Accelerated Development of Self-amplifying mRNA (saRNA) Vaccines using Microfluidics

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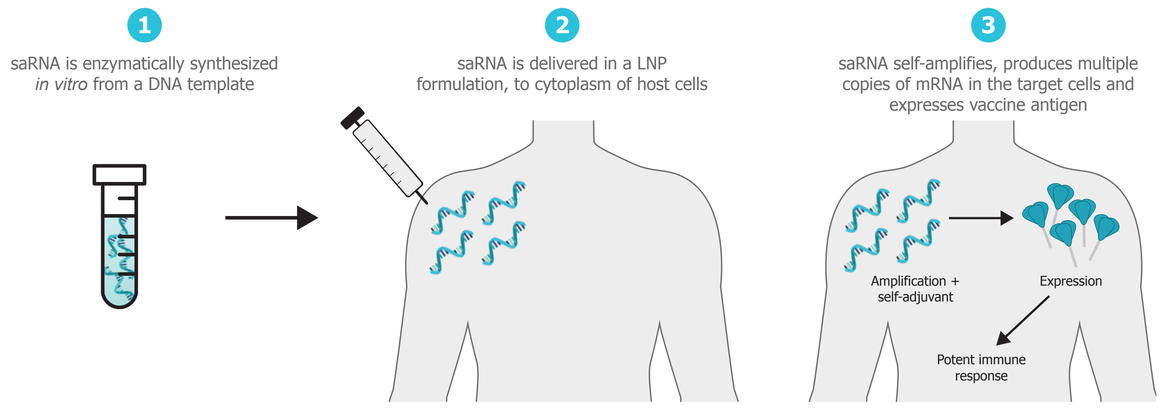
Introduction

- The COVID-19 pandemic has unveiled the potential of messenger RNA (mRNA) based vaccines as an ideal platform for pandemic response.
- RNA vaccines mimic antigen structure and expression similar to natural infection without causing the disease, but allowing body to produce antibody response against future infection.
- saRNAs have the potential for antigen sparring since lower doses elicit effective immune response compared to non-replicating mRNA vaccines.
- saRNAs are inherently more fragile than mRNA and are prone to degradation. Typical high shear manufacturing methods affect the potency.
- Herein, we showcased the utility of microfluidics to enable low shear, rapid screening of preclinical candidates and the swift advancement to GMP-enabling studies.

How do saRNA-LNP vaccines work?

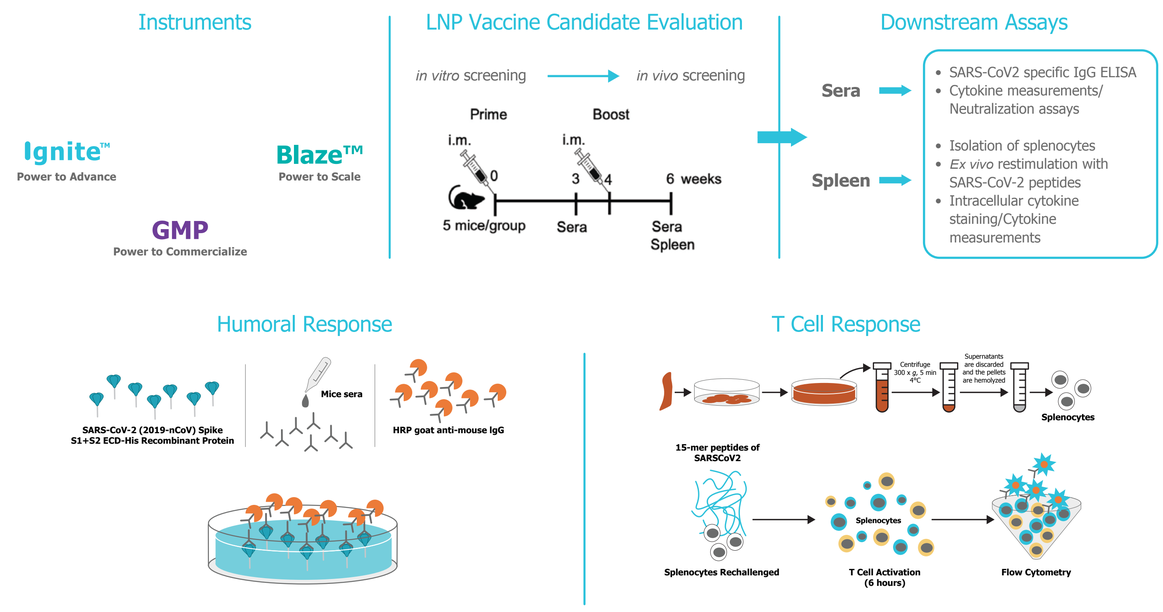


Objectives

- Production of Self-Amplifying mRNA (saRNA) SARs-CoV-2 Vaccine
- Evaluate cellular and humoral responses of the vaccine candidates
- Evaluate the efficacy of the vaccine candidates in a SARS-CoV-2 Hamster challenge model

Methods

- SARS-COV-2 full length spike protein encoded self-amplifying RNAs were encapsulated in Lipid nanoparticles using low shear NxGen microfluidics platform.
- Two RNA-vaccine candidate formulations (LNP-1 and LNP-2) manufactured using the PNI Manufacturing Platform (Ignite™, Blaze™, GMP NanoAssemblr®) were assessed for scalability.

