Lipid Nanoparticles (LNP) for mRNA Cancer Vaccine

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Abstract

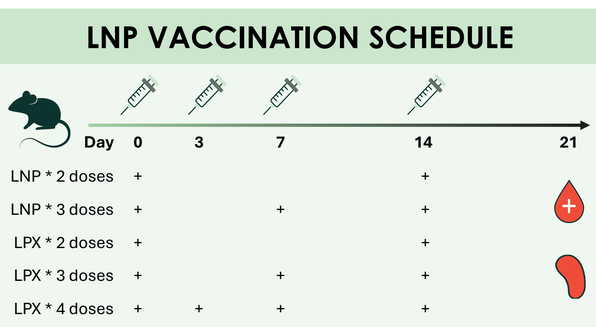
Following successful mRNA vaccines against COVID-19, mRNA-based vaccines, in combination with checkpoint inhibitors, have emerged as promising immunotherapy to combat cancer as demonstrated by current advanced-stage clinical trials. An effective and safe delivery platform is essential for mRNA cancer vaccines, enabling mRNA delivery to antigen (Ag) presenting cells leading to Ag expression and efficient Ag-specific immunogenicity capable of irradicating cancers.

With Acuitas' leading expertise in LNP technology, we have been advancing LNP for development of mRNA-based cancer vaccines. We have demonstrated that uridine-based mRNA encoding a tumour antigen, formulated with ALC-315™, the ionizable lipid in Covid-19 Vaccine COMIRNATY®, triggers strong CD8 T cell responses than N1-methylpseudouridine-based mRNA. A "prime and 2 boosts" mRNA vaccination regimen resulted in >40% Ag-specific CD8⁺ T cell responses, which were equivalent between 10 µg and 0.2 µg doses.

Subsequent studies assessed potency of Acuitas' ALC-315™ LNP compared to that of other non-viral lipid-based delivery platforms currently in clinical development[1]. 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. While plasma antibody data indicated Th1-biased immune response by both vaccines, Acuitas LNP maintained a more balanced Th1/Th2 ratio.

In addition to clinical evidence on efficacy and safety of COMIRNATY® to protect against infectious disease, our findings indicate that Acuitas' LNP are also a robust mRNA delivery technology for cancer vaccine development. Ongoing studies are assessing LNP capability to induce effective immunogenicity against syngeneic neo-antigens capable of eradicating tumours.

LNP VACCINATION SCHEDULE



1. ALC-315™ with Uridine-Based mRNA Induces Strong Cellular Response at a Very Low Dose

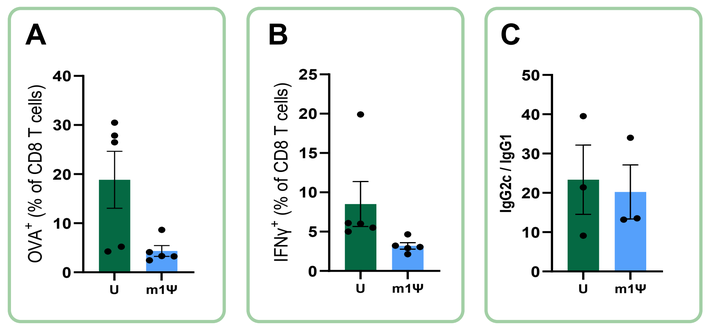


Fig. 1. Comparison of cellular & humoral responses to uridine and N1-methylpseudouridine-based mRNA-LNP. OVA-specific CD8 T cell and humoral responses in spleen @ Day 21 following i.m. vaccination with 2 doses of 10 µg LNP-OVA encoding mRNA (uridine or N¹Ψ -based mRNA) (**A-C**) or 3 doses of uridine-based mRNA (**D-F**). % MHC-I dextramer⁺ CD8 T cells (**A**); IFN-γ secretion by splenic CD8 T cells after ex vivo restimulation with OVA SIINFEKL peptide and intracellular staining (**B**); OVA-specific IgG2c/IgG1 ratio (**C**). % MHC-I dextramer⁺ CD8 T cells (**D**); IFN-γ secretion after ex vivo restimulation with OVA SIINFEKL peptide and intracellular staining (**E**); polyfunctional CD8 T cell analysis based on IFN-γ, TNF-a, CD107a and IL-2 expression (**F**).

