PRECISION NANOSYSTEMS
Create Transformative Medicines™

The Fundamentals of Developing mRNA-Based Therapeutics

Fueled by the success of the messenger ribonucleic acid (mRNA)-based COVID-19 vaccines, RNA-based medicines are poised to revolutionize the pharmaceutical industry. These therapeutics have provided the definitive proof of this new modality, demonstrating its safety and efficacy and breaking new ground as the first mRNA drugs to gain regulatory approval[1,2]. This has catalyzed interest and investment across the industry, driving companies big and small to enter the mRNA space. Key players in the industry like BioNTech and Moderna are pushing the boundaries of RNA therapeutics beyond prophylactic vaccines towards developing breakthrough treatments for cancer, rare diseases and more.

There are some unique challenges and risks in each step of the drug development process for this rapidly growing class of drugs (Figure 1), and success lies in considering the path to commercialization at the earliest stage possible. Choosing the right partners for the journey can also make a significant difference. The pandemic has shown what is possible when the right technologies, partnerships and innovation all come together.

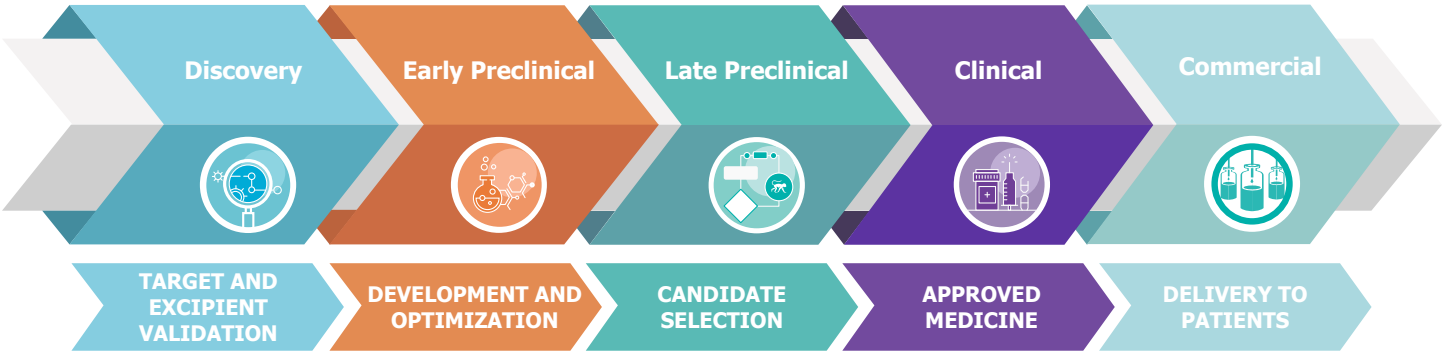


Figure 1. Five key stages in the development of LNP-based drug products.

INTRODUCTION

The unprecedented speed of the COVID-19 vaccine development was made possible in part because of the modular nature of mRNA, which can be rapidly tailored to encode almost any protein. Solving the delivery challenge was also critical to success. Currently, the leading mRNA COVID-19 vaccines all utilize lipid nanoparticle (LNP) technology to encapsulate and deliver the mRNA to the cytoplasm of the cells. These LNPs are likewise a modular technology, providing a more universal approach to RNA delivery with the ability to encapsulate different RNA species with minimal changes to their chemical characteristics[1].

The RNA and LNP components such as ionizable cationic lipid, cholesterol, helper lipid, polymers and stabilizers

need to be formulated in the correct ratio according to the target indication and must be manufactured in a consistent and scalable manner to meet regulatory requirements. LNP quality and efficacy are influenced by physicochemical properties, which are sensitive to the method of production. Microfluidic mixing is the gold standard for nanoparticle formulation, offering well-controlled mixing environments[3]. Next-generation microfluidic devices enable rapid and reproducible formulation of the mRNA-LNPs to produce highly homogenous batches of particles with a narrow size distribution and enhanced encapsulation efficiency using scalable and good manufacturing practice (GMP)-ready technology[2].

