Well Characterized Lipid Nanoparticle Library Accelerates Development of Next Generation Genomic Medicine

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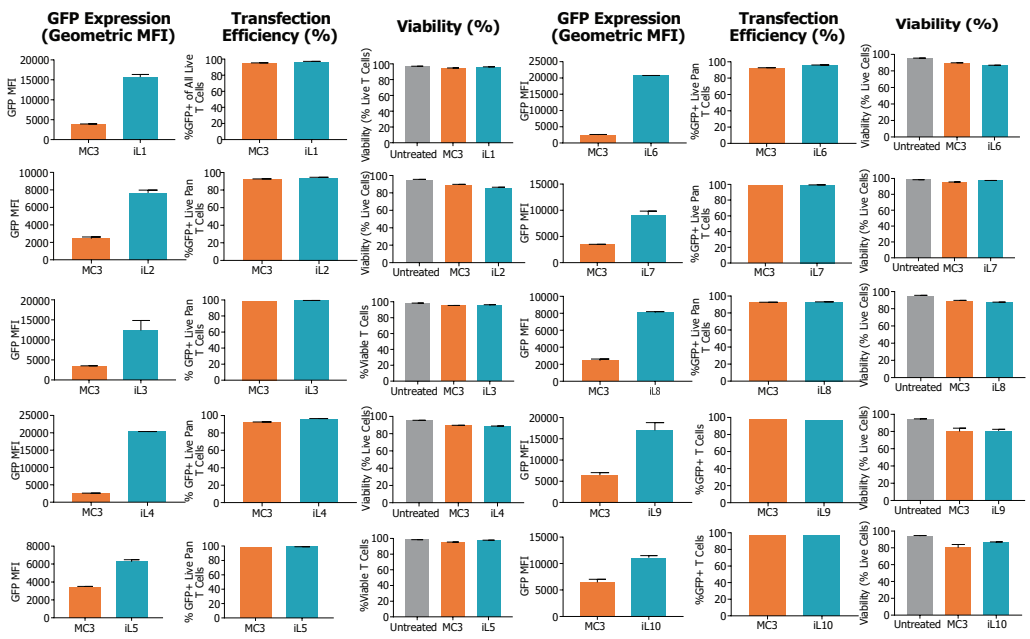
Introduction

- 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, and thus ionizable lipids with high encapsulation efficiency, high endosomal release that are non-toxic are essential for efficient clinical translation.
- The scarcity of ionizable lipids that are suitable for development of vaccines, cell and gene therapies continues to be a problem in advancing many potential therapeutic/vaccine candidates to the clinic.

Methods and Results

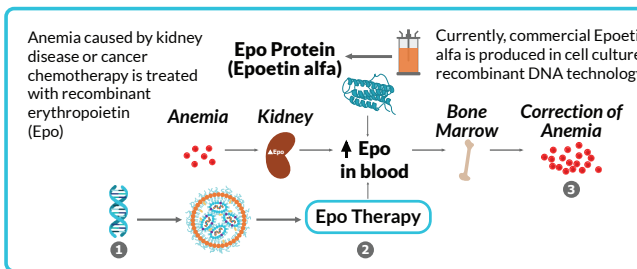
1. Non-Viral Delivery Systems Enable Cell Therapy



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

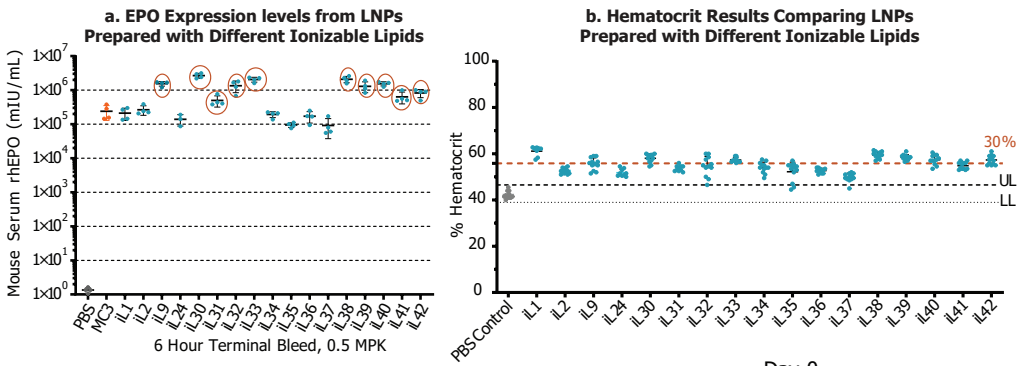
2. Proprietary LNPs Enable Erythropoietin Production *in vivo*

Anemia caused by kidney disease or cancer chemotherapy is treated with recombinant erythropoietin (Epo)

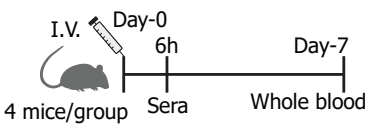


Currently, commercial Epoetin alfa is produced in cell culture using recombinant DNA technology

