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SPECIAL REPORT

Navigating GMP Manufacturing of mRNA-Lipid Nanoparticles

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Navigating Regulatory Complexities in the Clinical and Commercial Manufacturing of Lipid Nanoparticles

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Over the past few years, the remarkable speed in developing and approving the mRNA COVID-19 vaccines marked a momentous milestone in the biopharmaceutical industry. It showcased the industry's ability to respond effectively to a global health crisis, offering hope and protection to billions of people worldwide. A key factor in this success was the use of lipid nanoparticles (LNPs). They act as a delivery system for mRNA, protecting the fragile payload and facilitating its delivery into target cells. This platform technology enabled biopharmaceutical companies to adapt existing LNP knowledge and processes developed for other applications to create vaccines within an accelerated timeline.

However, navigating the regulatory landscape surrounding those developments has not been without its challenges. The urgency of the pandemic necessitated the use of emergency authorization pathways, such as the US Food and Drug Administration (FDA)'s Emergency Use Authorization (EUA) (**1**). Although those pathways expedited vaccine approval and deployment, they also present unique regulatory challenges as the use of LNPs expands to new therapeutic targets and applications.

Although LNPs are considered to be a platform technology, each LNP formulation can exhibit unique characteristics. Variations in lipid composition, surface modifications, and the nature of the encapsulated cargo can influence stability, pharmacokinetics, and safety profiles. Understanding which elements can be standardized across similar formulations and what is inherently unique to each LNP is crucial for efficient development and regulatory approval. Furthermore, the diverse applications of LNPs are both a blessing and a challenge. LNPs have demonstrated great versatility, acting as vehicles for different therapeutic domains, including vaccines, gene therapy, and immuno-oncology. However, such versatility entails complex regulatory considerations because each therapeutic area has unique requirements, safety profiles, and efficacy benchmarks. What's suitable for a vaccine might not align with expectations for a gene-therapy product, for example; therefore, developers must adapt their approaches accordingly.

That becomes particularly relevant when a company must decide whether to enter a traditional phase 1 trial or a combined phase 1–2 trial, a critical step that requires careful consideration. Whereas phase 1 trials mainly focus on assessing safety and determining the right dosage levels, phase 1–2 trials allow for simultaneous evaluation of safety and an initial look at efficacy. Determining which path to take is influenced by factors such as the therapeutic mechanism of action (MoA), the disease target, and the regulatory guidance specific to a given therapeutic domain — further emphasizing the nuanced nature of LNP-based therapeutic development.

Because LNPs represent a rapidly growing area in the biopharmaceutical industry, regulatory agencies continue to update their guidelines for evaluating and approving these innovative therapies. Keeping up with the changing standards, engaging in early and ongoing communication with regulatory authorities, and proactively addressing potential issues linked to emerging requirements all are important strategies for navigating the regulatory landscape successfully. Below are additional insights into strategies associated with successful clinical and commercial manufacturing of LNP-based therapeutics.

Standardizing Chemistry, Manufacturing, and Controls (CMC)

Given the considerable number of variables in the biomanufacturing workflow, establishing regulatory mechanisms can help to standardize CMC approaches for LNPs. By providing clear guidelines for consistent LNP manufacturing practices, drug developers can meet regulatory requirements effectively by ensuring product quality and patient safety. Similarly, establishing a robust analytics foundation is central to characterizing the critical quality attributes (CQAs) of each LNP formulation. The CQAs encompass a number of parameters, including particle size, stability, encapsulation efficiency, and other attributes specific to the modality.

A well-documented and comprehensive data package emphasizing analytical precision helps a developer to implement a systematic and risk-based strategy for process characterization and control — thus preventing regulatory delays and inquiries. Manufacturers can identify and address formulation-related challenges by assessing and mitigating risks associated with LNP formulations throughout development stages — even before entering the investigational new drug (IND)–enabling phase or in parallel with clinical trials. Such an approach enhances the overall efficiency and effectiveness of LNP-based therapeutic development.