Here we will examine the key stages in the development of RNA-LNP drugs in the United States. The general progression of the phases is similar in other regions of the world, but the specifics may vary based on guidance from the relevant regulatory authority, such as the European Medicines Agency (EMA) in Europe or the National Medical Products Administration (NMPA) in China. We highlight the benefits of enabling technology platforms such as the NanoAssemblr®, part of Precision NanoSystems' Genomic Medicine Toolkit that can accelerate the entire continuum from discovery to commercial production using a controlled microfluidic approach.

KEY STAGES IN DRUG DEVELOPMENT

During drug development, getting from A to Z as quickly and efficiently as possible can save years and millions of dollars. Under the Quality by Design (QbD) framework, a new product is designed to meet pre-identified quality objectives[4]. Establishing a Target Product Profile, or TPP, from the outset streamlines development efforts by providing a clear end goal. The TPP is a document that outlines a molecule's intended use, target populations and the desired 'profile' or characteristics of a target product. A well-defined TPP can help a company estimate the market potential of a product and establish an effective product development strategy to reach the desired commercial outcome. If prepared properly, a TPP can help address issues early in the product development process and prevent late-stage development failures. In the regulatory context, including a TPP in the information package can facilitate communication with regulatory authorities and stakeholders by presenting all the relevant medical and scientific information relevant to the company's drug program.

1. DISCOVERY

Discovery often begins with target identification—choosing an appropriate biological target that has a modulating role in a disease. Data from genome sequencing and bioinformatic analysis has led to the discovery of both coding and non-coding genes that can be associated with illness and targeted for therapeutic purposes. A good disease target should be "druggable" or accessible to the proposed drug candidate, resulting in a drug that is efficacious, safe and meets clinical and commercial requirements[5]. Once the target gene has been identified, target validation data confirms it is relevant to the disease and that modulating it will have the desired therapeutic

effect. Success depends on having physiologically relevant *in vitro* cell-based disease models and phenotypic assays that can be qualified with *in vivo* results.

After molecular target validation, screening of active pharmaceutical ingredients (APIs), excipients and formulation parameters is required to identify promising lead RNA drug candidates that have the desired characteristics. Fast, reproducible nanoparticle production at low volumes can support rapid evaluation of different parameters to inform the rational design of nanomedicines in the discovery space. The ability to execute screens at low volumes minimizes the use of API and nanoparticle excipients, which at the discovery stage, may be limited in availability, expensive to acquire or laborious to produce.

Microfluidic mixing is employed across the NanoAssemblr platform for preclinical and clinical scale production, providing exceptional process control and consistency. Because the same microfluidic technology is used across the instruments, the process is also easily scalable over several orders of magnitude from µL volumes to tens of liters to support the entire drug development pipeline from discovery to commercialization. It can greatly expedite workflows if the previously optimized conditions identified during preclinical development can be conserved and replicated at large scales, overcoming the need to re-define these parameters in later developmental stages.

The NanoAssemblr Spark™ is an efficient screening platform suitable for the discovery phase, enabling rapid, controlled and reproducible manufacturing of nanoparticles at volumes of up to 250 µL. At this stage, mRNA sequences, length, capping and nucleotide modifications can be assessed. In addition, mRNA-LNP formulation parameters including polyethylene glycol (PEG) content, N/P ratio (the ratio between cationic amines in the lipid excipient and the anionic phosphates on mRNA) and choice of the cationic/ionizable lipid and lipid mix concentration can be screened systematically to evaluate their impact on nanoparticle attributes[6]. The goal at the end of the discovery phase is to have several lead formulations that can advance to preclinical development for further formulation development and optimization. Promising formulations produced on the Spark can be applied directly to cells in culture for downstream *in vitro* functional screening or physical characterization.

