Scale Up and Manufacturing of Self-amplifying RNA-LNPs for a COVID-19 Vaccine Using the NanoAssemblr® GMP System

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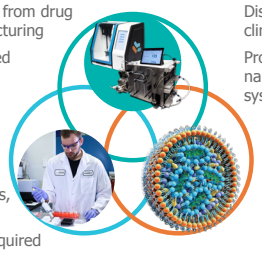
PNI Provides Full Stack Genetic Medicine Product Development

- **Proprietary Drug Product Manufacturing Platform**
  - Instruments & consumables from drug discovery to clinical manufacturing
  - Proprietary microfluidic-based manufacturing
- **Proprietary Delivery Platform**
  - Discovery reagents to clinical licensing
  - Proprietary lipid nanoparticle delivery systems
- **Expertise, Execution & Co-Development**
  - Discovery services, co-development partnerships, and clinical development
  - Comprehensive expertise required to develop a genetic medicine



Developing Vaccine in 9 Months from Project Funding

- **October 2020** — $18.2 million was received from SIF
- **November 2020 (on going activity)** — Process development manufacturing scale up
- **July 2021** — Hamster challenge study in using the Clinical Lead
- **Sept/Oct 2021 (est.)** — $18.2 million was received from SIF
- SARS-CoV-2 in vivo and in vitro: Lead candidate selection — **November 2020–January 2021**
- Engineering run for initiating safety tox study — **May 2021**
- GMP batch production — **July 2021**
- RNA Medicine Biomanufacturing Centre manufacturing 240 millions vaccine dose/years — **2023**

Canadian Strategic Innovation Fund (SIF)

- Last October PNI received $18.2 million from Canadian Strategic Innovation Fund (SIF) to develop cost-effective made-in-Canada COVID-19 self-amplifying RNA (saRNA) vaccine
- PNI also received a contribution of CAD $25.1 million through SIF to build an RNA Medicine Biomanufacturing Centre with the goal of manufacturing 240 millions vaccine dose/years

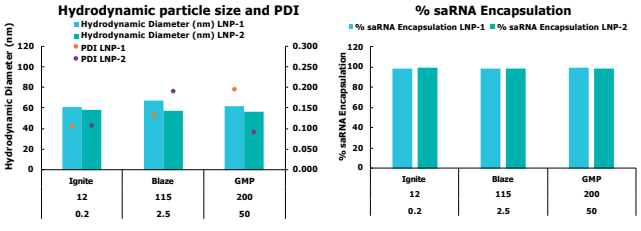
Objective

- To scale up SARS-CoV-2 self-amplifying RNA-Lipid nanoparticle using PNI developed ionizable lipid and formulation from 0.2, to 50 mg scale using PNI's Manufacturing Platform (Ignite™, Blaze™, GMP NanoAssemblr®)
  - A phase I trial for saRNA Vaccine requires 50 mg scale
- To optimize the down-stream processing (TFF/Sterile filtration)
  - TFF type, Material, MWCO
  - Processing buffers
  - TFF shear rate
  - TFF scalability
- To select a lead saRNA-LNP formulation and define a manufacturing process based on formulation activity, stability, scalability, repeatability and critical process parameters

Simplified Scale-Up of mRNA-LNP Using NxGen™

	Ignite™	Blaze™	GMP
Mixers	NxGen™, NxGen w/in-line dilution	NxGen 400, NxGen 500	NxGen 500
Organic Phase	Lipid in Ethanol		
Aqueous Phase	RNA in aqueous		
Total Micromixing Maximum Volume	up to 20mL undiluted	up to 10L undiluted	up to 50L undiluted*
Flow Rate Ratio [Org : Aq]	3:1		
Total Flow Rate	12 mL/min	115 mL/min	200 mL/min
In-line Dilution Ratio (Buffer : Micromix volume)	3:1	3:1	3:1
Downstream Processing	Dialysis Cassette	TFF	TFF

