Optimized and Scalable LNP Composition for RNA Vaccine Delivery

Booth #40

Natalie Orr[1] (Presenting author), Mikayla Walsh[1], Cayden Yu[1], Victoria Hartman[1], Ian Johnston[1], Ashley Braun[1], Deepkanwal Singh Mundra[1], Ariel Zhang[1], Pierrot Harvie[1], Leanna Yee[1], Suraj Abraham[1], Nikita Jain[1], Anitha Thomas[1], Deo Tiwari[1], Samuel Clarke[1]

[1]Precision Nanosystems ULC, Vancouver, Canada

Contact us at:
Precision NanoSystems, Vancouver, BC, Canada
info@precision-nano.com

Introduction

- Lipid nanoparticle encapsulated RNA (RNA-LNP) vaccines have played a pivotal role in the global response to the COVID-19 pandemic[1] and are at various stages of clinical development against different infectious diseases[2].
- Access to potent ionizable lipids is limited and the expertise required to optimize scalable formulations present significant barriers to entry in this field.
- To overcome these challenges, we developed a novel ionizable lipid and a corresponding LNP composition, optimized for vaccine delivery and scalable from bench to clinic.

Objectives

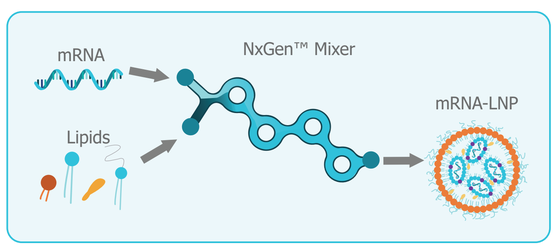
- Screen ionizable lipids and LNP compositions to determine the lead candidate for vaccine applications.
- Determine critical quality attributes (CQAs), *in vitro* efficacy and immunogenicity of lead LNP compositions.
- Investigate storage stability and scalability of the lead LNP candidate.

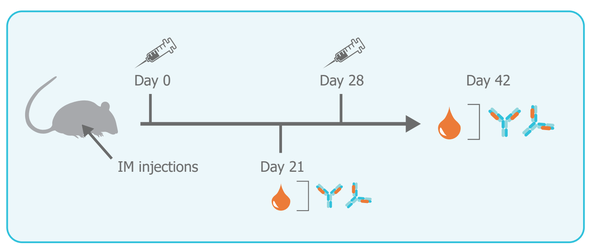
Methods and Results

RNA-LNP production: RNA-LNPs were prepared on the NanoAssemblr® Ignite™ system using a custom lipid mix including an ionizable lipid from the Precision NanoSystems Ionizable Lipid Portfolio. A total flow rate (TFR) of 20 mL/min, a flow rate ratio (FRR) of 3:1 (aq:org) and an N/P ratio of 8 were used, unless stated otherwise. Organic solvent was removed by dilution with a physiological buffer (pH 7.4) and LNPs were concentrated using 30 kDa Amicon® filters. LNPs were stored at -80°C in cryopreservation buffer for up to 6 weeks. To demonstrate the scalable nature of both the novel LNP composition and the mixing process, we took our lead SARS-CoV-2 saRNA-LNP vaccine candidate and prepared batch sizes of 30-150 mL at up to 48 L/h on NanoAssemblr® systems using NxGen™ mixing. These batches were concentrated and diafiltered by tangential flow filtration using a Cytiva T-series Centramate™ Delta cassette 30 kDa, 93cm².

RNA-LNP characterization and *in vitro* potency: RNA-LNP size and polydispersity (PDI) were determined by dynamic light scattering, and encapsulation efficiency by RiboGreen™ assay. *In vitro* protein expression in HEK-293 cells was determined by Western blotting for nCoV saRNA-LNPs, or by flow cytometry following treatment with eGFP mRNA- and saRNA-LNPs.

***In vivo* immunogenicity:** *In vivo* immunogenicity was investigated by intramuscular injection of BALB/c mice with SARS-CoV-2 saRNA and haemagglutinin (HA) (N1-methylpseudouridine (m1Ψ) modified and unmodified) mRNA LNPs, with a booster dose on day 28. Tail bleeds were performed on day 21 and mice were culled by cardiac puncture on day 42 followed by spleen removal. IgG titers and HA inhibition were assessed using sera, and cytokine response using splenocytes and a 12-plex LEGENDplex™ MU Th cytokine panel.





