In vivo CAR-M: Redirecting endogenous myeloid cells with mRNA for cancer immunotherapy

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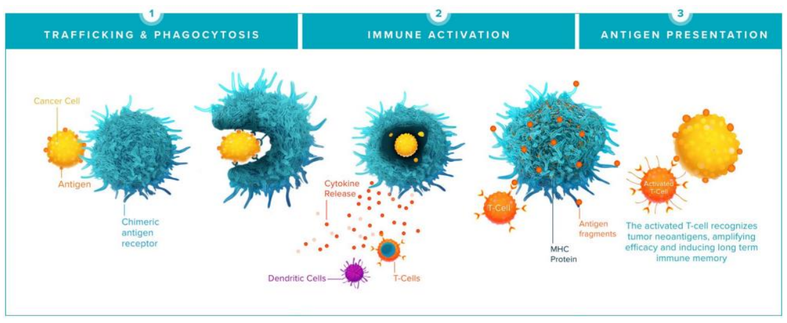
Introduction

Engineering myeloid cells for cancer immunotherapy

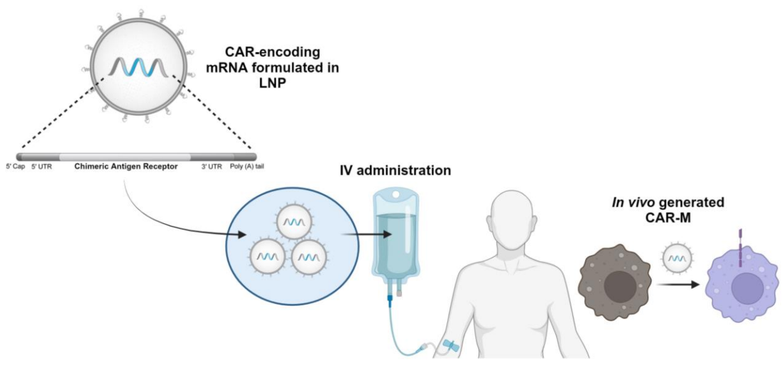
Macrophages, monocytes, and dendritic cells are sentinel cells of the innate immune system that play a central role in phagocytosis, inflammation, immune cell recruitment, and antigen presentation.

Ex vivo chimeric antigen receptor (CAR) macrophage and monocyte cell therapies have demonstrated robust anti-tumor immunity via targeted phagocytosis, cytokine/chemokine release, activation of the tumor microenvironment (TME), T cell recruitment, and epitope spreading in pre-clinical models.



Objectives

Here, we describe a novel strategy to deliver modified messenger RNA (mRNA) encapsulated in lipid nanoparticles (LNPs) to generate *in vivo* CAR-M (macrophages and monocytes), redirecting endogenous myeloid cells to exert targeted anti-tumor activity.



Results and conclusions

Human macrophages and monocytes engineered with CAR-encoding mRNA *in vitro* demonstrated high CAR expression and viability.

CAR expression conferred antigen specificity leading to target-specific proinflammatory cytokine secretion and tumor cell killing, with serial killing demonstrated upon tumor rechallenge.

CAR signaling upon antigen binding polarized macrophages toward M1, activating various pro-inflammatory innate immune pathways.

CAR expression following mRNA/LNP administration was predominantly observed in macrophages, monocytes, and dendritic cells compared to immune cells of non-myeloid origin in mice.

