Novel Lipids with Improved Activity for Prophylactic Vaccine Development

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Abstract

Introduction & Objectives

Acuitas' lipid nanoparticle (LNP) technology has been validated in humans through Pfizer-BioNTech's COMIRNATY®, which has protected billions of people from COVID-19 in more than 180 countries.

The LNP formulation used in COMIRNATY® is comprised of Acuitas' proprietary lipids, specifically the ionizable lipid ALC-315™ and the PEG lipid ALC-159™ – in addition to a DSPC helper lipid and cholesterol. To enable effective and innovative vaccines against increasingly challenging infectious disease applications, there is a need for continuous advancement in LNP technology. We achieved this by taking advantage of our comprehensive custom-made library of over 1,500 ionizable lipids, from which we rationally selected and screened a panel of new lipids to identify those with superior immunogenicity for next-generation vaccine development.

Materials & Methods

Using two antigen models derived from viral infectious diseases, H1N1 influenza (PR8) and SARS-CoV-2 (WT-1), we immunized mice with a prime-boost regimen and assessed antigen specific adaptive immune responses including neutralizing and hemagglutination inhibition (HAI) antibody titers, as well as cellular and B cell memory pools. Furthermore, reactogenicity and innate immune stimulation induced by mRNA LNP and *in vivo* expression studies were performed to further understand the antigen-specific adaptive vaccine immune response outcomes.

Results

We have identified ionizable lipids that induce significantly higher functional antibody titers than ALC-315™. Testing these lipids in multivalent LNP vaccine studies showed immunogenicity comparable to the respective monovalent LNP and, importantly, comparable immunogenicity to ALC-315™ at a 5-fold lower dose was achieved. Preliminary assessment of humoral response durability indicated that the newly identified LNP 3 induced a superior T cell response and B cell memory pool compared to ALC-315™. Assessment of reactogenicity in comparison to ALC-315™ showed that the new LNP were well tolerated. Interestingly, there was no correlation between the innate immune stimulation profile induced by LNP and vaccine potency. Antigen expression data showed higher expression in the secondary lymphoid organs and lower expression in the liver with newly identified lipids, compared to ALC-315™.

Conclusion

From our library of custom-made ionizable lipids, we have rationally identified new compounds that induce significantly higher virus-specific immunogenicity compared to ALC-315™. The potency of these LNP was confirmed in a multivalent vaccine setting, achieving equivalent activity to ALC-315™ at a 5-fold lower dose. Increased potency correlated with a higher antigen expression in secondary lymphoid tissues relative to the liver but did not correlate with innate immune stimulation profile.

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

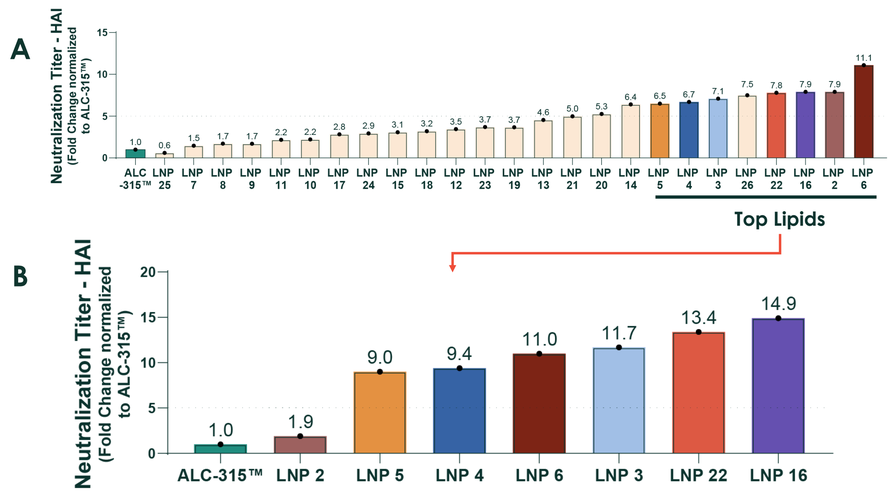

Fig. 1 – Lipid screening and ranking versus ALC-315™ - Serum HAI titers on Day 28 following prime/boost (D0, D14) vaccination of BALB/c mice (10/group) with **(A)** 0.2 µg or **(B)** 0.05 µg PR8 HA mRNA-LNP. To rank lipid performance, data is presented as fold change of mean HAI titer relative to ALC-315™

