LNP-RNA-Mediated Antigen Presentation Leverages SARS-CoV-2-Specific Immunity for Cancer Treatment

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INTRODUCTION

Lipid nanoparticle (LNP)-mRNA vaccines have demonstrated protective capability in combating SARS-CoV-2[1]. Their extensive deployment across the global population leads to the broad presence of T-cell immunity against the SARS-CoV-2 spike protein, presenting an opportunity to harness this immunological response as a universal antigen target for cancer treatment[2]. Herein, we design and synthesize a series of amino alcohol- or amino acid-derived ionizable lipids (AA lipids) and develop an LNP-RNA-based antigen presentation platform to redirect spike-specific T-cell immunity against cancer in mouse models[3]. First, in a prime-boost regimen, AA2 LNP encapsulating spike mRNA elicits robust T-cell immunity against the spike epitopes. Next, AA15V LNP efficiently delivers self-amplifying RNAs (saRNAs) encoding spike epitope-loaded single-chain trimer (sSE-SCT) MHC I molecules into tumor tissues, thereby inducing the presentation of spike epitopes. Our results show that a single intratumoral (i.t.) treatment of AA15V LNP-sSE-SCTs suppresses tumor growth and extends the survival of B16F10 melanoma tumor-bearing mice vaccinated with AA2 LNP-spike mRNA. Additionally, AA15V LNP-sSE-SCTs enable SE-SCT expression in *ex vivo* human glioblastoma and lung cancer samples, suggesting its potential in clinical translation.

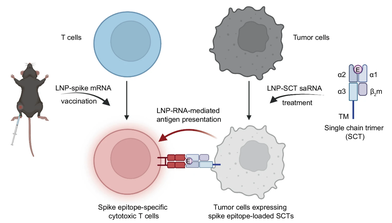

Figure 1. Illustration of LNP-RNA-mediated antigen presentation platform. E spike epitope, TM transmembrane domain.

METHODS

- Designed and optimized lipid nanoparticle formulations for intramuscular mRNA vaccination and intratumoral delivery.
- Constructed self-amplifying RNAs (saRNAs) encoding spike epitope-loaded single-chain trimer MHC I molecules (sSE-SCT) for cancer immunotherapy applications.
- Evaluated tumor growth and survival outcomes in vaccinated mouse models treated with AA15V-sSE-SCT.
- Explored the applicability of AA15V-sSE-SCT in human tumor tissue samples.

RESULTS

1. Amino-alcohol or amino acid-derived (AA) LNPs enable intramuscular mRNA delivery and elicit robust spike-specific T-cell responses.

We first designed a library of sugar-alcohol derived lipids to deliver mRNAs into JAWSII and C2C12 cells, followed by *in vivo* validation. Among them, AA2 LNPs demonstrated the highest delivery efficiency. Vaccination efficacy was confirmed by measuring spike-specific IgG titers and performing activation-induced marker assays on CD8$^+$ T cells in both blood and spleen.

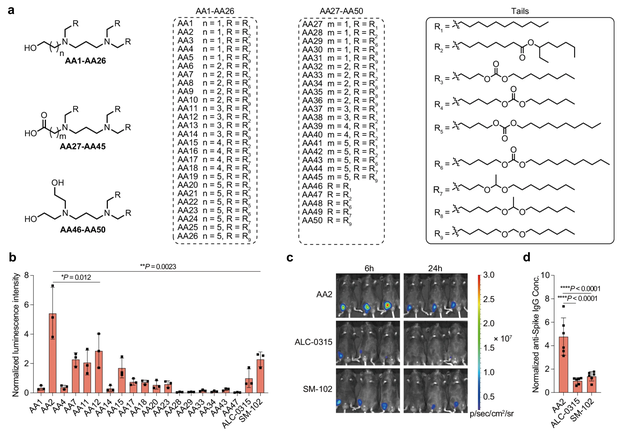

Figure 2. a Design and structures of AA lipids. b The luminescence intensity of the muscles in the lead AA LNP-FLuc mRNA-treated C57BL/6 mice normalized to the ALC-0315 LNP group. c Representative images of C57BL/6 mice i.m. treated with AA2 LNP-, ALC-0315 LNP-, or SM-102 LNP-Fluc mRNA. d Spike-specific IgG titer in blood drawn from C57BL/6 mice vaccinated with AA2 LNP-, ALC-0315 LNP-, or SM-102 LNP-spike mRNA.

