Development of LNP for mRNA Cancer Immunotherapy

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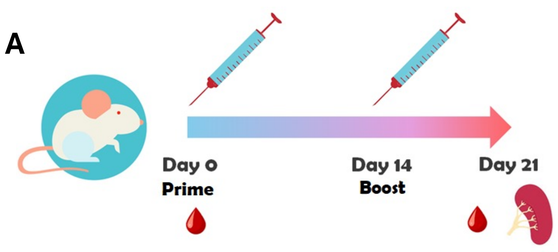
Abstract

Acuitas' mRNA lipid nanoparticle (LNP) technology has been clinically validated, as demonstrated by Alnylam's therapeutic for hereditary ATTR (hATTR) amyloidosis, ONPATTRO® and the Pfizer-BioNTech vaccine, COMIRNATY® which has protected billions of people from COVID-19 in more than 180 countries. The success of the Pfizer-BioNTech COMIRNATY® vaccine has demonstrated the potential of mRNA-LNP, not only for infectious disease vaccines but also potentially for cancer treatment. With continued advancements in genomic sequencing and immune modulation, mRNA-LNP cancer vaccines hold the potential to provide targeted, effective, personalized cancer treatments with less toxicity than current therapies. As clinical trials progress and more data becomes available, mRNA-based vaccines could revolutionize cancer therapy, by overcoming limitations of current treatments and offering new hope for patients with a variety of cancers.

Vaccination Schedule

Fig. A



To create effective and innovative LNP-based cancer vaccines, Acuitas has been advancing its LNP technology by integrating ionizable lipid screening. From our comprehensive, custom-made library of over 1,000 ionizable lipids, we have rationally selected a panel of 20 lipids to assess their relative activity as LNP mRNA cancer vaccines.

For the initial studies, the 20 LNP were formulated with nucleoside-modified OVA mRNA, incorporating N1-methylpseudouridine, and injected *i.m.* to mice in a prime-boost schedule. Antigen-specific adaptive immune responses including T cell responses, serum antibody titers/isotype profiles, and innate immune responses were assessed. Potent antigen-specific cellular responses were observed with the several lipids screened including ALC-0315, the ionizable lipid used in COMIRNATY®. The most active lipids induced a Th1 biased immune response, as judged by T cell poly-functionality and IgG isotype profiles, which is known to be favourable in the context of therapeutic cancer vaccines.

This initial activity assessment utilized the mRNA LNP formulation used in the Pfizer-BioNTech COMIRNATY® infectious disease vaccine. However, given that an aim of cancer vaccines to enhance activation of antigen-presenting cells, leading to stronger adaptive cellular immune responses, it was considered likely that the use of unmodified mRNA would be preferred due to its ability to trigger strong innate immune responses through the recognition of natural uridine residues by pattern recognition receptors such as Toll-like receptors.

For this reason, subsequent studies comparing the activity of modified and unmodified mRNA were conducted. As hypothesized, vaccination with LNP encapsulating unmodified mRNA resulted in significantly higher functional antigen-specific CD8 responses, as indicated by MHC dextramer/tetramer and intracellular cytokine staining. Furthermore, a direct comparison of antigen-specific immune responses showed robust cellular immunity following vaccination with both *i.m.*-administered mRNA LNP and *i.v.*-administered mRNA lipoplexes, an alternative mRNA cancer vaccine format in development.