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

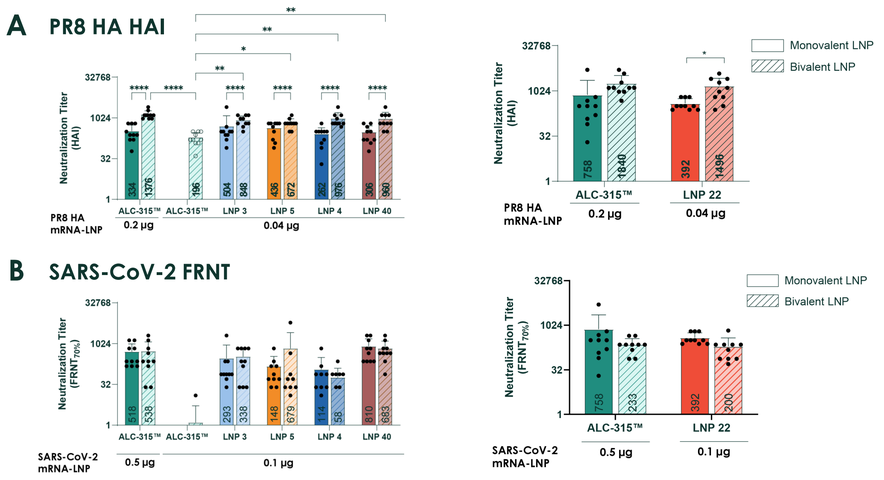

Fig. 2 – Multivalent LNP immunogenicity – Serum neutralization titers for **(A)** PR8 HA HAI and **(B)** SARS-CoV-2 FRNT on Day 28 following prime/boost (D0, D14) vaccination of BALB/c mice (10/group) with monovalent PR8 HA mRNA-LNP or SARS-CoV-2 RBD mRNA-LNP, & bivalent LNP (co-mixed monovalent LNP). ALC-315™ was dosed at 5x higher dose versus novel lipids. Differences between ALC-315™ and novel lipids assessed by 2-way ANOVA with Tukey's correction. Additionally, unpaired t-tests were used to compare monovalent and bivalent responses of individual lipids.

Novel Lipids Induce Higher Cellular Response than ALC-315™

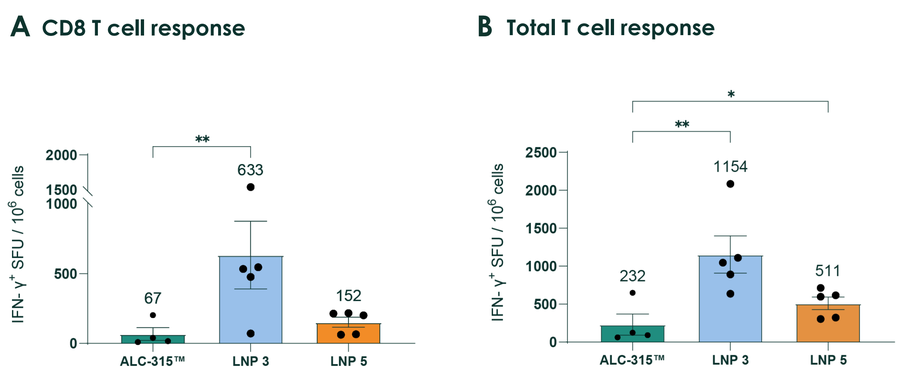

Fig. 3 - Cellular response induced by novel LNP. IFN-γ ELISpot of splenocytes at Day 28 following prime/boost (D0, D14), 0.2 µg PR8 HA mRNA-LNP. **(A)** ELISpot spot formation units (SFU) upon restimulation of MHC-I peptide IYSTVASSL. **(B)** ELISpot SFU upon restimulation of 15-mer MHC peptide pool. BALB/c, n=5.

LNP 3 Induces Higher Memory B cell & Humoral Response than ALC-0315™

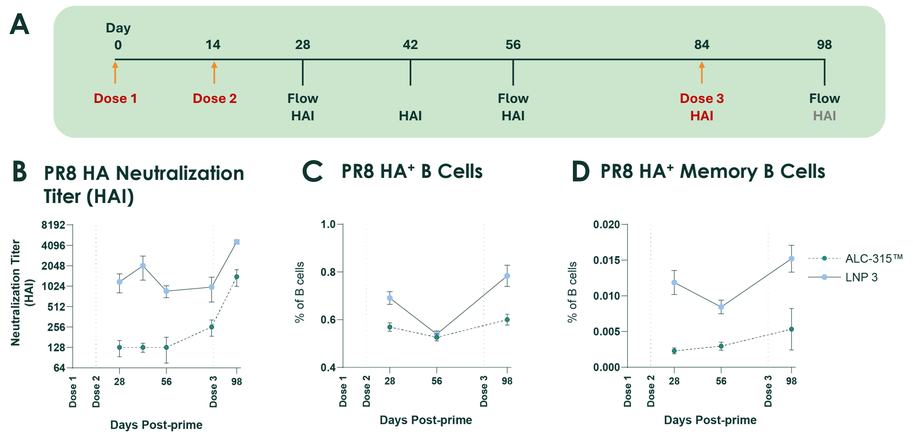

Fig. 4 - Durability of humoral response and memory B cells. (A) BALB/c mice (5/group) were vaccinated prime/boost/boost (D0, D14, D84) with 0.2 µg PR8 HA mRNA-LNP. **(B)** Serum HAI titers, **(C)** frequencies of PR8 HA specific B cells and **(D)** PR8 HA specific memory B cells (IgM^- IgD^- IgG^+)

