Advanced LNP Technology for Infectious Disease Vaccines

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Abstract

mRNA-LNP vaccines enable rapid development timelines and flexible manufacturing, allowing swift responses to emerging pathogens and viral variants. The success of COVID-19 mRNA vaccines demonstrated the transformative potential of this platform. However, increasingly complex and emerging infectious disease targets require continued innovation in LNP technology. More potent next-generation LNP systems could reduce mRNA dose requirements and enable multivalent vaccine formulations capable of targeting multiple pathogens or variants simultaneously.

LNP formulations were evaluated in influenza and SARS-CoV-2 mRNA vaccine models. Humoral response was assessed by neutralization assays, antigen-specific B cell memory response by flow cytometry, and bone-marrow IgG ELISpot. Cellular response was monitored by IFN-γ ELISpot and MHC tetramers flow cytometry. Prime-boost reactogenicity was assessed in Guinea pig. APC cell lines and the Ai14 Cre-Tomato reporter mouse model were used to investigate the mechanisms underlying differential LNP performance by assessing LNP uptake, antigen expression, and innate immune activation using flow cytometry and Olink proteomics.

In previously reported structure-activity-relationship (SAR) studies in which over 80 newly synthesized lipids were screened, we identified a dozen lead lipids with at least five-fold greater activity than ALC-315®. Herein, subsequent screening was undertaken to further refine favorable lipid SAR and to identify more potent derivatives. Building on an initially identified lead lipid, LNP29, we synthesized and evaluated 27 novel analogues, nine of them exhibited a further three to five-fold increase in potency compared with LNP29.

The potency of the novel lipids was further demonstrated in multivalent vaccine studies where significant dose sparing compared to ALC-315® was achieved. Humoral response durability assessment showed superiority of newly identified LNP as reflected by a larger antigen specific memory B-cell memory pool compared with ALC-315®. Importantly, higher functional antibody titers were sustained for over 10-months with a robust recall response characterized by significant increase in both humoral and cellular response following booster vaccination.

Furthermore, our data indicates that the next-generation LNP induces superior humoral and cellular immune responses compared with conventional adjuvanted recombinant protein. Preliminary studies to elucidate the mechanisms underlying the enhanced activity of new LNP relative to ALC-315®, indicate differential in payload expression and innate immune activation within secondary lymphoid tissues.

Collectively, these findings demonstrate that enhanced ionizable lipid potency can drive stronger *in vivo* antigen delivery and expression, and robust, durable protection while maintaining favorable reactogenicity and tolerability. These high potency LNP will provide a strong foundation for multivalent vaccines aimed at ensuring protection against multiple target strains and pathogens.

1 Next Generation Lipids Induce Significantly Higher HAI Antibody Titers than ALC-315®

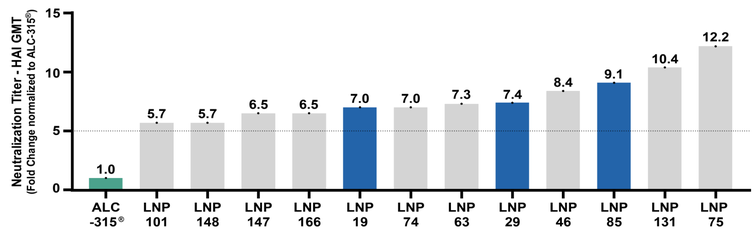


Fig. 1 – New Lipids Rank above ALC-315® based on HAI Titers - Serum HAI titers on Day 28 following prime/boost (D0, D14) i.m vaccination of BALB/c mice (10/group) with 0.2 µg PR8 HA mRNA-LNP. To rank lipid performance, data is presented as mean fold change in HAI titer relative to ALC-315®

2 SAR-Guided Design Identified More Potent Next Generation Lipids

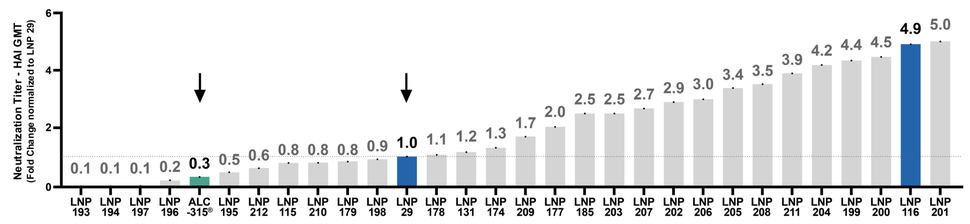


Fig. 2 – SAR refinement of Lead LNP Candidate - Analogs of LNP 29 were synthesized and tested in Prime/Boost vaccine setting: Serum HAI titers on Day 28 following prime/boost (D0, D14) i.m vaccination of BALB/c mice (10/group) with 0.05 µg PR8 HA mRNA-LNP. To rank lipid performance, data is presented as mean fold change in HAI titer relative to LNP 29

