IMMUNOWATCH

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NON VIRAL VECTORS

mabdesign
THE BIOTHERAPY EXPERT

INTRODUCTION

MabDesign's ImmunoWatch is a specialized intelligence newsletter focused on the evolving field of biologics. It has been designed to deliver to actors and stakeholders in the field, timely and high-value insights curated through the combined expertise of MabDesign and its network of contributors in scientific research, business intelligence, market analysis, and intellectual property.

Each edition is dedicated to a specific therapeutic area or trending class of biologics, providing a comprehensive overview of current trends and strategic developments. The content typically includes market research, financial and economic data, expert perspectives from academic and industrial teams, and an in-depth intellectual property analysis. The editorial direction is ensured by a chief-editor board of at least two field experts, with theme selection and content development overseen by MabDesign's permanent editorial team.

Finally, we would like to acknowledge the continued support and strategic oversight provided by MabDesign's Comité d'Orientation Stratégique et Scientifique de Filière (COSSF) for their significant contribution to the quality and relevance of each edition of ImmunoWatch.

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AGS

Manuel Vega
CEO

RNA- and other innovative molecules-based therapeutics are poised to transform medicine. Pharma companies have invested over **$32 billion** in the past few years to access RNA technologies—but delivering these molecules beyond the liver remains a critical bottleneck.

Current delivery platforms—**LNPs**, **mammalian EVs, and AAVs**—have all encountered major limitations, as highlighted at the RNA Leaders Congress in Basel, March 2025. As a result, dozens of promising therapeutic, gene therapy, and vaccine programs remain stalled, awaiting viable delivery solutions.

AGS Therapeutics (AGS-Tx) is pioneering **microalgae extracellular vesicles (MEVs)** as a universal delivery platform. MEVs combine two groundbreaking innovations:

1. **Disruptive delivery capabilities:** Non-invasive, tissue-targeted, payload-agnostic, biodegradable, re-dosable, non-immunogenic, MEVs overcome biological barriers that other systems cannot.

2. **Sustainable, scalable manufacturing:** Produced from Chlorella algae, MEVs require only water, light, and minerals, consume CO_2, and avoid solvents or animal materials. Compared to LNPs, mammalian EVs, or AAVs, MEVs are cost-effective and support equitable access to therapies.

All major advancements in MEV technology—including payload loading, biodistribution, administration routes, efficacy, safety, and manufacturing—have been developed and protected by AGS. The platform is now pre-industrialized and in-the-process-to-become GMP-compliant production through **AGS-M**, AGS's dedicated subsidiary, as no existing CDMO possesses the required expertise.

AGS' vision: delivery systems that are effective, disruptive, sustainable, cost-efficient, and accessible. **AGS' mission:** to establish MEVs as the new standard and unlock their full therapeutic and economic potential.

By addressing fundamental delivery challenges, MEVs could **reshape multiple therapeutic areas, and inspire a new generation of researchers and industry leaders.**

Editorial

Sezen Gul
Field Application Specialist

Audrey Nsamela
Co-Founder & CSO

The clinical triumph of RNA vaccines and the rise of gene editing have proven that we can reprogram biology to treat disease. But these revolutions share a common constraint: success depends not only on what we can design, but on what we can deliver. The ability to transport fragile genetic cargo safely, efficiently, and precisely into cells remains the true bottleneck of genetic medicine. Viral vectors opened the door, but their inherent limitations—immunogenicity, packaging constraints, and complex manufacturing—have made it clear that the future lies in non-viral delivery systems. Among these, lipid nanoparticles (LNPs) have emerged as the most transformative and versatile platform of our time.

The arrival of LNPs on the world stage was nothing short of transformative. They demonstrated that a synthetic, non-viral carrier could achieve clinical success at an unprecedented scale. Yet, as attention shifts from vaccines to a wider range of therapeutics, the cracks in the model are beginning to show. LNPs are effective, but not perfect. They need to be re-engineered for longevity, precision, and repeat dosing if they are to support the next generation of RNA and gene-editing nanomedicines.

