Accelerated development of self-amplifying RNA (saRNA) vaccines using scalable NxGen™ Microfluidics

Booth #40

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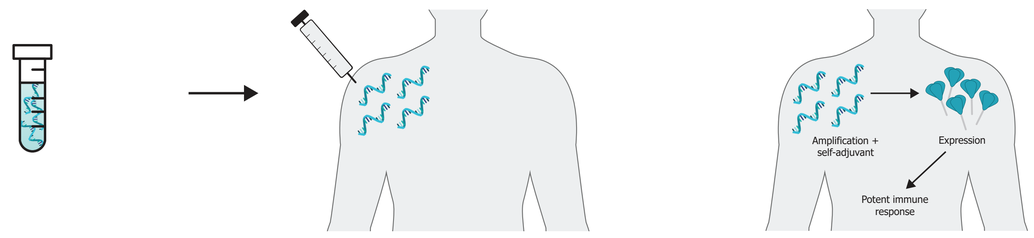
Introduction

- 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.
- The LNP downstream processing was also optimized using the Pall delta TFF cassette. These Pall cassette showed high flux as compared to competitor product.

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

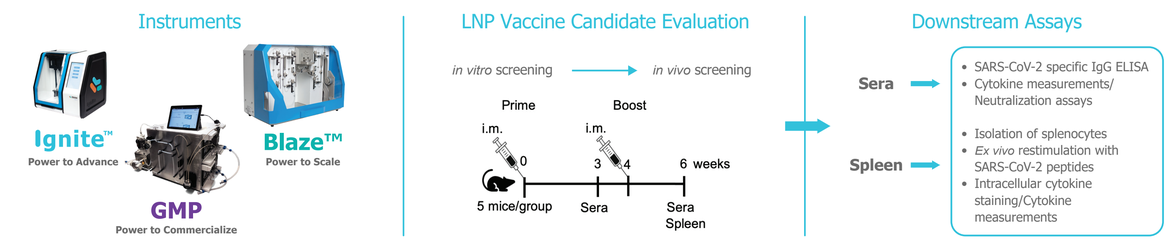


Objectives

- 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 Precision NanoSystems Manufacturing Platform (Ignite™, Blaze™, NanoAssemblr® GMP) were assessed for scalability.

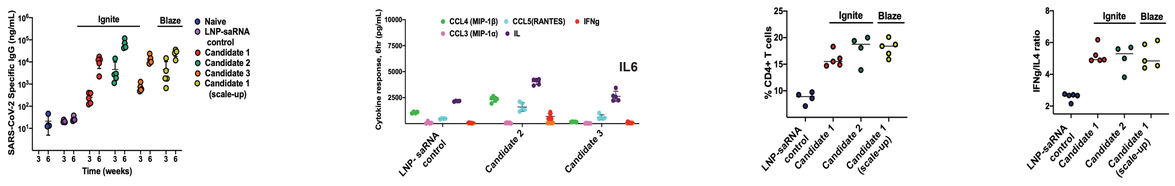


Manufacturing Process

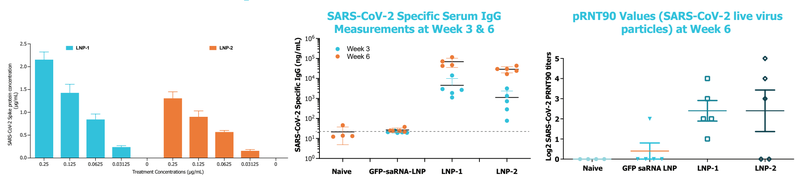
	Ignite™	Blaze™	GMP
NxGen™ Mixers	NxGen, NxGen w/in-line dilution	NxGen 400, NxGen 500	NxGen 500
Organic Phase	Lipid in Ethanol		
Aqueous Phase	RNA in aqueous		
Total Micromixing Maximum Volume	up to 20mL undiluted	up to 10L undiluted	up to 50L undiluted
Flow Rate Ratio [Org : Aq]	3:1		
Total Flow Rate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