1. We encoded human Epo in mRNA, packaged it in PNI LNP using NxGen™ Technology
2. Following IV injection, 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



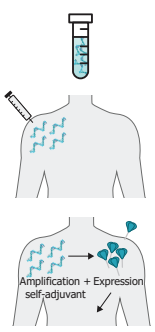
- EPO-encoded mRNA-LNP showed significant EPO expression levels at 6h in C57BL/6 mice following i.v administration of 0.5 mg/kg dose
- Post 7 days injection, EPP encoded mRNA LNP treated female C57BL/6 mice demonstrated ~20–40% increase in Hematocrit levels

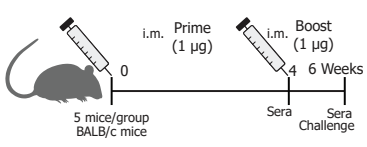


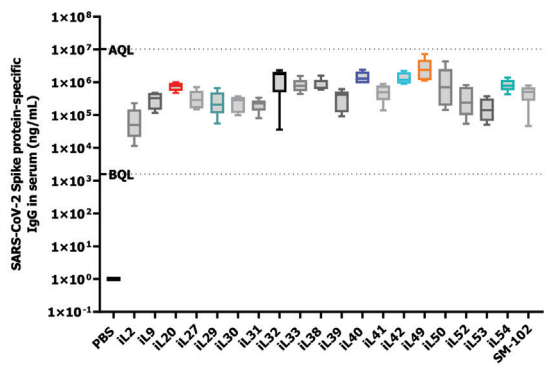
3. Proprietary LNPs Towards Developing Self-Amplifying mRNA (saRNA) Vaccines

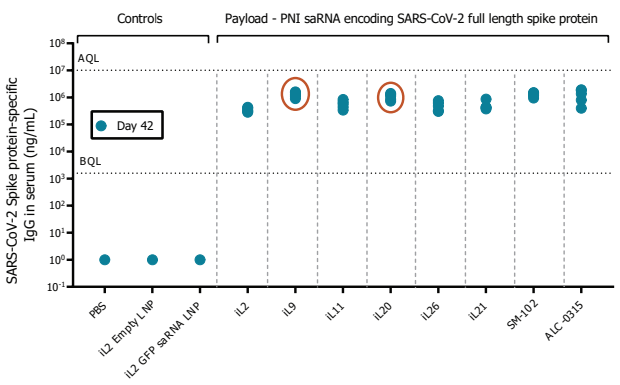
How do saRNA-LNP vaccines work?

1. saRNA is enzymatically synthesized *in vitro* from a DNA template
2. saRNA is delivered in a LNP formulation, to cytoplasm of host cells
3. saRNA self-amplifies, produces multiple copies of mRNA in the target cells and expresses vaccine antigen.





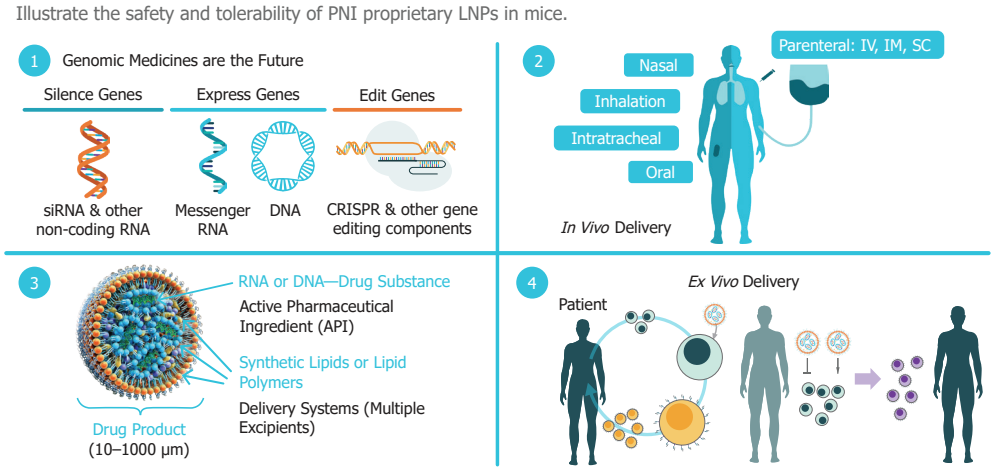




- The analysis for SARS CoV-2 spike protein specific antibodies, post two weeks boost (day 42), confirmed that many of the PNI lipids showed similar expression compared to clinically demonstrated SM-102 and ALC-0315.