2. ALC-315™ Induces More Potent Cellular & Humoral Responses than LPX

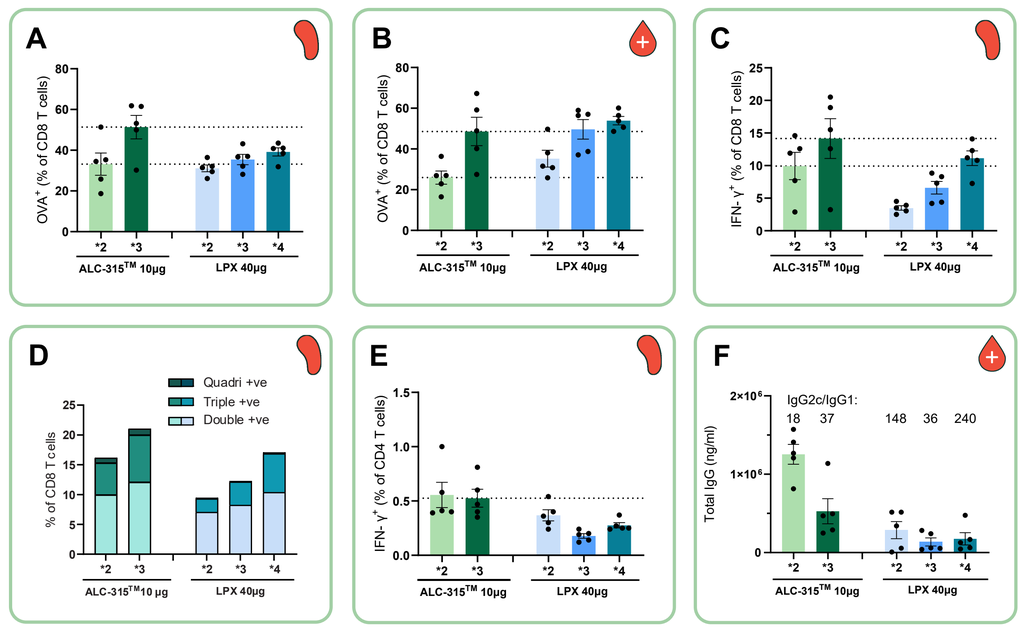


Fig. 2. Comparison of cellular and humoral responses between LNP- and LPX-based cancer vaccines. OVA-specific cellular and humoral responses induced @ Day 21 following i.m. vaccination with 2, 3 doses of 10 µg LNP, or 2, 3, 4 doses of 40 µg LPX (all formulated with uridine-based mRNA). % MHC-I dextramer⁺ CD8 T cells (**A** & **B**); IFN-γ secretion by splenic CD8 T cells after ex vivo restimulation with OVA SIINFEKL peptide and intracellular staining (**C**); polyfunctional CD8 T cell analysis based on IFN-γ, TNF-a, CD107a and IL-2 (**D**); IFN-γ secretion by splenic CD4 T cells after ex vivo restimulation with 15-mer OVA peptide pool (**E**); plasma OVA-specific IgG (**F**).