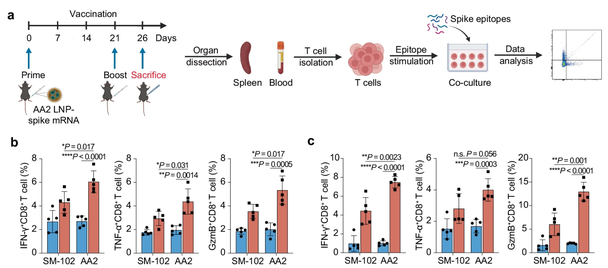

Figure 3. a Schematic depiction of activation-induced marker (AIM) assay of T cells isolated from spleen and blood of mice vaccinated with AA2 LNP- or SM-102 LNP-spike mRNA. b, c Spike epitope-specific activation of CD8$^+$ T cells isolated from the blood (b) and spleen (c) of the mice vaccinated with AA2 LNP- or SM-102 LNP-spike mRNA. Gzmb granzyme B, CE control epitope, SE spike epitope.

2. Treatment with AA15V LNP-sSE-SCTs redirects spike-specific T-cell immunity to treat tumors.

Next, we evaluated the delivery efficiency of AA LNPs in B16F10 melanoma cells, designed and constructed self-amplifying RNAs (saRNAs) encoding spike epitope-loaded single-chain trimer MHC I molecules (SE-SCTs). To evaluate the antitumor effects of spike-specific T-cell immunity against cancer cells expressing SE-SCT, we performed a single i.t. treatment in a B16F10 melanoma mouse model, where the mice received prime-boost immunizations of AA2 LNP-spike mRNA before tumor inoculation.

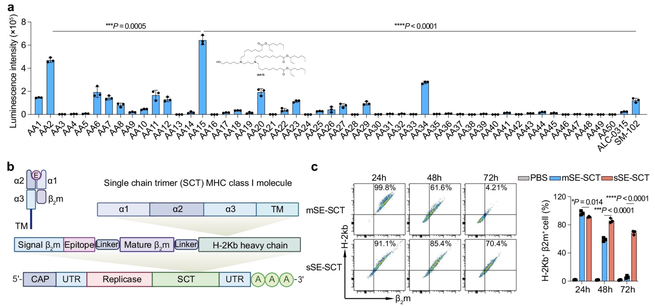

Figure 4. Screening of AA LNPs in B16F10 cells. b Construction of saRNA encoding MHC I single-chain trimer (SCT) containing spike epitopes. E spike epitope, TM transmembrane domain. c Dynamic expression of H-2Kb$^+$β2m$^+$ in B16F10 cells.

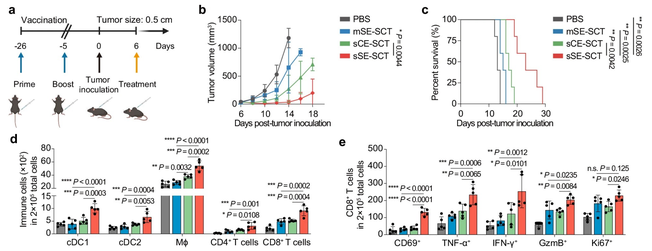

Figure 5. a Schematic of the treatment regimen in the B16F10 tumor model. b Tumor volumes in different groups. c Survival rates of the mice in the B16F10 tumor model. d Immune cell populations in tumor tissues. e Populations of primed CD8$^+$ T cells in tumor tissues.

3. Applicability of AA15V LNP-sSE-SCTs in human tumor samples

To explore the clinical translatability of AA15V LNP-sSE-SCTs, we examined AA15V LNP for the delivery of sSE-SCTs to human tumor tissues *ex vivo*.

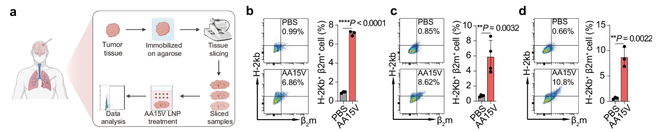

Figure 6. a Schematic depiction of *ex vivo* AA15V LNP-sSE-SCTs delivery in human tumor tissues. b Expression of H-2Kb$^+$β2m$^+$ expression in CD45$^-$ cells from pediatric glioma dissections after *ex vivo* treatment with AA15V LNP-sSE-SCTs. c, d H-2Kb$^+$β2m$^+$ in CD45$^-$ cells from two separate lung left lower lobe (LLL) adenocarcinoma specimens after *ex vivo* treatment with AA15V LNP-sSE-SCTs.

CONCLUSIONS

In summary, we have developed an LNP-RNA-based antigen presentation platform designed to redirect spike-specific T-cell immunity against cancer. The clinical potential of this platform is highlighted by two key aspects: first, AA2 LNP demonstrates superior efficacy for mRNA vaccine delivery *in vivo* compared to FDA-approved LNPs; second, AA15V LNP facilitates the delivery of sSE-SCTs to human cancer samples. Considering that a significant portion of the global population has already developed SARS-CoV-2 T-cell memory, this strategy provides a new avenue for cancer immunotherapy.

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