Identification of Novel Lipids with Improved Activity for Prophylactic Vaccine Development

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Abstract

Acuitas' lipid nanoparticle (LNP) technology has been validated in human vaccines with the Pfizer-BioNTech vaccine COMIRNATY®, which has protected billions of people from COVID-19 in more than 180 countries.

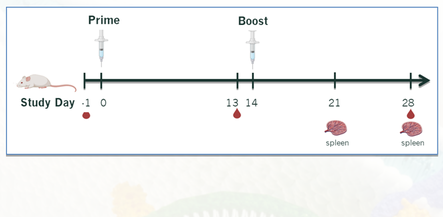
The COMIRNATY® LNP is comprised of Acuitas' proprietary lipids, specifically the ionizable lipid ALC-0315 and the PEG lipid ALC-0159, in addition to a DSPC helper lipid and cholesterol. To create more effective and innovative vaccines for progressively more challenging infectious disease applications, there is a need for continuous advancement in LNP technology. We achieved this by taking advantage of our comprehensive custom-made library of over 1,000 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.

Using three antigen models derived from viral infectious diseases, including H1N1 influenza (PR8), RSV (A2), and SARS-CoV-2 (WA-1), we have immunized mice in a prime-boost schedule and assessed antigen-specific adaptive immune responses including neutralizing / hemagglutination inhibition antibody titers. We have identified ionizable lipids that induce significantly higher functional antibody titers than ALC-0315. Tested in a multivalent vaccine targeting H1N1 influenza (PR8), RSV (A2), and SARS-CoV-2 (WA-1) viruses, identified lipids showed comparable immunogenicity in a multivalent LNP vaccine setting to the respective monovalent LNP.

Furthermore, we have assessed the T cell response, induced by selected lipids, to provide insights into the nature of the adaptive immune response as well as B cells memory response. In addition, the innate immune stimulation profile induced by LNP, antigen expression and reactogenicity data were investigated to understand the antigen-specific adaptive immune response outcomes induced by these novel LNP vaccines.

In summary, from our library of custom-made, rationally designed ionizable lipids, we have identified new compounds that induce significantly higher virus-specific immunogenicity compared to ALC-0315, which correlated with an increased secondary lymphoid tissue-to-liver ratio of antigen expression, but not with an innate immune stimulation profile.

Vaccination schedule



PR8 HA Vaccine Studies

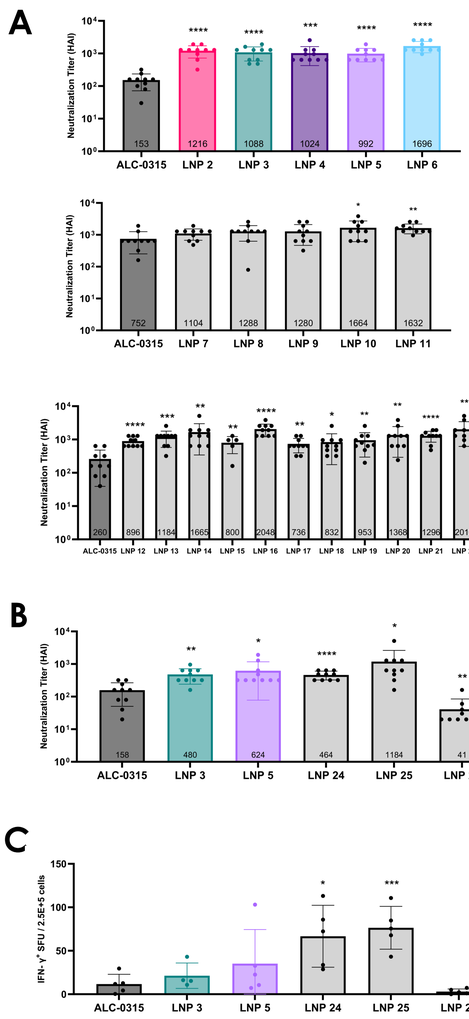


Fig. 1 - Lipid screening (LNP 2-26) against ALC-0315: Serum HAI titers 14 days following prime/boost vaccination of BALB/c mice (10/group) with 0.2 µg PR8 HA mRNA-LNP (studies: **A-B**). Following serum collection, spleens were collected from study **B** animals and after in vitro stimulation with CD8 T cell epitope (IYSTVASSL) IFNγ ELISPOT was performed (**C**).

