Solving Challenges in Manufacturing of mRNA Drug Products: Rapid Development and Scale-Up of a Model mRNA Therapeutic Encoding Erythropoietin

Suraj Abraham, Andrew Brown, Helena Son, Sree Talluri, Nikita Jain, Robert Young, Keara Marshall, Ben Versteeg, Maria Kerin, Mark Ma, James Ko, Lloyd Jeffs, Samuel Clarke, Anitha Thomas*

Contact us at:
Precision NanoSystems Inc, Vancouver, BC, Canada
info@precision-nano.com

Introduction

- Messenger RNA therapeutics have the potential to emerge as a platform technology for enabling transformative medicines such as protein replacement therapies and as rapid response vaccines against infectious diseases
- The development of gene therapies employing lipid nanoparticles (LNPs) for delivery of nucleic acids to cells is hindered by access to effective formulation reagents and scalable manufacturing methods
- NxGen is a novel microfluidic technology that provides reproducible, high-quality production of LNPs. NxGen can scale from mL/min to L/h production rates, allowing for mRNA LNP drug candidates to developed cost-effectively at small-scale, and then rapidly scaled-up for GMP manufacturing to meet clinical requirements
- GenVoy-Ionizable Lipid Mix (GenVoy-ILM™) is a research use reagent developed for use with NxGen to produce high-quality RNA-LNPs for genetic medicine applications

Objectives

- Showcase the rapid development and scale-up a model mRNA drug encoding for the therapeutic protein erythropoietin (EPO)
- Demonstrate the ease and robustness in scale-up manufacturing of mRNA-LNP using NxGen with minimal formulation process optimization
- Demonstrate the particle characteristics and in vivo activity of GenVoy-ILM compared to a clinical benchmark composition

Methods

Production: The mRNA-LNPs were prepared from the MC3 benchmark, D-Lin-MC3-DMA/DSPC/Cholesterol/DMG-PEG (50:10:38.5:1.5 mol%) or GenVoy-ILM (NWW0041, PNI) reagent and Epo-encoded mRNA (L-7209, Trilink) using NxGen mixers on the NanoAssemblr® Ignite, Blaze and GMP systems (PNI). Various process parameters are given in Table 1.

Downstream processing: The Epo mRNA-LNPs were concentrated using ultrafiltration (UF) with Amicon® Ultra 15 10kDa MWCO units (EMD Millipore) or tangential flow filtration (TFF) using KrosFlow® Research iII (Spectrum Labs) or Äkta™ Flux 6 (GE) TFF systems with 30kDa MWCO mPES membranes. TFF parameters are in given in Table 2. The final Epo mRNA-LNPs were sterile-filtered using 0.2 µm filters.

Characterization and Activity: The Epo mRNA-LNP size and polydispersity index (PDI) were determined by DLS (Malvern ZetaSizer). Surface pKa was determined by TNS assay. Encapsulation efficiency was measured using a Ribogreen-based RNA assay. For in vivo activity, female C57BL mice were treated intravenously with Epo mRNA-LNPs and serum was collected 6 h post injection via saphenous vein. The total serum Epo levels were evaluated using Quantikine® IVD Epo ELISA kit (Biotechne). Blood hematocrit levels were estimated 7 days post injection using microhematocrit tubes.

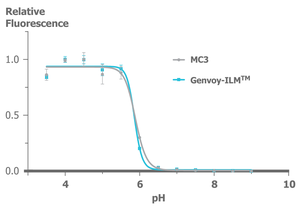
Conclusions

- We have demonstrated the generation of therapeutic levels of human recombinant EPO in mice using EPO-encoded mRNA LNPs
- NxGen technology enables rapid production and seamless scale-up of nucleic acid LNPs to accelerate drug development from bench-scale discovery research towards clinical-scale GMP manufacturing
- GenVoy-ILM can be utilized to produce high-quality RNA-LNPs for proof-of-concept and preclinical studies with any therapeutic mRNA
- GenVoy-ILM provides comparable performance to a clinical benchmark and can be used as a reference standard for any in-house RNA therapeutics development programs

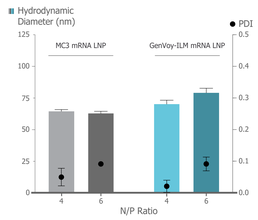
Results

GenVoy-ILM reagent and MC3 benchmark composition have equivalent physiochemical profile, particle characteristics and *in vivo* biological activity

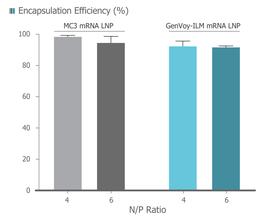
A) LNPs prepared using both GenVoy-ILM™ and the MC3 benchmark composition have similar surface pKa



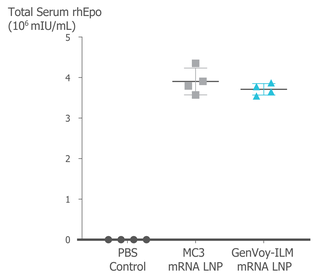
B) Equivalent particle size and polydispersity indices observed for Epo encoded mRNA-LNPs across different N/P ratios