Proactive Engagement with Regulators

Early engagement with regulatory authorities is an effective way to address potential risks and uncertainties in LNP drug development. Opportunities such as Type D meetings foster open communication between developers and regulators, leading to a mutual understanding of potential complexities that could arise and their solutions. Such early conversations help developers align their plans with the expectations of regulatory bodies, making the approval process smoother and more effective (Figure 1).

LNP drug development programs also can benefit from leveraging collaborative groups and projects inspired by initiatives such as Project Orbis (**2**), which has advanced the regulatory approval process for cell and gene therapies. Joint working groups can focus on standardizing LNP-specific guidelines and best practices, enhancing efficiency and consistency throughout the regulatory environment.

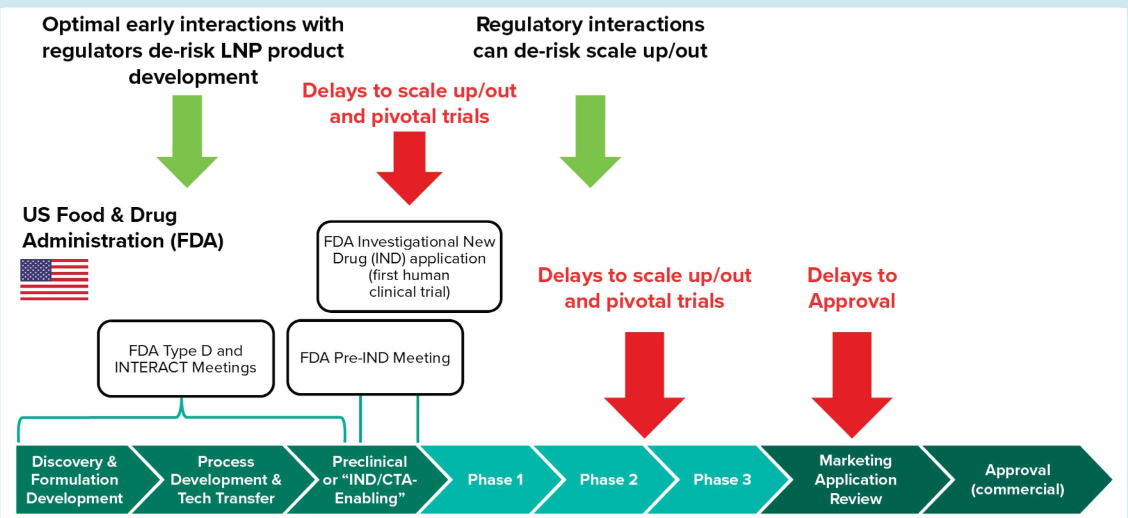
Unified Global Standards for Streamlined Approvals

Creating unified global regulatory standards can support LNP-based therapeutics to move easily through approval processes across different regions around the world. By synchronizing regulatory expectations, developers can minimize repeated efforts and speed up approvals. The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) is a quintessential global initiative, bringing together regulatory authorities and the pharmaceutical industry from across the globe, including the United States, Europe, Japan, Canada, among others. The primary goal of the ICH is to standardize pharmaceutical regulations. Such harmonization will improve efficiency in regulatory review processes, not only to support manufacturers, but also to ensure that patients have quick access to groundbreaking treatments no matter where they live.

Incorporating Single-Use Technology (SUT) in LNP Manufacturing

SUT holds great potential for improving flexibility and cost efficiency in the clinical and commercial manufacturing of LNP-based therapeutics. However, to implement SUT successfully and reduce associated risks during clinical and commercial production, companies need to consider carefully such factors as material compatibility, extractables and leachables (E&L), and supply chain resilience. Rigorous testing and documentation are essential to demonstrating the safety and quality of components and to meeting

Figure 1: Proactive engagement with regulators includes leveraging pre-IND meetings with the US Food and Drug Administration (FDA), getting recommendations for successful filing of applications, and building a collaborative relationship with the FDA and other agencies.



regulatory requirements, including standards of the US Pharmacopeial Convention (USP).

