Lipid Nanoparticles Enabling Potent Immune Response to mRNA Cancer Vaccines

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Abstract

Introduction & Objectives mRNA-based vaccines, particularly when combined with checkpoint inhibitors, have emerged as a promising therapeutic strategy for cancer, supported by encouraging outcomes from advanced-stage clinical trials. However, their clinical success depends on safe and efficient intracellular delivery of mRNA. Lipid nanoparticles (LNP) have become the delivery platform of choice because they efficiently target antigen-presenting cells, promote intracellular antigen expression, and induce potent antigen-specific immune responses that drive durable anti-tumor immunity. With Acuitas' leading expertise in LNP technology in various therapeutic areas, we have been advancing LNP for the development of mRNA-based cancer vaccines.

Materials & Methods Unmodified mRNA encoding OVA, Tumor-Associated Antigens (TAA) or Neoantigens, formulated with ALC-315®, the ionizable lipid in the COVID-19 vaccine COMIRNATY®, was tested in mice with a prime-two-boost vaccination regimen.

Antigen-specific adaptive immune responses, including T cell responses and their poly-functionality, plasma antibody titers, as well as efficacy in a tumor model were assessed in comparison to clinically advanced non-viral lipid-based delivery platforms.

Results We previously demonstrated that unmodified mRNA encoding OVA antigen formulated with ALC-315® triggered strong CD8 T cell responses. A prime-two-boost vaccination regimen induced >40% antigen-specific $CD8^+$ T cell responses, with comparable responses observed at doses of 10 µg and 0.2 µg. Subsequent studies assessed the potency of Acuitas' ALC-315® LNP compared to two non-viral lipid-based delivery platforms currently in advanced clinical development. Acuitas' LNP, at much lower doses, induced equivalent or superior cellular immunity as indicated by MHC tetramer and intracellular cytokine staining. In addition, Acuitas LNP induced higher poly-functional T cells as well as higher plasma antibody titers.

Beyond its activity with exogenous antigens, ALC-315® demonstrated potent induction of cellular immune responses against TAA and neoantigens following vaccination with concatemer mRNA vaccines. Importantly, Acuitas' LNP induced significantly stronger antigen-specific T cell responses than SM-102. Furthermore, *in vivo* efficacy studies showed that Acuitas' LNP effectively inhibited tumor growth and significantly prolonged survival in a murine tumor model.

In order to identify next generation LNP beyond ALC-315®, we screened additional lipids from our proprietary library and identified novel lipids with comparable or superior potency to ALC-315®. In parallel, mechanism-of-action studies are underway to identify key attributes associated with performance and to guide the rational design of next generation LNP for mRNA cancer vaccines.

Conclusion Overall, our data indicates that Acuitas' LNP are a robust mRNA delivery platform for cancer vaccine development which may outperform current lipid-based delivery platforms being evaluated in the clinic.

1 ALC-315® Based mRNA-LNP Vaccine Induces Comparable Potent CD8 T Cell Across 0.2 to 10µg Dose Range

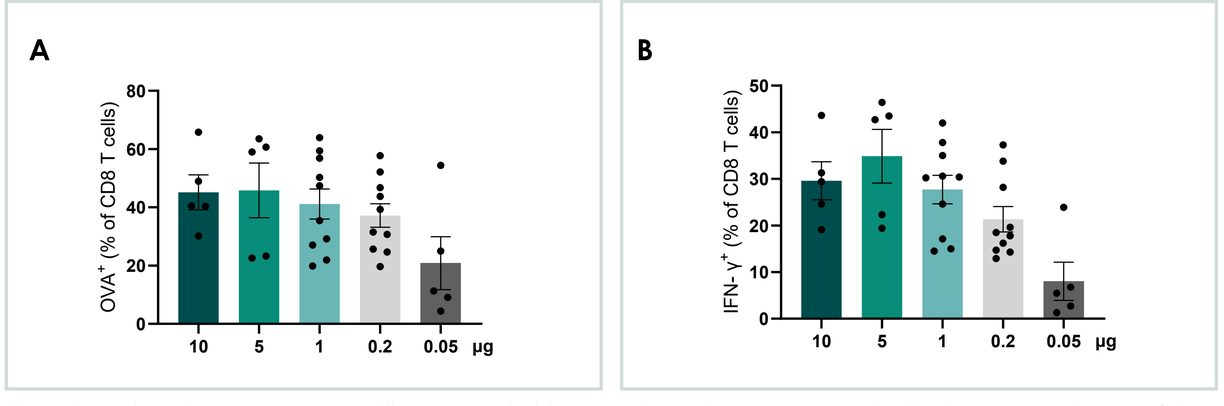


Fig. 1. Comparison of cellular responses to different doses of uridine-based OVA mRNA-LNP. OVA-specific CD8 T cell responses in spleen @ Day 21 following i.m. vaccination with 3 weekly doses of mRNA-LNP. % MHC-I SIINFEKL dextramer+ CD8 T cells (**A**); IFN-γ secretion after *ex vivo* restimulation with OVA SIINFEKL peptide and intracellular staining (**B**). Note: 5 doses were tested over 3 independant experiments (Exp.): Exp. 1 - 10, 5 & 1 µg; Exp. 2 - 1 & 0.2 µg; Exp. 3 - 0.2 & 0.05 µg.