One of the biggest sticking points is endosomal escape—the process by which the payload moves from the endosome into the cytosol. Even the best current formulations manage this step with low efficiency (~1-2%). The result is a constant trade-off between potency and safety: to get enough activity, we push doses higher than we would like. Recent work tuning lipid composition, tweaking particle morphology, or blending LNPs with extracellular vesicle components offer encouraging progress.

Another issue that has been hiding in plain sight is our reliance on PEGylation. Polyethylene glycol (PEG) lipids have given LNPs their stability and circulation time, but they also create new problems—most notably immune responses and reduced uptake after repeated exposure. The industry is now actively searching for better solutions. Alternatives like zwitterionic polymers, polysarcosine, or polycyclic imino ethers are being explored for their ability to stabilize without provoking the immune system. The next challenge will be scaling these materials without losing the reliability that made PEG such an attractive standard.

Perhaps the most exciting frontier lies in directing where these nanoparticles go. Systemic LNPs have a natural preference for the liver—a feature that has been both a blessing and a barrier. To reach other tissues, researchers are testing two main paths: first, active targeting through ligands such as antibodies or peptides, as demonstrated by recent T-cell-targeted LNPs for in situ CAR engineering developed by Capstan Therapeutics ; and second, passive targeting by adjusting the lipid composition through the Selective ORgan Targeting (SORT) approach. The latter has achieved lung, spleen, and other extrahepatic delivery by controlling nanoparticle surface interactions with serum proteins to direct receptor-mediated uptake. Both strategies point to a future where delivery can be programmed, not just discovered.

The next generation of LNP therapeutics will emerge from integrating these advances into coherent design frameworks. High-throughput in vivo screening, barcoded nanoparticle libraries, machine learning and automated manufacturing platforms are transforming what was once a slow, empirical process into a data-driven science.
But discovery is only half the battle. Translation is equally critical. Manufacturing reproducibility, regulatory clarity, and standardized quality control assays will determine how quickly these technologies reach the clinic. As the field matures, the focus is shifting from “can we make it work?” to “can we make it work at scale, reliably, and affordably?”.
The goal isn’t just better nanoparticles—it’s a new foundation for all of genetic medicine. The sooner we recognize that, the faster we can turn delivery from limitation into liberation.

SCIENTIFIC ARTICLES

Read the different inputs from the scientific community on Non Viral Vector

RETHINKING DELIVERY: THE RISE OF NON-VIRAL VECTORS

By MabDesign Market Research

The rapid expansion of novel biotherapies is redefining what is expected from delivery technologies. In vivo gene therapies, RNA and DNA vaccines, and oligonucleotide products all depend on delivery vectors capable of transporting nucleic acids or genome-editing complexes into target cells. In this landscape, the vector is no longer a simple vehicle; it directly shapes manufacturability, scalability, and ultimately clinical feasibility.

While viral vectors (AAV, lentivirus, adenovirus) enabled the first approved gene therapies, their limitations are increasingly constraining development: immunogenicity, cargo-size limits and complex, high-cost biological manufacturing. Due to these limits, there is a growing interest in delivery systems that are modular, scalable, and compatible with rapid iteration cycles, which is why non-viral vectors are now gaining strategic importance across the biopharmaceutical industry. They have reached a certain level of maturity, especially lipid nanoparticles (LNPs) that have demonstrated unprecedented scalability and global deployment through mRNA vaccines.

Nevertheless, the field is not limited to LNPs, and many innovative approaches coexist in the pipeline: plasmids, nanoparticles, transposons, bacterial vectors, etc. This technological diversity aligns with key industry needs: chemical or semi-synthetic manufacturing, modularity across payloads (mRNA, saRNA, siRNA, DNA, RNPs), design-to-CMC workflows, and scalable processes compatible with global supply chains.

Beyond this innovation dynamic, the market landscape is beginning to show similar signals, also suggesting that non-viral vectors are gaining strategic relevance for industrial players.

