A Holistic, End-to-end Approach to LNP-based Drug Development Both Derisks and Expedites the Development Process

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Introduction

- FDA approval of ONPATTRO® by Alnylam, Comirnaty® by BioNTech/Pfizer and Spikevax® by Moderna and the various clinical trials with mRNA-based drugs or vaccines have provided momentum to further develop lipid nanoparticle (LNP) based genetic medicines.
- Precision Nanosystems (PNI) offers an integrated suite of custom services and expertise to work with its platforms in scaling genetic medicines from proof-of-concept to the clinic.
- BioPharma Services at PNI pairs formulation scientists with analytical and bioassay development specialists to quickly develop formulations and scalable processes that enable the preclinical development of genetic medicines for our clients.

Team Highlights

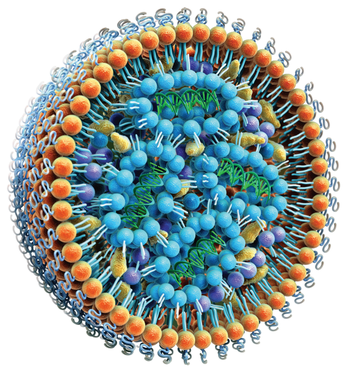
- >40 FTEs with advanced degrees
- Cumulative experience working on 26 clinical stage programs and the production of
- >44 GMP batches producing 2 approved drugs
- Experience across a broad range of drug payloads and carriers
- >160 partnered projects completed/or in progress

- Herein, we present several case studies that demonstrate how having these formulation development, analytical method development, and bioassay development capabilities under one roof is critical to the development of LNP-based genetic medicines. In the first case study, we showcase why having a robust bioassay can guide the formulation parameters as a project scales from pre-clinical to clinical formulation processes. In the second and third case studies, we highlight why going beyond the standard physicochemical characterization and drug release metrics is critical in understanding mechanisms that negate potency. Specifically, how ionizable lipids can inactivate RNA through newly discovered lipid modifications and how analytical and bioassay methods can diagnose or predict this issue.

- **Proprietary Drug Product Manufacturing Platform**

  Instruments & consumables from drug discovery to clinical manufacturing

  Proprietary microfluidic-based manufacturing



- **Proprietary Delivery Platform**

  Discovery reagents to commercial licensing

  Proprietary lipids and nanoparticle delivery systems

- **BioPharma Services**

  Formulation Development, Process Development, Scale-up, Manufacturing & Tech Transfer

  Full suite of Analytical and Bioanalytical method development Services

  Comprehensive expertise required to develop a genetic medicine

Case Studies

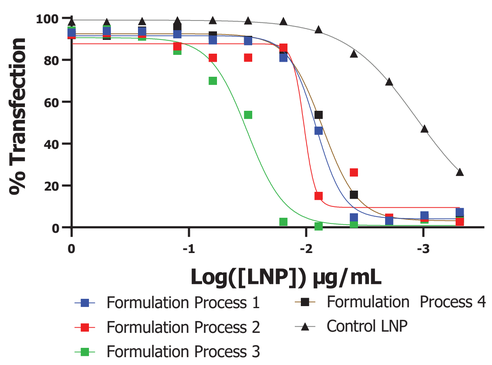
Case Study #1

Including *in vitro* potency in the development workflow elucidated an issue with a formulation process not predicted by traditional physical characterization

Physicochemical properties of LNP formulations

Sample ID	Parameters	Size (d.nm)	PDI	Encapsulation Efficiency (%)	Final mRNA concentration (mg/mL)
Formulation Process 1	20.7 mM lipid 115 mL/min	75	0.15	97	0.18
Formulation Process 2	20.7 mM lipid 150 mL/min	68	0.11	97	0.20
Formulation Process 3	60 mM lipid 115 mL/min	84	0.17	95	0.13
Formulation Process 4	60 mM lipid 150 mL/min	83	0.17	94	0.13

***In vitro* potency of each formulation**



Methods and Results

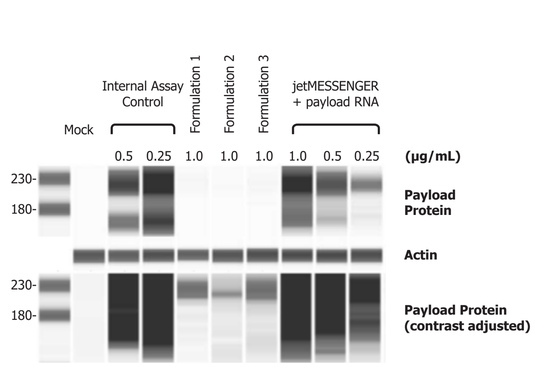
In this experiment, the mRNA payload was encapsulated in 4 LNPs containing PNI's proprietary ionizable lipid using the NanoAssemblr Ignite+. The lipid input concentration ranged from 20.7–60 mM, and the formulation total flow rate (TFR) from 115 mL/min to 150 mL/min. Formulations were downstream processed and analyzed for size, PDI, total RNA concentration, encapsulation efficiency (%EE). After characterization, formulations were screened for *in vitro* potency by treating cells with a dose-response of each test article. Payload expression was evaluated 24-hours post-transfection

