#260 EpCAM-CD3 Bispecific Antibody-Encoding mRNA Delivered by Lung Targeted Lipid Nanoparticles Suppresses Orthotopic Lung Tumor Growth

Dong Wang, Gang Liu, Yuhe Han, Maorong Fu, Tingting Li, Xiangnan Qiang, Shunchuan Zhang, Zhixiang Zhang, Letian Kuai
WuXi Biology, WuXi AppTec, China

WuXi AppTec

WuXi Biology

Introduction

The therapeutic use of bispecific T-cell engaging (BiTE) antibodies has shown great potential for treating malignancies. However, full exploitation of the potential of BsAbs is hindered by manufacturing challenges and short serum half-lives. In contrast, mRNA therapeutics have emerged as a powerful approach for treating a wide range of diseases. Their applications are increasingly linked to advancements in targeted delivery technologies and the production of mRNA encoded antibody is more flexible and cost-effective than the traditional method.

Method

We developed a lung selective organ targeting lipid nanoparticles (SORT LNPs)–formulated RNA (RNA-LNP) encoding a T cell–engaging bispecific antibody that binds the T cell marker CD3 and bivalently binds epithelial cell adhesion molecule (EpCAM), an epithelial antigen that is expressed on various solid tumors. We first performed In vitro flow cytometry analysis, which revealed that mRNA lipid nanoparticles (LNP) effectively mediated the killing of EpCAM positive tumor cells and activated human T cells. Then we established NCI-H441 lung orthotopic tumor model in PBMC humanized mice. We observed robust antitumor efficacy of mRNA LNP in this tumor model. To assess mRNA distribution, we quantified its content in various organs using qPCR. Results confirmed the lung targeting specificity of LNP. Moreover, we analyzed the activation of tumor-infiltrating T cells post mRNA LNP treatment. Finally, we conducted histopathological examination of various organs and we didn't find signs of adverse effects from LNP formulated mRNA administration.

Results

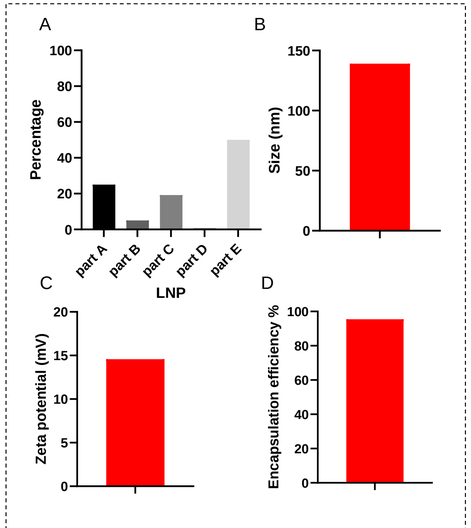

Figure 1. Preparation and characterization of mRNA LNP
(A) Molar ratio of the lipid components (B) particle size of mRNA LNP (C) Zeta potential of mRNA LNP (D) Encapsulation efficiency of mRNA LNP

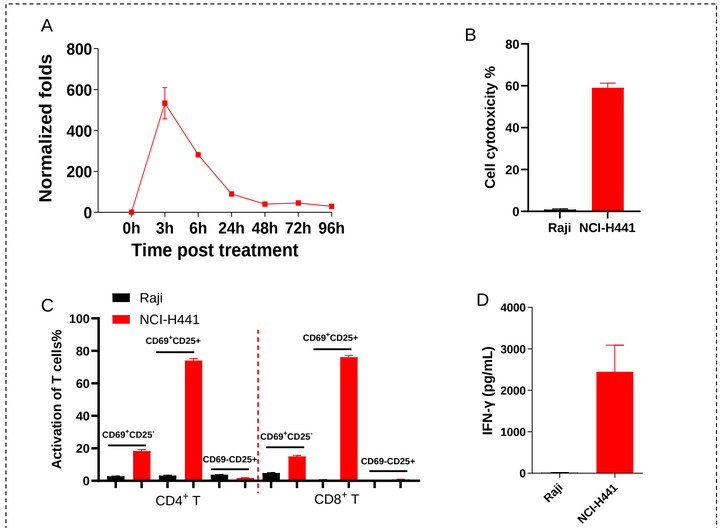

Figure 2. EpCAM-CD3 BsAb encoded mRNA is biologically active *in vitro*
Human PBMC was co-cultured with tumor cells and then treated with mRNA LNP for 48h (A) qPCR analysis of CD3 expression on different time points following mRNA transfection into H441 cells (B) Flow cytometry analysis of cytotoxicity (C) Flow cytometry analysis of T cell activation (D) ELISA analysis of IFNg concentration in supernatant