Critical Quality Parameters of the LNP Drug Product is Maintained Across Scales—From Discovery-to-Clinical Scale

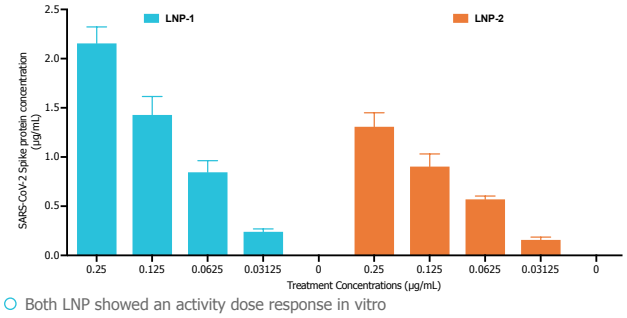


- SARS-CoV-2 self-amplifying RNA-LNP made with PNI proprietary ionizable lipid had similar size (~60 nm), polydispersity (<0.2) and encapsulation efficiency (>90%) across all scales tested with two different LNP

**The saRNA encoding a pre-fusion stabilized SARS-CoV-2 spike protein was kindly provided Dr Robin Shattock from Imperial College London*

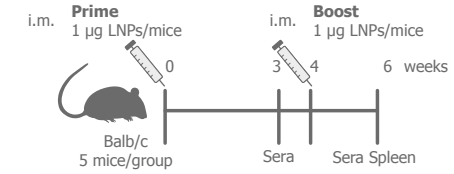
In Vitro Activity saRNA LNPs Using Two Novel Lipid Compositions

ELISA quantification of the SARS-CoV-2 spike protein expression in HEK-293 cells after transfection with saRNA LNPs



- Both LNP showed an activity dose response in vitro
- LNP-1 is more active than LNP-2 by less than 2-fold

In Vivo Testing of saRNA LNPs



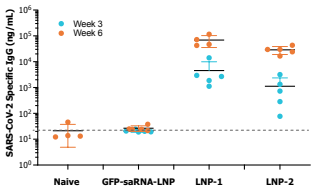
saRNA LNPs encoding a pre-fusion stabilized SARS-CoV-2 spike protein.

Sera
- SARS CoV2 specific IgG ELISA
- Cytokine measurements/Neutralization assays

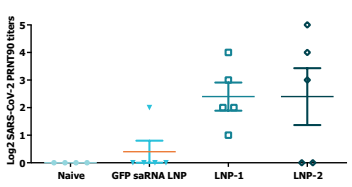
Spleen
- Isolation of splenocytes
- Ex vivo restimulation with SARS-CoV-2 peptides
- Intracellular cytokine staining/Cytokine measurements

In Vivo Activity of saRNA LNPs Using Novel Lipid Compositions

SARS-CoV-2 Specific Serum IgG Measurements at Week 3 & 6



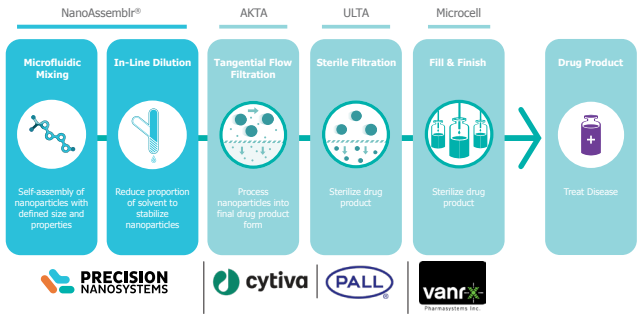
pRNT90 Values (SARS-CoV-2 real virus particles)



- Both LNP-1 and LNP-2 efficiently induced SARS CoV2 specific IgG response in mice.
- As observed in vitro LNP-1 showed slightly higher activity as compared to LNP-2.
- Both LNP-1 and LNP-2 generated neutralizing antibodies against the SARS-CoV-2 virus.