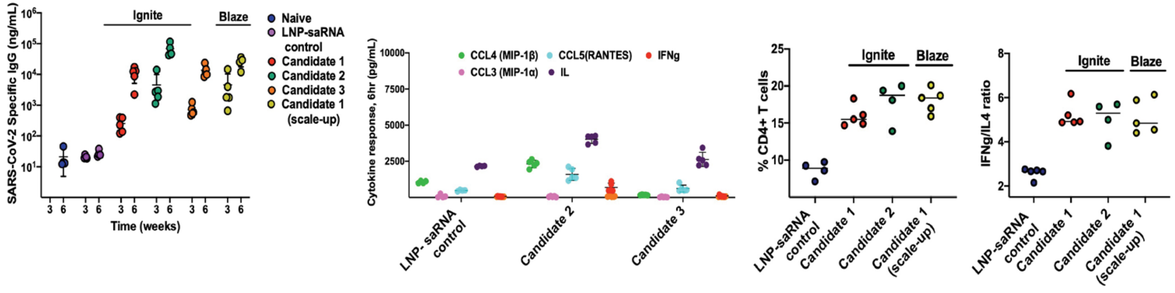


Manufacturing Process

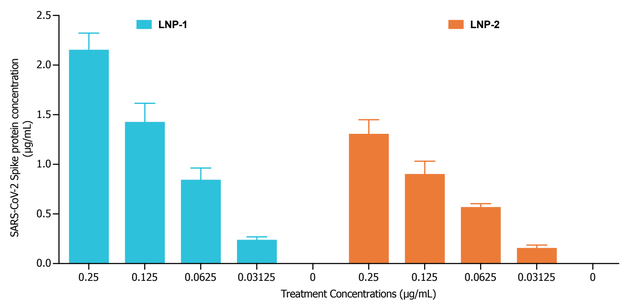
	Ignite™	Blaze™	GMP
Mixers	NxGen™, NxGen w/in-line dilution	NxGen 400, NxGen 500	NxGen 500
Organic Phase	Lipid in Ethanol		
Aqueous Phase	RNA in aqueous		
Total Micromixing Maximum Volume	up to 20mL undiluted	up to 10L undiluted	up to 50L undiluted*
Flow Rate Ratio [Org : Aq]	3:1		
Total Flow Rate	12 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

Results

1. Vaccine candidates manufactured using NxGen Microfluidics show effective Humoral and T Cell Responses

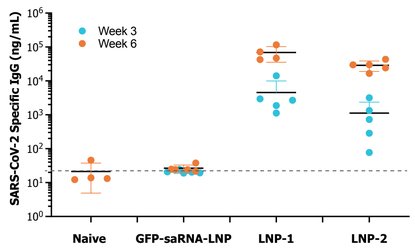


2. Two LNP compositions, LNP-1 and LNP-2 were selected for scale up based on *in vitro* and *in vivo* activity

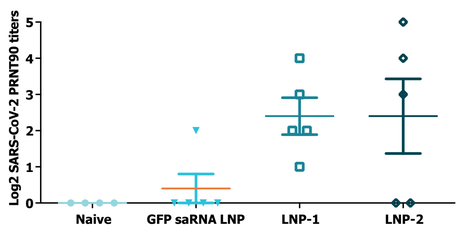


- Both LNPs showed an activity dose response *in vitro*
- LNP-1 is more active than LNP-2 by less than 2-fold *in vitro*

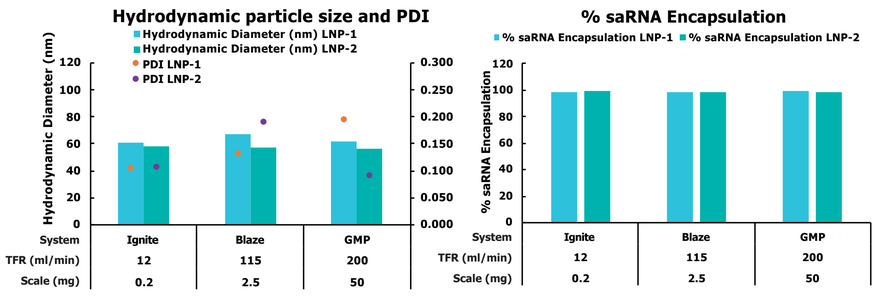
SARS-CoV-2 Specific Serum IgG Measurements at Week 3 & 6