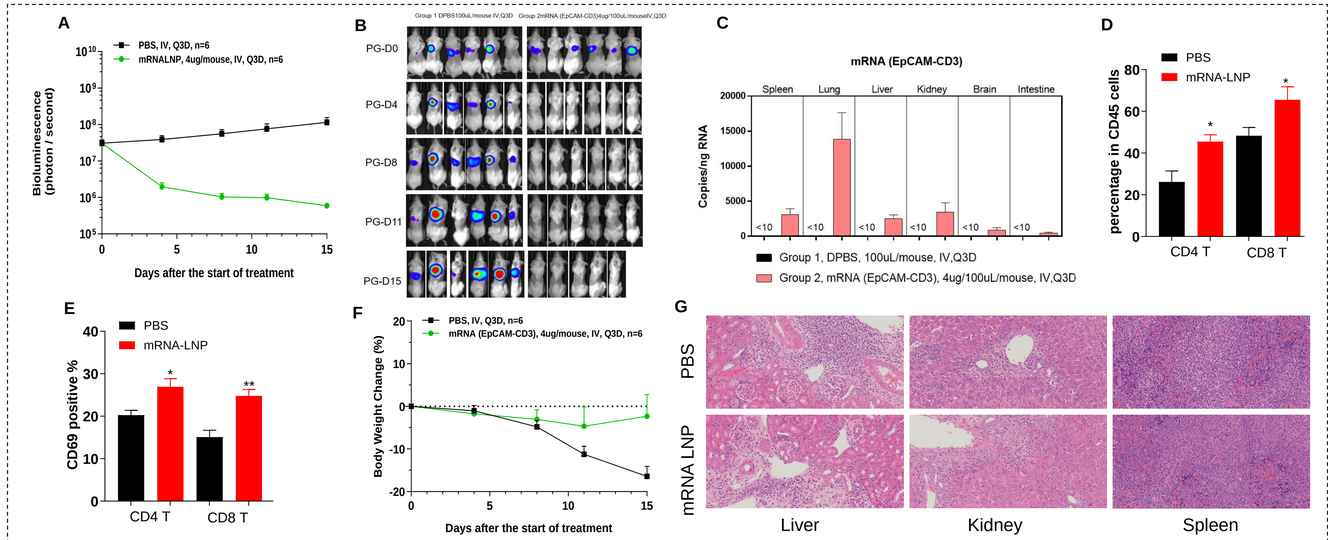

Figure 3. EpCAM-CD3 BsAb encoded mRNA LNP inhibited tumor growth *in vivo*
H441-Luc lung orthotopic tumor was established in NOG mice with human PBMC and treated with EpCAM-CD3 BsAb encoded mRNA LNP. (A) Tumor growth curve. (B) IVIS imaging of tumor bearing mice. (C) qPCR analysis of various organs. (D, E) Flow cytometry analysis of T cell percentage and activation in lung. (F) Body weight change of each group. (G) HE staining of various organs collected from H441 tumor-bearing mice

Conclusions

In this comprehensive preclinical evaluation, we demonstrated that mRNA-encoded bispecific antibody promoted the activation and cytotoxicity of human T cells, exhibiting significant inhibition of orthotopic lung tumor growth in vivo. These findings underscore the potential research value of mRNA-encoded CD3-EpCAM T cell engager in treating solid tumors, marking a potential shift in the clinical application of protein-based T-cell engagers.

References

1. Liu C, Shi Q, Huang X, Koo S, Kong N, Tao W. mRNA-based cancer therapeutics. Nat Rev Cancer. 2023 Aug;23(8):526-543. Epub 2023 Jun 13.
2. Bitounis D, Jacquinet E, Rogers MA, Amiji MM. Strategies to reduce the risks of mRNA drug and vaccine toxicity. Nat Rev Drug Discov. 2024 Jan 23.
3. Stadler CR, Bähr-Mahmud H, Celik L, Hebich B, Roth AS, Roth RP et al. Elimination of large tumors in mice by mRNA-encoded bispecific antibodies. Nat Med. 2017 Jul;23(7):815-817.
4. Wei T, Sun Y, Cheng Q, Chatterjee S, Traylor Z, Johnson LT et al. Lung SORT LNPs enable precise homology-directed repair mediated CRISPR/Cas genome correction in cystic fibrosis models. Nat Commun. 2023 Nov 11;14(1):7322.
5. Wang X, Liu S, Sun Y, Yu X, Lee SM, Cheng Q et al. Preparation of selective organ-targeting (SORT) lipid nanoparticles (LNPs) using multiple technical methods for tissue-specific mRNA delivery. Nat Protoc. 2023 Jan;18(1):265-291.

WuXi AppTec

Contact Us
USA: mahnaz_arjomand@wuxiapptec.com
EU/UK/Israel: dave_madge@wuxiapptec.com
Japan: fumio_itoh@wuxiapptec.com
Korea: sycho@wuxiapptec.com
China/Singapore/Australia: xu_longji@wuxiapptec.com

www.wuxibiology.com