- The four formulation parameters used in this experiment yielded very similar LNPs as measured by traditional physicochemical characterization assays
- However, Formulation Process 3 resulted in a significant potency shift relative to the other three
- By incorporating potency results, PNI was able to make a data-driven decision on how to appropriately scale this formulation

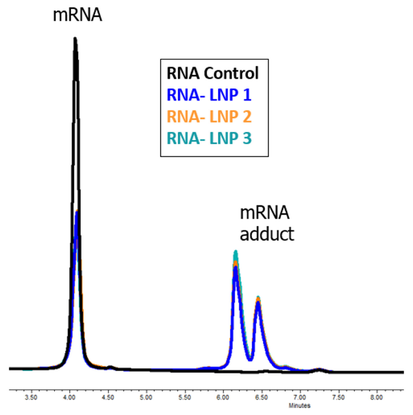
Case Study #2

Having Bioassay and Analytical capabilities in one group allowed PNI to quickly identify and diagnose a potency issue in a partnered program

***In vitro* potency of each formulation**



UHPLC-UV analysis of RNA from LNPs



Methods and Results

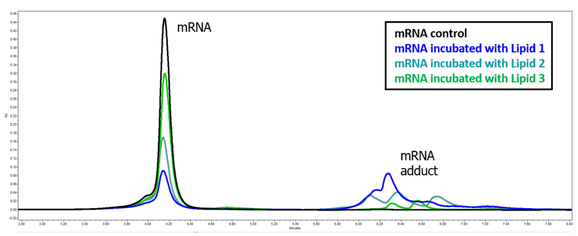
In this experiment, three different formulations were tested for *in vitro* potency via Western blot. As a control, the payload was transfected via a commercial transfection reagent. Cells were incubated for 24-hours in the presence of the different transfection conditions and then lysed and probed for the payload protein expression. RNA from formulated LNPs and from RNA control (no lipids) were extracted and analyzed by UHPLC-UV with absorbance at 260 nm.

- The payload RNA yielded robust protein expression when transfected with a commercial reagent, but not when formulated into LNPs containing the client's ionizable lipid
- Further characterization by the analytical team suggested that the payload RNA was partially inactivated by lipid adduct formation
- The ability of the Bioassay Development and Analytical team to quickly share reagents and results allowed PNI to diagnose an issue with the purity of the client's ionizable lipid, which would have caused substantial time-line delays if it was discovered at a later date

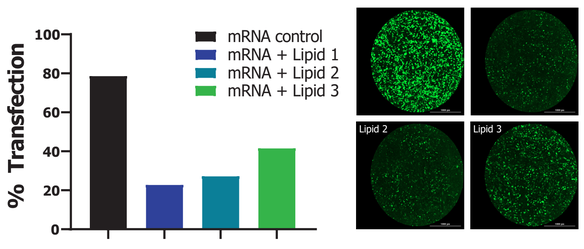
Case Study #3

Having both Bioassay and Analytical capabilities in one group allowed PNI to screen potential LNP components for adverse properties before formulation

Representative chromatogram of mRNA incubated with ionizable lipids



***In vitro* potency of each mRNA post-incubation with an ionizable lipid**



Methods and Results

In this experiment, six different ionizable lipids (in EtOH) were incubated with GFP mRNA (in NaOAc pH 5.3) in a 3:1 ratio (mRNA: lipid) for 1 week. The mRNA was extracted and analyzed via UHPLC-UV with absorbance at 260 nm. In parallel, the RNA from each incubation condition was transfected into BHK 570 cells, with potency assessed by GFP expression via fluorescence microscopy 24 hours later. Images represent GFP fluorescence at 1 µg/mL.

- Each ionizable lipid created lipid adducts in the GFP mRNA after 1 week of incubation, with Lipid 1 being the most reactive and Lipid 3 being the least reactive
- Both transfection efficiency and relative fluorescence were affected by mRNA adduct formation
- Lipid 1, which caused the most mRNA adduct formation, was reduced in activity by ~75%. Conversely, the least reactive lipid, Lipid 3, only reduced the RNA potency by ~50%
- Having the ability to rapidly screen lipid characteristics cross-functionally enabled PNI to identify potential problems before they became apparent later in the development process
- By incorporating potency results, PNI was able to make a data-driven decision on how to appropriately scale this formulation

Conclusion

- During the development of LNP-based medicines, it is equally important to monitor physicochemical characteristics of the drug product and substance in addition to the in vitro potency. This will allow the elucidation of underlying mechanisms which may impact the stability or efficacy of the formulation.
- The availability of high-throughput tools and workflows, such as high-throughput microscopy, and robust *in vitro* potency bioassays enables the quick evaluation of hundred of different formulation and process development variables.
- BioPharma Services at PNI allows partners to gain access to hundreds of person-years of experience in LNP drug development – including expertise in formulation development, process development, and analytical and bioassay method development – in addition to state-of-the-art equipment. This streamlines and de-risks the development of genetic medicines from the discovery to clinical stage.

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