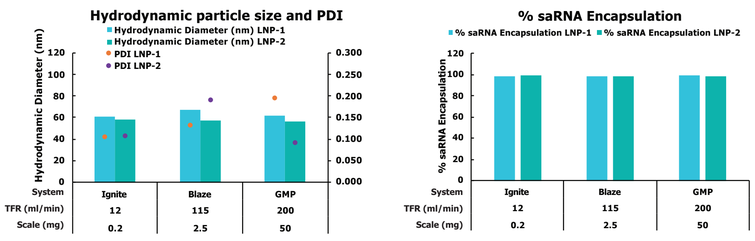


2. Two LNP compositions, LNP-1 and LNP-2 were selected for scale up based on *in vitro* and *in vivo* activity



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

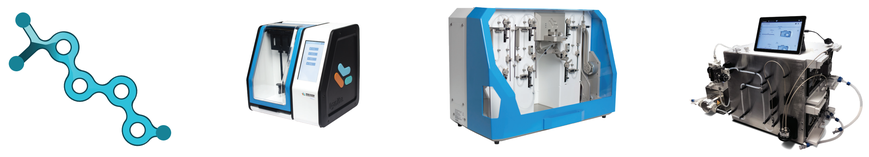
3. Self-amplifying RNA-LNP have equivalent Size, PDI and encapsulation across scales (Ignite-Blaze-GMP)



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

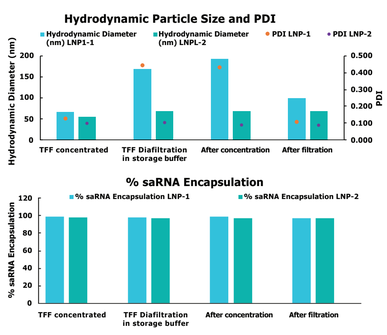
4. Scalability Across Platforms was achieved for LNP-1 and LNP-2

Simplified Scale-Up of mRNA-LNP Using NxGen™



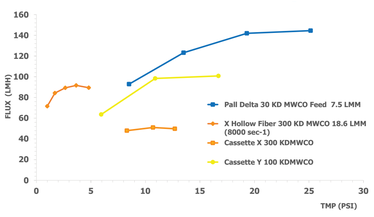
5. LNP composition was selected based on stability and robustness during TFF process

- Both LNP-1 and LNP-2 efficiently induced SARS-CoV-2 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 downstream processing (TFF)
- LNP-2 remained size stable during the downstream processing step and was selected as the lead for GLP tox and GMP manufacturing

6. LNP-2 Downstream processing optimization by flux excursion



Precision NanoSystems COVID-19 vaccine candidate in 3% ethanol was recirculated into the TFF product for 5 min at various feed flow rate prior to TMP increase in order to generate these flux excursion curve

TFF Product	Size		PDI		%saRNA Encapsulation	
	T=0	Final	T=0	Final	T=0	Final
Pall T-series Centramate™cassette, Delta part# DC030T01	69	94	0.16	0.19	99	99
Cassette X 300 KD MWCO	62	71	0.19	0.25	99	99
Cassette Y 100 KD MWCO	65	105	0.17	0.21	99	98
Hollow fiber X 300 KD MWCO	69	74	0.11	0.14	99	99

- Flux excursion demonstrated that the Pall T-series Centramate™cassette, Delta (DC030T01) was more effective in terms of permeate flux for LNP diafiltration as compared to competitor TFF product
- The Pall cassette can operate at higher TMP thus leading to a superior permeate flux without significant changes in LNP characteristics

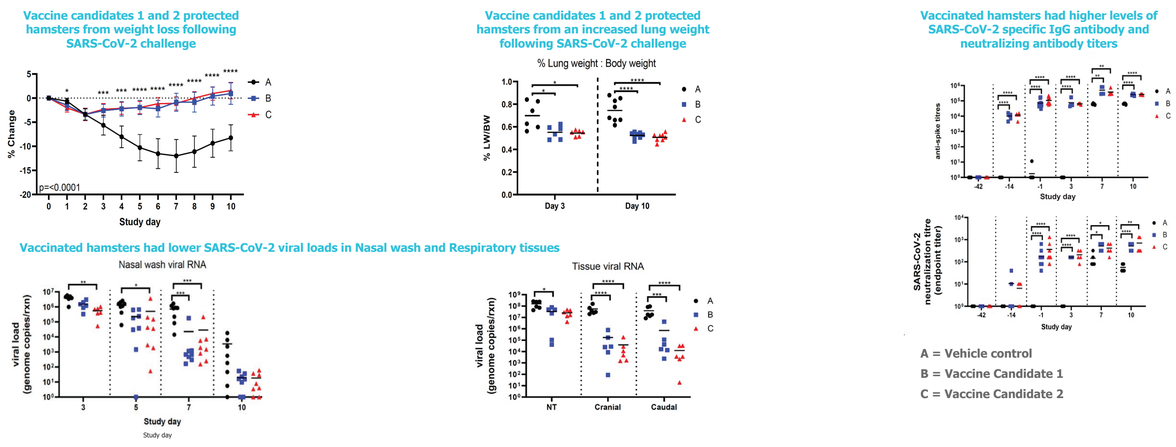
7. The Pall T-series Centramate™cassette, Delta performs better than a hollow fiber product at the 50 mg scale