3 Next Generation Lipids Enable a 5-Fold Lower Antigen Dose While Maintaining Comparable Antibody Titers Across a Multivalent Vaccine

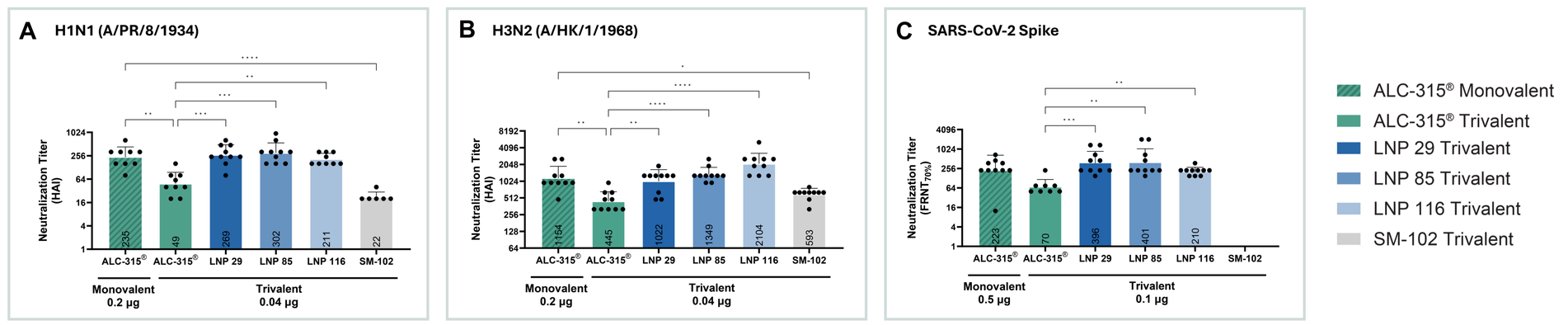


Fig. 3 – Multivalent LNP Immunogenicity – Serum neutralization titers for **(A)** H1N1 HAI, **(B)** H3N2 HAI, and **(C)** SARS-CoV-2 FRNT on Day 28 following prime/boost (D0, D14) i.m vaccination of BALB/c mice (10/group) with trivalent LNP (co-mixed monovalent LNP). ALC-315® monovalent LNP were dosed at 5x higher dose versus novel lipids and Spikevax LNP, SM-102.

4 Next Generation LNP Induce Stronger Humoral and Cellular Responses Compared to Adjuvanted Protein

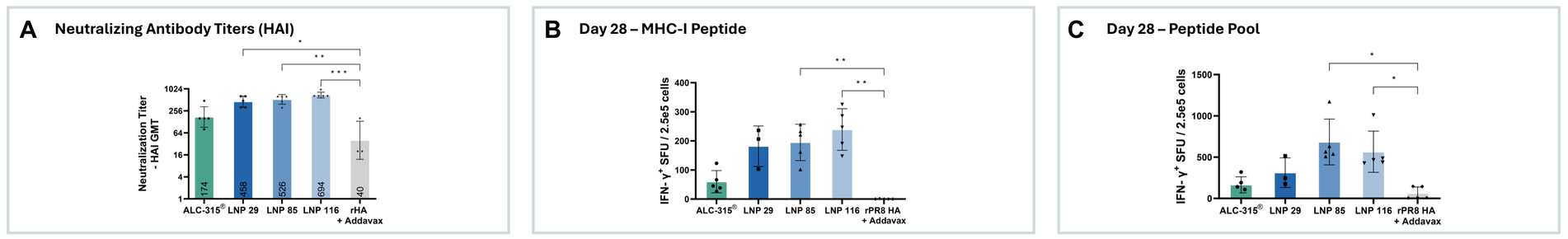


Fig. 4 - Humoral and cellular response induced by novel LNP compared to adjuvanted PR8 HA protein. BALB/c mice (5/group) were vaccinated prime/boost (D0, D14) i.m with 0.2 µg PR8 HA mRNA-LNP or 1 µg adjuvanted rPR8 HA. **(A)** Serum HAI titers at 28 days post prime vaccination. IFNγ ELISpot spot formation units (SFU) of splenocytes at Day 28 upon restimulation with **(B)** MHC-I peptide IYSTVASSL or **(C)** 15-mer MHC peptide pool.