1. Ionizable Lipid Screen

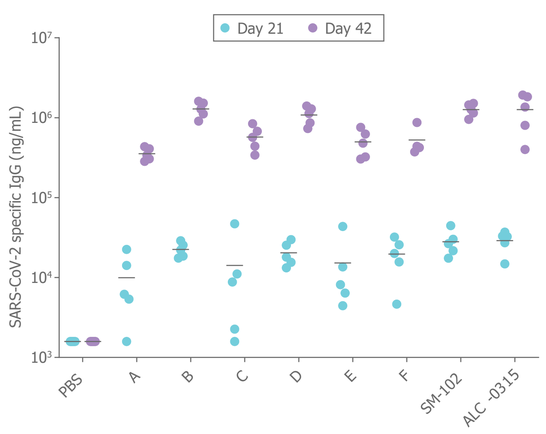


Figure 1. Ionizable lipid screen.
Graph shows antigen-specific IgG titers in BALB/c mice after immunization with saRNA-LNPs, encoding for the pre-fusion SARS-CoV-2 spike protein, on day 21 and day 42 of the immunization schedule. LNPs were prepared with select ionizable lipids from Precision NanoSystems' Ionizable Lipid Library (A – F), and two clinically relevant control ionizable lipids.

2. LNP Composition Screen (1)

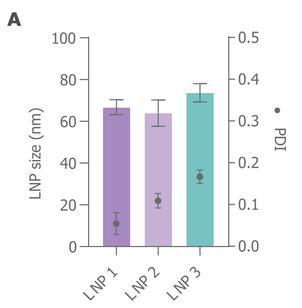


Figure 2. Lead LNP compositions, identified in previous screening and prepared using the selected ionizable lipid, were investigated using saRNA encoding for the pre-fusion SARS-CoV-2 spike protein.
Graphs **A)** and **B)** show the CQAs of nCoV spike protein saRNA-LNPs. Bars indicate the mean, and error bars show the SD where multiple batches were prepared.

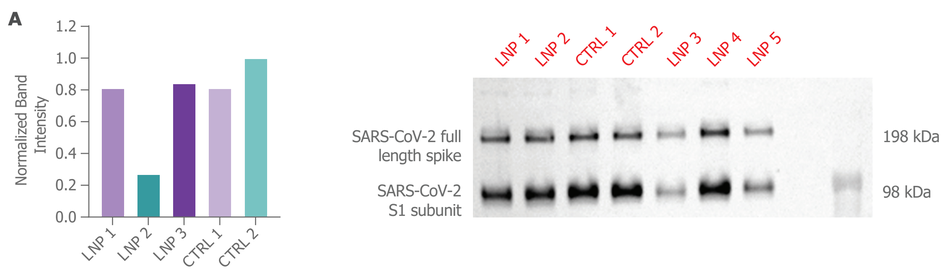


Figure 3. Potency and immunogenicity of lead LNP compositions in a SARS-CoV-2 vaccine application.
A) *In vitro* protein expression of HEK-293 cells when treated with lead LNP compositions and controls. Expression was determined by Western blot and the band intensities normalized using β-actin loading controls. **B)** Graph shows the SARS-CoV-2 spike protein-specific IgG titers in serum of BALB/c mice on days 21 and 42 of the immunization schedule. Whiskers show the maximum and minimum values. A one-way ANOVA was performed on day 42 log-transformed data, and Tukey's post hoc test was used to compare the immune responses of each group, with no significant differences observed ($p \leq 0.05$ *, $p \leq 0.01$ **).

3. LNP Composition Screen (2)

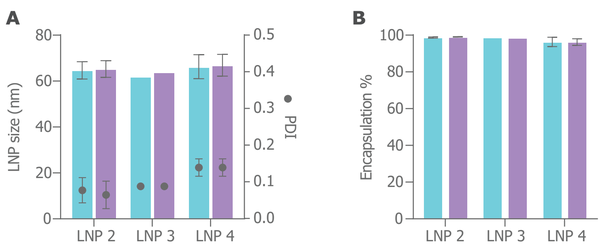


Figure 4. Lead LNP compositions were investigated using mRNA encoding for haemagglutinin (HA).
Graphs **A)** and **B)** above show the CQAs of HA mRNA-LNPs (m1 Ψ modified and unmodified). Bars indicate the mean, and error bars show the SD where multiple batches were prepared

