Next Generation LNP Technology for Infectious Disease Vaccines

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Abstract

The COMIRNATY® mRNA lipid nanoparticle (LNP) vaccine, which has protected billions of people from COVID-19, incorporates Acuitas' proprietary ionizable ALC-315™ and PEG ALC-159™ lipids as well as DSPC, and cholesterol. To address increasingly complex and emerging infectious disease targets, continued innovation in LNP design is essential. Next-generation LNP systems with higher-potency would enable dose reduction, and the development of multivalent vaccine formats.

To screen for potent LNP we have used influenza A H1N1 (PR8 HA) and SARS-CoV-2 (WT-1), as infectious disease antigens. Mice were immunized in a prime-boost regimen, and humoral responses were determined by neutralization assays with assessment of antigen-specific B cell memory response by flow cytometry. T-cell induction was measured following *ex vivo* stimulation of splenocytes and IFNγ ELISpot analysis. Reactogenicity studies were performed in Guinea pig to determine tolerability, and innate immune activation was investigated to help elucidate the mechanisms underlying antigen-specific adaptive immune response.

Previously, we have conducted two rounds of structure activity relationship (SAR) analysis, during which over eighty ionizable lipids were synthesized and evaluated in vaccine studies. From this effort, twelve candidates demonstrated greater than 5-fold higher neutralizing antibody titers compared to ALC-315™. A subsequent round was undertaken to further refine favorable lipid SAR and to identify more potent derivatives. Building on an initially identified lead lipid, we synthesized 27 novel analogues and evaluated them in follow-up immunogenicity studies with influenza A H1N1 (PR8 HA). Among these newly synthesized lipids, nine exhibited three to five-fold greater potency.

When evaluated in multivalent vaccine studies, novel lipids achieved comparable immunogenicity to ALC-315™ at 5-fold lower dose. Humoral response durability assessment further demonstrated superiority of new LNP as reflected in higher B cell memory pool compared to ALC-315 ™ and importantly higher functional antibody titers were sustained for at least 6-months following vaccination. Reactogenicity studies, in prime/boost settings, indicated that next generation LNP were well tolerated.

These next generation, high potency LNP will provide a strong foundation for multivalent vaccines aimed at ensuring robust protection against multiple target strains and pathogens whilst maintaining favorable reactogenicity and tolerability.

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

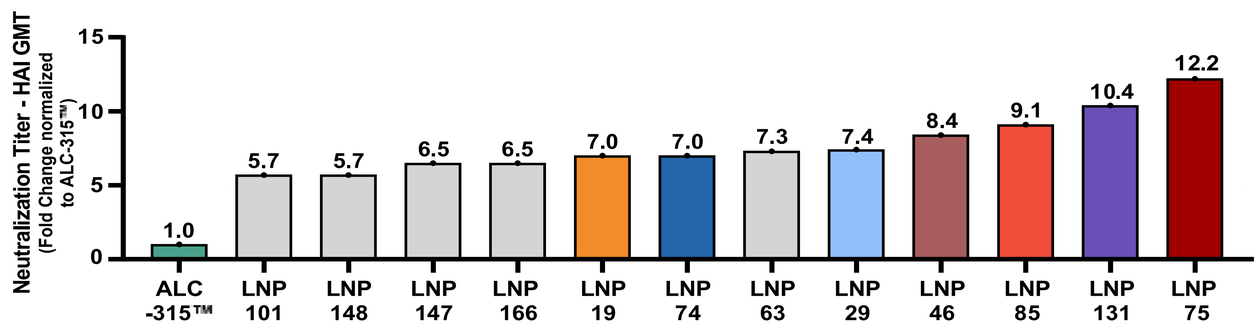

Fig. 1 – New Lipids Rank above ALC-315™ based on HAI Titers - Serum HAI titers on Day 28 following prime/boost (D0, D14) I.M vaccination of BALB/c mice (10/group) with 0.2 µg PR8 HA mRNA-LNP. To rank lipid performance, data is presented as mean fold change in HAI titer relative to ALC-315™

