

Lipid Nanoparticles Enabling Potent Immune Response to mRNA Cancer Vaccines

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Abstract

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-tumour 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.

We previously demonstrated that unmodified mRNA encoding OVA antigen formulated with ALC-315[®] triggered strong CD8 T cell responses. A prime-two boosts vaccination regimen induced >40% antigen-specific CD8⁺ T cell responses, with comparable responses observed at 10 µg and 0.2 µg doses. 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 assays. In addition, Acuitas' LNP induced higher poly-functional T cells as well as higher humoral response.

Beyond its potency with exogenous antigens, ALC-315[®] induced strong cellular immune responses against syngeneic tumour-associated antigens (TAA) in mice vaccinated with concatemer mRNA vaccine. To identify next generation LNP with superior cancer vaccines activity, we screened additional lipids from our proprietary library and identified new LNP inducing greater T cell response than ALC-315[®]. To decipher MoA of active LNP, efforts are underway to identify key attributes associated with performance and to guide the rational design of next generation LNP for mRNA cancer vaccines.

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

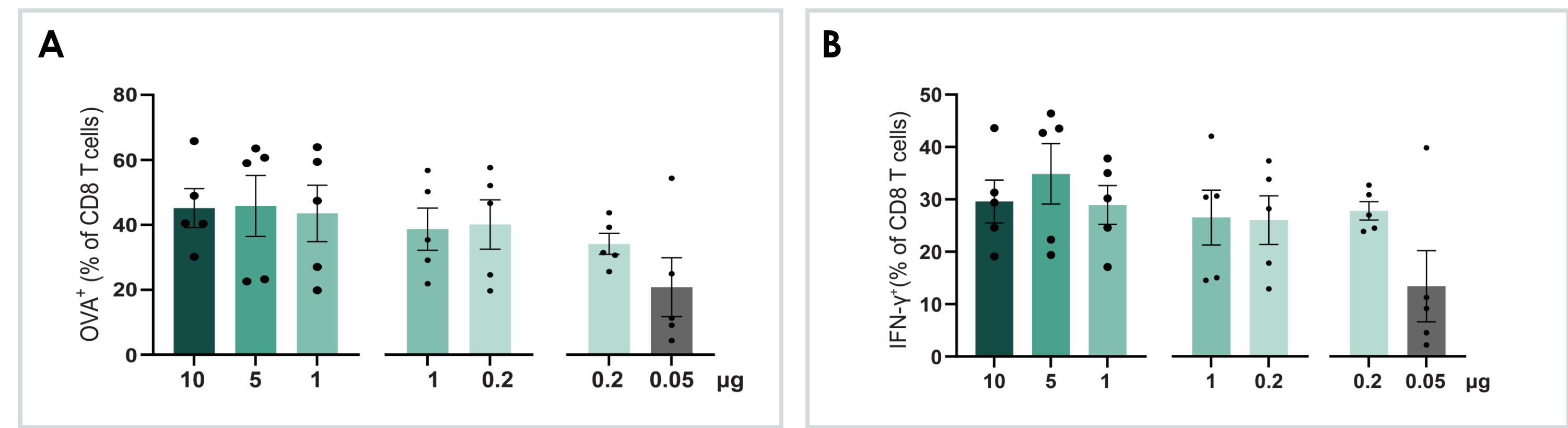


Fig. 1. Comparison of cellular responses to different doses of uridine-based OVA mRNA-LNP. OVA-specific CD8 T cell and humoral responses in spleen @ Day 21 following i.m. vaccination with 3 weekly doses of mRNA-LNP. % MHC-I SIINFEKL dextramer⁺ CD8 T cells (**A**); IFN-γ secretion after ex vivo restimulation with OVA SIINFEKL peptide and intracellular staining (**B**).