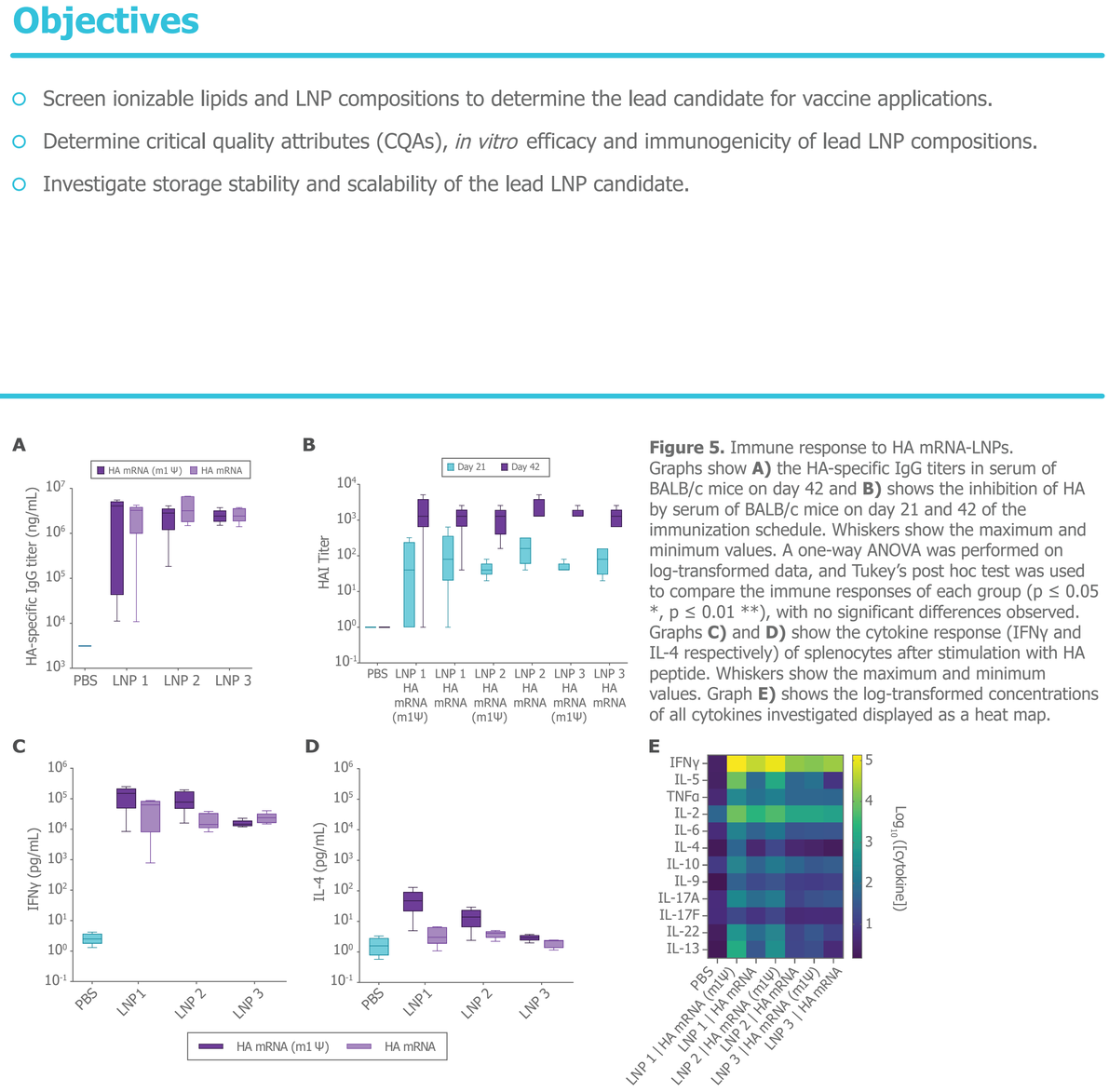


Figure 5. Immune response to HA mRNA-LNPs.
Graphs show **A)** the HA-specific IgG titers in serum of BALB/c mice on day 42 and **B)** shows the inhibition of HA by serum of BALB/c mice on day 21 and 42 of the immunization schedule. Whiskers show the maximum and minimum values. A one-way ANOVA was performed on log-transformed data, and Tukey's post hoc test was used to compare the immune responses of each group ($p \leq 0.05$ *, $p \leq 0.01$ **), with no significant differences observed. Graphs **C)** and **D)** show the cytokine response (IFNγ and IL-4 respectively) of splenocytes after stimulation with HA peptide. Whiskers show the maximum and minimum values. Graph **E)** shows the log-transformed concentrations of all cytokines investigated displayed as a heat map.

4. Storage buffer allows cryopreservation of lead candidate LNPs while retaining CQAs and *in vitro* potency over 6 weeks