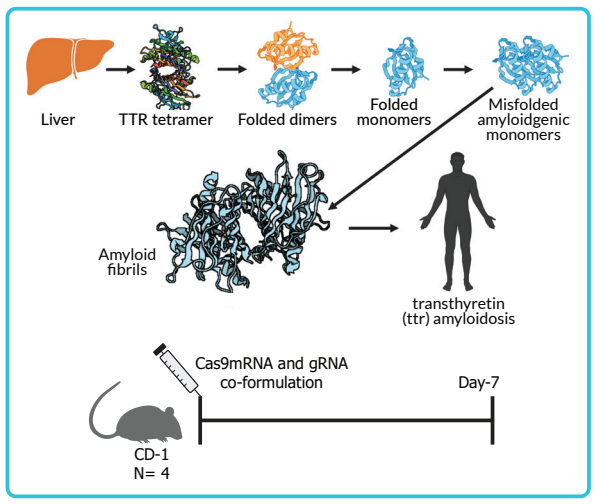
Objectives

- Demonstrate cell therapy applications for proprietary lipids using GFP encoded mRNA and compare their transfection with clinically approved lipid Dlin-MC3-DMA (MC3) 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 mice.
- Display PNI proprietary lipids for vaccine applications using self-amplifying RNA encoding for SARS-CoV-2 spike protein in comparison to SM-102 and ALC-0315 in mice.
- Showcase the PNI proprietary lipids for gene editing applications *in vivo.*
- Illustrate the safety and tolerability of PNI proprietary LNPs in mice.

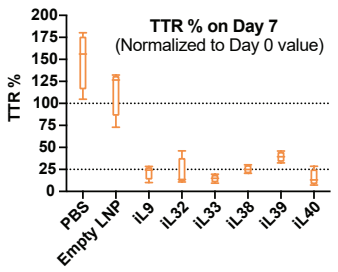


4. Novel Ionizable Lipids for Gene Editing Applications

a. TTR CRISPR Gene Editing – Preliminary Data with PNI Delivery System

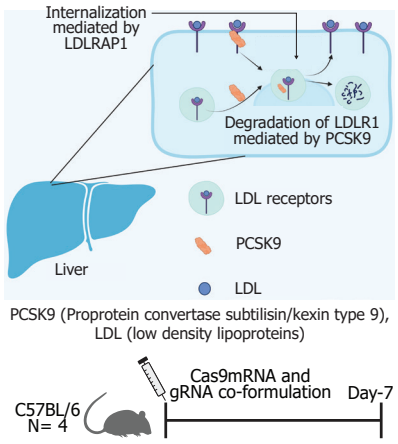


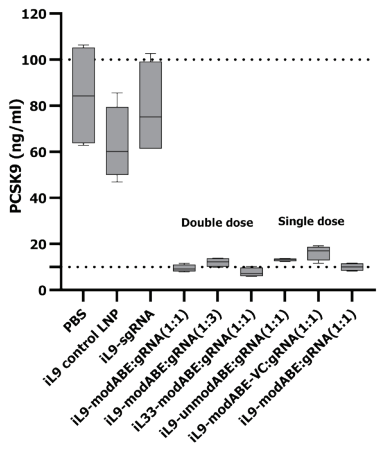
- A life-threatening genetic disease, caused by progressive accumulation of misfolded transthyretin (TTR) protein in tissues of nerves and heart
- Polyneuropathy & Myopathy can be resulted
- Leads to cardiovascular disorders
- Preliminary results show more than 75% reduction in TTR protein levels with majority of tested lipids with one injection (3 mg/Kg)



b. Base Editing - PCSK9 Knock Down - Preliminary Data with PNI Delivery system

- Caused by mutations in LDLR, PCSK9, and or APOB genes
- Two missense mutations in PCSK9 were associated with an autosomal dominant form of hypercholesterolemia
- Gain-of-function variants of PCSK9 reduces LDLR levels in liver, thereby inducing hypercholesterolemia - higher LDL cholesterol levels in plasma





5. Tolerability of Proprietary LNP Administered IV in Mice

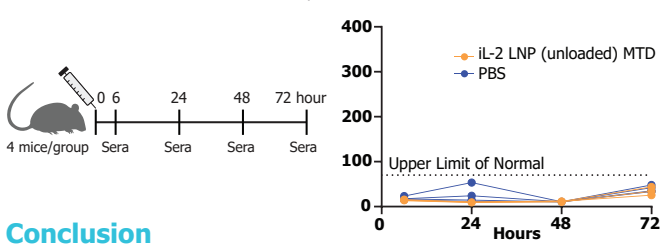
72h study CD-1 male mice 6wk old n=4/LNP LNP	0-72 h post dose		72 h post dose			
	Clinical Observations		Necropsy		Liver Histology	
	Moderate Dose	High Dose	Moderate Dose	High Dose	Moderate Dose	High Dose
Control PBS	Normal		Normal		Normal	
SM-102	Mild signs recover w/in 6h	Moderate signs lasted full 72h	Normal	¾ Normal ¼ Euthanized @ 1h	Normal	Slight apoptosis & inflammation
ALC-0315	Mild signs recover w/in 72h	Mild to moderate for 6-72h	Normal	¾ Normal ¼ 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 occured with greater frequency in the LNPs with SM-102 and ALC-0315, than compared to a matched mg/kg dose of Precision NanoSystems 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.



Conclusion

- Non-viral lipid nanoparticle delivery systems show significant promise in the field of genomic medicine.
- Precision NanoSystems 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 vaccine.
- The Precision NanoSystems 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.

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