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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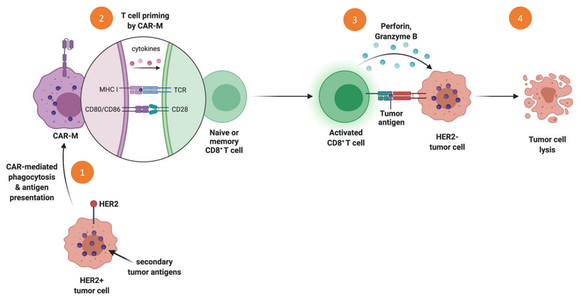
INTRODUCTION

We previously developed human chimeric antigen receptor macrophages (CAR-M) and demonstrated that adoptive cell transfer of CAR-M into xenograft models of human cancer controls tumor progression and improves overall survival.[1] The immunosuppressive tumor microenvironment and heterogenous antigen expression of solid tumors imposes a significant challenge onto cell therapies. In order to model the interaction of CAR-M with the TME and the endogenous immune system, we established a fully immunocompetent syngeneic mouse model of CAR-M therapy.

First, a surrogate murine CAR-M (mCAR-M) system was generated using Ad5f35 and murine bone marrow derived macrophages. mCAR-M were phenotypically and functionally evaluated in vitro and found to mirror their human counterparts. In vivo, mCAR-M exerted potent anti-tumor activity, reprogrammed the TME, primed T cells against tumor neoantigens, induced epitope spreading, and led to long term T cell memory against the tumor. Given that CAR-M are professional antigen presenting cells that prime anti-tumor T cell immunity, we evaluated the combination of CAR-M and a PD-1 blocking antibody in a PD-1 monotherapy resistant model. The combination led to improved tumor control and significantly enhanced overall survival.

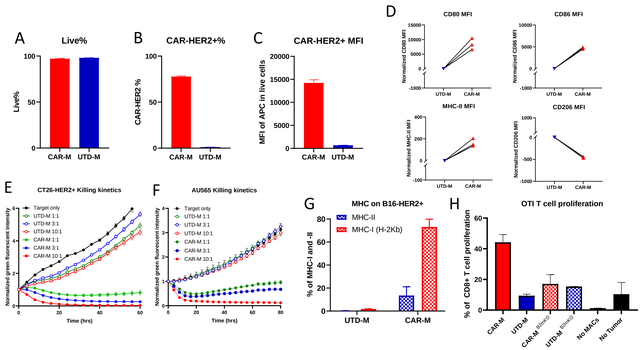
In summary, these data demonstrate that in immunocompetent hosts CAR-M engage endogenous immunity, leading to epitope spreading, enhanced activity, and resistance to antigen negative relapse.

CAR-M antigen presentation to CD8+ T cells



1. CAR mediated phagocytosis leads to capture of antigen positive (i.e. HER2) tumor cells.
2. CAR-M process and present secondary antigens that are unique to the cancer (i.e. neoantigens) to T cells.
3. Primed T cells identify MHC peptide complexes on the surface of tumor cells. Those tumor cells can be antigen (i.e. HER2) positive or negative.
4. CAR-M primed T cells lyse tumor cells.

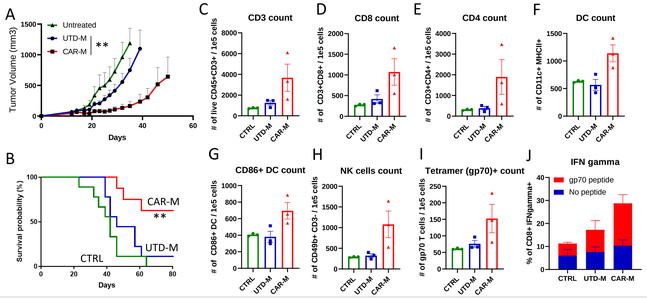
Functional & phenotypic analysis of murine CAR-M



