Lipid Nanoparticle Library for Non-Viral Delivery of mRNA and saRNA Towards Vaccine and Cell & Gene Therapy Applications

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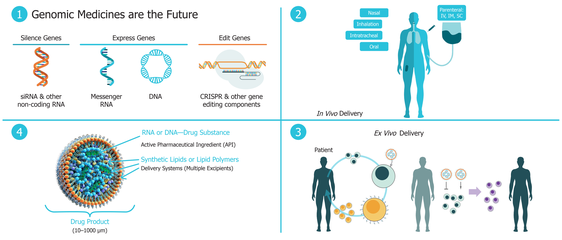
Introduction

- Recent FDA approval of ONPATTRO® by Alnylam, Comirnaty® by BioNTech/Pfizer and Spikevax® by Moderna and the various clinical trials with mRNA-based drugs or vaccines have provided momentum to further develop lipid nanoparticle (LNP) based genetic medicines.
- Ionizable amino lipids are a major constituent of LNPs for delivering nucleic acid therapeutics (e.g., DLin-MC3-DMA in ONPATTRO®, ALC-0315 in Comirnaty®, SM-102 in Spikevax®).
- Efficient ionizable lipids are needed that are non-toxic, can effectively encapsulate nucleic acids, and can help with endosomal release.
- The scarcity of ionizable lipids that are suitable for vaccination, cell and gene therapies continues to be a problem in advancing many potential therapeutic/vaccine candidates to the clinic.

Objectives

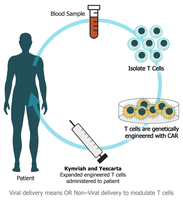
- Showcase the utility of PNI's proprietary ionizable lipids and LNP compositions across cell therapy, gene therapy and vaccine applications.
- Demonstrate cell therapy applications for proprietary lipids using GFP encoded mRNA and their transfection comparison with clinically approved lipid Dlin-MC3-DMA in human primary T cells.
- Demonstrate protein replacement applications for proprietary lipids using Epo encoded mRNA and their potency comparison with clinically approved Dlin-MC3-DMA in C56BL6 mice.
- Demonstrate vaccine applications for PNI proprietary lipids using self-amplifying RNA encoding for Hemagglutinin and SARS-CoV-2 spike protein in comparison to SM-102 and ALC-0315 in multiple animal models.



1. Non-Viral Delivery Systems Enable Cell Therapy

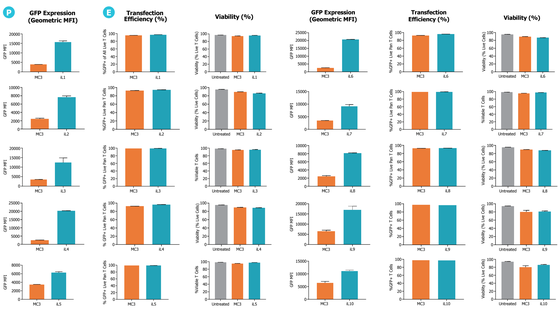
Why do LNP enable efficient genetic engineering?

- Dense packaging of payload
- Protects nucleic acid from degradation
- Uptake via endogenous pathways
- Efficient gene delivery
- Very low cellular toxicity



Viral delivery means OR Non-Viral delivery to modulate T cells

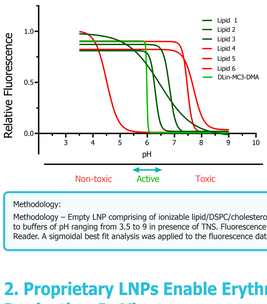
a. GFP Expression in Primary Human T Cells Using PNI proprietary LNPs encoded with GFP mRNA



- MC3 (Dlin-MC3-DMA) – Ionizable lipid used as an excipient in FDA approved Onpattro™. It was used as benchmark lipid for comparative evaluation of potency of proprietary PNI lipids
- Cell viability was >90% as compared to untreated cells
- Higher GFP MFI and transfection efficiency relative to MC3 was observed with PNI lipids

Methodology:
LNPs were formulated with CleanCap® eGFP mRNA (TriLink, Catalog# L-7601) using NanoAssemblr® Spark™. Size: 80-120 nm, PDI: <0.2, %EE: >85% GFP MFI, transfection efficiency, and viability were measured by flow cytometry at 48 hours post treatment. Treatment details - 0.5 µg of mRNA/125K T cells/0.25 mL media containing 100 ng/mL IL-2 and 1 µg/mL of ApoE.