2. EARLY PRECLINICAL DEVELOPMENT

Preclinical development aims to inform clinical trial design and satisfy regulatory requirements for regulatory filings, such as an investigational new drug (IND) or clinical trial application[7]. Key goals include identifying a lead candidate formulation, analytical and bioanalytical methods development, qualification to ensure comparability across scales and executing IND-enabling studies (non-GLP and GLP) required for an IND application including pharmacology, pharmacokinetics (PK) and toxicology assessments. For LNP-based drug development, one of the primary goals in early preclinical stages is formulation development and optimization. Orthogonal design of experiments (DOE) methodology is often employed to rapidly explore the design space for LNP compositions and process parameters[6,8]. Quick formulation runs allow for an efficient systematic approach to finding lead genomic medicine candidates, which need to be optimized for each indication

and application. The NanoAssemblr Ignite™ (volumes of up to 20 mL and flow rates of up to 20 mL/min) and Ignite+™ (up to 60mL at up to 200 mL/min) enable the controlled and precise assembly of LNPs to model clinically relevant parameters and processes at low volumes to support early preclinical studies. It can be used along with GenVoy-ILM™, an LNP reagent mix optimized for the delivery of mRNA.

These formulations are then tested in relevant *in vitro* and *in vivo* models, the results of which help developers select the best performing formulations to advance for further optimization. These studies also help to establish preliminary target performance and quality specifications of the LNP-based drug, or the critical quality attributes (CQAs). A CQA is a physical, chemical, biological or microbiological property or characteristic that should be controlled within an appropriate limit, range or distribution to ensure the desired product quality[9].

Formulation optimization involves fine-tuning the LNP lipid composition since alterations in the chemical structure and ratio of the LNP components affect its delivery efficiency, potency and biodistribution. Access to a well-characterized LNP portfolio, such as Precision NanoSystems' proprietary ionizable lipids, allows for rationally designed LNP formulations that are fit-for-purpose. Process optimization includes optimization of critical process parameters (CPPs) such as total flow rate (TFR), flow rate ratio (FRR), temperature and dilution, which can affect the physicochemical characteristics of the resulting nanoparticles[3,10,11]. All of these parameters collectively play vital roles in controlling the size, morphology and homogeneity of particles, which are important CQAs for LNP-based drugs. In addition, early process development studies as well as stability testing experiments based on manufacturing, storage and downstream processing conditions can be initiated.

By the end of early preclinical testing, the specific analytical methods and biological assays to measure CQAs will be established with 2-3 lead candidates advancing to late preclinical development. The acceptable ranges for product CQAs defined here will be critical to ensure that quality metrics are maintained during late preclinical process development where changes in CPPs during scale-up can impact product CQAs.

3. LATE PRECLINICAL DEVELOPMENT

The goals of late preclinical development include building a comprehensive drug safety profile and executing early Chemistry, Manufacturing and Controls (CMC) studies as part of the Quality Management System (QMS) framework to support GMP manufacture[12]. The NanoAssemblr Blaze™ and Blaze+™ allow rapid scaling of the selected lead candidate nanoparticle formulation in late preclinical stages and can manufacture between 10 mL and 10 L of formulation at flow rates of up to 115 mL/min. These batch volumes are suitable to support confirmatory preclinical *in vivo* toxicology studies in secondary animal species.

Other considerations during late preclinical development include evaluation and qualification of raw materials and process consumables with the regulatory compliance required for clinical testing in anticipation of later developmental stages to mitigate risk and potential delays.

Material produced on the Blaze and Blaze+ is suitable for process development of both the upstream and downstream processes in late-preclinical testing. Optimization of manufacturing processes from material preparation to buffer exchange, filtering and analytics, at scales and

under conditions representative of the clinical/commercial setting, ensures CQAs are maintained during scale-up. This can enable direct protocol transfer of formulation and process parameters without significant investment into additional engineering/production runs for comparability testing prior to technology transfer to GMP manufacturing.

