Accelerating the Development of Transformative Nanomedicines with NxGen™ Microfluidics Technology
The Precision NanoSystems R&D team has demonstrated the development of a **model mRNA-LNP therapeutic** from discovery to scale-up production, with minimal formulation and process optimization.

1. Background & Overview
2. NxGen Microfluidics for Scalable Manufacture of LNPs
3. Formulation and Process Development of Model mRNA Drug
Our Vision

To accelerate the creation of transformative medicine that significantly impacts human well being.
PNI’s Clients are Developing Novel Drugs to Tackle Diseases with Significant Unmet Medical Need

~350 NanoAssemblr® Instruments Deployed Worldwide

>90 Academic Accounts

>100 Industry Accounts Including Top 25 Pharma

>150 Publications featuring NanoAssemblr® technology

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<th>Vaccines</th>
<th>Cell Therapy &amp; Regenerative Medicine</th>
<th>Immuno-Oncology</th>
<th>Targeted Therapeutics</th>
<th>Small Molecule Delivery</th>
<th>RNA &amp; DNA Therapeutics</th>
<th>CRISPR &amp; Gene Editing</th>
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Core Technologies Overview

1. Nanoparticle Drug Delivery Technology

Genvoy-ILM™ RUO Reagents

2. NxGen Microfluidics Nanoparticle Manufacturing Technology

NanoAssemblr® Platform:
Spark™, Ignite™, Blaze™, and cGMP
GenVoy-ILM Lipid Nanoparticles for Nucleic Acid Encapsulation & Delivery

Background & Overview
GenVoy-ILM has been validated for gene silencing and mRNA-mediated gene expression applications.
NxGen Microfluidics for Scalable Manufacture of LNPs
Nanoparticles are More Complex than Previous Generations of Drugs

- **Aspirin**
  - 21 atoms
  - 1 molecule

- **Somatropine**
  - ~3,000 atoms
  - 1 molecule

- **Herceptin**
  - ~25,000 atoms
  - 1 molecule

- **RNA LNP**
  - ~17,000,000 atoms
  - ~100,000 molecules

- 1 nm
- 10 nm
- 100 nm
Optimal Nanoparticle Products are Achieved by Controlling the Self-Assembly Process
Microfluidics leverage non-turbulent flow and rapid mixing for control over nanoparticle self-assembly.
The Evolution of NxGen Microfluidics

**High Energy Techniques**
- Limited applications
- Difficult to reproduce
- Harsh process conditions
- Difficult to scale

**In-Line Macromixing**
- Limited applications
- Difficult to reproduce
- Gentler process conditions
- Improved Scalability

**Microfluidic Approaches**
- Some scaling challenges remain
- Mixers are difficult to make
- Non-turbulent process conditions

**NxGen Microfluidics**
- Easy to Scale
- Mixers are Easy to make
- Potential multi-mixer integration opens possibilities
- Reproducible
- Reproducible
- Non-turbulent process conditions

**Precise** - Non-turbulent particle formation to ensure the most reproducible results for a wide range of nanoparticle types

**Scalable** - More than 25X single mixer throughput simplifies scaling up while maintaining particle quality and batch-to-batch reproducibility

**Innovative** - Platform designed to rapidly take ideas to patients
NxGen Microfluidics Designed for All Stages of Development

Discovery and Development

- **Spark™**
  - Lead Candidates

Preclinical Development

- **Ignite™**
  - Optimized Formulations

Clinical Development

- **NxGen Blaze™ & Blaze+™**
  - Clinical Candidate
- **GMP**
- **Clinical Data**

**Screen**
Rapidly prepare low-volume nanoparticle formulations with a push of a button
- 25-250 μL

**Develop**
Rationally optimize a wide range of nanomedicine formulations
- 1-20 mL

**Advance**
Efficiently scale bench formulations for expanded preclinical studies
- 10 mL - 10 L

**Break Ground**
Confidently transfer nanomedicine manufacturing to cGMP environment
- > 20 L / h

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Formulation and Process Development of Model mRNA Drug
We developed, formulated and scaled up a model messenger RNA (mRNA) therapeutic

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

1. We encoded human Epo in mRNA, packaged it in an LNP using GenVoy-ILM and NxGen Technology

2. The LNP delivers the mRNA to liver cells which express EPO protein, which stimulates red blood cell production

3. We observed an increase in red blood cell production in mice with consistent results across scales and NanoAssemblr instruments

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

**Background**
NxGen Mixer and Classic Mixer Compared for LNP Production

- **Mixers**
  - Classic (SHM)
  - NxGen

- **Ignite**
  - 1-20 mL
  - 3 inlets

**NxGen is the evolution of microfluidic nanomedicine**
Epo-encoded mRNA-LNPs prepared with either mixer had equivalent size, polydispersity and mRNA encapsulation.

Epo mRNA-LNP using GenVoy-ILM had similar size (~75 nm), polydispersity (<0.1) and encapsulation efficiency (>90%) across NxGen and SHM.
Following i.v. administration in mice, Epo-encoded mRNA-LNP using GenVoy-ILM had similar Epo levels in serum and hematocrit increase across NxGen and Classic.
De-risking Manufacturing Process of mRNA-LNP Using NxGen Technology
Philosophy of our process: modeling GMP process at earlier scales

Reduce risk during transition from Research to Development and accelerate timelines to IND
<table>
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<tr>
<th></th>
<th>Ignite</th>
<th>Blaze</th>
<th>GMP</th>
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</thead>
<tbody>
<tr>
<td>Mixers</td>
<td>NxGen, NxGen w/in-line dilution</td>
<td>NxGen 400, NxGen 500</td>
<td>NxGen 500</td>
</tr>
<tr>
<td>Org. Phase</td>
<td>12.5 mM GenVoy-ILM in Ethanol</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aq. Phase</td>
<td>0.174 mg/mL CleanCap 5moU Epo mRNA in RNA formulation buffer (pH 7.0)</td>
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<tr>
<td>Total micromixing volume</td>
<td>4 mL</td>
<td>25, 55 mL</td>
<td>325 mL</td>
</tr>
<tr>
<td>FRR [Org : Aq]</td>
<td>3:1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TFR</td>
<td>12 mL/min</td>
<td>60 mL/min 90 mL/min</td>
<td>200 mL/min</td>
</tr>
<tr>
<td>In-line dilution ratio</td>
<td>3:1</td>
<td>3:1, 2:1</td>
<td>3:1</td>
</tr>
<tr>
<td>(Buffer:Micromix volume)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Downstream processing</td>
<td>UF</td>
<td>UF or TFF</td>
<td>UF or TFF</td>
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Experimental Design
Epo-encoded mRNA-LNP using GenVoy-ILM had similar size (~70 nm), polydispersity (<0.1) and encapsulation efficiency (>90%) across all scales.
mRNA-LNP Have Equivalent Protein Production and Therapeutic Activity Across Scales

Following i.v. administration in mice, Epo-encoded mRNA-LNP using GenVoy-ILM had similar Epo levels in serum and hematocrit increase across all scales.
Philosophy of our process: modeling GMP process at earlier scales

Reduce risk during transition from Research to Development and accelerate timelines to IND
Thank you for Listening!

If you have any additional questions, please reach out to your regional PNI representative, or send them to info@precision-nano.com

Or go to our website:
www.precisionnanosystems.com