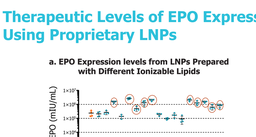
b. Effect of pKa on Transfection Efficiency and Cell Viability



- Proprietary lipids showed higher GFP MFI and transfection efficiency relative to MC3 (benchmark, used in Onpattro™).
- Overall, there is the correlation between activity and pKa of ionizable lipids in T cells.
- If the pKa of lipid lies outside the active range, it would either not transfect at all OR viability is compromised.

Methodology:
Methodology – Empty LNP comprising of ionizable lipid/DSPC/cholesterol/PEG-lipid (50/10/38.5/1.5) were formulated using Spark™. LNP were added to buffers of pH ranging from 3.5 to 9 in presence of TNS. Fluorescence intensity was measured in a BioTek™ Synergy™ H1 Fluorescence Microplate Reader. A sigmoidal best fit analysis was applied to the fluorescence data, and the pKa was measured as the pH at half-maximal fluorescence intensity.

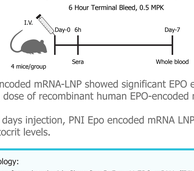
2. Proprietary LNPs Enable Erythropoietin (EPO) Production *In Vivo*



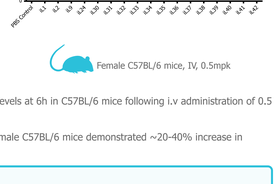
1. We encoded human Epo in mRNA, packaged it in PNI LNP using NxGen™ Technology
2. PNI LNP delivers the mRNA to liver cells which express Epo protein stimulating red blood cell production
3. We observed an increase in red blood cell production in mice

Therapeutic Levels of EPO Expression and Hematocrit Levels in Mice Using Proprietary LNPs

a. EPO Expression levels from LNPs Prepared with Different Ionizable Lipids



b. Hematocrit Results Comparing LNPs Prepared with Different Ionizable Lipids

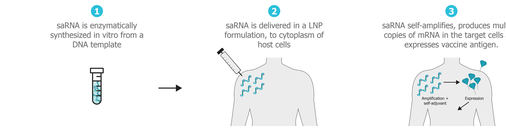


- EPO-encoded mRNA-LNP showed significant EPO expression levels at 6h in C57BL/6 mice following i.v administration of 0.5 mg/kg dose of recombinant human EPO-encoded mRNA LNP
- Post 7 days injection, PNI Epo encoded mRNA LNP treated female C57BL/6 mice demonstrated ~20-40% increase in Hematocrit levels.

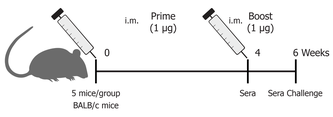
Methodology:
LNPs were formulated with CleanCap® 5 moU EPO mRNA (TriLink, Catalog # L-7209) using NanoAssemblr® Ignite™.
Size: 80-100 nm, PDI: <0.2, %EE: >85
Mice were dosed at 0.5 mg/kg iv. Serum EPO levels were measured 6h post administration of EPO encoded mRNA LNP using ELLA™ Simple™ Plex automated ELISA (Protein Simple). Blood hematocrit levels were estimated 7 days post injection using microhematocrit tubes.

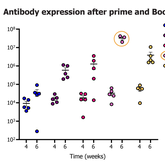
3. Proprietary LNPs Towards Developing Self-Amplifying mRNA (saRNA) Vaccines

How do saRNA-LNP vaccines work?

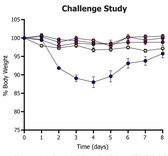


a. Proprietary LNPs Enhance Immunogenicity of Hemagglutinin saRNA





- PNI iL1 saRNA-LNP induced highest antibody level expression (> 108 ng/mL)
- Antibody responses generated after boost were much higher with proprietary LNP when compared to pABOL
- Challenge Study – Loss in body weight correlated to amount of antibodies generated and no weight loss was seen for groups that were saRNA-LNP treated

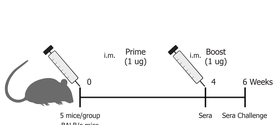


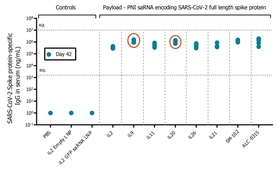
Methodology:
LNPs were formulated with Hemagglutinin saRNA using NanoAssemblr® Ignite™
Size: ~80 nm, PDI: ~0.1, %EE: >85

Collaboration with Imperial College London (Prof. Robin Shattock & Anna Blakney)

Blakney et al., Journal of Controlled Release 338 (2021) 201-210

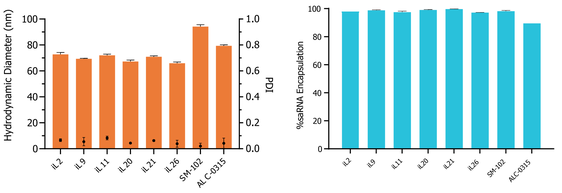
b. Proprietary Lipids Generate Equivalent Amount of SARS-CoV-2 Spike Protein Specific IgG Compared to Clinically Demonstrated Lipids