Cellular / Humoral Responses and Innate Cytokine Stimulation Induced by mRNA-LNP

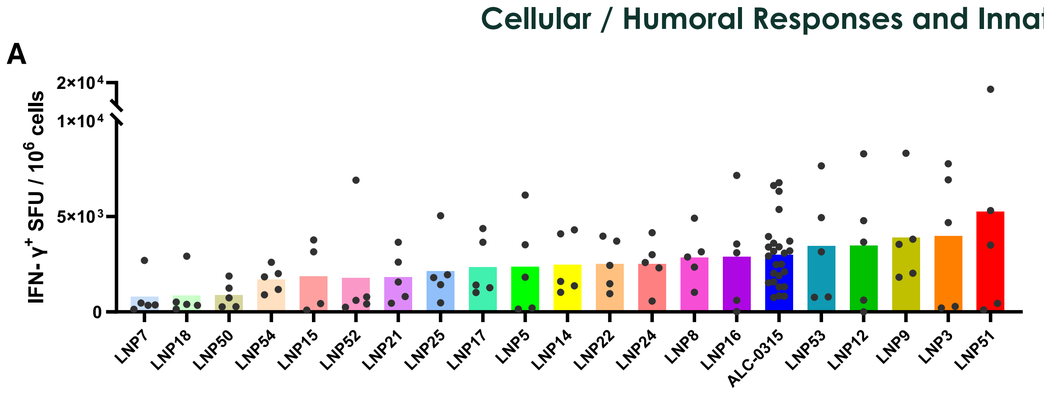


Fig. 1A. Cellular Response Rank of 21 screened LNP: LNP were formulated with modified OVA mRNA, and 1µg RNA-LNP dose was injected *i.m.* in a prime-boost schedule. Splenocytes were restimulated *ex-vivo* with MHC-I SIINFEKL peptide, and antigen specific T cells secreting IFN-γ were assessed by ELISpot assay.

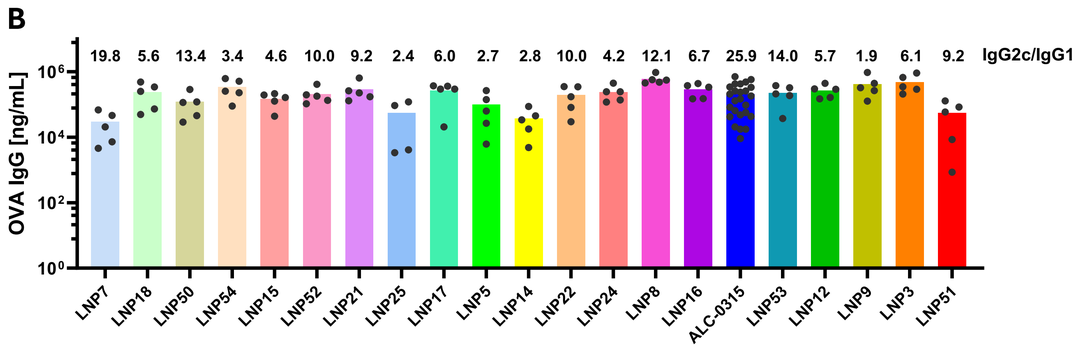
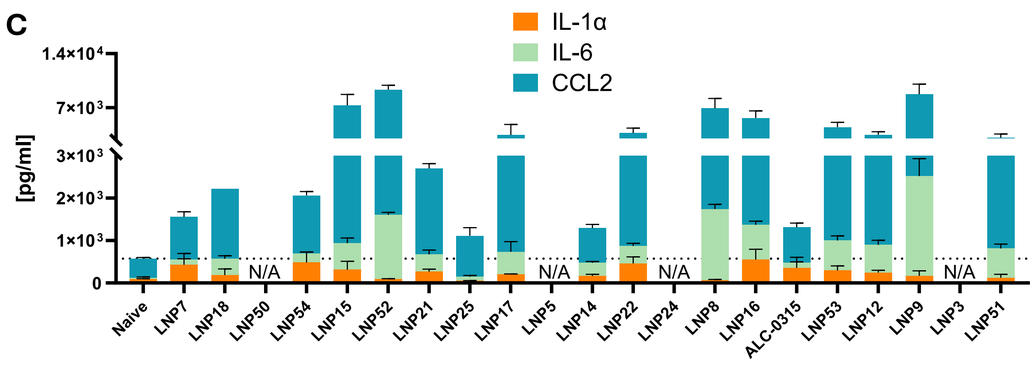
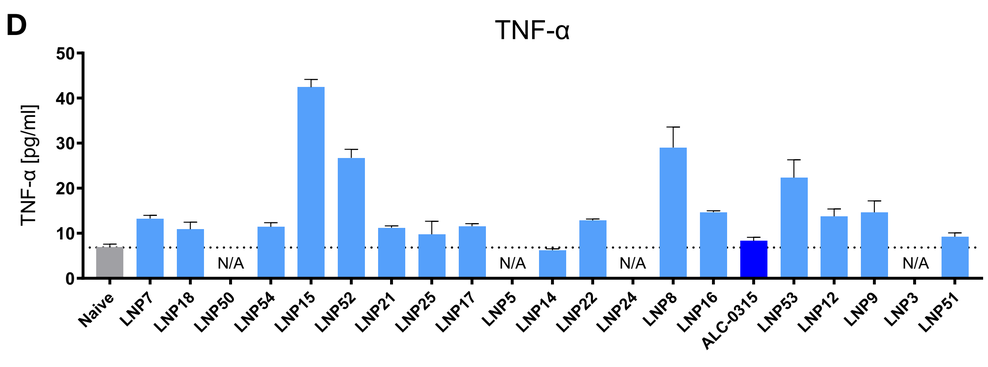


