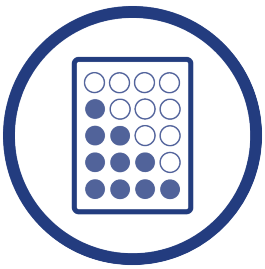
TOP 10 INSIGHTS

Lowering the Lipid Barrier to RNA-LNP Therapeutics

PRECISION NANOSYSTEMS
now part of Cytiva

Precision NanoSystems recently hosted a Panel Discussion, bringing together industry leaders in the field of genomic medicine and nanoparticles to discuss current and future trends. There were great conversations on developing LNP formulations that maximize performance and deliver commercial success of nucleic acid nanomedicines.



1. Screening LNP Formulations

Start with the target product profile in mind, ensuring that the efficacy and safety assays accurately reflect that profile. The first step is to focus on the ionizable lipid, refining the pKa to be in an effective range to be active. Screen for other excipients as well (stabilizer, cholesterol, helper phospholipids) each with their own role for delivery aspects, uptake, and endosomal release. The formulation and N/P ratio should meet the target product profile for safety, efficacy, and stability, tested empirically and validated *in vivo*. In addition, the LNP formulation must also consider manufacturability and scale-up processes, even in an early preclinical stage.

In vitro screening is a great opportunity to refine LNP formulation quality, including identifying the Critical Quality Attributes (CQAs) for a formulation (size, distribution, N/P ratios, encapsulation efficiency). This type of screening can also assess stability, changes in LNP properties, and RNA degradation, however it is not predictive of *in vivo* results and is mainly used for qualitative studies. Efficacy studies must always be done *in vivo*, as is a crucial component that cannot be skipped when screening and optimizing LNP formulations.

2. LNP Formulation Optimization

When developing a new product or technology, there is consideration of where to take risks. Many emerging biotech companies are taking on risk by advancing a new biology or new therapeutic area, and therefore are looking to de-risk in other areas such as the drug delivery formulations. Working with a true collaborative partner with expertise on the drug substance and the drug product can offer optimized formulations that decrease dosing requirements and manufacturing burden, reducing the risk of the technology platform, allowing developers to focus on the therapeutic aspects. Often the new biology contains inherent risk or heavy competition, so it is imperative to move quickly to validate and get to market first.

3. Stability

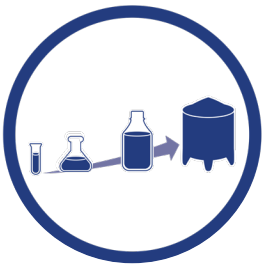
Stability is a compounding effect of ionizable lipid and other excipients, dependent on the application and LNP formulation. Studies to assess long term stability are necessary to monitor the Critical Quality Attributes over time and how well the nucleic acid is protected within the LNP.

Complex analytics are required to assess drug performance, stability, and cargo degradation with many factors playing a role, including storage temperature, excipient interactions, and formulation process. At room temperatures, there is a risk of interactions and lipid adduct formation, while at -80°C, an optimized cryobuffer and freeze/thaw procedures can introduce complexity to the formulation.

USP guidelines around mRNA LNPs vaccines that offer tools and techniques to consider:

- www.uspnf.com/sites/default/files/usp_pdf/EN/USPNF/usp-nf-notices/mrna-vaccine-chapter.pdf

LNP formulations are currently in liquid form, not a classic solid oral dosage pill that can sit on the shelf. This limitation was acceptable during the pandemic, with -80°C conditions tolerated, however the next generation of therapeutics will need to advance the technology into more conventional storage and delivery solutions, which is the next big CMC challenge.



4. LNP Formulation Scale Up

Develop the LNP formulations with the end in mind, in addition to the formulation performance and nanoparticle assembly process, consider regulatory hurdles, clinical trial design, and CMC considerations in early preclinical phases.

Hot Tip: Always procure additional RNA, should any problems arise with a batch, it can take months to have another batch made at scale.

Nanoparticles are surface active, designed to interact with membranes with flexibility and mobility. When manufactured in large volumes or under GMP conditions, they can be impacted by filtration processes or filling. Identifying the CQAs early and maintaining good analytics to verify and validate unit operations of the particle and composition is key for successful scale up. It is recommended to have an internal expert to monitor the analytics or partner with experts than can provide guidance and oversee the scale up.

The FDA requires analytical data about RNA and LNP condition throughout the process, often in the form of comparability studies. It is crucial to maintain formulations from preclinical studies to GMP safety studies and First in Human studies, ensuring that any process changes haven't impacted the potency.