2 ALC-315® Based mRNA-LNP Vaccine Induces Stronger Cellular & Humoral Responses than LPX and SM-102

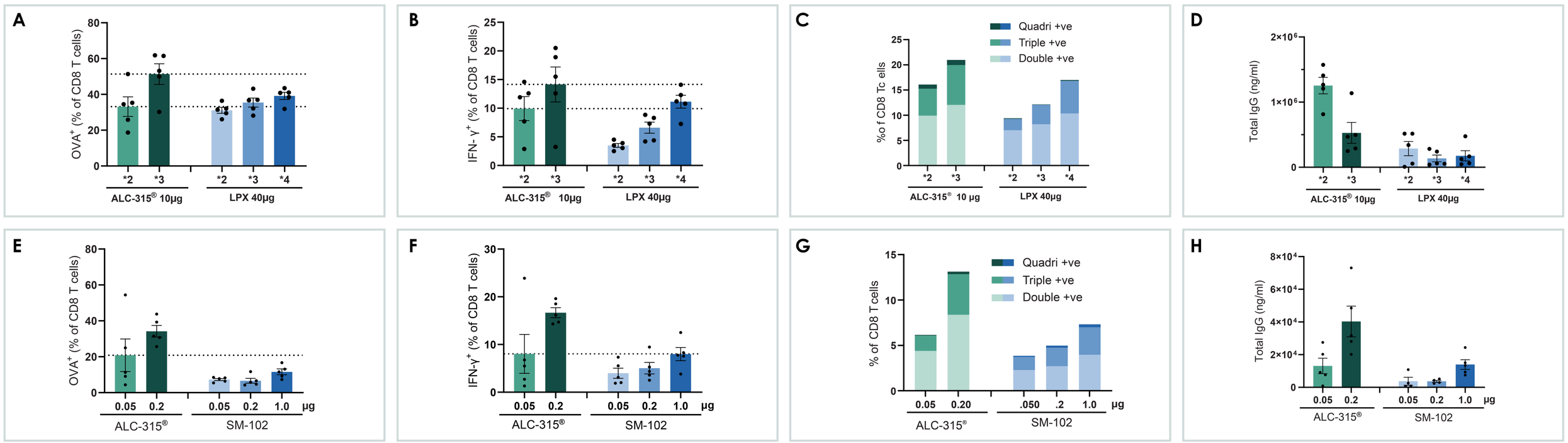


Fig. 2. Comparison of cellular and humoral responses between ALC-315® & LPX (A-D) or SM-102 (E-H). OVA-specific cellular and humoral responses induced @ Day 21 following: i.m. vaccination of 10 µg mRNA-LNP (on Day 0 & 14, or Day 0, 7 & 14), or 40 µg mRNA-LPX (on Day 0 & 14, or Day 0, 7 & 14, or Day 0, 3, 7 & 14) (**A-D**); i.m. vaccination with 3 weekly doses of mRNA-LNP (**E-H**). % SIINFEKL dextramer+ CD8 T cells (**A, E**); IFN-γ secretion by splenic CD8 T cells after *ex vivo* restimulation with OVA SIINFEKL peptide and intracellular staining (**B, F**); polyfunctional CD8 T cell analysis based on IFN-γ, TNF-a, CD107a and IL-2 (**C, G**); plasma OVA-specific IgG (**D, H**).