Fig. 1B. Humoral Response of 21 screened LNP: Serum samples were assessed for total anti-OVA IgG, IgG2c and IgG1 antibodies by ELISA. Bars report total IgG titer, and numbers on the top of the bars indicate IgG2c/IgG1 ratio to evaluate Th bias of immune response.





Figs. 1C & 1D. Innate Cytokine Stimulation induced by screened LNP: Serum was sampled 4 hours after *i.m.* prime with LNP-OVA mRNA formulated @ 1µg OVA mRNA-LNP dose. Serum analytes were quantified with Olink T48 platform. Figures reported highest induced analytes: IL-1α, IL-6, CCL2 levels (C) and TNF-α level is shown (D). Dotted line represents cytokine level in unvaccinated group.

Optimal Dose Finding

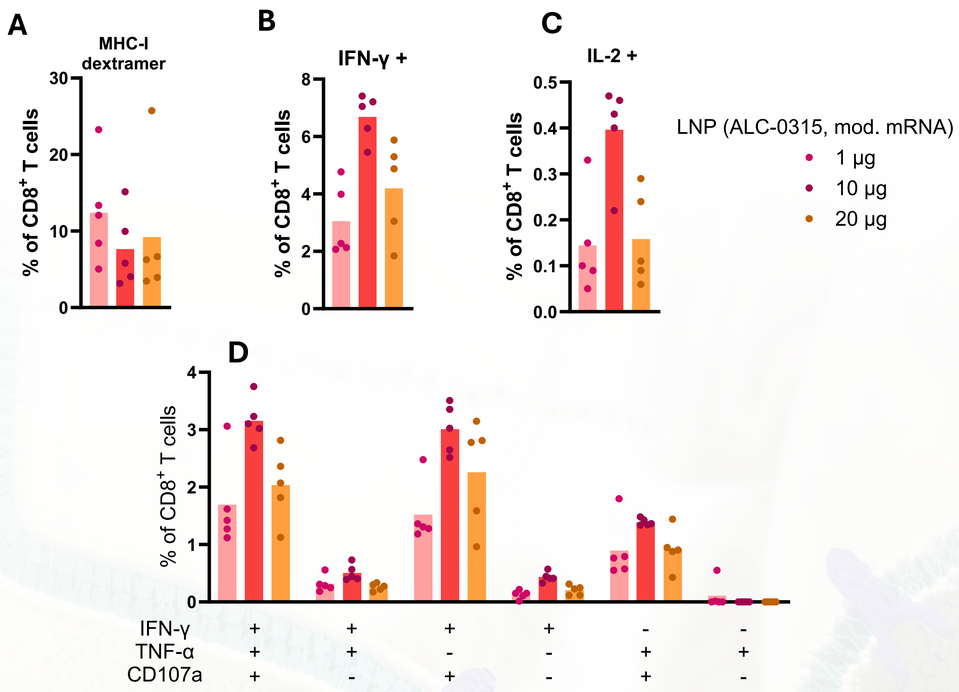


Fig. 2. LNP mRNA vaccine dose response: OVA-specific CD8 T cells response induced following *i.m.* vaccination with 1, 10 or 20 µg LNP-OVA mRNA (mod). **A**: CD8 MHC-I dextramer; **B** and **C**: IFN-γ and IL-2 secretion after *ex vivo* restimulation with OVA SIINFEKL peptide and intracellular staining for IFN-γ, TNF-α and CD107a. **D**: CD8 T cells polyfunctionality analysis.