3. ALC-315™ Induces More Potent Cellular & Humoral Responses than SM-102

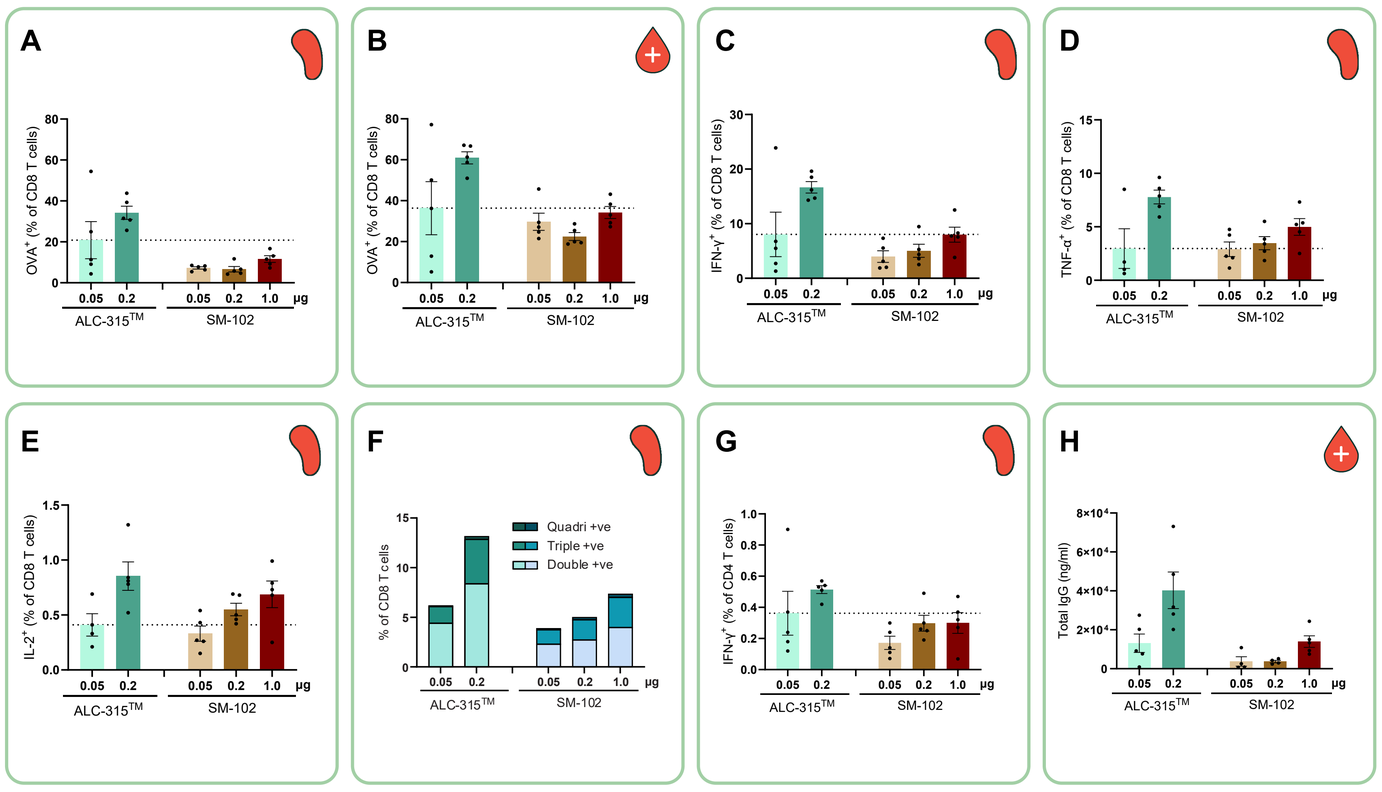


Fig. 3. Comparison of cellular & humoral responses between Acuitas LNP and SM-102 mRNA-LNP vaccines. OVA-specific cellular and humoral responses induced @ Day 21 following i.m. vaccination with 3 doses of LNP-OVA uridine-based mRNA: % MHC-I dextramer⁺ CD8 T cells (**A** & **B**); IFN-γ (**C**), TNF-a (**D**), and IL-2 (**E**) secretion by CD8 T cells after ex vivo restimulation with OVA SIINFEKL peptide; polyfunctional CD8 T cell analysis based on IFN-γ, TNF-a, CD107a and IL-2 expression (**F**); IFN-γ secretion by splenic CD4 T cells after ex vivo restimulation with 15-mer OVA peptide pool (**G**); plasma OVA-specific IgG (**H**).