Utilizing DOE and multivariate data analysis (MVDA) are core tools for implementing QbD in process development to achieve process understanding and set acceptable limits for CPPs and CQAs. Producing candidate LNP formulations on the Blaze and Blaze+ allows developers to verify the process parameters optimized at the Ignite/ Ignite+ scale also work at a larger scale. This can include execution of flow mapping studies to optimize FRR, testing to verify dilution ratio and dilution process and monitoring pressure to assess formulation scalability.

In downstream processes, filterability is a key concern for LNPs both in the final formulation stages (i.e., tangential flow filtration) and fill-finish (sterile filtration) operations because the encapsulated mRNA-LNP intermediates are shear sensitive. Therefore, the choice of TFF consumables such as the selection of hollow fiber or flat sheet cassettes, the molecular weight cut off threshold, transmembrane pressure (TMP) and the filter membrane material needs to be carefully evaluated together with the processing conditions of each operation to maximize process efficiency while minimizing impacts to product quality (i.e., LNP size and average size distribution)[11]. By the end of this developmental phase, all upstream and downstream CPPs at scale will be defined and ready for technology transfer.

Finally, acceptance criteria and specifications for the drug substance, reagents and buffers and drug product release criteria should be established and ready for transfer to the GMP environment. Formulation stability studies should be initiated with material produced with representative manufacturing processes to evaluate drug product stability under defined storage conditions. By the end of this developmental stage, developers will have all the necessary information to select the strongest nanomedicine candidate that will progress to clinical trial evaluation.

4. CLINICAL DEVELOPMENT

Before IND submission, engineering batches and feasibility studies should be conducted at scale to verify that the process is robust and consistent. These studies can help to narrow CPP ranges and mitigate risks before submission to regulatory agencies or executing Process Performance Qualification (PPQ) batches as part of process validation. This information helps to finalize the criteria for drug substance acceptance and drug product release. These activities can be executed on the NanoAssemblr GMP System, which is designed for large-scale cGMP production of nanoparticles. At full capacity, the GMP System can produce up to 50L of RNA-LNPs with a flow rate of up to 200mL/min. The batch volumes produce sufficient materials for early clinical phase evaluation.

By the end of clinical development, the master batch record (MBR) will be drafted and the first GMP batch run for Phase I studies manufactured. Based on the clinical trial design, future batch size requirements for later stage clinical testing can be determined. As well, tech transfer to the clean room environment (GMP equipment/process) ensures readiness for advancing the RNA therapeutic towards commercialization. The GMP System comes with installation and operational qualification (IQ/OQ)

and service packages to streamline tech transfer and ensure easy setup with minimal downtime. Its single-use fluid path and accompanying material traceability report also simplify tech transfer since they overcome the need for cleaning validation and documentation. Analytical method development and qualification are also important parts of the technology transfer process.

The information from clinical development along with data collected from all the preclinical studies are submitted in the IND to the regulatory authorities for review and approval to begin clinical trials. The IND is a dynamic document that builds continually over time and will continue to expand to include data from all clinical studies done from phase to phase as more data and process knowledge is gained[12].

Clinical testing encompasses studies, or trials, that are done in people with specific goals for each phase of testing (Table 1). As the developers design the clinical study, they consider what they want to accomplish for each of the different Clinical Research Phases[13].

Table 1. *Overview of clinical trial phases I-IV for mRNA-based therapeutics for common diseases.*