pRNT90 Values (SARS-CoV-2 live virus particles) at Week 6



3. Self-amplifying RNA-LNP have equivalent Size, PDI and encapsulation across scales (Ignite-Blaze-GMP)



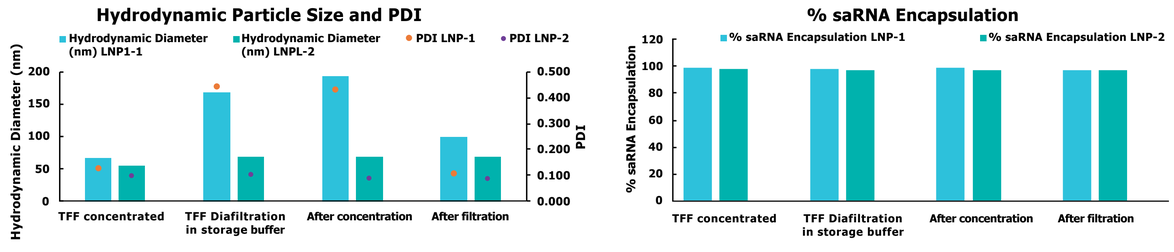
SARS-CoV-2 self-amplifying RNA-LNP made with PNI proprietary ionizable lipid had similar size (~60 nm), polydispersity (<0.2) and encapsulation efficiency (>90%) across all scales tested with two different LNP

4. Scalability Across Platform was achieved for LNP-1 and LNP-2

Simplified Scale-Up of mRNA-LNP Using NxGen™

5. LNP composition was selected based on stability and robustness during TFF process

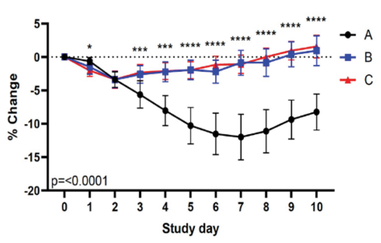
- Both LNP-1 and LNP-2 efficiently induced SARS CoV2 specific IgG response in mice
- As observed *in vitro* LNP-1 showed slightly higher IgG response as compared to LNP-2
- Both LNP-1 and LNP-2 generated neutralizing antibodies against the SARS-CoV-2 virus



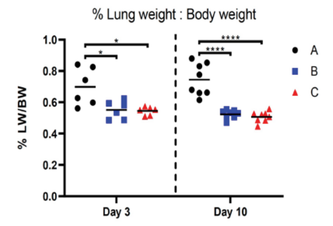
- At the 50 mg scale LNP-1 showed particle size increase during the down-stream processing (TFF)
- LNP-2 remained size stable during the down stream processing step and was selected as the lead for GLP tox and GMP manufacturing

6. Vaccine candidates protected hamsters in a SARS-CoV-2 challenge study

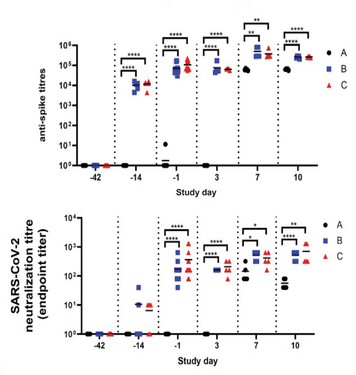
PNI Vaccine candidates 1 and 2 protected hamsters from weight loss following SARS-CoV-2 challenge



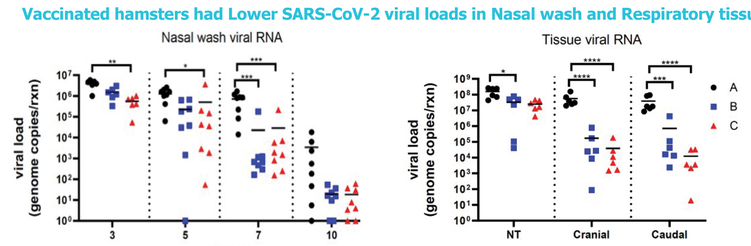
PNI Vaccine candidates 1 and 2 protected hamsters from an increased lung weight following SARS-CoV-2 challenge



Vaccinated hamsters had higher levels of SARS-CoV-2 specific IgG antibody and neutralizing antibody titers



Vaccinated hamsters had Lower SARS-CoV-2 viral loads in Nasal wash and Respiratory tissues



A = Vehicle control
B = Vaccine Candidate 1
C = Vaccine Candidate 2

Conclusions

- LNP based Vaccine candidates manufactured using PNI NanoAssemblr® platform showcased effective cellular and humoral immune response.
- SARS-CoV-2 self-amplifying RNA-LNP made with PNI proprietary ionizable lipid had similar Critical Quality Attributes (CQAs) such as size (~60 nm), polydispersity (<0.2) and encapsulation efficiency (>90%) across all scales tested with two different LNP compositions.
- Downstream processing time and particle stability during large scale TFF should be considered as a critical parameter during scale up.
- Vaccine candidates made with PNI proprietary ionizable lipid and NxGen™ microfluidic platform protected hamsters in a SARS-CoV-2 challenge study.

Acknowledgments

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