4. ALC-315™ Induces Strong Cellular Response Against Syngeneic Tumour Antigens

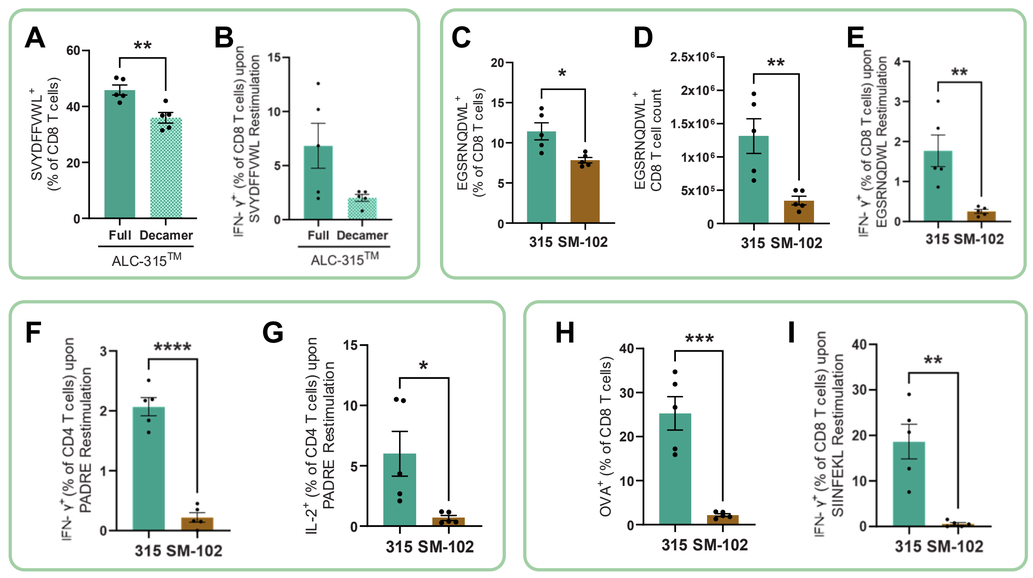


Fig. 4. Cellular responses against syngeneic antigen. Tumour associated antigen-specific cellular responses in spleen induced @ Day 21 following i.m. vaccination with 3 doses of 5 µg LNP-uridine-based mRNA encoding full melanoma TRP2 protein, TRP2 epitope decamer (**A** & **B**) or melanoma poly-epitope (Pmel/gp100, Dct/Trp2, Pbk, Trp1, Obsl1, Plod2, Ints11, Kif18b, Atp11a, Trp53, PADRE and SIINFEKL control) (**C-I**). % Trp2 tetramer⁺ CD8 T cells (**A**); IFN-γ secretion by CD8 T cells after ex vivo restimulation with Trp2 peptide (**B**); % (**C**) and cell count (**D**) of gp100 tetramer⁺ CD8 T cells, and IFN-γ secretion by CD8 T cells after ex vivo restimulation with gp100 peptide (**E**); IFN-γ (**F**) and IL-2 (**G**) secretion by CD4 T cells after ex vivo restimulation with PADRE; % OVA MHC-I dextramer⁺ CD8 T cells (**H**) and IFN-γ secretion by CD8 T cells after ex vivo restimulation with SIINFEKL (**I**).

