample while helper T cells, DC, neutrophils and monocytes were greatest in the combo group.

Conclusion

- In vitro, CAR-M killed target cells, secreted pro-inflammatory cytokines, promoted MHC-I expression on tumor cells and enhanced T cell-mediated recognition of target cells
- In vivo, CAR-M reduced tumor burden, prolonged overall survival in syngeneic models of HER2+ tumors, and induced long-term anti-tumor immune response against antigen-negative relapses
- CAR-M reprogrammed the TME and primed T cells against secondary antigens
- CAR-M + anti-PD-1 combo improved tumor control, prolonged survival and synergistically modulated the TME and TCR repertoire diversity in a HER2+ solid tumor model.

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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