Simplifying and Unifying the LNP Manufacturing Process

Given the increasing complexity and variability with increasing batch size, scaling up LNP processes from small-scale development at milliliter volumes to commercial manufacturing batches measured in hundreds of liters is challenging. This often involves working with numerous contractors that require significant project and operational management. End-to-end technology providers help to streamline operations and reduce the number of contractors involved, boosting efficiency and accelerating timelines. Precision NanoSystems (now part of Cytiva) offers integrated solutions for end-to-end process insight across the entire manufacturing workflow, enabling access to a full single-use manufacturing line encompassing RNA drug substance to the final RNA–LNP drug product from a single point of contact.

Overcoming the challenges in LNP clinical and commercial manufacturing requires a well-rounded approach. The relatively recent success and advances in LNP technology add a layer of regulatory intricacy, requiring thorough planning, built-in flexibility, robust analytics, and active dialogue with regulators. Successfully navigating the obstacles that inevitably will arise is key to advancing groundbreaking therapies with the potential to revolutionize patient care. By implementing the strategies discussed herein, companies can work together to accelerate approvals and ensure that innovative treatments will reach patients as soon as possible.

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GMP Manufacturing of mRNA-LNP Drug Products

Navigating Scale, Process Controls, and Personalized Medicine

Lloyd Jeffs, Laura Ho, and Reshma Kapatrala

Delivering an active pharmaceutical ingredient (API) into a target disease site remains a significant challenge in drug development. Lipid nanoparticles (LNPs) act as a drug-delivery system that can encapsulate mRNA and enable it to enter the target cells, where it is used by ribosomes as a template for protein synthesis. This technology has played a pivotal role in the success of the mRNA COVID-19 vaccines, opening up new possibilities and paving the way for future RNA-based therapies and vaccines. However, as the manufacturing technologies for RNA-LNPs evolve, navigating both the associated challenges and opportunities will be critical to achieving successful commercial production.

Good manufacturing practices (GMPs) for RNA-LNP drug products present a multifaceted undertaking that encompasses scale, analytics, and process controls. Scaling up from bench to clinical and commercial production increases both the complexity of a manufacturing process and the number of variables that must be managed across the production workflow. Analytics and process controls underpinned by advanced automation platforms play a fundamental role in maintaining the precision and consistency of manufacturing operations. Early consideration of GMP requirements will become increasingly important to achieving cost-effective manufacturing, meeting regulatory standards, and ensuring patient safety as LNPs expand to new therapeutic areas, including personalized medicine. A flexible RNA-LNP manufacturing workflow that leverages advancements in production, automation, and digital technologies can ensure the successful development of RNA vaccines and therapeutics.

Scaling Strategies for GMP and Commercial Manufacturing

A key challenge during scale-up to clinical and commercial manufacturing is ensuring a consistent and reproducible manufacturing process. Variations in production at larger scales can affect a formulation's stability and efficacy, requiring careful engineering and raw-material control. Moreover, as production scale increases, advanced equipment and specialized facilities become critical factors for maintaining product quality attributes.

Implementation of advanced technologies such as automated platform solutions provides a flexible approach to enable small-scale modeling of unit operations that can predict performance at scale and thus accelerate process optimization. Additionally, early investigation of process parameters helps companies establish process ranges and critical process controls to ensure GMP compliance in manufacturing that meets the requirements of a defined target product profile (TPP). Ultimately that will prevent program delays.

Investing in flexible capital-expenditure (CapEx) equipment and instruments can help biopharmaceutical manufacturers adapt their facilities to meet changing needs, which is important in a rapidly evolving market. Platform technologies that include single-use components enable standardized manufacturing workflows and rapid configuration for different products across a range of scales.