5. Novel LNP Identification with Comparable Activity to ALC-315™

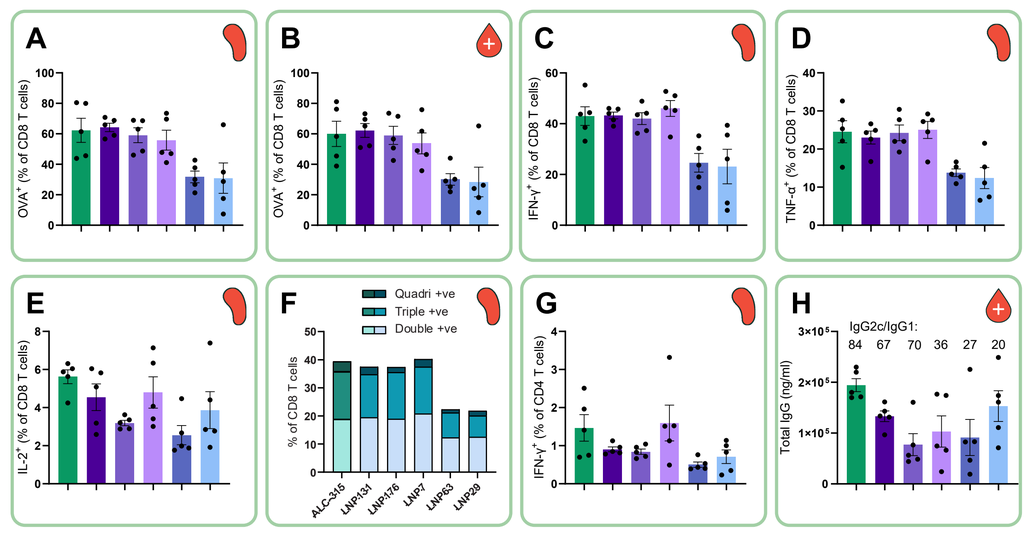


Fig. 5. Identification of novel LNP with equivalent immune activity to ALC-315™. OVA-specific cellular and humoral responses induced @ Day 21 following i.m. vaccination with 3 doses of 1 µg LNP-OVA uridine-based mRNA: % MHC-I dextramer⁺ CD8 T (**A** & **B**); IFN-γ (**C**), TNF-a (**D**), and IL-2 (**E**) secretion by CD8 T cells after ex vivo restimulation with OVA SIINFEKL peptide; polyfunctional CD8 T cell analysis based on IFN-γ, TNF-a, CD107a and IL-2 (**F**); IFN-γ expression by CD4 T cells after ex vivo restimulation with 15-mer OVA peptide pool (**G**); plasma OVA-specific IgG, IgG2c/IgG1 ratio shown above bars (**H**).

Summary

1. Uridine-based mRNA induced significantly stronger CD8⁺ T-cell responses than N1-methylpseudouridine modified mRNA, highlighting the importance of robust type I IFN signaling for APC maturation and efficient antigen presentation during T-cell priming. Despite potentially lower antigen expression from uridine-based mRNA, ALC-315™-formulated mRNA vaccine remained highly potent, sustaining strong CD8⁺ T-cell responses across a 0.2 and 10 µg dose range.
2. At 1/4th of LPX vaccine dose, and with fewer dosing occasions, Acuitas' LNP elicited better CD8 T cell response. Furthermore, humoral responses is significantly higher with a more balanced Th1-biased antibody profile with Acuitas' LNP.
3. Compared to SM-102, LNP used in Spikevax®, Acuitas' LNP at 20-fold lower dose induced equivalent T cell response. At 5-fold lower dose Acuitas' LNP is significantly more potent than SM-102. Same trend is observed for humoral response.
4. ALC-315™ was able to break tolerance and induce strong CD8 cellular response against syngeneic tumour-associated antigen which was superior to SM-102.
5. We have identified novel LNP inducing potent immune responses equivalent to ALC-315™.
6. Further studies are ongoing to screen proprietary LNP potency using neoantigen models, to demonstrate the ability of Acuitas' LNP platform to induce effective anti-cancer immune responses capable of eradicating tumours.

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1. Lena M. Kranz et al. Systemic RNA delivery to dendritic cells exploits antiviral defence for cancer immunotherapy. Nature. 2016 Jun 16;534(7607):396-401. doi: 10.1038/nature18300.