Gene therapies: a market still dominated by viral vectors, but a diversifying pipeline

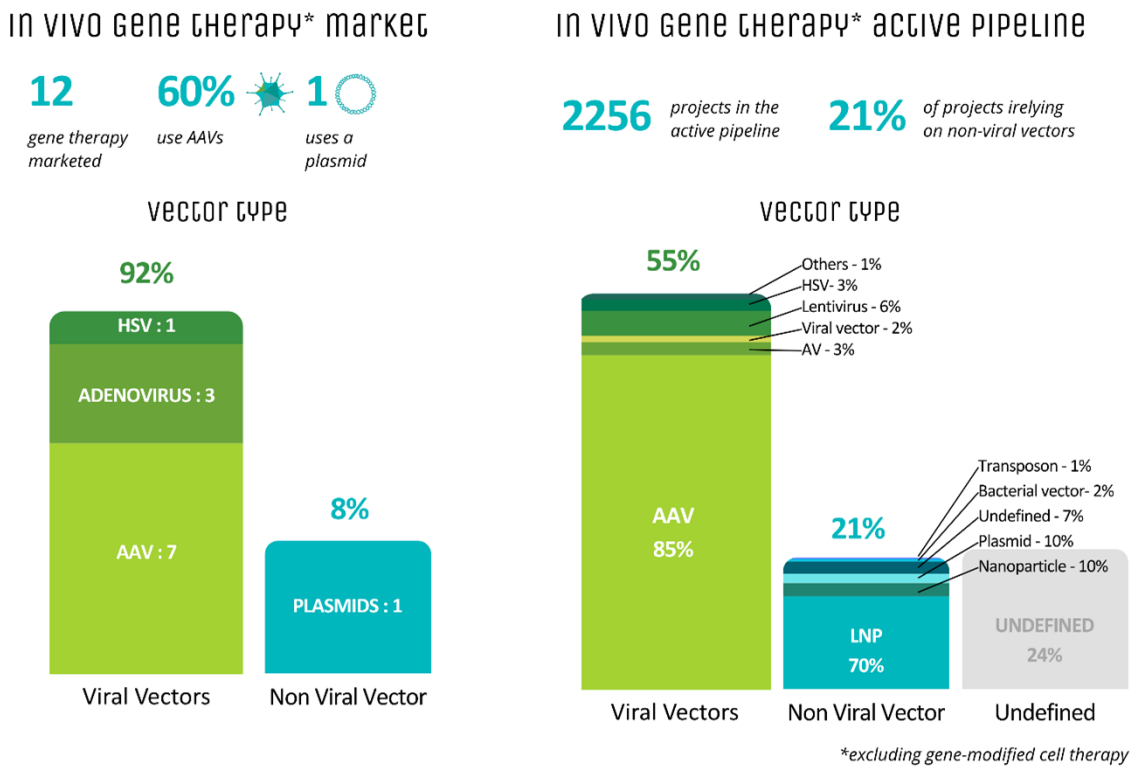
Only one example of non-viral gene delivery on the market…

The current delivery landscape for gene therapies remains overwhelmingly dominated by viral vectors. Among the 12 gene therapy products commercially available, only one relies on a non-viral vector, using plasmid DNA for in vivo gene delivery : Neovasculgen, Artgen biotech, approved solely in Russia for peripheral arterial disease. All other approved gene therapies are based on AAV (60% of the total), lentiviral or adenoviral platforms, reflecting the historical reliance on viral systems for clinical-grade gene transfer.

This near absence of non-viral vectors on the gene therapy market is the result of important constraints currently hampering clinical translation of this technology: lower in vivo transfection efficiency compared with AAV or lentiviral systems; short and unpredictable duration of expression; and challenges in achieving robust tissue targeting beyond local or intramuscular administration.

Due to these limitations, the only example of a marketed gene therapy product using a non-viral vector is in a setting with modest delivery requirements : Neovasculgen—targeting a localized peripheral vascular disease—relies on plasmid DNA in a context where transient, locally delivered expression is sufficient, and is furthermore approved only on a national market. Consequently, the non-viral approach has yet to prove its clinical efficacy for more demanding settings in gene therapy.

… but a dynamic pipeline of innovative approaches emerging



The active pipeline in in vivo gene therapy is rich, with more than 2256 projects in development. As seen on the market, viral vectors still dominate, accounting for 55 % of active programs, with AAV alone representing 85 % of this category. Yet non-viral platforms now constitute 21 % of the pipeline, driven primarily by lipid nanoparticles (70 % of projects using non-viral vectors). Nevertheless, many other approaches are emerging, such as plasmids (10%), nanoparticles (10 %), bacterial vectors (2%), transposons (1%), etc. This diversification of delivery modalities signals that innovative efforts are invested towards modular, manufacturable non-viral delivery systems. Importantly, 24% of gene-therapy programs—mostly at early stages—do not specify the vector type, which limits the precision of this analysis.