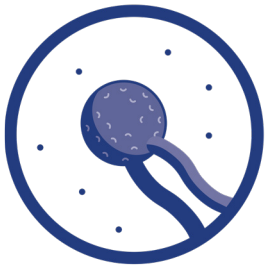
PNAS article demonstrates that LNPs are a perfect delivery system for nucleic acid, showing for the first time scalable liposomal technology that can be commercialized. It discusses that fundamentally LNPs are a complex technology that can be processed at scale (tangential flow filtration, sterile filtration, fill & finish).

- Geall AJ et al. Nonviral delivery of self-amplifying RNA vaccines. Proc Natl Acad Sci U S A. 2012 Sep 4;109(36):14604-9.

5. Control Fraction of Empty LNPs

Good manufacturing processes and optimized formulations provide well encapsulated lipid nanoparticles and can eliminate empty LNPs. Nanoparticle tracking analytics including nanoFCM are used to optimize the formulation process, ensuring complete encapsulation efficiency with protected payload.

In a regulatory context, this would manifest in a toxicity issue or batch-to-batch consistency, which can be addressed in the CMC data, supported by potency assays and good analytics.



6. Biodegradability Regulatory Concerns

Non-biodegradable lipids will need a biological rationale for the disease target patient population, justifying the risk over the benefits provided. Safety will need to be established, along with answers to questions about duration of the LNP and lipids. Regulatory bodies may require additional studies to investigate if the LNP does not degrade quickly.

For biodegradable lipids, regulators will require biodistribution studies to identify the cargo delivery and duration.

As more data becomes available, regulators are gaining a better understanding of RNA-LNP therapeutics, however the route of administration, dosing regimen, and application affect the formulations. It is important to understand the assumptions before developing anything new and providing scientific data to support any novel therapeutics or modalities.

7. Safety of Excipients / Drug Substance

The FDA inactive ingredient guide includes a variety of inactive compounds, ranges and routes, which can be used as a benchmark. Should any lipids or ranges be listed with references on previous use, it is a very powerful argument, in toxicity and CMC risk assessments.

- www.fda.gov/drugs/drug-approvals-and-databases/inactive-ingredients-approved-drug-products-search-frequently-asked-questions

8. LNPs / Ionizable Lipids Considered Excipients

Regulators around the world are considering ionizable lipids and LNPs as excipients, as the LNP mechanism of action is quite uniform, regardless of composition or ratios. Novel excipients will require justification, including how it is produced, characterized, its purity profile, if they are any animal components etc. There will be a high level of scrutiny and proof will be needed to satisfy the regulatory body that it is well-characterized.

9. IND Filing Tips & Tricks

Prepare early. What will be communicated to the FDA? Build a strong scientific rationale and plan experiments to substantiate it. Clinical trial design is often a stumbling block, plan the study design early and work backwards to generate the supporting data (safety, tolerability, immunogenicity etc).

Be scientific. Include data to support the filing with detailed explanations, because ultimately the FDA are scientists and want a clear scientific rationale for the choices made.

Leverage the pre-IND meeting. Include as much CMC data as possible, as it will be 70% of the final submission. Provide specifications and if the data isn't available yet, outline exactly what it will look like in the IND filing.

Ask de-risking questions. Ask key questions about the technology, safety/toxicology studies and get buy-in during the pre-IND meeting and build a relationship with the FDA.

Cover all the bases. Include a compatibility study, formulation analytics from preclinical to patients, the full CMC process.

10. Internal Development vs Outsourcing

When developing a new therapeutic using non-viral delivery with LNPs, there is a choice for formulation, in-house development or outsourcing. Typically, building an internal team and capabilities is a 2–3 year discovery process, acquiring the technology (lipids, formulations, services) through a partner is a great alternative to accelerate programs.

> *Precision NanoSystems offers a diverse, ionizable catalytic library with advantages in terms of licensing and pricing, but they also offer the formulation side and end-to-end service. They can do all of the pre-clinical work, the scale up for you and you can focus on your biology and in vivo testing and accelerate your program forward.*
>
> *– Dr. Andy Geall, Replicate Bioscience*

Panel Discussion Summary

The LNP landscape is dynamic, as more therapeutics enter into clinical trials for different applications, awareness will increase in the RNA-LNP field and push technology into new areas, which will create new opportunities for development.

Precision NanoSystems has diverse portfolio of well-characterized **ionizable lipids** with varying levels of biodegradability. We are constantly developing differentiated novel compositions and have already showcased safety and activity in vaccine models and also cell/gene therapy.

Our BioPharma Services team partners with developers to provide optimized compositions for testing in a specific biological model, based on our formulation expertise. Once *in vivo* testing is performed, we work collaboratively to iterate the LNP formulations and process development, with supporting analytics and CMC guidance.

Contact Us
Phone: 1-888-618-0031
info@precison-nano.com
precisionnanosystems.com