Equally important during development, scale-down strategies can offer several benefits. By carefully designing and characterizing scale-down models, manufacturers can gain insights into process behavior, identify potential challenges, and establish critical process controls before scaling up. This approach reduces the risk of making costly errors, accelerates process development, and helps companies to ensure that their final products will meet quality and safety standards. The NanoAssemblr family of formulation systems from Precision NanoSystems (now part of Cytiva) leverages NxGen mixing technology to minimize process development when changing scales to produce uniform and reproducible LNPs (**1**). The NanoAssemblr Ignite and Ignite+ platforms enable formulation development and optimization at volumes of 1–60 mL; the Blaze and Blaze+ enable process development of upstream and downstream steps at volumes of 20 mL–10 L (**2, 3**).

Clinical trials and commercialization also can benefit from scale-down ability, which is used in evaluating different dosage levels and patient populations. Manufacturers can simulate and adjust their processes to accommodate variations in drug dosage, ensuring that a pharmaceutical product can be tailored to the specific needs of different patient populations. However, addressing the challenges posed by scaling down production requires a combination of advanced manufacturing technologies, robust quality-control (QC) measures, diligent regulatory compliance, and specific facility considerations.

Process and Analytical Insights for GMP Manufacturing

Establishing comprehensive analytics provides a solid foundation for consistent, scalable, and efficient GMP manufacturing. Analytics and stability studies include testing a product's identity, size, purity, potency, and stability. Advanced analytics can be used to predict how RNA-LNP formulations will behave in specific patient populations, allowing adjustments to optimize efficacy while minimizing adverse effects. These tests are also crucial to assessing the shelf-life and performance of RNA-LNP formulations for use in personalized medicine.

Ensuring sufficient sample quantities required for conducting such comprehensive tests throughout a manufacturing process and for final product release is important in determining batch size. GMP standards require strict QC measures in which batch data play a central role. Batch documentation serves as a meticulous record of processes, materials, and quality measures, ensuring that each batch adheres to the highest safety and efficacy standards. This is like a "manufacturing GPS" guiding companies through regulatory compliance and helping them fine-tune processes to deliver top-notch therapies to patients.

Advantages of Automated Platform Technologies

Integrating automated technologies for mRNA-LNP drug manufacturing can bring numerous benefits by streamlining development and manufacturing, minimizing human error, and providing precise control over critical operations. Real-time monitoring, data analytics, and algorithms facilitate process optimization and QC.

Automated systems generate comprehensive data records and traceability logs, capturing every manufacturing process step as it happens. The availability of detailed data facilitates quality assurance (QA) and troubleshooting if they are required in response to problems that arise. Digitized batch records and electronic systems ensure data integrity, simplify regulatory compliance, and enhance transparency throughout a manufacturing workflow.

Additionally, automated platforms are designed to accommodate large-scale manufacturing requirements. Manufacturers can maintain product integrity and quality by automating critical processes at increasing volumes with multiple batches. Angela Johnson (Cytiva's global regulatory and compliance leader) asserts, "The more certainty that regulators have in an established process — with robust data, deployment of modern CMC, non–paper-based sensors, and analytics — the more likely it is that they will be assured that their products are safe and work as intended, preventing process errors and reducing testing requirements for personalized medicine."

The NanoAssemblr commercial formulation system from Precision NanoSystems is an automated, single-use system for clinical and commercial production of LNPs under current good manufacturing practice (CGMP) conditions (**4**). The system enables operational flexibility and standardized manufacturing of genomic medicines through an automated workflow of priming, calibration, formulation, and in-line dilution. A single-use flow path helps boost production by enabling efficient changeovers and minimizing downtime that otherwise would be needed for sanitizing and performing cleaning validation. Leveraging single-use technology facilitates the manufacture of multiple LNP products within the same facility.

Facility Considerations

Facility design is critical to ensuring sterile manufacturing processes through contamination control, environmental control, and workflow optimization. Cleanrooms for such operations should be classified based on standards from the International Organization for Standardization (ISO), with continuous environmental monitoring to track critical parameters such as temperature and humidity, thus enabling precise environmental control throughout manufacturing. Material handling and storage procedures also should be in place to prevent batch failures, cross-contamination, and degradation of raw materials.