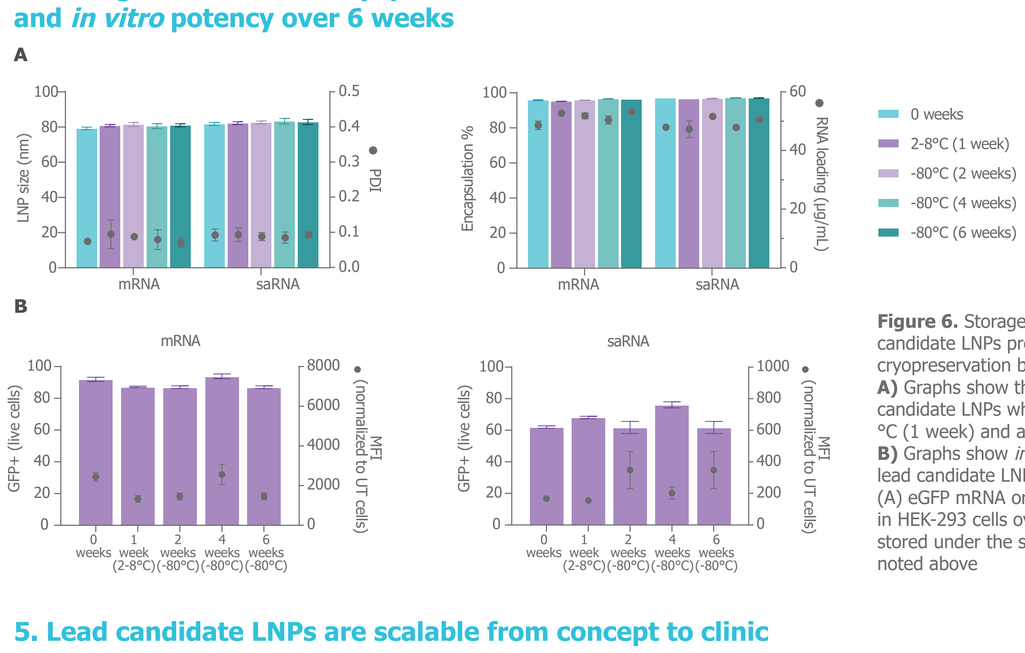


Figure 6. Storage stability of lead candidate LNPs prepared with cryopreservation buffer
A) Graphs show the CQAs of lead candidate LNPs when stored at 2-8 °C (1 week) and at -80 °C (6-weeks).
B) Graphs show *in vitro* potency of lead candidate LNPs encapsulating (A) eGFP mRNA or (B) eGFP saRNA in HEK-293 cells over 6-weeks when stored under the same conditions noted above

5. Lead candidate LNPs are scalable from concept to clinic

	LNP Size (nm)	PDI	Encapsulation %
Ignite 20 mL/min	80.1	0.143	94.4
Ignite+ 200 mL/min	77.6	0.154	94.0
Blaze 115 mL/min	79.0	0.170	95.3
GMP 12 L/hr	69.8	0.178	95.3
Commercial Formulation System 48 L/hr	73.4	0.135	97.0
Modular Commercial Formulation Skid 48 L/hr	73.2	0.176	95.0

Table 1. CQAs of saRNA-LNPs prepared using NanoAssemblr® NxGen™ technology at different scales.
Table shows LNP size, PDI and encapsulation of lead candidate saRNA-LNPs prepared at the maximum flow rates of each system.

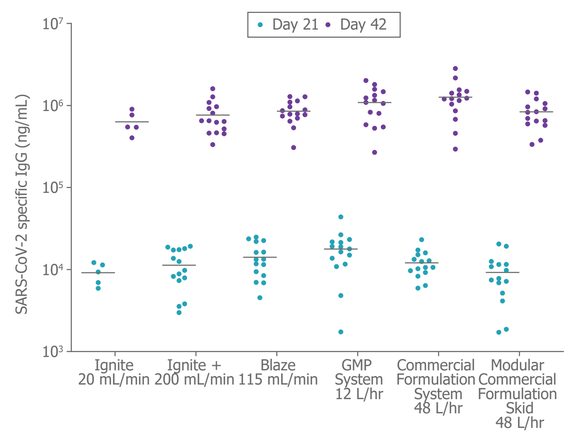


Figure 7. Immunogenicity of saRNA-LNPs prepared using NanoAssemblr® NxGen™ technology at different scales.
Graph shows the SARS-CoV-2 spike protein specific IgG titers in serum of BALB/c mice treated with lead candidate saRNA-LNPs prepared at the maximum flow rates of each system. Lines show the median response. A one-way ANOVA on log-transformed data was performed, and Tukey's post hoc test was used to compare the immune response to LNPs prepared with different systems ($p \leq 0.05$ *, $p \leq 0.01$ **), with no significant differences observed between instruments on day 42.

Conclusion

- Novel LNP formulations were screened for physicochemical attributes, *in vitro* potency and *in vivo* immunogenicity in vaccine applications, and the lead LNP candidate selected as the main component in Cytiva's RNA delivery LNP kit.
- LNP CQAs and potency were maintained using NxGen™ mixers up to cGMP scale, showcasing how this formulation can fast-track and de-risk drug development timelines.
- Leverage Precision NanoSystem's expertise and pre-optimized formulations to accelerate pre-clinical drug development activities, including antigen screening and *in vivo* proof-of-concept studies for vaccine applications.

Download