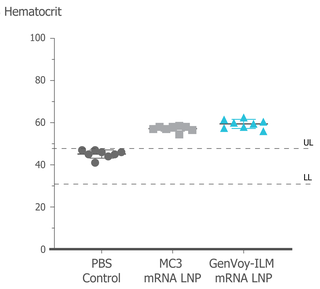
C) Equivalent encapsulation efficiency observed for Epo encoded mRNA-LNPs across different N/P ratios



D) Comparable serum Epo expression demonstrated from mRNA-LNP prepared with GenVoy-ILM and MC3 benchmark composition

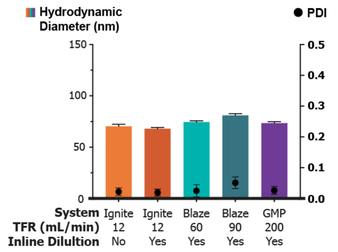


D) Equivalent elevation of hematocrit observed for GenVoy-ILM and MC3 benchmark composition

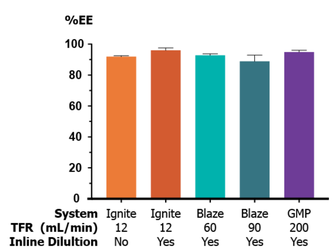


Scalable manufacturing of mRNA-LNP drug candidates using a single NxGen mixer, enabling large scale production with minimal development time

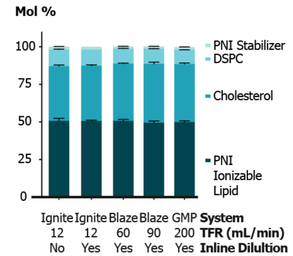
A) Equivalent size and PDI achieved across the scales and systems



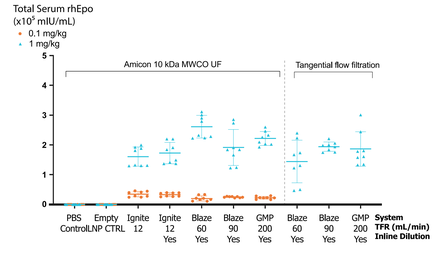
B) Equivalent encapsulation achieved across scales and systems



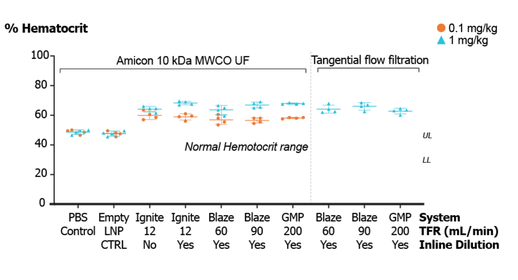
C) Composition maintained across scales and systems



D) Treatment induced serum Epo expression across various scales



E) Treatment stimulated hematocrit increase showing equivalency across various scales



Instruments used in this study

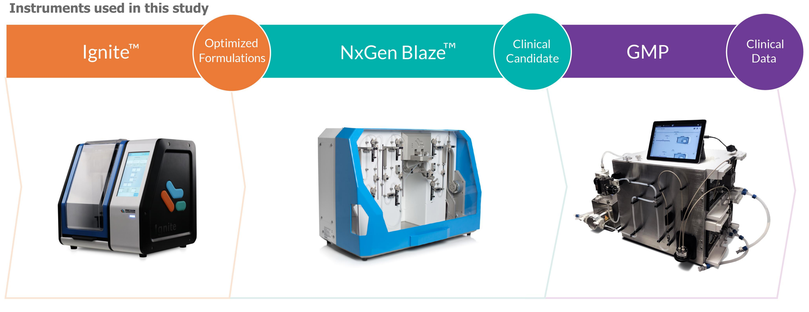


Table 1. Formulation process parameters used in Epo mRNA-LNP manufacturing with different NanoAssemblr Systems and NxGen Mixers

NanoAssemblr System	Ignite™	Blaze™	GMP
Mixers	NxGen, NxGen in-line dilution	NxGen 400D, NxGen 500D	NxGen 500D
Org. Phase	12.5 mM GenVoy-ILM™ in Ethanol		
Aq.Phase	0.174 mg/mL CleanCap® 5moU Epo mRNA in RNA formulation buffer (pH 7.0)		
Total micromixing volume	4 mL	20, 25, 55 mL	325 mL
Flow Rate Ratio (FRR) [Org : Aq]	3:1		
Total Flow Rate (TFR)	12 mL/min	12, 60 & 90 mL/min	200 mL/min
In-line dilution ratio (Buffer:Micromix volume)	3:1	3:1, 3:1, 2:1	3:1
Downstream processing	UF	UF or TFF	UF or TFF

*UF – Ultrafiltration, TFF – Tangential Flow Filtration

Table 2. TFF process parameters for downstream processing of Epo mRNA-LNPs

Process parameters	KrosFlow® Research iII TFF System	GE Äkta™ Flux 6 TFF System
Input Diluted mRNA LNP Volume (mL)	600 - 800	2000 - 8000
Sample Reservoir Temperature (°C)	8 – 12	14 - 17
Feed Flow Rate (mL/min)	40 – 130	1500 -1700
Expected Shear Rate (s^{-1})	4000	6000
Average TMP (psi)	7	4 - 5
Average Permeate Flux Rate (LMH)	60	-
Q_p (gm/min)	-	500
Total Run Time (min)	55 - 63	15 - 35
Final processed mRNA-LNP Volume (mL)	15 – 17.5	100 - 150

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