Overall, these figures confirm a clear trend towards non-viral vectors in in vivo gene therapies. Their adoption remains more limited in ex vivo gene-modified cell therapies, where only one marketed product (Encelto) uses a plasmid vector, and 70 pipeline programs rely on non-viral delivery. However, when focusing specifically on in vivo CAR-T approaches, around 25% of pipeline projects already involve non-viral vectors — highlighting that, much like in conventional in vivo gene therapies, non-viral delivery technologies are becoming an increasingly important component of innovative strategies.

Nucleic-acid-based products : the reign of LNPs

Non-viral vectors have become a central component of modern vaccine technologies, with the rise of nucleic-acid–based vaccines. While traditional viral-vector vaccines do not require a delivery vector, next-generation vaccines rely on delivery systems capable of transporting mRNA or DNA into target cells. These include lipid nanoparticles (LNPs) for mRNA vaccines and naked plasmid DNA for DNA-

based vaccines—both of which enable rapid design, scalable manufacturing and flexible antigen switching, without the constraints associated with viral backbones.

There are currently 25 mRNA-based vaccines on the market, the vast majority of which target COVID-19—a direct consequence of the unprecedented acceleration of mRNA vaccine development during the pandemic. Only one marketed product falls outside this category, targeting Respiratory-syncytial virus (RSV, mRESVIA®). All approved mRNA vaccines rely on encapsulation of the mRNA payload into lipid nanoparticles (LNPs). In parallel, only a single DNA vaccine is commercially available, ZyCoV-D in India, which uses a plasmid as its non-viral delivery vector.

Lipid nanoparticles (LNPs) have become the foundational delivery technology for all marketed mRNA vaccines because they address the key biological and pharmaceutical challenges associated with administering naked mRNA : LNPs protect the RNA from rapid extracellular degradation, enable efficient cellular uptake, and promote endosomal escape—steps essential for the RNA to reach the cytosol and be translated into antigen. Beyond these biological functions, LNPs offer major industrial advantages: they are fully synthetic, compatible with rapid scale-up, and easily adaptable to new antigen sequences, which proved essential during the COVID-19 pandemic. Their high tolerability, safety, ease of design and manufacturing have made them the vector of choice for mRNA vaccines, and they remain the most advanced non-viral platform in terms of both clinical validation and global deployment.

Nucleic acid-based products

Vaccines

25
mRNA vaccines marketed

100%
use LNPs

1
DNA vaccine (in India only)

1
uses a plasmid

Oligonucleotides

23
oligonucleotides marketed

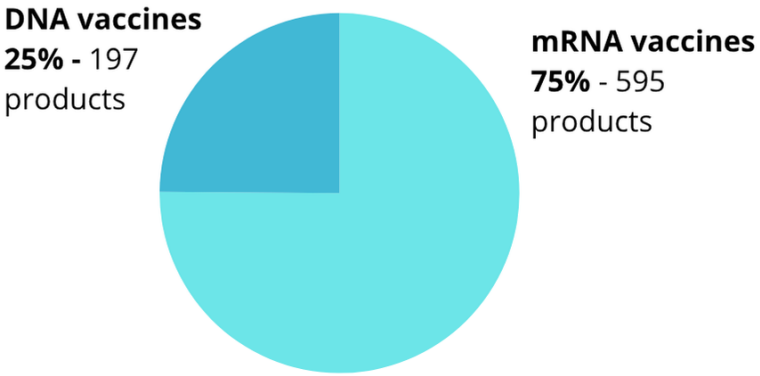
1
uses LNPs

Beyond vaccines and gene therapies, non-viral delivery systems are also used in a limited subset of oligonucleotide drugs, although the landscape is heterogeneous. Among 23 marketed products, patisiran (Onpattro) is the only example formulated in lipid nanoparticles (LNPs), enabling protection of siRNA from nucleases, hepatic delivery, and endosomal escape. It was also the first RNA-based therapy to reach the market in 2018. It is worth noting that several siRNA and antisense drugs that followed patisiran are GalNAc-conjugated, enabling more precise hepatocyte targeting, at lower dose, and with subcutaneous administration. GalNac is not a vector per se in the sense of a carrier system, as it is a small-molecule ligand that provides cell-specific targeting to the liver but does not encapsulate or protect the oligonucleotide like a nanoparticle would : it represents the ligand-based delivery approach, which has emerged as the preferred approach for approved oligonucleotide therapeutics.