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

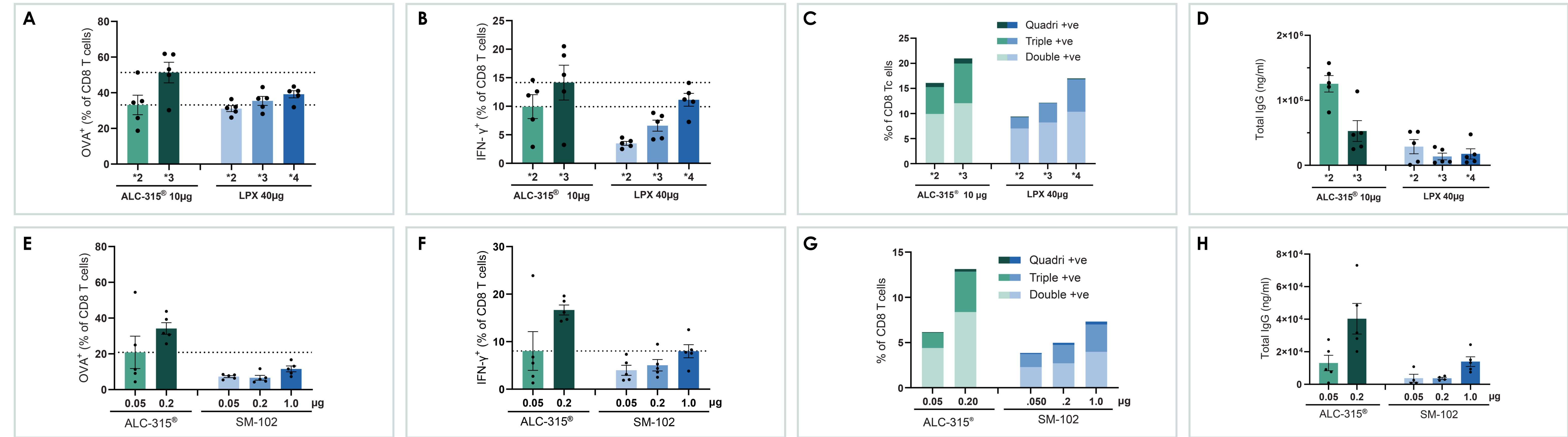


Fig. 2. Comparison of cellular and humoral responses between ALC-315[®] & LPX (A-D) or ALC-315[®] & SM-102 (E-H). OVA-specific cellular and humoral responses induced @ Day 21 following i.m. vaccination of 10 µg mRNA-LNP (on Day 0 & 14, or Day 0, 7 & 14), or 40 µg mRNA-LPX (on Day 0 & 14, or Day 0, 7 & 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-α, 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