5 Next Generation Lipids Induce Higher Cellular & Memory B cell Responses than ALC-315®

ALC-315® LNP 19 LNP 29

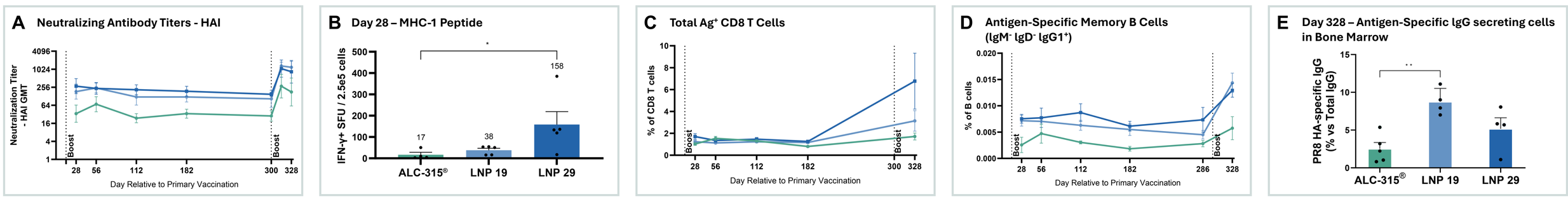


Fig. 5 - Cellular response and durability of humoral response induced by novel LNP. BALB/c mice (5/group) were vaccinated prime/boost/boost (D0, D14, D300) i.m with 0.2 µg PR8 HA mRNA-LNP. **(A)** Serum HAI titers at 28-328 days post prime vaccination. **(B)** IFNγ ELISpot spot formation units (SFU) of splenocytes at Day 28 upon restimulation with MHC-I peptide IYSTVASSL. Frequencies of PR8 HA specific **(C)** CD8 T cells and **(D)** memory B cells (IgM^- IgD^- IgG^+) at 28-328 days post prime vaccination. **(E)** Frequencies of PR8 HA specific IgG-secreting cells in bone marrow at 11 months.