LNP Processing Time	PALL Delta cassette 1000cm² MWCO 30KD	Hollow Fiber 1600cm² MWCO 500KD
TFF conc step (6.0X – 6.5X)	~33min	~20 min
Diafiltration in storage buffer	~22min (4DV)	~37 min
Final conc step	~5min	~6 min
Total time	1h	1h 07min

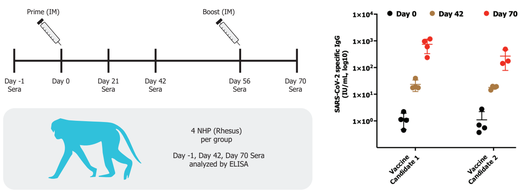
	Size (nm)		PDI		% Encap.	
	Cassette	Hollow Fiber	Cassette	Hollow Fiber	Cassette	Hollow Fiber
8X dilution	65	59	0.172	0.104	99	99
TFF concentrated 6.0X – 6.5X	73	56	0.256	0.091	99	98
TFF dilution buffer (4DV)	83	69	0.278	0.098	98	97
TFF final collected	92	68	0.343	0.083	98	97
Sterile filtered	77	69	0.17	0.076	98	97

- The total TFF time on a Pall delta 30 KD is 7 min faster with a smaller filter as compared to processing time on a larger hollow filter
- Both TFF filters lead to comparable particles characteristics
- The LNP from the Pall cassette were sterile filtered on a Pall ECV and the LNP from the hollow fiber TFF were sterile filtered on a Sartopore 2

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

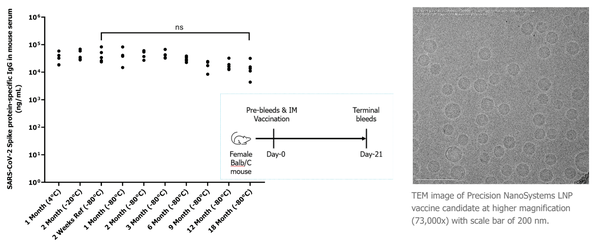


9. Immune response of vaccine candidates in NHP



- Vaccine candidates showed high SARS-CoV-2 specific IgG titres in NHPs
- Significant difference in IgG expression at Day 42 (post prime) was observed compared to pre-bleeds and the response was amplified post booster (Day 70)

10. Long term storage stability of vaccine candidate and morphology



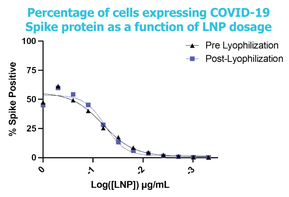
TEM image of Precision NanoSystems LNP vaccine candidate at higher magnification (73,000x) with scale bar of 200 nm.

- Vaccine candidate was stable up to 12-months for similar immune response in animals
- All the LNPs throughout this study were found in the range of 70-75 nm with encapsulation efficiency more than 95%

11. Precision NanoSystems' Lyophilized saRNA-LNP Vaccine Candidate Retains Activity

- Similar particle characteristics and *in vitro* potency following lyophilization cycle

	Before Lyophilization	After Lyophilization
Size (d.nm)	71	89
PDI	0.074	0.09
% saRNA encapsulation	97	95
EC50 (ug/mL)	0.063	0.057



BHK 570 cells transfected in a 96-well plate with SARS-CoV-2 saRNA LNP in a dose response manner from 1 to 0.00049 ug/mL

Conclusion

- LNP based Vaccine candidates manufactured using Precision NanoSystems NanoAssemblr® platform showcased effective cellular and humoral immune response.
- SARS-CoV-2 self-amplifying RNA-LNP made with Precision NanoSystems proprietary ionizable lipid had similar Critical Quality Attributes (CQAs) such as size (~70 nm), polydispersity (<0.2) and encapsulation efficiency (>90%) across all scales tested with two different LNP compositions.
- Precision NanoSystems COVID-19 vaccine candidate protected hamsters in a SARS-CoV-2 challenge study and showed high SARS-CoV-2 specific IgG titres in NHPs.
- SARS-CoV-2 self-amplifying RNA LNP Vaccine candidates showed long term stability and is compatible with a lyophilization process.
- Downstream processing time and particle stability during large scale TFF should be considered as a critical parameter during scale up.
- Pall product showed faster downstream processing time as compared to other membrane tested.

Acknowledgments

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