Unit Operations for mRNA-LNP Production

**Includes potential partners for downstream processing*



NanoAssemblr GMP System

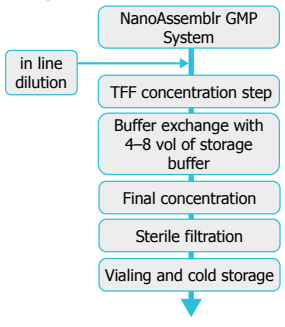
Microfluidic based non-turbulent flow, constant pressure and even flow at 200 mL/min. 20 L in 100 min.

- Configurable & modular system that is fit-for-purpose & phase appropriate
- Continuous flow enables manufacturing scales from 200 ml to >50 L
- Fully disposable fluid path reduces risk and cost (e.g., reduced cleaning validation)
- GMP System fluid path integrated to downstream TFF system enabling a closed system
- Quality documentation meets cGMP requirements
- Customer support with technology transfer and IQ/OQ of GMP System

Down-Stream Processing: Tangential Flow Filtration

Tangential Flow Filtration is used to remove the ethanol from the LNP and exchange the formulation buffer for a relevant storage buffer

- Multiples factors are taken in consideration when developing the down-stream processing step:
  - Type of cartridges
    - hollow fibers vs plate
  - Material of these cartridges
    - PS vs mPES
  - Molecular weight cut-off
    - 100–500KD typically used
  - Cartridge surface area
  - Shear rate
    - The flow rate in a single of fibers divided by radius of those fibers
  - Transmembrane Pressure (TMP)
    - The driving force for liquid transport through the ultrafiltration membrane

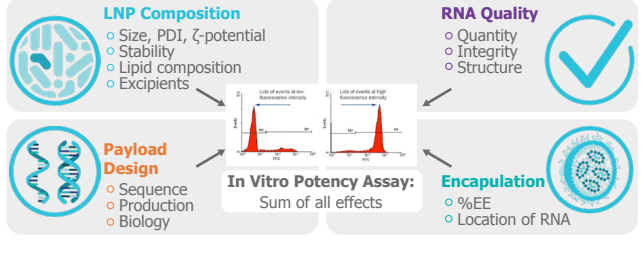


Effect of Shear Rate on TFF Processing Time

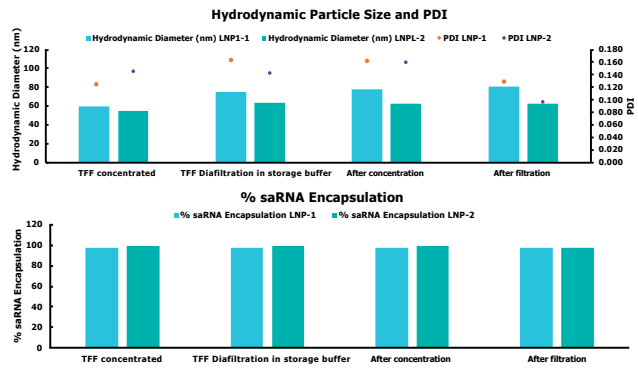
TFF steps	4000 sec-1	6000 sec-1	8000 sec-1
TFF concentration step	105 min	40 min	43 min
Diafiltration in buffer #1 (4V)	30 min	15 min	15 min
Diafiltration in Buffer #2 (4V)	50 min	70 min	50 min
Total processing time	185 min	125 min	108 min

- 2.5 mg batch size and 300KD MWCO
- TMP of 4 PSI was maintained for each conditions