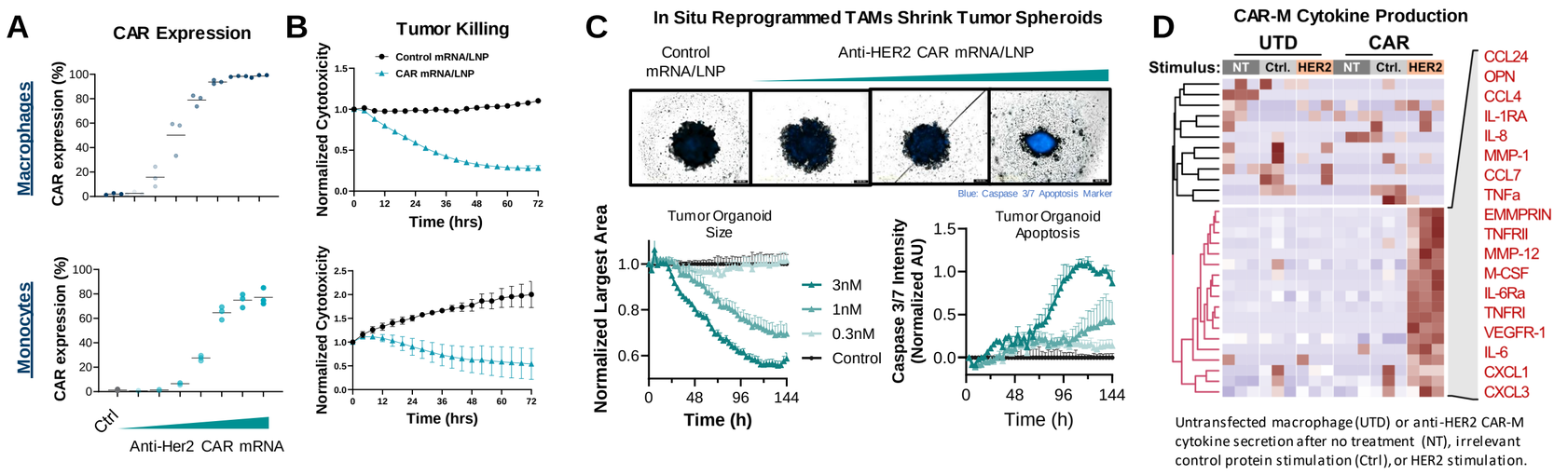
In vivo, regional and systemic administration of CAR-encoding mRNA led to significant tumor regression in subcutaneous and systemically disseminated metastatic solid tumor models, respectively.

Repeat administration of mRNA/LNP was well tolerated.

- ✓ CAR-M can be directly produced *in vivo* and directed against tumor associated antigens using mRNA/LNP technology.
- ✓ This *in vivo* CAR-M platform offers a novel off-the-shelf solution to cancer immunotherapy and has the potential to be applied to numerous target antigens and indications.

mRNA/LNP transfection generates highly functional CAR-M

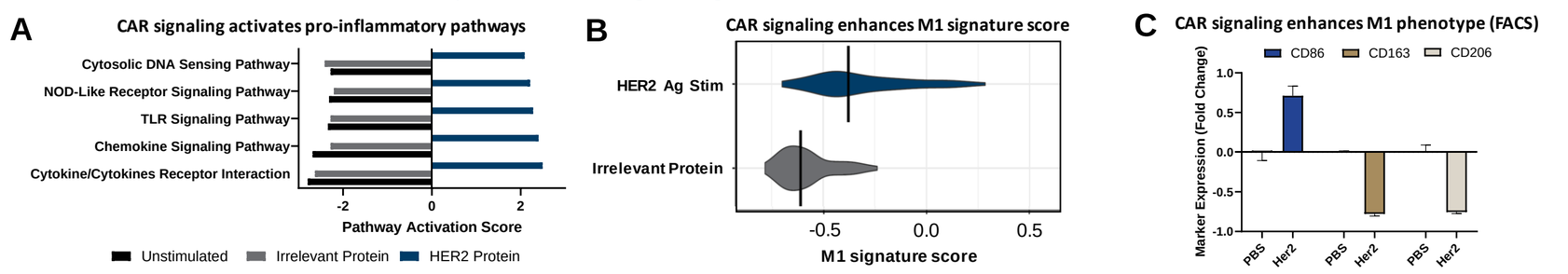
Engineering human macrophages (top) and monocytes (bottom) with anti-HER2 CAR mRNA/LNP leads to titratable CAR expression (**A**) and target-specific killing against HER2+ tumor cells (**B**). Direct *in situ* reprogramming of TAMs with anti-HER2 CAR mRNA/LNP in tumor spheroids leads to robust anti-tumor activity (**C**). mRNA/LNP CAR-M produce a repertoire of pro-inflammatory cytokines and chemokines upon target engagement (**D**).