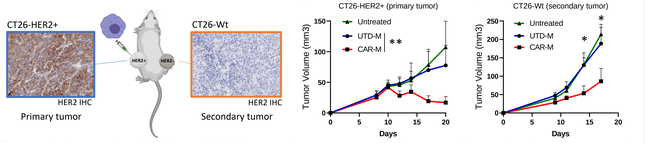
Murine bone marrow derived macrophages were engineered to express an anti-HER CD3-zeta based CAR using Ad5f35 with high viability and efficiency (A-C). Ad5f35 imparted a sustained pro-inflammatory phenotype with increased expression of the M1 markers CD80, CD86 and MHC-II and reduced expression of the M2 marker CD206 (D). CAR-M, but not untransduced macrophages (UTD-M), eradicated murine CT26-HER2+ (E) and human AU565 (HER2+) cancer cells (F). Co-culture of CAR-M with B16-HER2+ cancer cells increased MHC-I and MHC-II expression on the surface of tumor cells (G). CAR-M that had been exposed to B16-OVA+HER2+ primed OTI CD8 T cell proliferation in an MHC-I dependent manner (H).

CAR-M shrink tumors, improve overall survival, reprogram the TME, and prime T cells

CT26-HER2+ tumors were implanted s.c. in immunocompetent BALB/c mice. Tumors were treated with intratumoral injections of CAR-M or UTD-M starting 15 days post tumor inoculation. CAR-M significantly reduced tumor progression (A) and increased long term survival (B) compared to control groups. CAR-M treatment increased CD3+, CD8+ and CD4+ T cell infiltration (C-E). An increased in activated CD86+ dendritic cells (DC) (F, G) as well as NK cells (H) was also observed. CAR-M enhanced tumor antigen-specific (gp70+) CD8 T cell infiltration in the TME (I). Ex vivo restimulation of tumor-infiltrating lymphocytes (TILs) with the $gp70_{423-431}$ peptide revealed that TILs from CAR-M-treated mice produced more IFN gamma (L) in response to the gp70 antigen, indicating that enhanced antigen presentation had occurred in the CAR-M group.

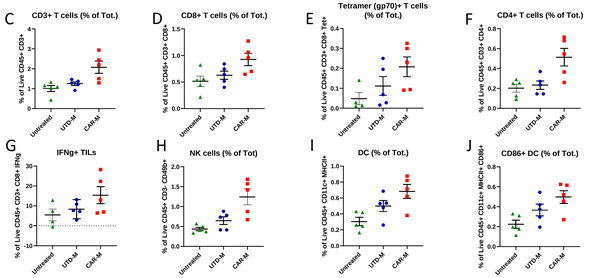


CAR-M induce epitope spreading



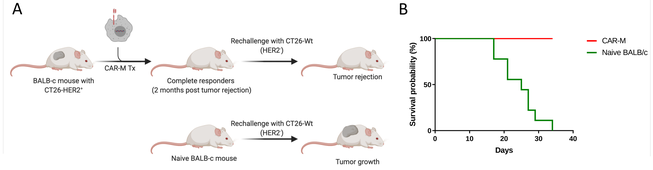
To test whether localized injection of CAR-M would induce a systemic anti-tumor effect against abscopal tumors (i.e. tumors that are outside of the treated field), we implemented a model in which BALB/c mice were co-engrafted with CT26-HER2+ on the left flank (primary tumor) and CT26-Wt on the right flank (secondary tumor). Ten days after primary tumor inoculation, mice received local CAR-M or UTD-M in the primary tumor. 75% of HER2+ primary tumors were rejected (A). CAR-M treatment led to significant inhibition of the growth rate of the HER2(-) secondary tumor, suggesting expansion of a systemic anti-tumor immune response (B).