SAR-Guided Design Identified More Potent Next Generation Lipids

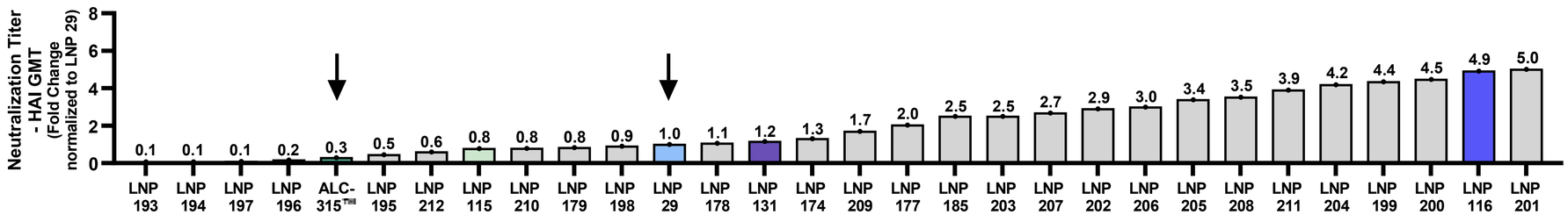

Fig. 2 – SAR refinement of Lead LNP Candidate - Serum HAI titers on Day 28 following prime/boost (D0, D14) i.m vaccination of BALB/c mice (10/group) with 0.05 µg PR8 HA mRNA-LNP. To rank lipid performance, data is presented as mean fold change in HAI titer relative to LNP 29

Next Generation Lipid Potency is Comparable to ALC-315™ at 5x Lower Dose

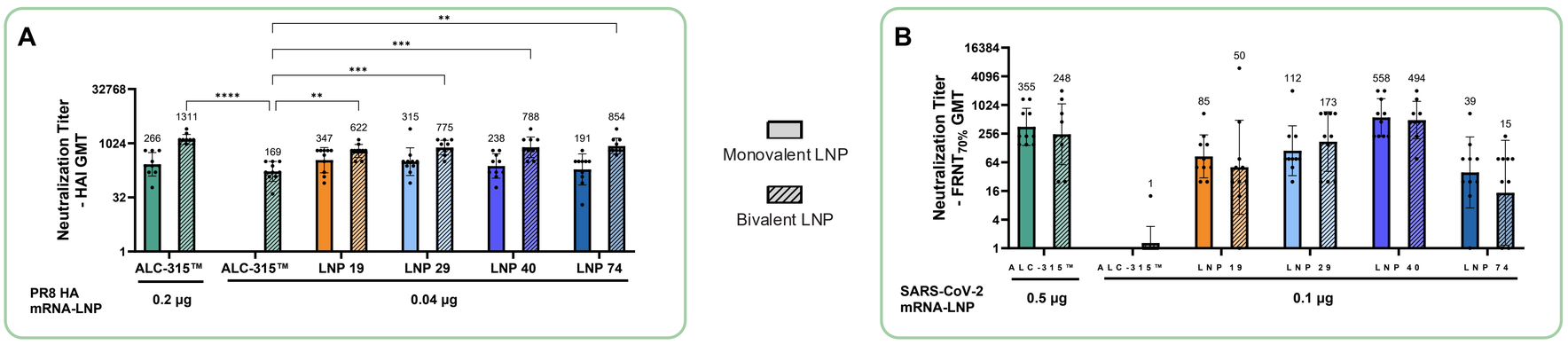

Fig. 3 – Multivalent LNP Immunogenicity – Serum neutralization titers for **(A)** PR8 HA HAI & **(B)** SARS-CoV-2 FRNT on Day 28 following prime/boost (D0, D14) i.m vaccination of BALB/c mice (10/group) with monovalent PR8 HA mRNA-LNP or SARS-CoV-2 RBD mRNA-LNP, & bivalent LNP (co-mixed monovalent LNP). ALC-315™ was dosed at 5x higher dose versus novel lipids.