Large-scale manufacturing of RNA-LNPs often involves using large quantities of 100% ethanol, a flammable solvent, to dissolve lipids. Thus, strict attention must be paid to safety, which necessitates compliance with standards such as Europe's ATmosphere EXplosibles (ATEX) certification or those from the International Electrotechnical Commission for Explosive Atmospheres (IECEx) to prevent and mitigate associated risks. Careful consideration of these factors in designing manufacturing suites and selecting equipment can help companies establish robust and compliant manufacturing environments for RNA-LNP drug production.

The above general considerations must be adapted to specific regional regulations and the nature of a given RNA-LNP drug product.

Establishing a Manufacturing Partnership

Large-scale LNP drug production is a complex and resource-intensive process. Partnering with an established technology and service provider helps developers leverage their expertise in technology transfer, optimization of manufacturing processes, and assistance with CMC regulatory submissions for CGMP operations. That can be crucial when the goal is to produce billions of doses — as with the mRNA COVID-19 vaccines — or to develop tailored RNA-LNP therapies for personalized-medicine demands. Manufacturing partners have established infrastructures that can adapt drug sponsors' processes to different scales, thus effectively reducing development costs and accelerating products to market.

Establishing a manufacturing partnership also reduces risks to ensure supply chain resilience. Interruptions and failures in the supply chain can trigger noncompliance with regulatory authorities such as the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA). Supply-chain security helps to guarantee consistent and dependable production flow and acts as a safety net to ensure uninterrupted production in case of unforeseen circumstances.

Experienced manufacturers also can engage with regulatory agencies early to gain insight into the technologies and innovations needed to accelerate development before standardization can be established. Moreover, partnering accelerates drug development and delivers adaptive strategies to support changing industry needs. Precision NanoSystems offers an end-to-end manufacturing workflow for clinical and commercial nanomedicine production, including instrumentation, lipids, and services across all stages of development.

LNP technology undoubtedly will influence the future of oncology treatment, infectious disease response, and cell and gene therapy development. For that future, the industry will require a combination of advanced yet cost-effective manufacturing technologies. Navigating the intricacies of this evolving manufacturing landscape will require integration of scaling strategies, automated platforms, process controls, and analytical insights to enable innovation for new therapeutic areas.

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How Single-Use Technology Is Transforming mRNA-LNP Drug Manufacturing

Laura Ho and Reshma Kapatrala, with Joe Makowiecki

Genomic medicine is a groundbreaking field that harnesses the power of genetic information to revolutionize healthcare by uncovering crucial insights into genetic predispositions for diseases and drug responses. Such information enables personalized medicine approaches, in which treatment plans are tailored to each patient's specific genetic makeup for targeted therapies that offer improved outcomes and minimize adverse effects. As technology advances, genomic medicine is poised to unlock new possibilities for precision healthcare and transform medicine.

The successful application of lipid nanoparticle (LNP) technology is a significant development in genomic medicine and has supported development of mRNA therapies and vaccines, with COVID-19 vaccines as a notable example. LNPs typically consist of four components: ionizable lipids, phospholipids, cholesterol, and PEGylated lipids (fused with polyethylene glycol). Those components self-assemble into nanoparticles, creating protective shells around their therapeutic cargo, such as mRNA molecules. Such protective encapsulation enables those molecules to navigate a patient's body and reach target cells — making LNPs a safe and effective drug delivery system for many applications, including cell and gene therapy as well as drug delivery.

In biomanufacturing, single-use technology (SUT) eliminates contamination risks, optimizes drug analysis efficiency, and enables cost-effective research and drug development. Using it can accelerate advancements in innovative biopharmaceutical areas such as personalized healthcare.

