Identification of Novel Lipids With Improved Activity for Prophylactic Vaccine Development

Thomas Chamberlain, Fan Yan, Jennifer Moon, Polina Blagojevic, Kyle Stephenson, Paulo Lin, Steve Arns, Ying Tam, Ghania Chikh

Acuitas Therapeutics, Vancouver, BC, Canada

ABSTRACT

Introduction & Objectives: Acuitas' lipid nanoparticle (LNP) technology has been successfully validated in human vaccines as demonstrated by the Pfizer-BioNTech COVID-19 vaccine, COMIRNATY®, which has protected billions of people in more than 180 countries.

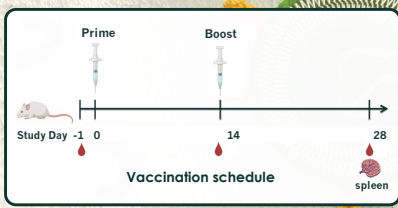
To create more effective and innovative vaccines to address progressively more challenging infectious disease applications, there is a need for continuous advancement in LNP technology through the integration of ionizable lipid screening.

The COMIRNATY® LNP composition consists of ALC-0315 and ALC-0159, Acuitas' proprietary ionizable and PEG lipids respectively, as well as helper lipids. From our comprehensive custom-made library of over 1,000 ionizable lipids, we have rationally selected a panel of new lipids to identify those with superior activity for vaccine development.

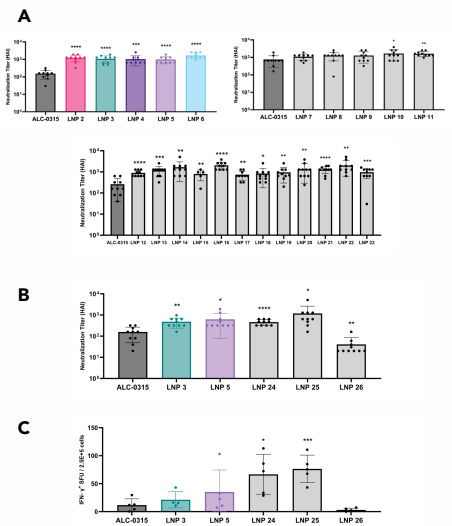
Material & Methods: Using three antigen models derived from viral infectious diseases, H1N1 influenza (PR8), RSV (A2) and SARS-CoV-2 (WA-1), we have immunized mice in a prime-boost schedule and assessed antigen specific adaptive immune responses including neutralizing antibody titers. Furthermore, we have assessed the T cell response induced by selected lipids providing insights into the nature of the adaptive immune response and potential for long-term immunity. Moreover, innate immune stimulation induced by LNP and biodistribution were assessed to further understand the antigen-specific adaptive vaccine immune response outcomes.

Results: We have identified ionizable lipids that induce significantly higher immunogenicity than ALC-0315, as evidenced by antibody neutralization titers. Additionally, the T cell response, innate immune stimulation, and biodistribution of selected ionizable lipids have also been reported.

Conclusion: From our library of novel, rationally designed ionizable lipids, we have identified new compounds that induce significantly higher virus-specific immunogenicity compared to ALC-0315. Follow-up studies will compare potency of the identified lipids, assess their reactogenicity and immunogenicity in a multivalent vaccine targeting H1N1 influenza (PR8), RSV (A2), and SARS-CoV-2 (WA-1) viruses.

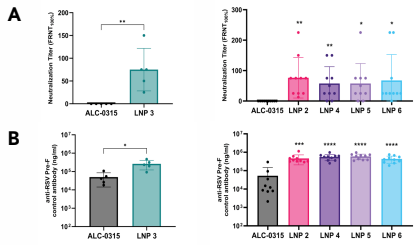


PR8 HA Vaccine Studies



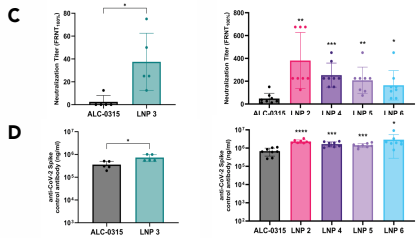
Lipid screening (2-26) against ALC-0315. Serum HAI titers 14 days following prime/boost vaccination of BALB/c mice (10/group) with 0.2 μg PR8 HA mRNA-LNP (studies: **A-B**). Following serum collection, spleens were collected from study **B** animals and after in vitro stimulation with CD8 T cell epitope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

RSV – Pre-F Vaccine Studies



Lipid screening (2-6) against ALC-0315. Serum neutralization (**A**) and IgG (**B**) titers 14 days following prime/boost vaccination of BALB/c mice (8 or 5/group) with 0. 2μg RSV–Pre-F mRNA-LNP.

SARS-CoV-2 RBD Vaccine Studies



Lipid screening (2-6) against ALC-0315. Serum neutralization (**C**) and IgG (**D**) titers 14 days following prime/boost vaccination of BALB/c mice (8 or 5/group) with 0. 5μg SARS-CoV-2 RBD mRNA-LNP.

RESULTS

PR8 HA vaccine studies: From our extensive library of custom made and rationally designed lipids, we have screened 25 compounds with PR8 HA antigen against ALC-0315 over four studies. Neutralization antibody (nAb, HAI) data resulted in several lipids with a significantly higher HAI titers than the benchmark (**Fig. A-B**).