Untransfected macrophage (UTD) or anti-HER2 CAR-M cytokine secretion after no treatment (NT), irrelevant control protein stimulation (Ctrl), or HER2 stimulation.

CAR engagement leads to CAR-M polarization toward M1 phenotype

Target antigen stimulation of human CAR-Macrophages *in vitro* leads to activation of pro-inflammatory transcriptomic pathways **(A)** and polarization toward the M1 phenotype based on gene expression **(B)** and flow cytometry **(C)**. Macrophages were transfected with mRNA/LNP CAR then stimulated for 24 hours with an irrelevant protein or the target antigen HER2.

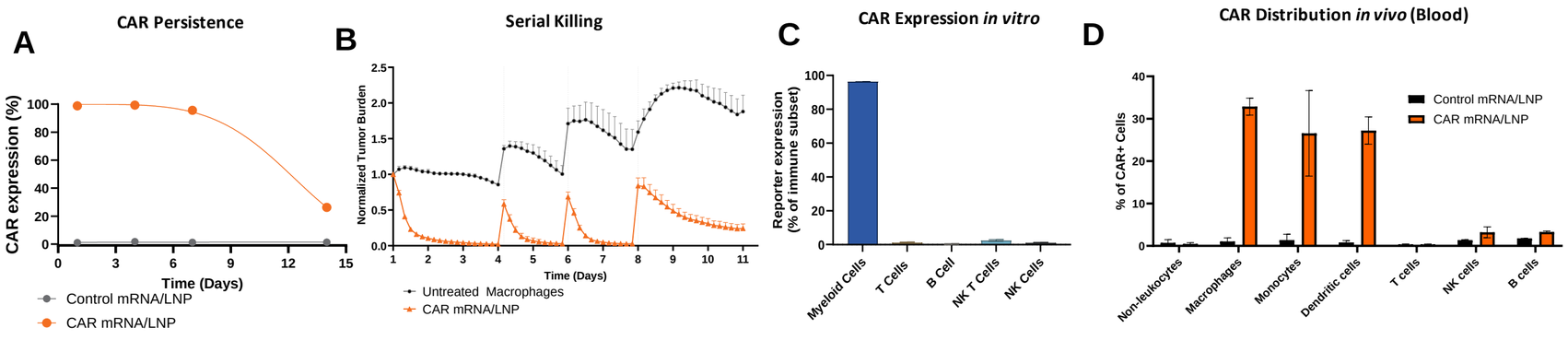


CAR expression and serial killing capacity following a single mRNA/LNP CAR transfection

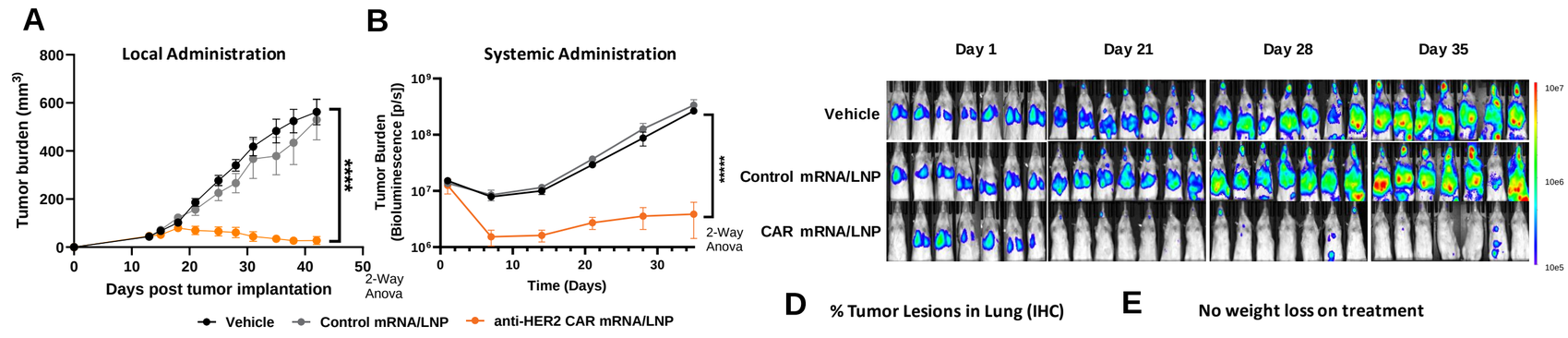
Evaluation of CAR expression *in vitro* for 2 weeks **(A).** HER2+ tumor rechallenge assay shows mRNA/LNP CAR-M serial killing capacity **(B).**