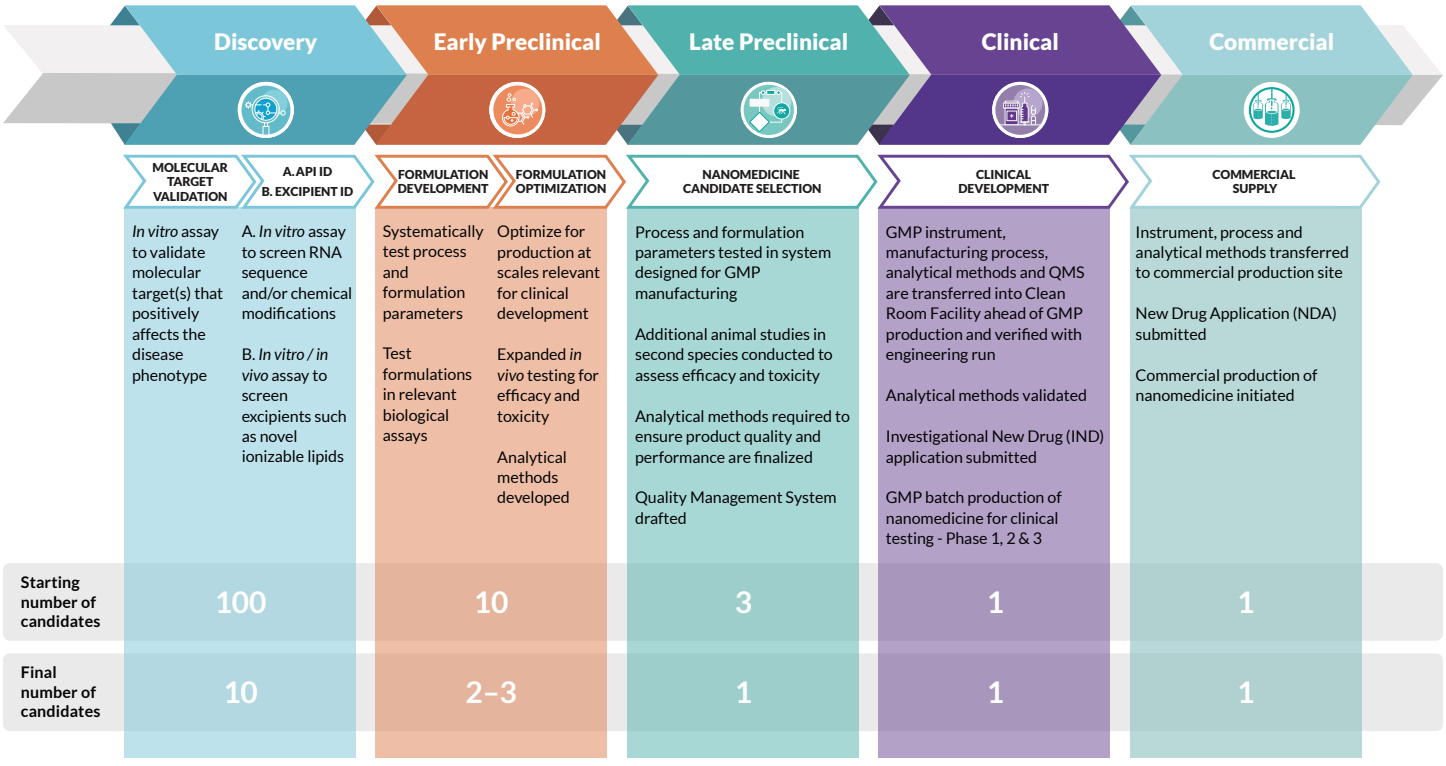
Clinical Trial Phase	Details
Phase I	**Study Participants:** 20 to 100 healthy volunteers or people with the disease/condition **Length of Study:** Several months **Purpose:** Safety and dosage Phase I studies assess the drug safety, PD and ADME and aims to find the highest dosage that can be given safely without causing severe side effects.
Phase II	**Study Participants:** Up to several hundred people with the disease/condition. **Length of Study:** Several months to 2 years **Purpose:** Efficacy and side effects Phase II studies provide researchers with additional drug safety and efficacy data. Researchers use these data to refine research questions, develop research methods and design new Phase III research protocols.
Phase III	**Study Participants:** 300 to 3,000 volunteers who have the disease or condition **Length of Study:** 1 to 4 years **Purpose:** Efficacy and monitoring of adverse reactions Phase III, also known as pivotal studies, provides most of the safety data. These larger and longer studies are more likely to show long-term or rare side effects, enabling medication labeling and instructions for proper drug use.
Phase IV	**Study Participants:** Several thousand volunteers who have the disease/condition **Purpose:** Safety and efficacy Phase IV trials are carried out after the drug has been approved by FDA during the Post-Market Safety Monitoring. These trials look for side effects that were not seen in earlier trials and may also study how well a new treatment works over a long period of time.

