

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

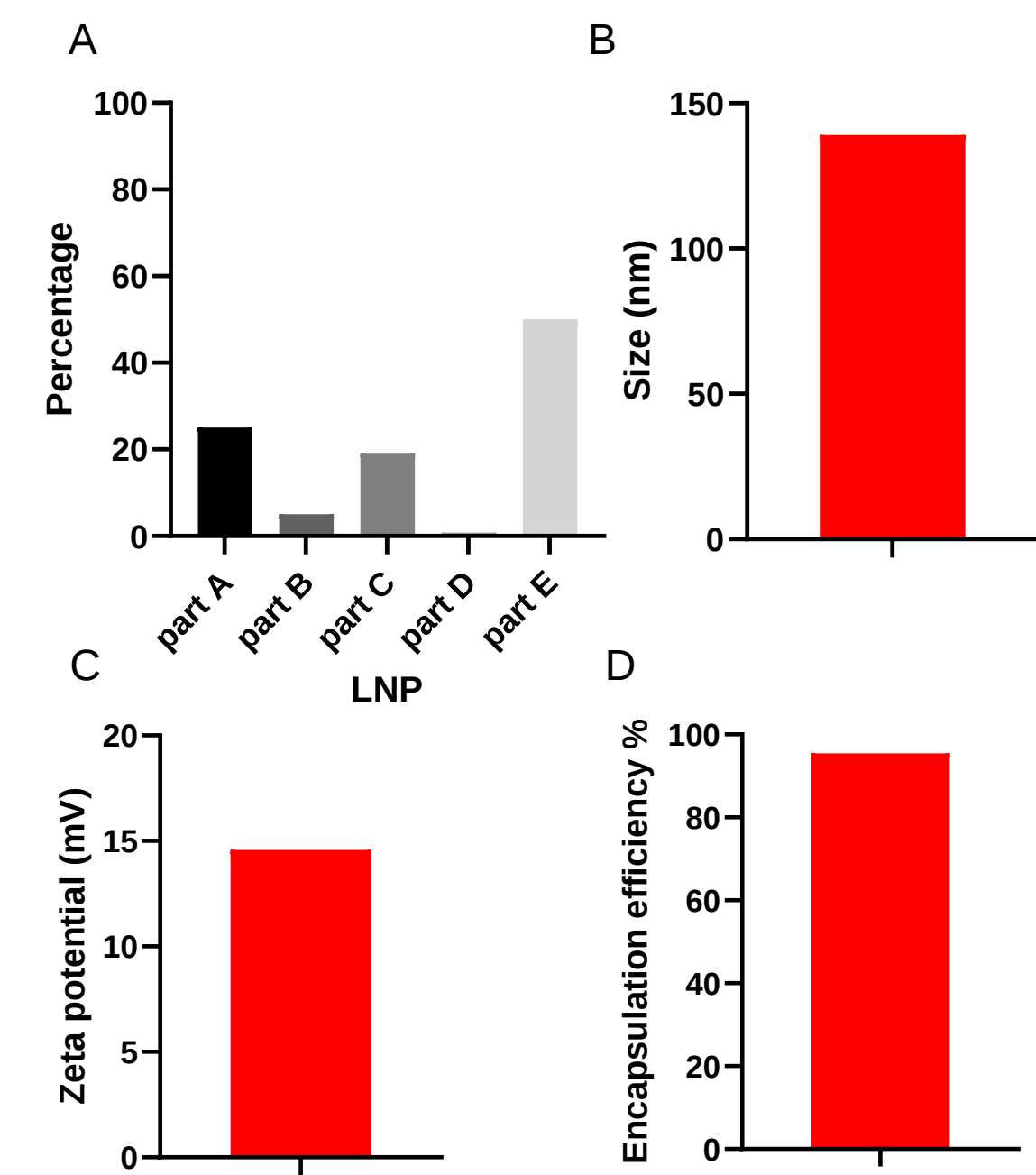


Figure 1. Preparation and characterization of mRNA LNP

(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

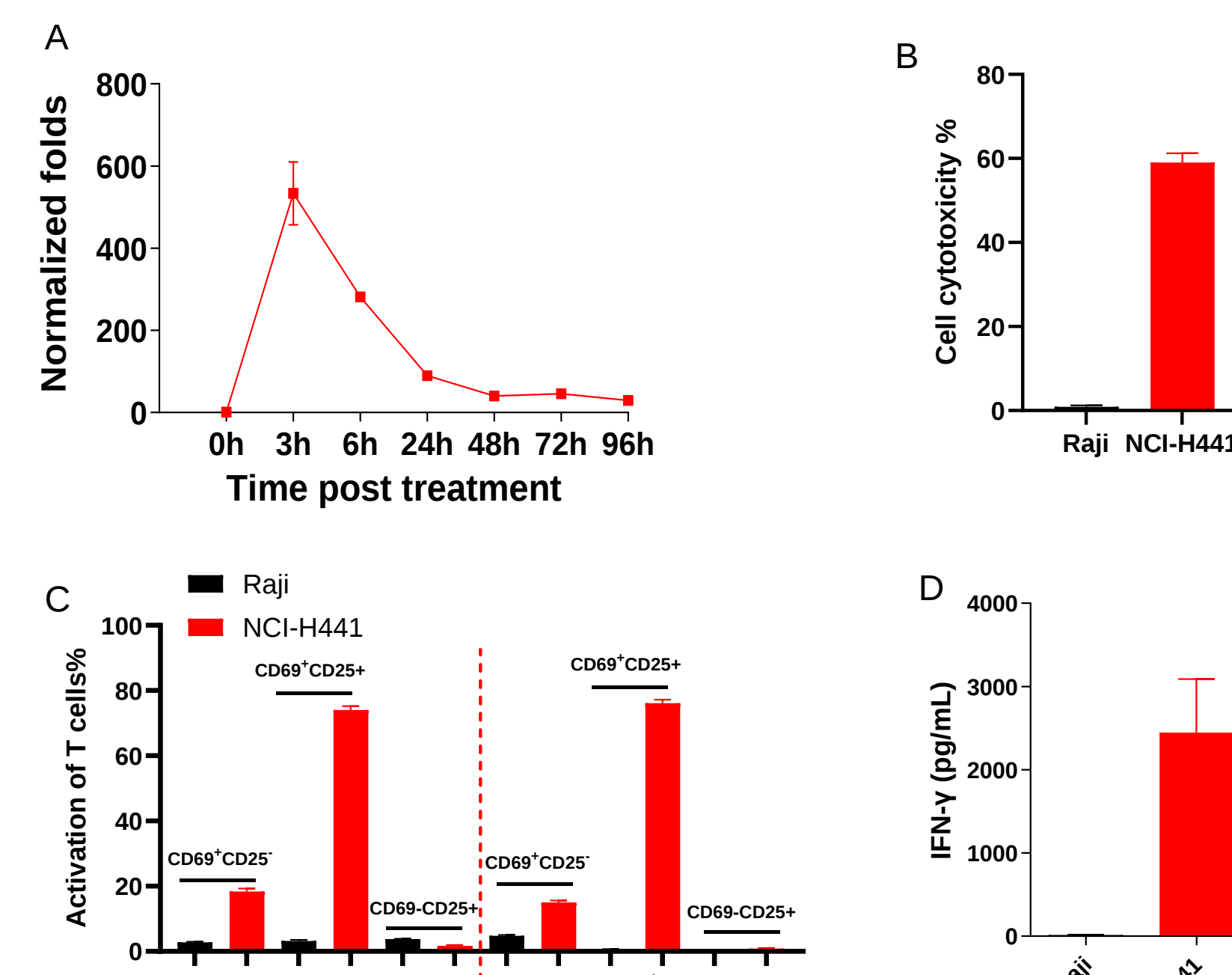


Figure 2. EpCAM-CD3 BsAb encoded mRNA is biologically active *in vitro*