Several Novel Lipids Produce Equally Favorable Reactogenicity Profiles as ALC-315™

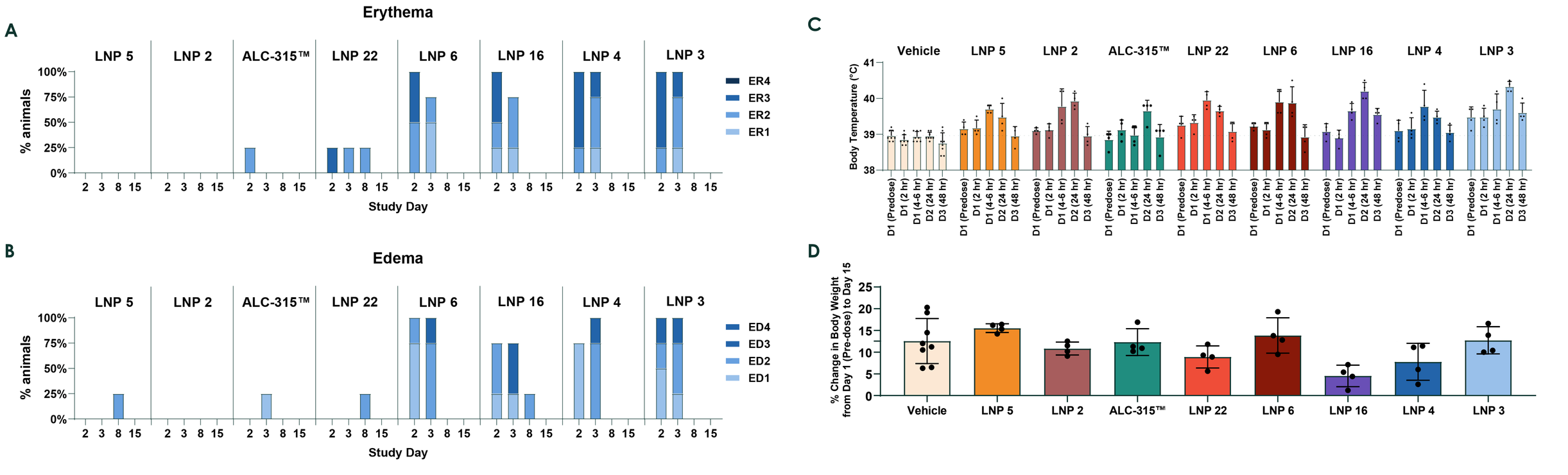

Fig. 5 - Reactogenicity studies: Hartley guinea pigs (4/group) were administered a single IM dose of 30 µg PR8 HA mRNA-LNP. Injection site erythema **(A)** and edema **(B)** were assessed by modified Draize Scoring with scores peaking at Day 2-3 post-injection and resolving in most instances by Day 5-8. Microchip body temperatures **(C)** were captured and % changes in body weight **(D)** determined from Day 1 (pre-dose) to Day 15.

LNP Immunogenicity does not correlate with Innate Immune Response

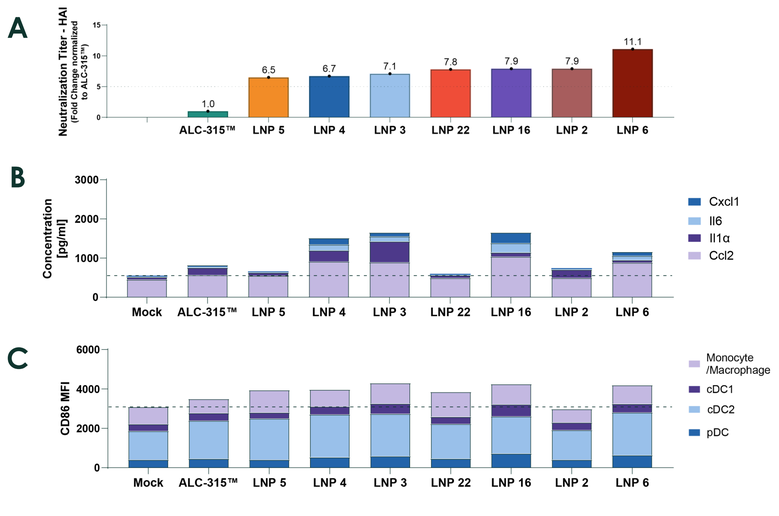

Fig 6. – Innate stimulation studies: **(A)** LNP immunogenicity ranking based on lipid screening at 0.2 µg PR8 HA LNP. **(B)** Cytokine levels in sera at 6h, & **(C)** CD86 activation (flow cytometry) in lymph node 24-hours post 0.2 ug dose. BALB/c mice (n=10 (A), n=3 B,C)).