Many approved oligonucleotide therapeutics do not rely on complex vectors because their small size combined with extensive chemical modifications confer sufficient stability, target affinity and pharmacokinetic properties, especially when administered by appropriate routes (intrathecal or subcutaneous for many ASO/siRNA).

Nucleic-acid based vaccines pipeline

792 *mRNA & DNA vaccines in the active pipeline*



The rapidly expanding pipeline of nucleic-acid–based vaccines — 595 mRNA and 197 DNA candidates currently in development — is accelerating innovation in non-viral delivery systems. Because these vaccines rely on synthetic RNA or plasmid DNA, their feasibility depends largely on efficient vectors capable of protecting nucleic acids, enabling cell entry, and supporting scalable manufacturing. For mRNA vaccines, lipid nanoparticles (LNPs) remain the dominant approach: they protect RNA from degradation, enhance cellular uptake, and enable cytosolic delivery. DNA vaccines, while less mature clinically, increasingly rely on improved non-viral strategies such as electroporation or polymeric formulations to overcome low transfection efficiency.

Interestingly, of the 792 products in the pipeline, 234 are therapeutic vaccines targeting indication in oncology : 163 mRNA vaccines, and 71 DNA vaccines. A substantial proportion of these projects are using non-viral vectors, mostly LNPs for mRNA vaccines and plsamids for DNA vaccines.

Overall, the size of the mRNA/DNA vaccine pipeline is creating strong industrial pressure to optimize modular, rapidly deployable non-viral vectors, reinforcing their strategic relevance well beyond COVID-19 vaccines.

Innovations and trends : tomorrow's non-viral vectors

Beyond development of the "active" product, vector engineering itself is becoming a strategic priority for developing projects in gene therapy and nucleic-acid-based products. While non-viral vectors commercial footprint is still limited compared to viral platforms, they represent an innovative field with a lot of potential for clinical translation. Indeed, as payloads diversify and regulatory pressure on viral vectors increases, non-viral delivery technologies are becoming a central competitive lever. All innovations converge towards a common goal: delivering increasingly diverse nucleic-acid payloads efficiently, safely, and at industrial scale.

Next-generation LNPs

Whether it is for in vivo gene therapy or for nucleic-acid-based vaccines, new generation LNPs are currently the hot topic. Current innovation focuses notably on ionizable lipid engineering: new ionizable lipids aim to balance potency, biocompatibility and manufacturability. Most developments target improved endosomal escape, reduced inflammation, and optimized pharmacokinetics. Another critical limitation of first-generation LNPs is their natural liver tropism. Targeted LNPs — via lipid tuning, surface ligands or altered PEG-lipid compositions — aim to redirect delivery to other tissues, such as the lung, CNS, spleen, muscle, immune cells, etc. This represents one of the most active R&D frontiers in the entire field of non-viral vectors. Efforts are also being made regarding thermostable and manufacturing-optimized LNPs. Lyophilizable LNPs and lipid compositions compatible with higher-temperature storage would respond to global manufacturing constraints highlighted during COVID-19.

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Emerging biological carriers

Extracellular vesicles (EVs) and exosome-mimetic nanoparticles represent a nascent but high-interest area. They offer many advantages, namely natural biocompatibility, immune stealth and endogenous tropism, but face major challenges in scalable manufacturing, reproducibility, and loading efficiency.

Interestingly other types of EVs are investigated as biological carriers, such as microalgae extracellular vesicles (MEVs), combining the biocompatibility of exosomes with the scalability of microbial systems, and offering a promising platform for RNA and DNA delivery.

