

Cellular Therapies

1514

IN VIVO CAR-M: REDIRECTING ENDOGENOUS MYELOID CELLS WITH MRNA FOR CANCER IMMUNOTHERAPY

¹Bindu Varghese*, ²Simone Mori, ³Stefano Pierini, ³Asen Bagashev, ³Yumi Ohtani, ³Rashid Gabbasov, ³Kayleigh Ross, ³Shuo Huang, ³Amanda Bona, ³Sherly Merdiana, ³Kate Slovik, ³Alison Worth, ³Karan Nagar, ³Robert Saporito, ³Lauren Shaw, ³Michael Ball, ³Rehman Qureshi, ³Nicholas Minutolo, ³Kevin Tosh, ³Claudia Lee, ²Christine Lukacs, ²Lin Guey, ³Michael Klichinsky, ³Thomas Condamine. ¹Carisma Therapeutics, Natick, MA, USA; ²Moderna, Cambridge, MA, USA; ³Carisma Therapeutics, Philadelphia, PA, USA

Background 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. Genetically redirecting myeloid cells against tumor-associated antigens represents a novel strategy for cancer immunotherapy. *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. Here, we describe a novel strategy to deliver modified messenger RNA (mRNA) encapsulated in lipid nanoparticles (LNPs) to generate CAR-M (macrophages and monocytes) directly *in vivo*.

Methods Human and murine primary monocytes and macrophages were utilized to characterize mRNA/LNP driven CAR expression kinetics and anti-tumor functionality *in vitro* using flow cytometry, live cell imaging, single cell RNA sequencing (scRNAseq), and cytokine release assays. The efficacy, safety, and myeloid cell tropism of CAR-encoding mRNA/LNP were evaluated *in vivo* in syngeneic and humanized mouse models of solid tumors. Tissues were collected and CAR expression along with dynamic changes in immune cell frequency and phenotype were assessed using flow cytometry and scRNAseq. All animal studies were conducted in accordance with the Animal Welfare Act and Public Health Service Policy on Humane Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee at the Wistar Institute.

Results Human macrophages and monocytes engineered with CAR-encoding mRNA/LNP *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. The myeloid tropism of the LNP was demonstrated both *in vitro* and *in vivo*, with significant CAR expression 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/LNP led to significant tumor regression in subcutaneous and systemically disseminated metastatic solid tumor models, respectively. Treated animals tolerated repeat mRNA/LNP administration with no signs of toxicity.

Conclusions These data demonstrate that CAR-M can be directly produced *in vivo* and directed against tumor associated antigens using mRNA/LNP technology. This novel cancer immunotherapy platform offers an off-the-shelf solution that has the potential to increase access to CAR-based therapies and can be applied to numerous target antigens and indications.

Ethics Approval All animal studies were conducted in accordance with the Animal Welfare Act and Public Health Service

Policy on Humane Care and Use of Laboratory Animals and approved by the Institutional Animal Care and Use Committee at the Wistar Institute.

<http://dx.doi.org/10.1136/jitc-2023-SITC2023.1514>