Reactogenicity Correlates with Innate Immune Response

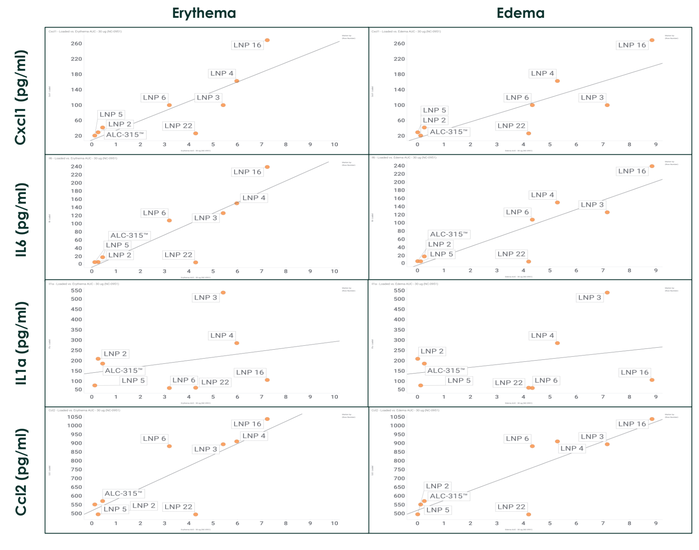

Fig 7. – Correlation between erythema & edema (AUC_{D1-15}) in guinea pigs & cytokine production at 6 hrs in BALB/c mice administered single IM doses of 30 µg, and 0.2 µg PR8 HA mRNA-LNP, respectively.

Higher Expression in Secondary Lymphoid Organs for Novel Lipids

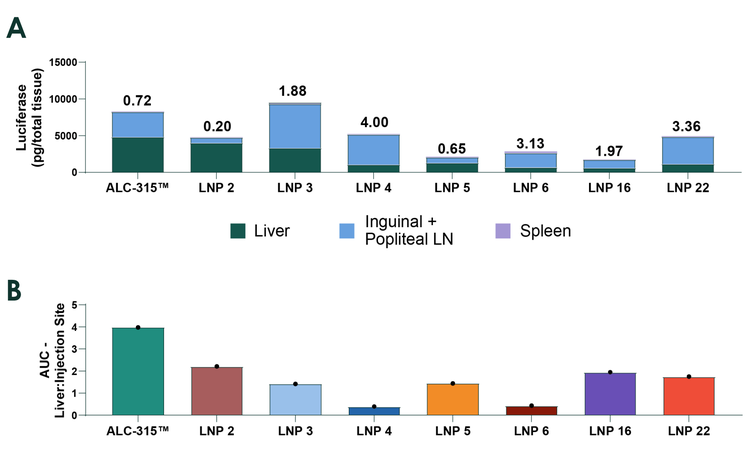

Fig. 8 - Biodistribution studies: (**A**) Luciferase expression in organs harvested 4h post-IM w/0.2 µg FLuc mRNA-LNP (# above bars: lymph node (LN) + spleen-to-liver ratio. (**B**) AUC liver-to-injection ratio following IM with 2 µg FLuc mRNA-LNP. Live whole-body imaging was performed at 4-, 24- and 48-hours post-dose (after 48h no signal was detected in the liver). BALB/c, n=3 (A&B)

Summary

1. Initial IM lipid screening using a PR8 HA mRNA-LNP model identified 11 new lipids that induce at least 5x higher HAI titers than ALC-315™. Potency of 6 out of 7 lipids was confirmed at 5-fold lower dose level (Fig.1).
2. 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.2).
3. Preliminary novel lipid T cell and humoral response assessments indicate higher cellular and B cell (humoral and B-cell memory pool) responses than ALC-315™ (Fig.3 & 4).
4. Several potent novel lipid candidates induce favorable reactogenicity profiles equivalent to ALC-315™ (Fig.5).
5. Innate immune response induced by LNP (rodents) tend to correlate with their reactogenicity (GP), however there is no correlation with adaptive & innate immune response (Fig.6 & 7).
6. *In vivo* expression is higher in secondary lymphoid organs than in the liver, as well as higher local expression than in the liver for novel potent lipids compared to ALC-315™ (Fig. 8)

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