3 ALC-315® Based mRNA-LNP Vaccine Elicits Potent Cellular Response Against Syngeneic TAA & Neoantigens

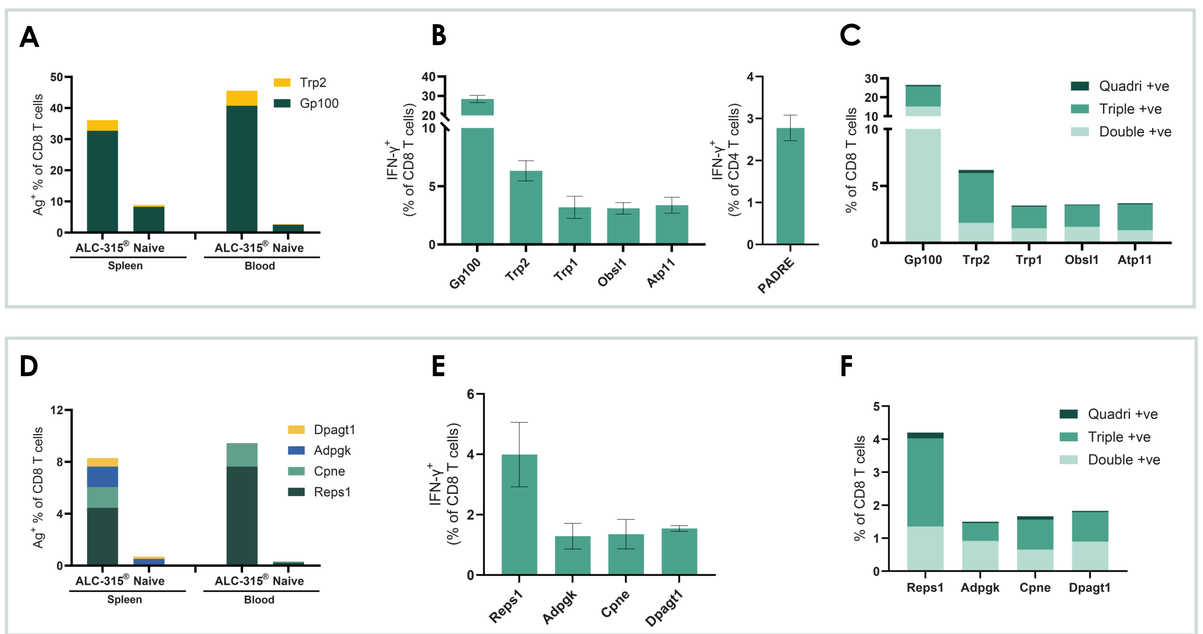


Fig. 3. Cellular response against syngeneic tumour-associated antigens (TAA) & neoantigens. Antigen-specific cellular response induced @ Day 21 following i.m. vaccination with 3 weekly doses of 5 µg mRNA-LNP concatemer encoding B16-F10 melanoma epitopes (3 TAA: Pmel/gp100, Dct/Trp2, Trp1 & 7 neoantigens: Pbk, Obsl1, Plod2, Ints11, Kif18b, Atp11a, Trp53) and PADRE (**A-C**) or MC38 colon adenocarcinoma neo-epitopes (Reps1, Adpgt, Cpne, Dpagt1, Aatf, Irga, Med12) (**D-F**). % antigen specific CD8 T cells in spleen and blood (**A, D**); IFN-γ secretion by CD4 T cells (PADRE) or CD8 T cells (all other antigens) after *ex vivo* restimulation with indicated peptides (**B, E**); polyfunctional CD8 T cell analysis based on IFN-γ, TNF-a, CD107a and IL-2 (**C, F**).