Analysis of the TME of the HER2(-) tumor revealed that CAR-M treatment increased CD3+, CD8+ and CD4+ T cell infiltration (C, D and F). CAR-M treatment enhanced the infiltration of tumor antigen-specific (gp70+) CD8 T cells in the HER2(-) abscopal tumor (E), indicating epitope-spreading had occurred. Ex vivo restimulation of abscopal TILs with $gp70_{423-431}$ peptide revealed that TILs from CAR-M-treated mice produced more IFNg (G). Additionally, an increased infiltration of NK cells (H) and activated DCs (I, J) was also observed in the HER2(-) secondary tumors of CAR-M treated mice.

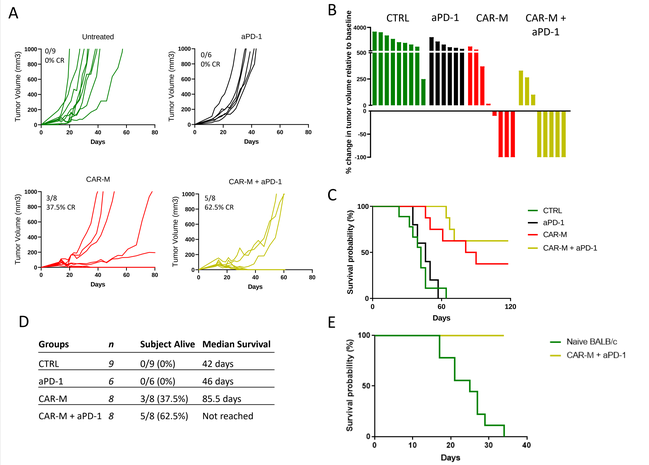


CAR-M protect against antigen negative relapse

To assess whether CAR-M therapy induces long-term anti-tumor immunity and protects mice from antigen negative relapse, mice with CT26-HER2 tumors that achieved complete responses (CRs) post anti-HER2 CAR-M therapy were re-challenged with CT26-Wt (A). While naïve mice succumbed to disease within 35 days, 100% of mice with previous CAR-M mediated CRs rejected antigen negative rechallenge (B), indicating long-term tumor protection against antigen-negative relapse.



CAR-M/anti-PD1 combination improves outcomes



Groups	n	Subject Alive	Median Survival
CTRL	9	0/9 (0%)	42 days
aPD-1	6	0/6 (0%)	46 days
CAR-M	8	3/8 (37.5%)	85.5 days
CAR-M + aPD-1	8	5/8 (62.5%)	Not reached

To potentiate the anti-tumor immune response induced by CAR-M therapy in the CT26-HER2+ model, we combined CAR-M with concurrent anti-mPD-1 antibody administration. We administered aPD-1 four times, at 3-day intervals, starting at day 14. Macrophage therapy initiated on day 15. Anti-PD-1 monotherapy did not impact tumor progression (A). The addition of aPD-1 to CAR-M significantly improved tumor control (A) and extended overall survival (B-D). In a HER2(-) rechallenge model, complete responders of the CAR-M + aPD-1 combination therapy also rejected HER2(-) tumors 2 moths later, demonstrating long term immunological memory (E).

*=P<0.05; **=P<0.01; ***=P<0.001

CONCLUSION

- A fully immunocompetent animal model was established for the evaluation of CAR-M in a clinically relevant system.
- CAR-M induced MHC-I and MHC-II expression on tumor cells and effectively cross-presented tumor derived antigens to CD8+ T cells.
- CAR-M reduced tumor burden and prolonged overall survival in syngeneic models of HER2+ tumors. Anti-HER2 CAR-M induced epitope spreading and led to an abscopal effect against HER2(-) tumors.
- CAR-M reprogrammed the TME (increased infiltration of T cells, DCs, NK cells, and B cells within the tumor) and primed T cells against secondary antigens in vivo.
- CAR-M therapy vaccinated mice against tumor recurrence and prevented antigen negative relapse.
- The combination of CAR-M with anti-PD1 blockade led to synergistic tumor control and significantly increased overall survival.

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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