5. COMMERCIAL PRODUCTION

This phase of RNA-LNP drug development aims to establish commercial production requirements for later stage clinical studies (Phase III/IV) and transfer the comprehensive information package to the commercial manufacturing site. Process and analytical method validation for commercial scale production are also completed.

The New Drug Application (NDA) is the final step formally taken by a drug developer which is submitted to the Food and Drug Administration (FDA) for market approval. The data gathered during the animal studies and human clinical trials of an Investigational New Drug (IND) become part of the NDA. Preision NanoSystems offers a customizable modular NanoAssemblr system suitable for commercial manufacturing under cGMP conditions with a scalable number of mixing modules to meet throughput requirements with flow rates of up to 1.6 L/min. The vertically scalable platform for RNA-LNP production provides a streamlined mechanism to meet all the end goals of each stage of nanomedicine development from beginning to end (Figure 2).

Figure 2. *Summary of each drug development stage and key end-of-stage criteria towards market approval.*



As RNA technologies continue to evolve, the key will be to focus on flexible facilities design to future-proof manufacturing capacity. Choosing modular equipment that can be rapidly configured for a range of RNA products at different scales and single-use fluid paths can allow manufacturers to easily modify their facilities to adapt to diversifying needs in a cost-effective manner[14]. This supports efficient changeover between products to increase facility flexibility as well as reducing the risk of contamination between batches and products. Modular equipment that can stand alone or be nested into existing facility space could make a geographically distributed, decentralized manufacturing model for RNA therapeutics more feasible, which is especially attractive for RNA vaccine development to enable production at the point of need.

6. ANALYTICS

However well-controlled and robust the manufacturing process, it is necessary to monitor and confirm that CQAs are maintained within appropriate limits throughout all developmental stages[14]. The manufacturing process for RNA-LNP drugs involves the formation of complex nanostructures from purified RNA and lipid species, all of which require analytical testing for in-process controls, product release and stability programs. As such, developing fit-for-purpose analytics is essential to ensure the quality, purity, potency, safety and stability of RNA-LNP drugs with the resolution and speed needed to operate in a manufacturing setting[3,10,11]. Partnering with experts with deep technical knowledge of genomic medicines, such as Precision NanoSystems' Biopharmaceutical Services, can accelerate the qualification of RNA and lipid raw materials as well as the characterization of the LNP drug product using advanced analytical methodologies. The nanoparticle properties such as particle size, polydispersity (PDI) and drug encapsulation efficiency (EE%) are some of the key in-process parameters that should be evaluated across all developmental stages to guide formulation and process development since they can impact final biological function (Table 2).

Table 2. In-process analytical methods important for nanoparticle characterization

Test	Method
RNA Identity/Integrity	Capillary Electrophoresis or Bioanalyzer
Particle Size/PDI	Dynamic Light Scattering
RNA Content/ Encapsulation	RiboGreen Assay
Lipid Content	UPLC-CAD

CLOSING REMARKS

RNA-LNP-based therapeutics represent an exciting new medical paradigm, but in the absence of a standardized process uncertainty exists surrounding the best approaches to drug discovery process development and manufacturing. Planning a product development strategy early on with a technology partner with specialized

expertise in the production of RNA-LNP drugs, who understands all steps of the drug development process and the regulatory environment, and supports a complete genomic medicine workflow including microfluidic instruments, LNP delivery reagents, lipids and services can accelerate timelines and pave the way for success. Ensuring the development of a scalable, reproducible process following appropriate GMP principles can help drug developers achieve consistent quality throughout all stages of the project, reduce variability in the formulation development process, as well as bridge toxicity studies.