Enhanced Cellular Response with Unmodified mRNA-LNP

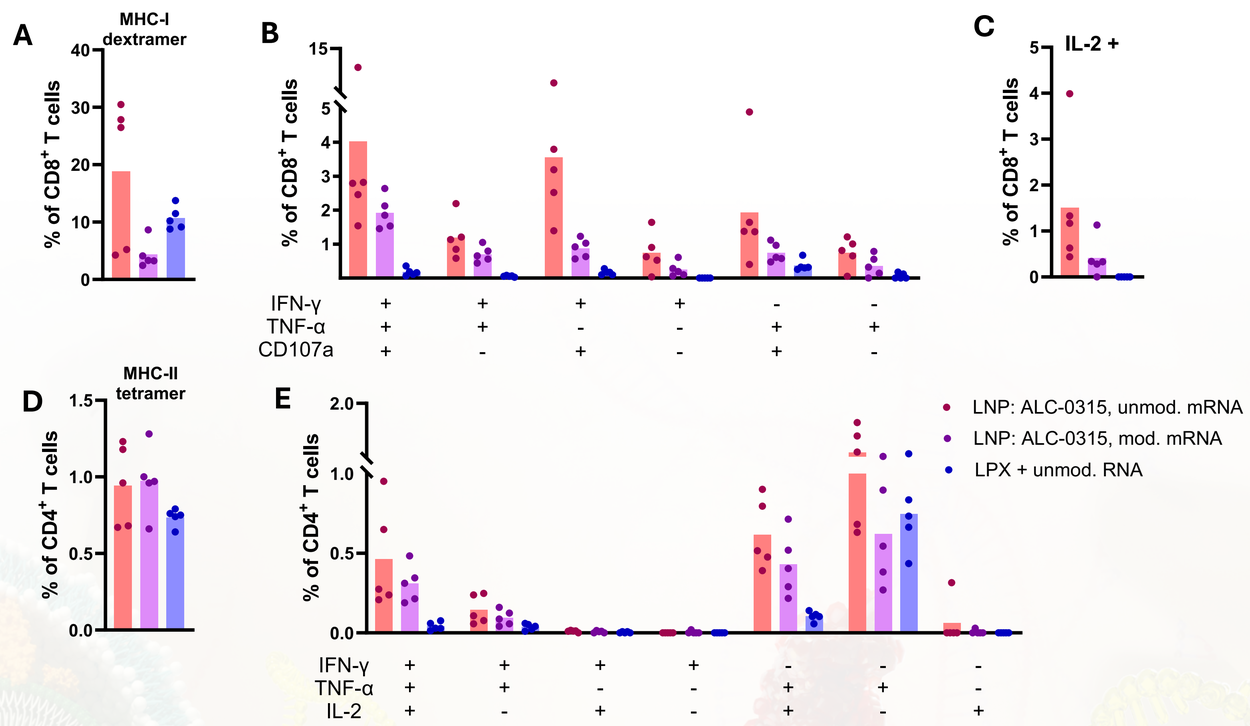


Fig. 3. Unmodified mRNA LNP immunogenicity and comparison to Lipoplex (LPX): OVA-specific CD8 T cells following prime-boost vaccination with unmodified or modified mRNA LNP (ALC-0315) (*i.m.*) or unmodified mRNA LPX (*i.v.*). **A**: Enumeration of antigen-specific CD8 T cells by dextramer staining; **B & C**: Characterization of cytokine secreting antigen-specific T cells (IFN-γ, TNF-α and CD107a, polyfunctional analysis) & IL-2 following *ex vivo* restimulation with SIINFEKL peptide; **D**: Enumeration of antigen-specific CD4 T cells by tetramer staining; **E:** Characterization of cytokine secreting antigen-specific CD4 T cells (IFN-γ, TNF-α, IL-2, and polyfunctional analysis) following *ex vivo* restimulation with OVA peptides pool.