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

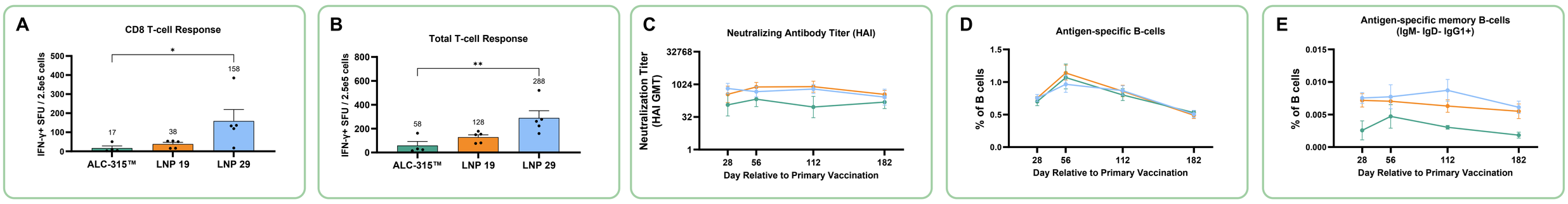

Fig. 4 - Cellular response and durability of humoral response induced by novel LNP. BALB/c mice (5/group) were vaccinated prime/boost (D0, D14) i.m with 0.2 µg PR8 HA mRNA-LNP. IFNγ ELISpot spot formation units (SFU) of splenocytes at Day 28 upon restimulation with **(A)** MHC-I peptide IYSTVASSL or **(B)** 15-mer MHC peptide pool. **(C)** Serum HAI titers, and frequencies of **(D)** PR8 HA specific B cells and **(E)** PR8 HA specific memory B cells (IgM^- IgD^- IgG^+) at 28-182 days post prime vaccination.

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

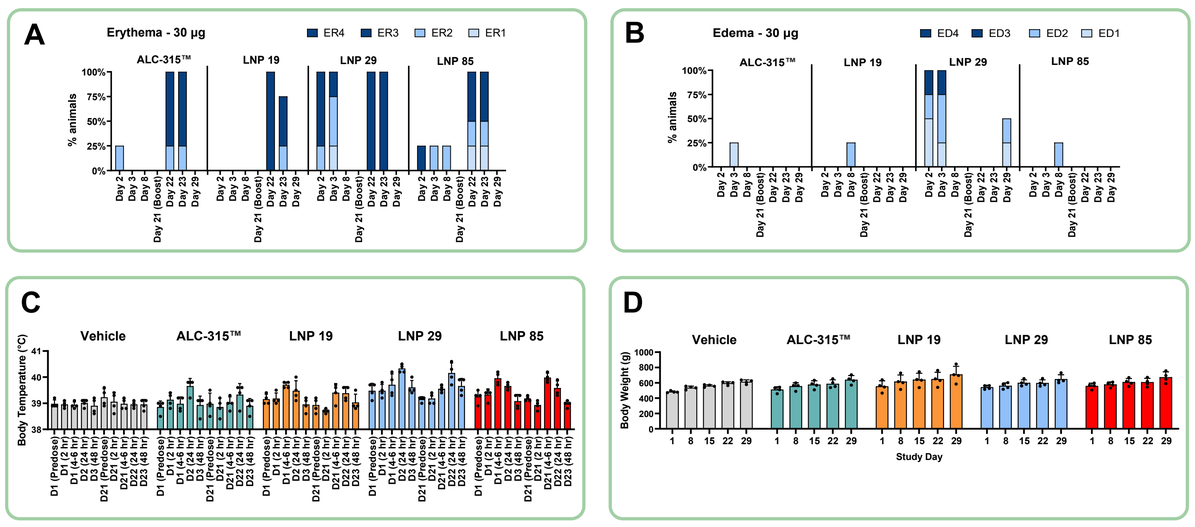

Fig. 5 - Reactogenicity studies: Hartley Guinea Pigs (4/group) were vaccinated prime/boost (D1, D21) i.m with 30 µg PR8 HA mRNA-LNP. Injection site Erythema **(A)** and Edema **(B)** were assessed by modified Draize Scoring. Microchip body temperatures **(C)** and Body weights **(D)** were captured.