4 ALC-315® Based mRNA-LNP Vaccination Significantly Inhibits B16-F10 Melanoma Tumour Growth

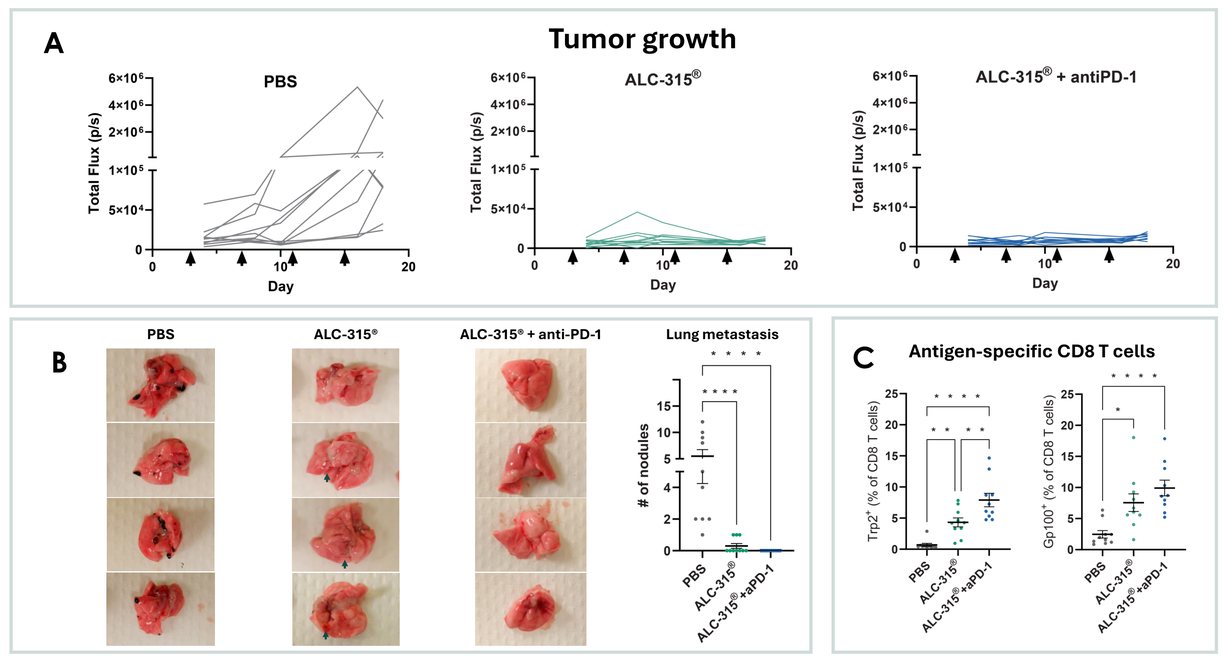


Fig. 4. Cancer vaccine efficacy in mouse melanoma model. 1 x 10^5 B16-F10-Luc2 cells were inoculated intravenously on Day 0. On Day 3, 7, 11, 15, two groups received i.m. vaccination with 5 µg mRNA-LNP concatemer encoding B16-F10 melanoma TAA & neoantigen epitopes (as indicated in Fig. 3 legend) or vehicle for PBS group. On Day 2, 7, 11, and 15, one of the vaccinated groups also received i.p. injection with 200 µg PD-1 antibody (clone: RMP1-14) while the other 2 groups received i.p. injection of PBS. On Day 4, 8, 10 ,16 and 18, all mice were administered D-luciferin substrate solution i.p. and imaged using an exposure time of 60s. Bioluminescence values were quantified by measuring total photon flux (photons/second) in chest regions and plotted for individual animals to show the tumor burden in PBS and treatment groups (**A**). At termination on Day 18, lungs were collected and counted for melanoma metastasis nodules (**B**). % antigen specific CD8 T cells was assessed in blood (**C**).

5 Novel LNP Demonstrates Superior Potency to ALC-315® For mRNA Cancer Vaccine

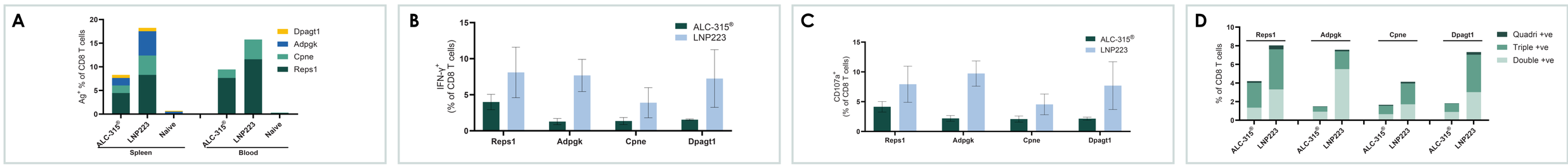


Fig. 5. Identification of novel LNP with superior potency than ALC-315®. Tumour neoantigen-specific cellular response in spleen induced @ Day 21 following i.m. vaccination with 3 weekly doses of 5 µg mRNA-LNP encoding a concatemer of MC38 colon adenocarcinoma neo-epitopes (Reps1, Adpgt, Cpne, Dpagt1, Aatf, Irgq, Med12). % MHC-I dextramer+ CD8 T in spleen and blood (**A**); IFN-γ secretion (**B**) and CD107a expression (**C**) by CD8 T cells after *ex vivo* restimulation with indicated peptides; polyfunctional CD8 T cell analysis based on IFN-γ, TNF-a, CD107a and IL-2 (**D**).

Summary

1. ALC-315®-formulated mRNA vaccine demonstrates strong $CD8^+$ T-cell responses across a 0.2 ~ 10 µg dose range (Fig. 1).
2. At 1/4th of LPX dose & with fewer vaccination occasions, Acuitas' LNP elicits greater CD8 T cell and humoral responses (Fig. 2 A-D); compared to SM-102, Acuitas' LNP @ up to 20-fold lower dose achieves equivalent or superior CD8 T cell response; the trend is the same for humoral response (Fig. 2. E-H).
3. ALC-315® induces strong and broad CD8 T cell responses in 2 murine cancer models against syngeneic antigens delivered in concatemer mRNA constructs containing a mixture of TAA and neoantigens (B16-F10) or neoantigens alone (MC38). Importantly T cell response was induced against majority of antigenic epitopes including CD4 T cells against PADRE (Fig. 3).
4. ALC-315®-based mRNA-LNP vaccination significantly inhibited B16-F10 melanoma tumour growth in a therapeutic setting, with enhanced tumour inhibition when combined with anti-PD-1. This benefit was associated with increased antigen-specific CD8+ T-cell responses, which were further elevated by combination therapy.
5. Newly identified LNP induces superior multi-functional CD8 T cell response against MC38 neoantigens compared to ALC-0315 (Fig. 5).

   Validation of Acuitas' LNP efficacy and key attributes to guide next generation LNP for mRNA cancer vaccine design is ongoing.

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