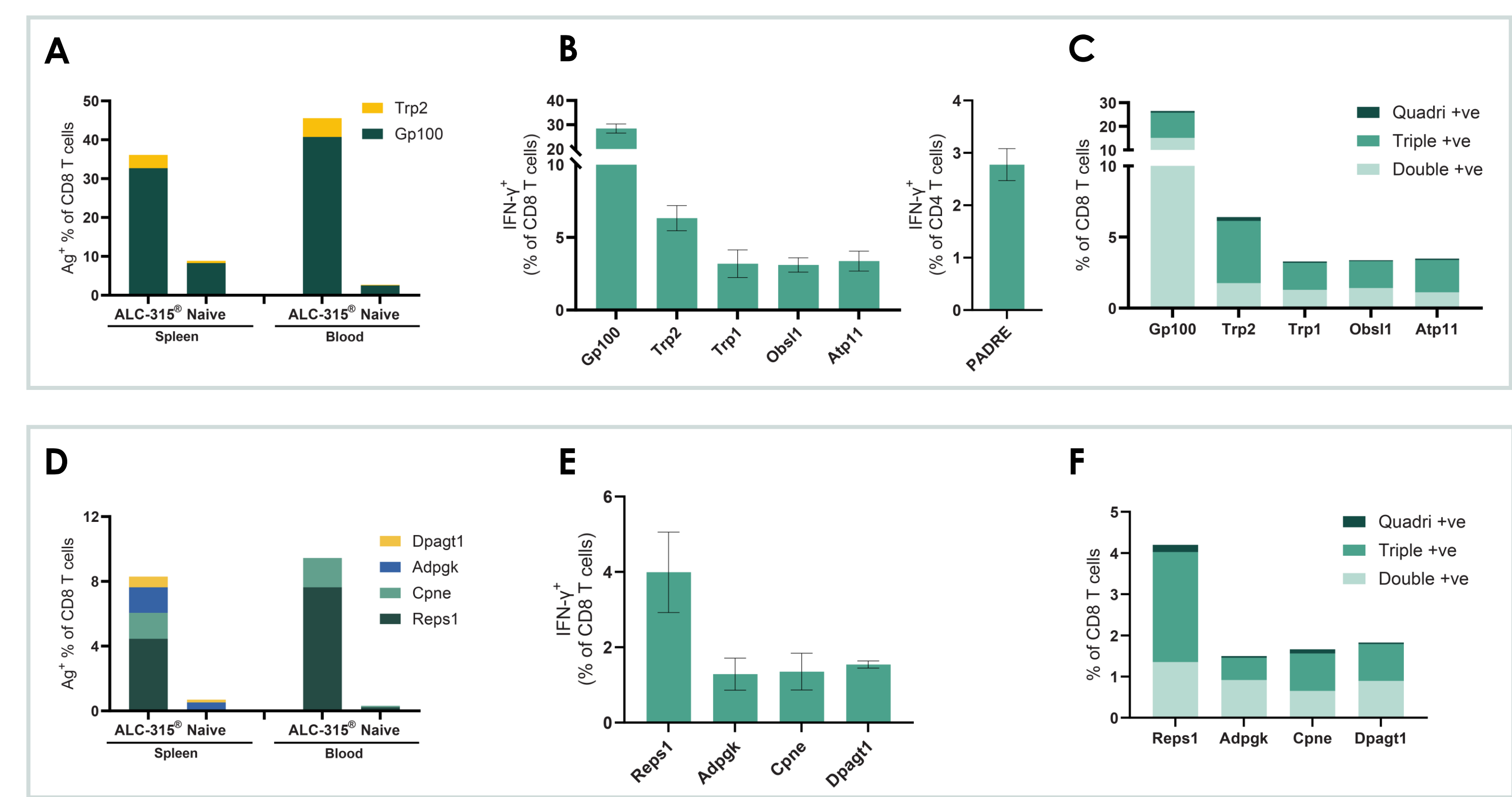


Fig. 3. Cellular response against syngeneic tumour Associated Antigens (TAA) & neoantigens. Antigen-specific cellular response in spleen induced @ Day 21 following i.m. vaccination with 3 weekly doses of 5 µg mRNA-LNP concatemer encoding B16F10 melanoma epitopes (3 TAA: Pmel/gp100, Dct/Trp2, Trp1 & 7 neoantigens: Pbk, Obsl1, Plod2, Ints11, Kif18b, Atp11a, Trp53) and PADRE (**A-C**) or MC38 colon adenocarcinoma neo-epitopes (Reps1, Adpgk, Cpne, Dpapt1, Aatf, Irgg, 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-α, CD107a and IL-2 (**C, F**).