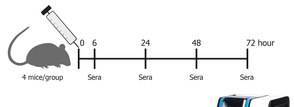


Methodology:
LNPs were formulated with PNI saRNA encoding SARS-CoV-2 full length spike protein using NanoAssemblr® Ignite™
Size: 70-100 nm, PDI: <0.2, %EE: >85%

- Sera after 2 weeks post boost (day 42), were isolated and analyzed using ELISA for Spike protein specific antibodies.
- PNI iL9 and iL20 showed similar amount of SARS CoV-2 Spike protein specific IgG compared to clinically demonstrated SM-102 and ALC-0315.

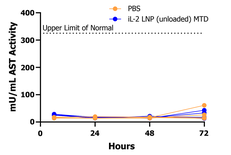


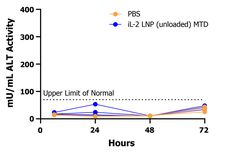
4. Tolerability of Proprietary LNP Administered IV in Mice



Methodology:
Unloaded (empty) LNPs were formulated using NanoAssemblr® Ignite™.
Size: 65-90 nm, PDI: <0.2. Endotoxin levels were measured before administration to mice and were found to be below limit of detection (LOD = 5 EU/mL)

CD-1 Mice were dosed i.v. at two escalating doses. Clinical symptoms were observed at regular intervals. Animals were euthanized at end of 72h, necropsy was performed.
Serum ALT, AST levels were tested at regular intervals following the unloaded LNP administration. All LNPs were made with the same composition except for the choice of ionizable lipid. Dosing was based on the ionizable lipid dose.





72h study
CD-1 male mice
6wk old
n=4/LNP

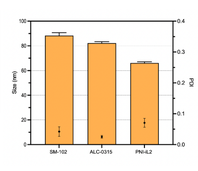
LNP	0-72 h post dose		72 h post dose			
	Clinical Observations		Necropsy		Liver histology	
	Moderate Dose	High Dose	Moderate Dose	High Dose	Moderate Dose	High Dose
Control PBS	Normal		Normal		Normal	
SM-102	Mild signs recover w/in 6h	Moderate signs lasted full 72h	Normal	↑ % Nonmal ↑ % Eutheroid @ 3h	Normal	Slight apoptosis & inflammation
ALC-0315	Mild signs recover w/in 72h	Mild to moderate for 6-72h	Normal	↑ % Normal ↑ % Enlarged Spleen	Mild apoptosis & inflammation	Mild apoptosis & inflammation
PNI iL2	Normal	Mild signs recover w/in 6h	Normal	Normal	Slight apoptosis & inflammation	Slight apoptosis & inflammation

Normal — Slight — Mild — Moderate — Severe

Clinical observations were relatively higher, lasted longer, and occurred with greater frequency in the LNPs with SM-102 and ALC-0315, then compared to a matched mg/kg dose of PNI LNP.

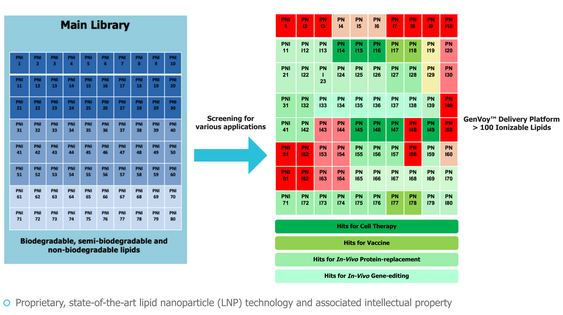
Necropsy were normal for most groups.

Liver histology showed relatively higher apoptosis and inflammation in the LNP containing ALC-0315 compared to all other groups at matched doses.



- PNI iL2 LNP showed similar or better safety profile compared to dose matched LNPs with SM-102 and ALC-0315 in a blinded study.

5. Proprietary Lipid Library: Summary and Future Perspectives



- Proprietary, state-of-the-art lipid nanoparticle (LNP) technology and associated intellectual property
- Novel lipids and compositions designed and optimization for established use cases (i.e., vaccine, gene therapy, cell therapy) as well as emerging applications

Conclusions

- Non-viral lipid nanoparticle delivery systems show significant promise in the field of genomic medicine
- PNI has developed a proprietary ionizable lipid library comprising more than 100 lipids with diverse pKa for different applications including cell therapy, protein replacement, gene therapy and RNA vaccines.
- The PNI lipid technology enables the targeted delivery of nucleic acids to specific cells and tissues and can help to accelerate the development of genomic medicines for a wide-range of diseases.