RSV – Pre-F Vaccine Studies

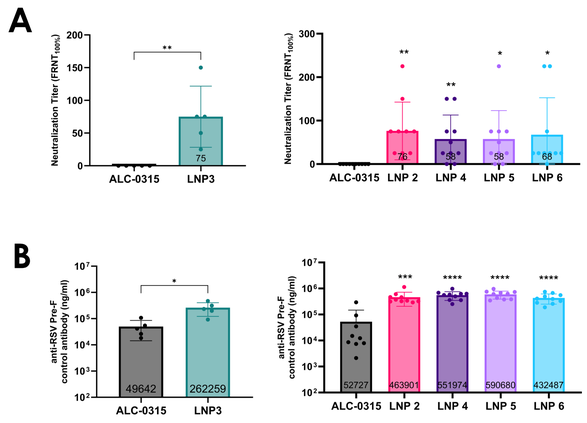


Fig. 2 - Lipid screening (LNP 2-6) against ALC-0315: Serum neutralization (**A**) and IgG (**B**) levels 14 days following prime/boost vaccination of BALB/c mice (10/group) with 0.2 µg RSV–Pre-F mRNA-LNP.

SARS-CoV-2 RBD Vaccine Studies

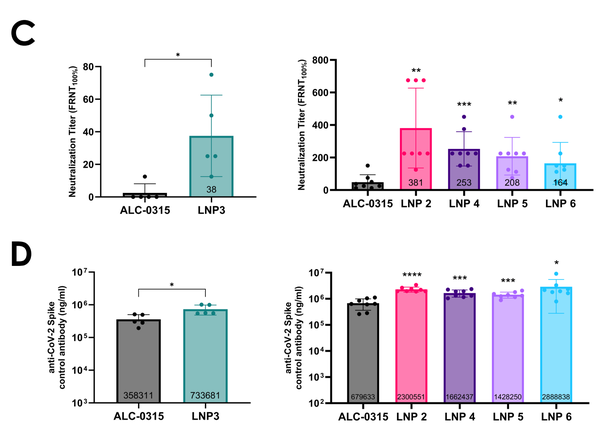


Fig. 2 - Lipid screening (LNP 2-6) against ALC-0315: Serum neutralization (**C**) and IgG levels (**D**) 14 days following prime/boost vaccination of BALB/c mice (10/group) with 0.5 µg SARS-CoV-2 RBD mRNA-LNP.

Lipid Rank Potency

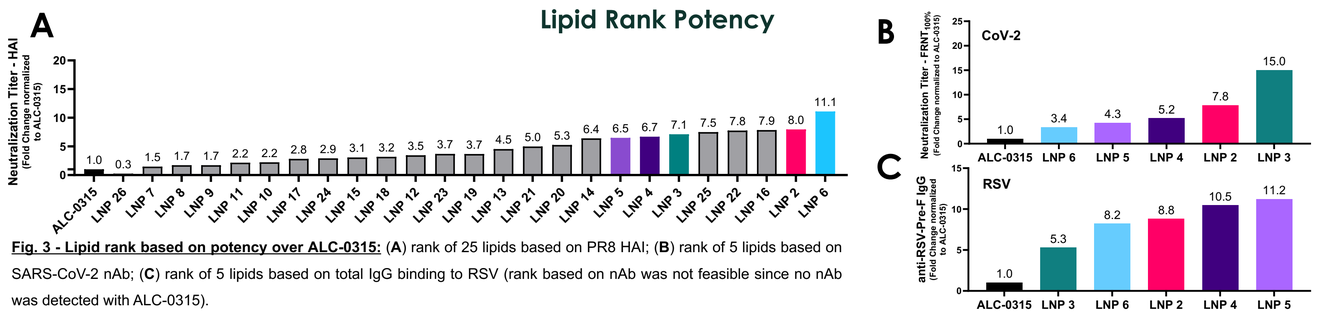


Fig. 3 - Lipid rank based on potency over ALC-0315: (**A**) rank of 25 lipids based on PR8 HAI; (**B**) rank of 5 lipids based on SARS-CoV-2 nAb; (**C**) rank of 5 lipids based on total IgG binding to RSV (rank based on nAb was not feasible since no nAb was detected with ALC-0315).