Reactogenicity, but not LNP Immunogenicity, Correlates with Innate Immune Response

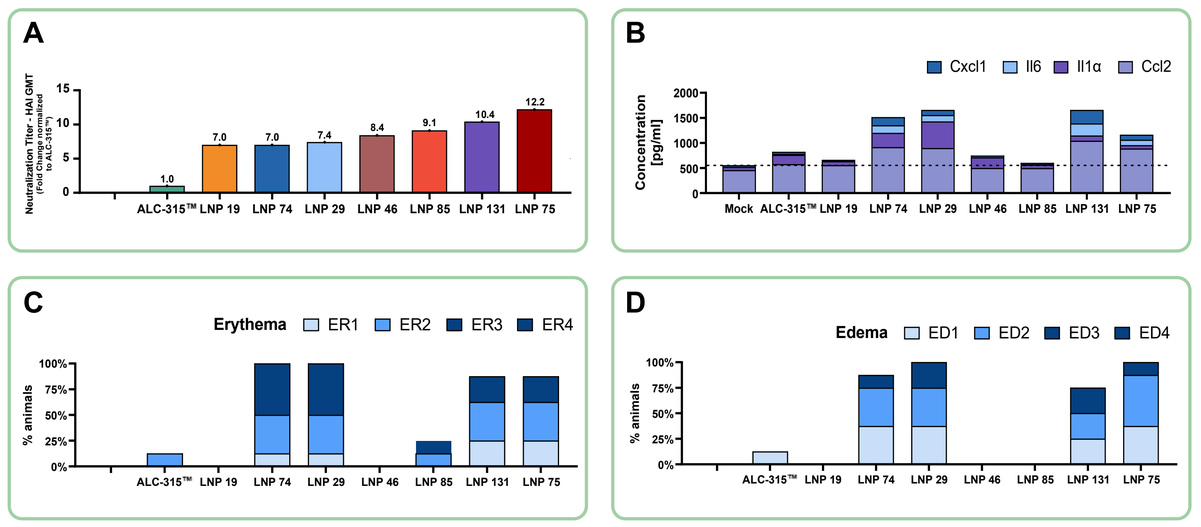

Fig. 6 – Innate Immune Studies: **(A)** LNP immunogenicity ranking based on lipid screening at 0.2 µg PR8 HA mRNA-LNP in BALB/c mice (n=10/group). **(B)** Cytokine levels in sera at 6 hours post 0.2 µg PR8 HA mRNA-LNP dose in BALB/c mice (n=3). Injection site erythema **(C)** and edema **(D)** in Guinea Pig dosed with 30 µg PR8 HA mRNA-LNP. (Sum of Day 2 and 3 Draize Score post-Prime dose).

Summary

1. Initial two rounds of SAR modeling identified 12 new lipids that induce at least 5x higher PR8 HAI titers than ALC-315™ (Fig.1).
2. SAR refinement of LNP 29, a next generation candidate, identified additional lipids with 3-5-fold increased potency over LNP 29 (Fig.2).
3. Potency of identified lipids is demonstrated in a bivalent vaccine format where, at 5-fold lower antigen dose, equivalent titers to ALC-315™ were induced and were comparable between mono- and bivalent vaccines (Fig. 3).
4. Next generation lipids outperform ALC-315™ : stronger T cell responses, more potent humoral immunity that persisted over 6 months and enhanced memory B-cell formation compared to ALC-315™ (Fig 4).
5. Several potent next generation lipid candidates induce favorable reactogenicity profiles equivalent to ALC-315™ (Fig. 5).
6. As expected, LNP-induced Innate immune response in rodents, correlate with reactogenicity in guinea pigs, however they do not correlate with adaptive immune response (Fig. 6).

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