As this new drug modality expands to new therapeutic areas, there remains room for further continued innovation. Improving thermostability and designing LNPs with tissue specificity will improve overall RNA drug delivery with the potential to reduce dosage levels while also reducing off-target effects to improve safety/toxicity profiles. Developing the LNPs systematically utilizing the NanoAssemblr platform by screening libraries, optimizing parameters and then modeling the full unit operations at scale offers an efficient strategy to facilitate these goals and de-risk future manufacturing. The vertically scalable production platform provides developers with a reliable drug development path from discovery to commercialization.

REFERENCES

1. Webb C, Ip S, Bathula NV, et al. Current Status and Future Perspectives on mRNA Drug Manufacturing. Mol Pharm. 2022;19(4):1047-1058. doi:10.1021/acs.molpharmaceut.2c00010
2. Semple SC, Leone R, Barbosa CJ, Tam YK, Lin PJC. Lipid Nanoparticle Delivery Systems to Enable mRNA-Based Therapeutics. Pharmaceutics. 2022;14(2):398. Published 2022 Feb 11. doi:10.3390/pharmaceutics14020398
3. Precision NanoSystems. Seamless scale up of siRNA-LNP formulations using the NanoAssemblr® platform. Application note: sirnalnpsc-up-AN-0918
4. Cocco P, Ayaz-Shah A, Messenger MP, West RM, Shinkins B. Target Product Profiles for medical tests: a systematic review of current methods. BMC Med. 2020;18(1):119. Published 2020 May 11. doi:10.1186/s12916-020-01582-1
5. Lansdowne LE. Exploring the Drug Development Process. Published March 13, 2020. Technology Networks. https://www.technologynetworks.com/drug-discovery/articles/exploring-the-drug-development-process-331894 Accessed August 2, 2022.
6. Precision NanoSystems. Robust low-volume production for screening high-value nanoparticle materials. Application note: mrnaspark-AN-1018
7. Mirasol F. Biopharma Industry is Seeing Merit in Early Preclinical Strategies. BioPharm International, November 2021;34 (11):36–37.
8. Nag K, Sarker MEH, Kumar S, et al. DoE-derived continuous and robust process for manufacturing of pharmaceutical-grade wide-range LNPs for RNA-vaccine/drug delivery. Sci Rep. 2022;12(1):9394. Published 2022 Jun 7. doi:10.1038/s41598-022-12100-z
9. ICH Q8 (R2) – Pharmaceutical Development, September 2009
10. Roces CB, Lou G, Jain N, et al. Manufacturing Considerations for the Development of Lipid Nanoparticles Using Microfluidics. Pharmaceutics. 2020;12(11):1095. Published 2020 Nov 15. doi:10.3390/pharmaceutics12111095

11. Precision NanoSystems. Accelerating The Development And Scale-Up Of mRNA Vaccines. Cell & Gene. Published March 2, 2022. https://www.cellandgene.com/doc/accelerating-the-development-and-scale-up-of-mrna-vaccines-0001 Accessed August 2, 2022
12. Cytiva. Basics of Process Development for Biotherapeutics. https://www.cytivalifesciences.com/en/us/solutions/emerging-biotech/knowledge-center/biopharma-process-development-introduction Accessed August 2, 2022
13. Food and Drug Administration (FDA). The Drug Development Process, Stage 3: Clinical Research. https://www.fda.gov/patients/drug-development-process/step-3-clinical-research Accessed August 2, 2022
14. Cameau E, Zhang P, Ip S, et al. Process & analytical insights for GMP manufacturing of mRNA lipid nanoparticles. Published July 2, 2022. Cell & Gene Therapy Insights 2022; 8(4), 621–635 doi: 10.18609/cgti.2022.095

Precision NanoSystems and the logo are trademarks of Precision NanoSystems or an affiliate doing business as Precision NanoSystems. NanoAssemblr, GenVoy, NxGen, are trademarks of Precision NanoSystem. or an affiliate doing business as Precision NanoSystems. For local office contact information, visit https://www.precisionnanosystems.com/contact-us

© 2022 Precision NanoSystems. All Rights Reserved.