4 Therapeutic Vaccination with ALC-315[®] Based mRNA-LNP Reduces Tumour Growth and Improves Animal Survival

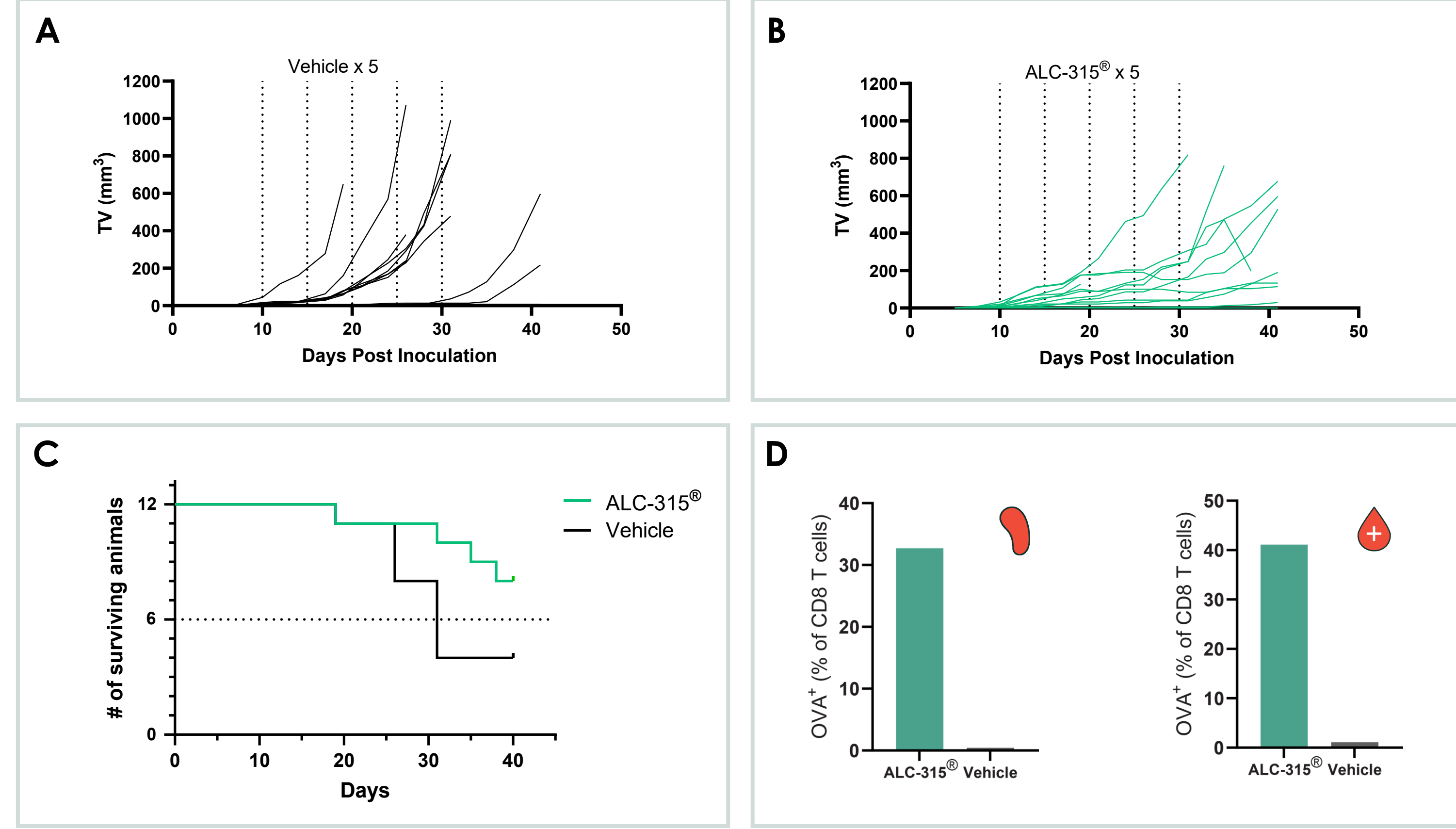


Fig. 4. Mouse melanoma model therapeutic vaccine. 5 x 10⁵ B16-F10-OVA#1-G12 cells were inoculated subcutaneously into the lower back of mice on Day 0. At Day 10, mice receive 1st i.m. vaccination with 1 µg mRNA-LNP encoding OVA or vehicle every 5 days for 5 doses. Tumour volume (TV) calculated by L X W² / 2 mm³. (**A, B**); animal survival (**C**); % MHC-I dextramer⁺ CD8 T cells on Day 20, 5 days following 2nd dose (**D**).

5 Novel LNP Demonstrate Superior Potency to ALC-315[®] For mRNA Cancer Vaccine

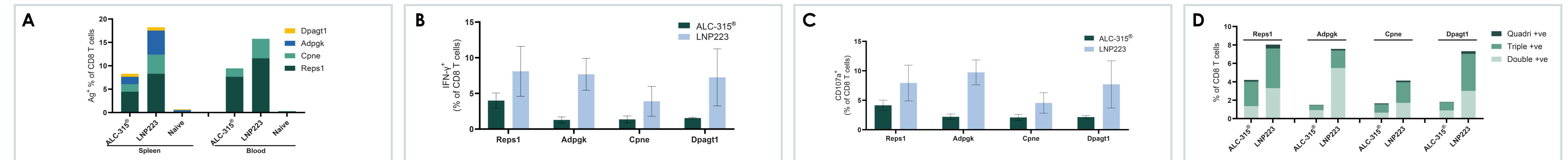


Fig. 5. Identification of novel LNP with superior potency than ALC-315[®]. Tumour neoantigen-specific cellular response in spleen induced @ Day 21 following i.m. vaccination with 3 weekly doses of 5 µg mRNA-LNP encoding a concatemer of MC38 colon adenocarcinoma neo-epitopes (Reps1, Adpgk, Cpne, Dpapt1, Aatf, Irgg, 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-α, CD107a and IL-2 (**D**).

Summary

- 1 ALC-315[®]-formulated mRNA vaccine demonstrates potency with sustained 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 @ 20 and 5-fold lower doses achieves respectively equivalent and 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 response against 2 models of mouse syngeneic antigens designed in concatemer mRNA constructs containing mixture of TAA and neoantigens (B16F10) or neoantigens only (MC38). Importantly T cell response was induced against majority of antigenic epitopes including CD4 T cells against PADRE (Fig. 3).

- 4 Despite initiating treatment 10 days after tumour implantation, ALC-315[®] based mRNA-LNP vaccination inhibited tumour growth & improved survival, demonstrating therapeutic efficacy in an established tumour model (Fig. 4).
- 5 Newly identified LNP induces superior multi-functional CD8 T cell response than ALC-315[®] against MC38 neoantigens (Fig. 5)

Additional efforts to validate Acuitas' LNP efficacy and to identify key attributes guiding next generation LNP for mRNA cancer vaccine design are ongoing.

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