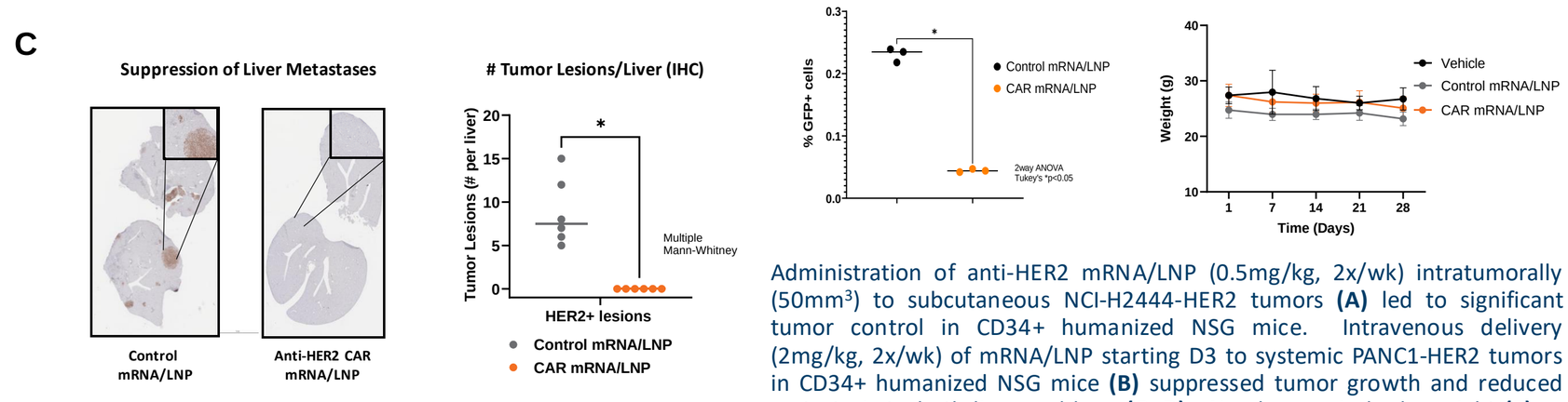
mRNA/LNP administration preferentially transfects myeloid cells *in vivo*

mRNA/LNP delivery leads to preferential expression of CAR on myeloid cells in human PBMCs *in vitro* **(C)** or in Balb/c mice *in vivo* **(D)**.



In vivo CAR mRNA/LNP treatment leads to significant tumor control





Administration of anti-HER2 mRNA/LNP (0.5mg/kg, 2x/wk) intratumorally (50mm^3) to subcutaneous NCI-H2444-HER2 tumors **(A)** led to significant tumor control in CD34+ humanized NSG mice. Intravenous delivery (2mg/kg, 2x/wk) of mRNA/LNP starting D3 to systemic PANC1-HER2 tumors in CD34+ humanized NSG mice **(B)** suppressed tumor growth and reduced metastasis to both liver and lung **(C, D)**. No changes in body weight **(E)** or any other clinical observations were reported in any groups.

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Disclosures: SM, CL and LG are employees of Moderna, Inc., and may hold stock/stock options in the company.