Innate Stimulation Studies

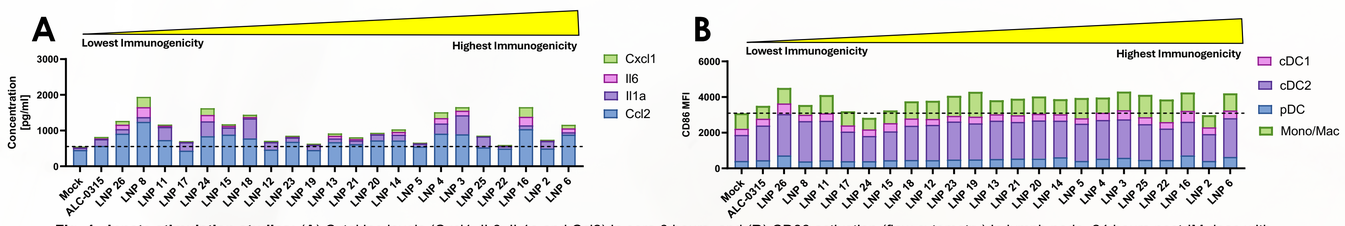


Fig. 4 - Innate stimulation studies: (**A**) Cytokine levels (Cxcl1, IL6, IL1a and Ccl2) in sera 6 hours, and (**B**) CD86 activation (flow cytometry) in lymph node, 24 hours post-IM dose with 0.2 µg PR8 HA LNP in BALB/c mice (3/group). Dotted line represents cytokine level in negative control group.

Multivalent LNP (PR8 HA/RSV- Pre-F/SARS-CoV-2 RBD)

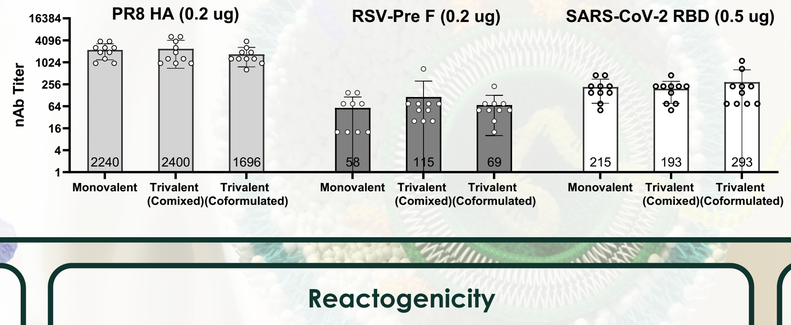


Fig. 5 - Multivalent immunogenicity with LNP 3: Serum neutralization titers 14 days following prime/boost vaccination of BALB/c mice (10/group) with either Monovalent LNP 3, co-mixed Trivalent LNP 3, or co-formulated Trivalent LNP 3.

Biodistribution

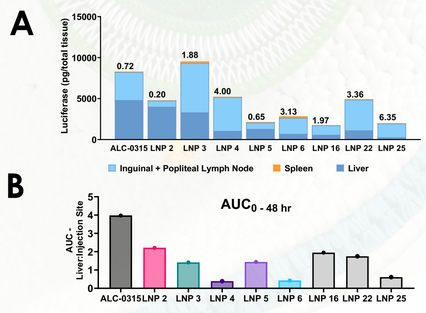


Fig. 6 - Biodistribution studies: (**A**) Luciferase expression in organs harvested 4 hours post-IM administration of BALB/c mice (3/group) with 0.2 µg FLuc mRNA-LNP. Numbers above the bars indicate ratio of Lymph Node/Spleen to Liver expression. (**B**) AUC Liver/Injection ratio following IM administration of BALB/c mice (3/group) with 2 µg FLuc mRNA-LNP. Live whole-body imaging was performed at 4, 24 and 48 hours post-dose.

Reactogenicity

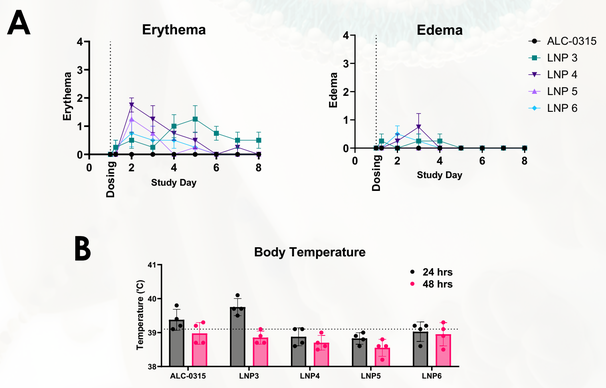


Fig. 7 - Reactogenicity studies: (**A**) Draize scoring of injection site Erythema and Edema, (**B**) Rectal body temperature following single IM-administration of Hartley Guinea Pigs (4/group) with 10 µg PR8 HA mRNA-LNP.

Memory B Cells Responses

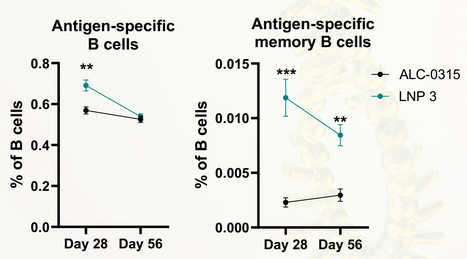


Fig. 8 – Memory B cells responses: Splenocytes harvested at Day 28 and Day 56 following prime/boost vaccination in BALB/c mice (5/group) with 0.2 µg PR8 HA mRNA LNP were stained with two different fluorescently labelled recombinant PR8 HA conjugates. Antigen-specific B cells ($CD45^+$ $CD19^+$ $PR8^+$), and among them IgM^- IgD^- IgG^+ $PR8^+$ memory B cells responses were assessed.