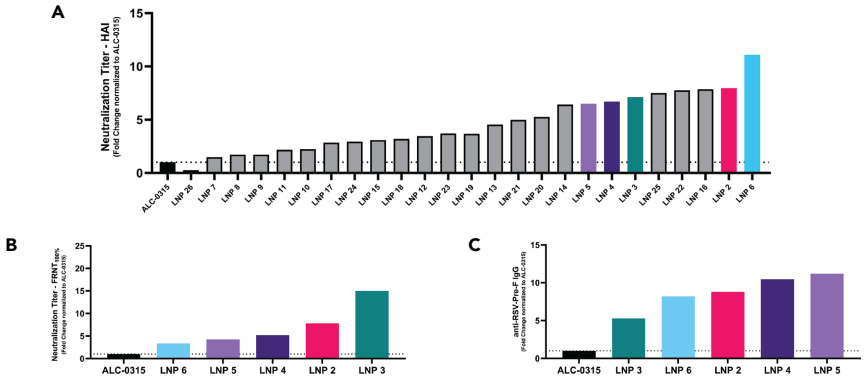
Similarly, preliminary T cell response assessment identified lipids inducing higher CD8 T cell response than ALC-0315 (**Fig. C**).

RSV-Pre-F and SARS-CoV-2 RBD vaccine studies: To confirm that potency of identified lipid over ALC-0315 is independent of the antigen identity, we selected five of these lipids and assessed them with RSV-Pre-F and SARS-CoV-2 RBD antigens.

nAb titers against RSV and SARS-CoV-2 were significantly superior to benchmark (**Fig. A & 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. B & D**).

Lipids Potency Ranking: Lipid ranking potency of the 25 lipids screened with PR8 HA based on HAI titers indicate that 11 out of the 25 screened lipids induced five to 11-fold higher nAb titers than ALC-0315. Similarly, three and five out of the five lipids screened with SAR-CoV-2 RBD and RSV-Pre-F respectively induced 5.2 to 15–fold higher nAb / IgG titers than the benchmark.

Lipids Potency Ranking

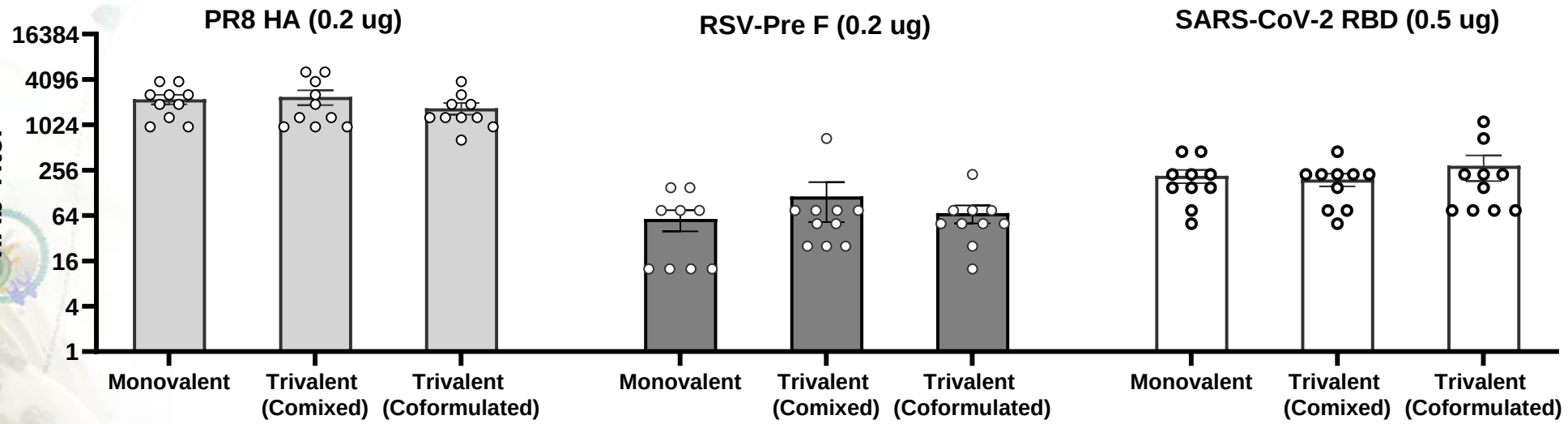


Lipid ranking based on potency over ALC-0315: (**A**) rank of 25 lipids based of PR8 HAI titers; (**B**) rank of 5 lipids based on SARS-CoV-2 nAb; (**C**) rank of 5 lipids based on total IgG binding to RSV (rank based on nAb was not feasible since no nAb was detected with ALC-0315)

SUMMARY

- Screening our library of rationally designed ionizable lipids, we have identified new compounds that induce significantly higher virus-specific immunogenicity compared to ALC-0315.
- Importantly, we have demonstrated the enhanced potency of these lipids with three antigens indicating that this improved immunogenicity is antigen independent.
- To better understand the mechanism of the improved immunogenicity of the identified lipids, their innate immune stimulation as well as LNP biodistribution, is currently being assessed.
- Future studies will determine and rank potency of identified lipids and characterize their T cell immune response.
- Data generated from this screening will be used to build a structure activity relationship (SAR) database to identify the motif (s) that drives potent activity for vaccine against viruses.

Multivalent mRNA-LNP Vaccine



Multivalent mRNA Vaccine. Serum HAI and neutralization titers 15 days following prime/boost vaccination of BalBc mice (10/group) with monovalent PR8 mRNA-LNP3 (0.2 μg), RSV-PreF mRNA-LNP3 (0.2 μg), SARS-CoV2 mRNA-LNP3 (0.5 μg) or trivalent vaccine either co-mixed post individual formulation (0.2 μg PR8 mRNA-LNP3 + 0.2 μg RSV-PreF mRNA-LNP3 + 0.5 μg SARS-CoV2 mRNA-LNP3) or co-formulated.

ACUITAS THERAPEUTICS