Analytical Methods Are Critical for Developing RNA-LNP Medicines

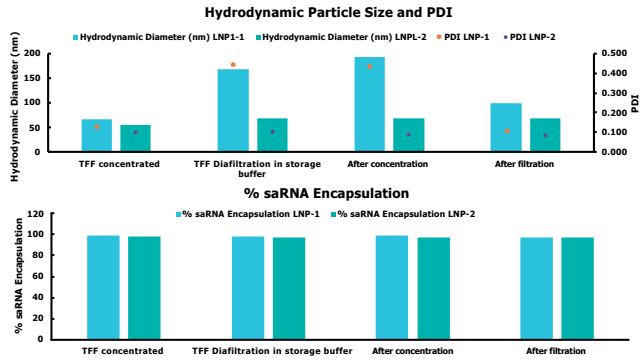


Simplified Scale-Up of mRNA-LNP Using NxGen™



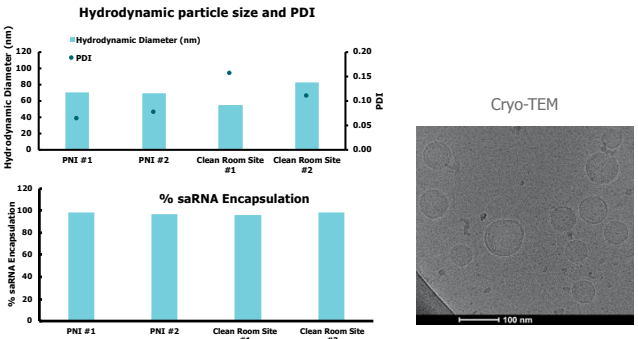
- At the 2.5 mg scale LNP-1 showed a slight particle size increase during the TFF process

Process Scalability at the 50 mg Scale



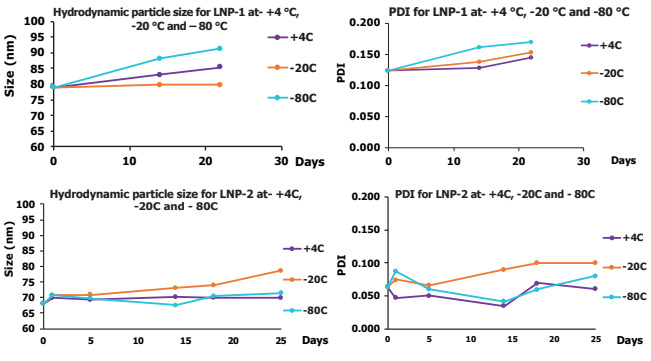
- At the 50 mg scale LNP-1 showed particle size increase during the down-stream processing
- LNP-2 remained size stable during the down stream processing step and was selected as the lead for GLP tox and GMP manufacturing

In Vivo Activity of saRNA LNPs Using Novel Lipid Compositions



- Four successful batches were made at clinical scale at three different sites
- Minor variations in the particle size may be explained by the use a different saRNA lots
- Dense core particle observed by Cryo-TEM
- Currently being tested in a GLP tox study

LNP-1 vs LNP-2 Pilot Stability



- No changes in saRNA encapsulation following 25 days storage for all the conditions tested
- After 25 days storage LNP-1 showed a 10 nm particle size growth at -80C and a 5nm increase at +4 °C
- LNP-2 is size stable at -80 °C and +4 °C and showed a 10 nm size increase at after 25 days at -20 °C

Conclusion

- Two RNA-vaccine candidate formulations (LNP-1 and LNP-2) were investigated using the PNI Manufacturing Platform (Ignite™, Blaze™, GMP NanoAssemblr®)
- At small scale (0.2 and 2.5 mg), both LNP-1 and LNP-2 had comparable particle characteristics
- However, LNP-1 was difficult to scale and showed sub-optimal stability upon storage as compared to LNP-2
- LNP-2 was selected for further development based on scalability, manufacturability and stability
- Four 50 mg batches of SARS-CoV-2 self-amplifying RNA-LNP-2 were made at 3 different sites showing the robustness of the PNI GMP NanoAssemblr® platform
- LNP-2 is currently being tested in a GLP tox studies to support phase I trial commencing in 2021