SUTs Accelerate Genomic Medicine Development Timelines

Speed to market is critical; however, difficulties arise in producing a commercial mRNA-LNP drug product. SUT has gained attention in the field for its ability to facilitate reliable and robust manufacturing processes. Single-use systems help companies to maintain sterile and controlled environments. Because any kind of contamination can compromise both the safety and effectiveness of LNPs, it is essential to prevent cross-contamination between batches.

Joe Makowiecki (business and product development leader for Enterprise Solutions at Cytiva) says this situation now reminds him of when SUT was in its nascency for monoclonal antibody (mAb) manufacturing around 2005. "We had to do a lot of convincing on the benefits of single-use equipment over the existing stainless-steel (clean and re-use) infrastructure. There was a lot of cynicism and questions like: 'Why would you want single-use? What do you do with the plastics that are generated? Does it have the same capabilities as the existing stainless-steel systems? And beyond that, what does it offer that differentiates it?' That's the sort of conversation we had back then. It was a stainless-steel world, and we were introducing a new paradigm."

Almost 20 years later, the benefits of SUT are well recognized in manufacturing mAbs and in other biopharmaceutical products. As RNA technology evolves from mRNA to self-amplifying RNA (saRNA) and circular RNA (circRNA), the encapsulation technologies also become more targeted and complex. Makowiecki says that operational flexibility in the associated manufacturing operations will become a prerequisite for developers and that modularized single-use equipment will play a critical role.

The risk of contamination for genomic medicines is a significant concern, leading to loss of therapeutic activity and regulatory delays or rejections because of safety concerns. Single-use components, such as disposable mixers, tubing, and filters, minimize the risk of contamination and reduce the need for cleaning, maintaining, and validating reusable equipment. That can benefit biopharmaceutical companies by decreasing the need for time-consuming and labor-intensive work. In addition to the reduced risk of contamination, enhanced product quality and safety and simplified validation processes help to ensure compliance with regulatory requirements while accelerating speed to market.

Implementing SUT in mRNA-LNP manufacturing means that extractables and leachables (E&L) become a consideration. Manufacturers follow guidelines such as the US Pharmacopeia (USP) chapter ‹665› and the BioPhorum Operations Group (BPOG) protocol for testing of product-contacting plastic components.

Cost efficiency is another critical factor driving SUT adoption. Although the consumables cost of single-use components can be higher than that of reusable equipment, the overall cost-efficiency becomes evident over time. Disposable components eliminate the need to invest in equipment cleaning, maintenance, and validation procedures. Additionally, the risk of batch failure and associated downtime can be reduced significantly, providing long-term savings of both time and money.

Scale-Up and Scale-Out with SUT

As the demand for LNPs and mRNA-based therapeutics grows, it is crucial to have a scalable manufacturing process that can adapt quickly to different production volumes. SUTs offer consistent, reproducible performance, minimizing the potential for variability and the risk of batch failure as a process transitions from preclinical to GMP manufacturing. SUTs also allow manufacturers to scale production volumes easily up or down by adjusting the number of single-use bioreactors, mixing bags, or other single-use components used — unlike stainless-steel equipment, which requires larger investment. Such equipment modularity was a significant factor in setting up manufacturing lines around the world swiftly and efficiently during the COVID-19 pandemic (**1**). Another advantage comes in expediting technology transfer, whether new lines are added inside an existing facility or new facilities are established elsewhere. That makes SUT an efficient, reliable, and attractive option for manufacturers implementing mRNA-LNP production at different scales.

Given the pace at which genomic medicine is advancing, adopting single-use technologies and platforms into multiproduct and multiprocess workflows enables manufacture of multiple mRNA products in the same facility. Precision NanoSystems, now part of Cytiva, empowers its NanoAssemblr family of products with NxGen technology to generate optimal particles reproducibly through a single-use mixer across scales. The NanoAssemblr commercial formulation system enables clinical and commercial production of LNPs by leveraging a single-use flow path, shortening both turnaround times and time to market for mRNA-based vaccines and therapeutics.

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