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 IFN γ concentration in supernatant

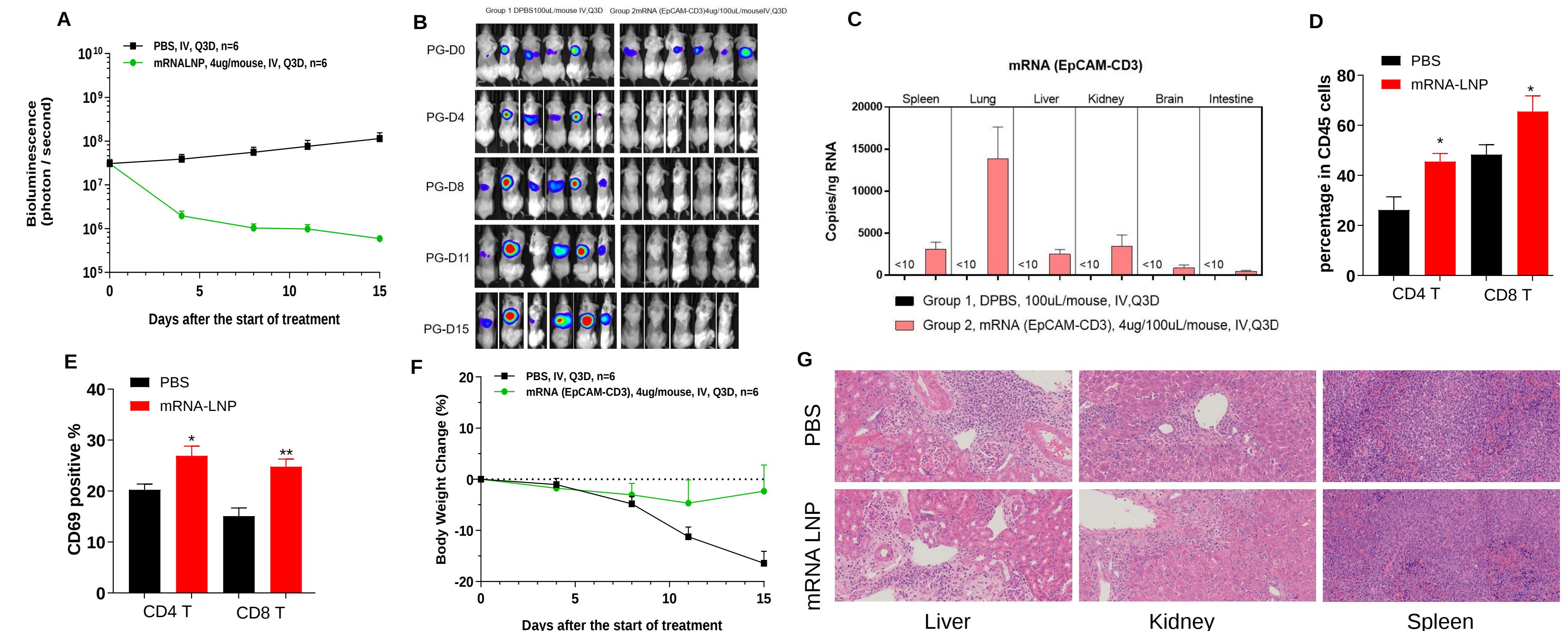


Figure 3. EpCAM-CD3 BsAb encoded mRNA LNP inhibited tumor growth *in vivo*

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

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