Results

PR8 HA Vaccine Studies: Hemagglutination assay inhibition (HAI) data resulted in several lipids inducing significantly higher nAb titers than the benchmark (**Fig. 1A & B**). Similarly, preliminary T cell response assessment identified lipids inducing significantly higher CD8 T cell response than ALC-0315 (**Fig. 1C**).

RSV-Pre-F and SARS-CoV-2 RBD Vaccine Studies: Similarly, LNP that were significantly potent with PR8 antigen model compared to benchmark, significantly improved potency (nAb titers) for both RSV and CoV-2 compared to benchmark (**Fig. 2A & C**). Given that neutralization assay condition didn't detect any RSV nAb titers with ALC-0315 and very minimal with SARS-CoV-2, IgG levels were assessed and confirmed induction of antigen binding Ab with ALC-0315 (**Fig. 2B & D**).

Lipid Rank Potency: Lipid rank potency of the 25 lipids screened with PR8 HA based on HAI indicate that 11 out of the 25 screened lipids induced 5 to 11-fold more nAb than ALC-0315 (**Fig. 3A**). Similarly, 3 and 5 out of the 5 lipids screened with SAR-CoV-2 RBD (**Fig. 3B**) and RSV-Pre-F (**Fig. 3C**) respectively induced 5.2 to 15–fold higher nAb/IgG levels than the benchmark.

Innate Stimulation Studies: Representative stacked cytokine plots (IL1a, IL6, Ccl2 and Cxcl1) and APC activation indicate that innate immune stimulation triggered by screened lipids were upregulated by most and least vaccine-potentiating LNP, suggesting no correlation between innate immune stimulation and vaccine adaptive immune response (**Fig. 4A & B**). Interestingly, ALC-0315 was among lipids inducing the least cytokines and APC activation.

Multivalent LNP Immunogenicity: Using ionizable LNP 3, both co-mixing monovalent LNP (H1N1 PR8 HA, RSV A2 Pre-F, and SARS-CoV-2 WA.1 RBD) into a trivalent LNP or co-formulating mRNA into a trivalent LNP show comparable nAb/HAI titers to their respective monovalent LNP.

Biodistribution (Expression): Data indicate that while for ALC-0315, expression was higher in the liver than secondary lymphoid organs, 6 out of 8 new potent lipids lead to higher secondary lymphoid organ expression relative to liver (**Fig. 6A**). Similarly, all novel lipids produced a lower ratio of liver to injection site expression than ALC-0315 (**Fig. 6B**).

Reactogenicity: Data indicate that while ALC-0315 induce minimal local (erythema & edema) and systemic reaction (body temperatures), minor to moderate local reaction was observed with novel lipids (**Fig. 7A**). Body temperature was transiently elevated at 24 hours for ALC-0315 and LNP 3 and returned to baseline by 48 hours (**Fig. 7B**).

Memory Cell Responses: Plasma and memory B cells were monitored to assess if identified potent lipids over ALC-0315 lead to higher memory B cells pool. Interim data indicate that LNP 3 induce significantly higher memory B cells pool than ALC-0315, and the trend is the similar for plasma B cells notably at an earlier time point (**Fig. 8**).

Summary

- Screening from our library of custom-made, rationally designed ionizable lipids, we have identified new compounds that induce significantly higher virus-specific immunogenicity compared to ALC-0315.
- Importantly, we have demonstrated potency of identified lipids with 3 antigens indicating superiority over ALC-0315 is antigen independent.
- Furthermore, utilizing LNP 3, immunogenicity in a multivalent vaccine targeting H1N1 influenza (PR8), RSV (A2), and SARS-CoV-2 (WA-1) viruses was comparable to the respective monovalent LNP.
- Expression biodistribution data indicated higher secondary lymphoid exposure than liver expression with newly potent lipids compared to ALC-0315, which might have contributed to superior activity.
- Innate immune stimulation studies suggest that screened LNPs differentially induce innate immune stimulation without correlation with adaptive immune response to vaccine.
- Reactogenicity studies suggest that novel LNP produce minor to moderate injection site reactions compared to ALC-0315. This is not surprising given their higher immunogenicity which should allow to dose lower, compared to ALC-0315.
- Preliminary memory B cells assessment indicate that new potent LNP 3 lipid induced higher memory B cells pool, compared to ALC-0315.
- Data generated from this screening will help to build a SAR database to identify lipid structures favourable for prophylactic infectious diseases vaccines.