6 Next Generation Lipids Induce Stronger Payload expression and APC activation

Vehicle ALC-315® LNP 29 LNP 85 LNP 116

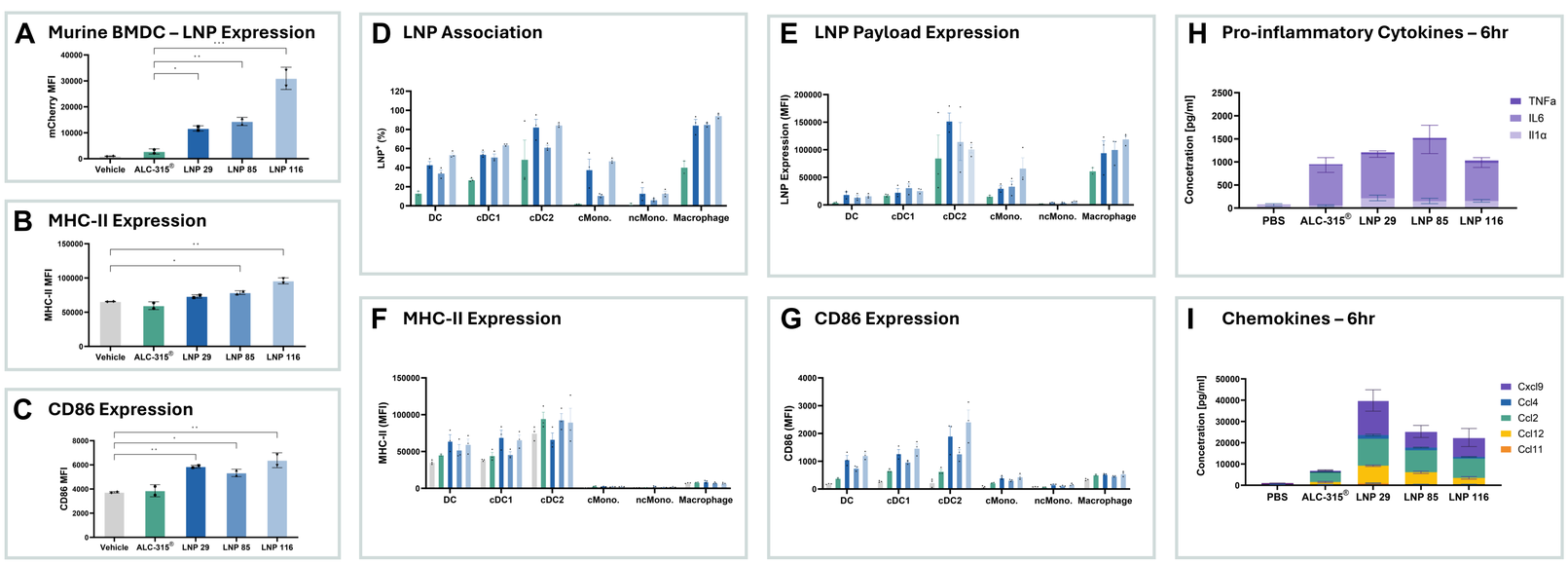


Fig. 6 - *in vitro* and *in vivo* LNP expression and cell activation. Murine bone-marrow derived DCs (BMDC) were incubated with LNP (1 µg/ml) for 24 hours. **(A)** LNP payload expression (mCherry) and cell activation markers **(B)** MHC-II and **(C)** CD86 were assessed by flow cytometry. Ai14 mice (n=3) were vaccinated i.m with 5 µg Cre mRNA-LNP. **(D)** LNP association (DiD), **(E)** LNP payload expression (tdTomato) and cell activation markers **(E)** MHC-II and **(F)** CD86 were assessed in APC populations in the spleen 24 hours post-dose. **(H)** Pro-inflammatory cytokines and **(I)** chemokines were assessed in serum 6 hours post i.m vaccination.

7 Next Generation Lipids Are Well Tolerated with Mild Transient Local and Systemic Reactogenicity

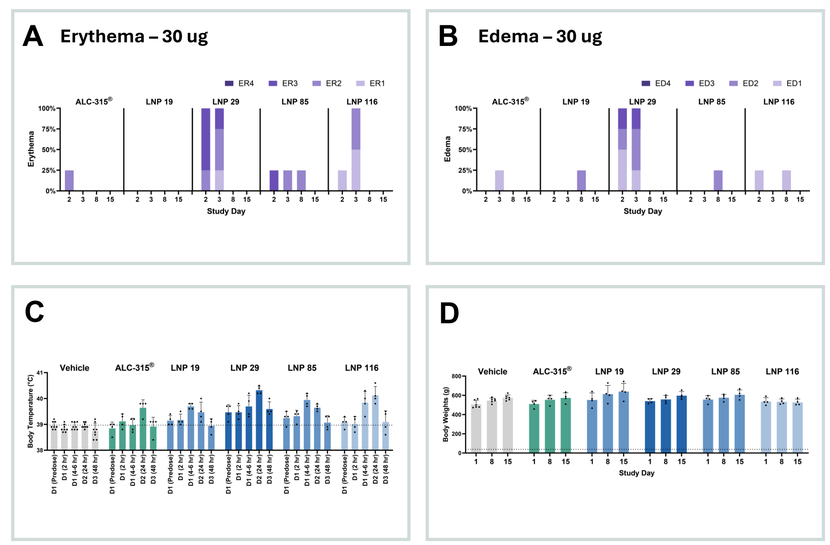


Fig. 7 - Reactogenicity studies: Hartley Guinea Pigs (4/group) were vaccinated i.m with 30 µg PR8 HA mRNA-LNP. Injection site **(A)** erythema and **(B)** edema were assessed by modified Draize Scoring. **(C)** Microchip body temperatures and **(D)** body weights were captured.

Summary

1. Initial rounds of SAR modeling identified 12 new lipids that induce at least 5x higher PR8 HAI titers than ALC-315® (Fig. 1). SAR refinement of LNP 29, a next generation candidate, identified additional lipids with 3-5-fold increased potency over LNP 29 (Fig. 2).
2. Potency of identified lipids is demonstrated in a trivalent vaccine format where, at 5-fold lower antigen dose, equivalent titers to ALC-315® monovalent LNP were induced for each of the antigen (Fig. 3).
3. Next generation lipids outperform adjuvanted protein vaccine for both humoral and cellular immune response (study is ongoing to assess longevity of the immune response) (Fig. 4).
4. In addition to enhanced and long-lasting humoral response, next Gen lipids outperform ALC-315® in terms of memory B-cell pool that persisted over 10 months with a significant recall upon a boost (Fig. 5).
5. Mechanistic studies suggest that the superior potency of next-generation LNP is associated, at least in part, with enhanced antigen expression and cellular activation compared with ALC-315® as demonstrated in both *in vitro* and *in vivo* models (Fig. 6)
6. Next Gen lipid candidates induce favorable reactogenicity profiles equivalent to ALC-315® (Fig. 7).

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