Results

Potent Cellular and Humoral Responses Induced by mRNA-LNP:

- To screen for potent lipid for cancer vaccine development, we rationally selected 21 ionizable lipids from our library, formulated in LNP with N1-methylpseudouridine OVA mRNA and immunized mice (**Fig. A**). Immune response data assessed by evaluation IFN-γ producing CD8 T cells identified several active LNP. 14 out of the 21 LNP including ALC-0315 generated potent CD8 T cell response ranging between 2,100 and 5,200 spots per 10^6 cells (**Fig. 1A**).
- Furthermore, serum humoral response analysis indicated significant anti-OVA IgG induction by all LNP. Based on IgG2c/IgG1 ratio, all LNP induced Th1 biased immune responses known to be favourable for anti-cancer immunity (**Fig. 1B**). There was no correlation between antibody and T cell response with exception of lowest titers and lowest T cell response was induced by same LNP (**Figs. 1A & 1B).**

Innate Cytokine Stimulation:

- Innate immune stimulation was analyzed 4h after prime using Olink T48 platform measuring 43 immune mediators relevant in inflammation, immune response and immuno-oncology.
- Several analytes including IL-1α, IL-6, CCL2 and TNF-α were upregulated by LNP that induced both high and low adaptive immunogenicity (**Figs. 1C & 1D**). Overall data indicate that screened LNP differentially activate the innate immune response with some lipids being more active and others silent, even among the most vaccine-potentiating LNP, suggesting no correlation between innate immune stimulation and vaccine adaptive immune response.

Improving mRNA-LNP Induced Cellular Response:

- To identify optimal mRNA-LNP vaccine dose, we formulated ALC-0315 with modified mRNA, and treated mice with either 1, 10 or 20 µg mRNA dose (**Fig. A**). All LNP were well tolerated, with no clinical observations noted during the study in-life phase. Based on CD8 T cells MHC-I dextramer, a similar level of T cells were induced by all 3 doses (**Fig. 2A**); however, based on T cell functionality, assessed by cytokine secretion following *ex vivo* restimulation, 10 µg mRNA-LNP dose induced the highest IFN-γ and IL-2 CD8 T cells frequency (**Figs. 2B & 2C**). Polyfunctionality T cells levels for IFN-γ, TNF-α and CD107a was also the highest in 10 µg dose (**Fig. 2D**).
- Subsequentially, ALC-0315-encapsulating modified mRNA or unmodified mRNA were put into a side-by-side comparison with unmodified mRNA-lipoplex (LPX), a developing vaccine format that has previously been demonstrated to induce robust immune responses against cancer antigens. Same prime-boost schedule was applied for all 3 vaccines, but LPX was administrated at a higher dose (30 µg *i.v.*) than both LNPs (10 µg *i.m.*). ALC-0315 with unmodified RNA induced higher frequency of antigen-specific CD8 T cells than the other two vaccines (**Fig. 3A**), which was echoed by frequencies of triple and double producers of IFN-γ, TNF-α and CD107a (**Fig. 3B**), as well as IL-2^+ CD8 T cells (**Fig. 3C**).
- CD4 T cell responses were also assessed using MHC-II tetramer and stimulating peptides. Although there was no significant difference in antigen-specific CD4 T cell frequency (**Fig. 3D**), ALC-0315 with unmodified RNA still induced highest cytokine secretion by CD4 T cells (**Fig. 3E**).

Summary

- Using modified mRNA OVA, we have identified several potent lipids among the 20 selected from our library, in addition to ALC-0315 used in COMIRNATY®. A potent cellular response, and a positive humoral response with favourable Th1 bias, were induced demonstrating mRNA-LNP as a promising candidate for therapeutic cancer vaccine.
- Innate immune stimulation studies suggest screened LNP differentially induce innate immune stimulation, with no correlation with adaptive immune response to vaccine. Interestingly, ALC-0315 was among lipids inducing the least cytokines. Of note, modified mRNA was used in these studies; different outcomes might be obtained with unmodified mRNA given innate immune stimulation following interaction with Pattern Recognition Receptor (PRR) including TLR and RIG-I.
- Significantly higher CD8 T cell response was obtained with unmodified mRNA LNP compared to modified mRNA LNP, indicating the importance of stronger inflammatory response to enhance APCs' maturation and antigen presentation for an effective and functional T cell response.
- Compared to lipoplex, LNP used at 1/3rd of the dose elicited comparable or better cellular response; furthermore, CD8 T cells' functionality based on cytokines and CD107a expression was significantly better with LNP.
- Having demonstrated the potential of Acuitas' proprietary lipid for cancer vaccine development using the exogenous antigen model, further development will be undertaken. Furthermore, LNP effectiveness will be assessed with syngeneic neoantigens to demonstrate the ability of Acuitas' LNP to break tolerance and induce effective anti-tumour immune response capable of irradicating tumours.