Conclusion

Although today the impact of non-viral vectors on the market remains modest compared with viral vectors, the rapid expansion of nucleic-acid vaccines, RNA therapeutics and gene therapy is reshaping strategic priorities across the industry. As payloads diversify and regulatory pressures intensify, delivery will increasingly determine what is clinically and industrially feasible. In this context, non-viral vectors are no longer peripheral technologies but central enablers of the next wave of genetic medicines, with the potential to unlock applications that viral platforms cannot reach—biologically, technologically or economically.

Sources :

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Notes : Some viral based gene therapies are classified as oncolytic virus by GlobalData and thus excluded from this analysis.

NON-VIRAL DELIVERY OF INNOVATIVE THERAPEUTICS.

Manuel Vega
CEO,AGS Therapeutics

1. Viral-Based Delivery Systems [1-13]

In the field of gene and cell therapy, viruses have long played a central role as delivery vehicles. Their natural ability to enter host cells and insert genetic material has been cleverly repurposed by scientists, who now engineer viruses to act as vectors rather than pathogens. By stripping away their harmful properties and equipping them with therapeutic DNA or RNA sequences, researchers have transformed viruses into powerful tools for medicine.

Among the different viral platforms, Adeno-Associated Viruses (AAVs) stand out as the most widely used. These are small, non-pathogenic viruses that have gained enormous popularity for in vivo applications. Their appeal lies in their relatively low immunogenicity and their capacity to drive long-term expression in non-dividing cells. One important limitation, however, is their restricted packaging capacity of approximately 4.7 kilobases.

Lentiviruses (LVs), derived from retroviruses, form another important class of vectors. Unlike AAVs, lentiviruses integrate their genetic payload into the host genome, ensuring stable and long-lasting expression. This property makes them particularly valuable in ex vivo applications, such as in the engineering of CAR-T cells, where persistence and reliability of expression are critical. At the same time, integration carries risks, including insertional mutagenesis, which has been observed in earlier retroviral-based therapies.

Adenoviruses occupy yet another niche. These larger DNA viruses can accommodate a significantly bigger genetic payload than AAVs—up to 37 kilobases in some configurations—and are therefore useful for delivering more complex constructs. However, their relatively strong immunogenicity has historically limited their clinical adoption.

Beyond these three mainstream systems, there are additional viral platforms under investigation. For example, Herpes Simplex Virus (HSV) is being explored for its unique ability to deliver very large genes, particularly in therapies targeting the nervous system. Likewise, non-lentiviral retroviruses, though important in the early history of gene therapy, have largely been replaced because of safety concerns related to insertional mutagenesis.

Despite their many advantages, viral-based delivery systems come with important limitations and challenges. Perhaps the most fundamental of these is the restricted payload capacity already mentioned for AAVs. Immunogenicity is another critical issue: patients may mount strong immune responses against viral capsids or even against the therapeutic transgene. In the case of AAVs, high systemic doses have been linked to liver toxicity and, in some cases, severe adverse events.

Another challenge is non-redosability. Once a patient has been exposed to an AAV, their immune system often prevents safe or effective re-administration. On the production side, the manufacturing of viral vectors is notoriously complex and expensive. Scaling up, especially for AAVs, requires advanced facilities and specialized expertise, creating bottlenecks that affect both accessibility and regulatory timelines.

Biodistribution also presents significant hurdles. Viral vectors often struggle to reach certain tissues—such as the retina, the central nervous system, or solid tumors—without resorting to invasive administration techniques. For integrating vectors like lentiviruses, the concern shifts to insertional mutagenesis, since integration into the host genome carries the risk of disrupting vital genes or inadvertently activating oncogenes.

Finally, the field faces broader regulatory and supply constraints. The global capacity to manufacture viral vectors remains limited, creating competition between clinical programs, delaying trials, and slowing down the path to commercialization.

Viral-based delivery systems have been the workhorses of modern gene and cell therapy, enabling breakthroughs that were unimaginable only a few decades ago. Yet, they also embody a set of challenges—technical, biological, and logistical—that continue to drive the search for safer, more versatile, and more scalable alternatives.

In summary

Viral Vectors	
Payload Capacity	Limited (AAV ≤ 4.7 kb; LVs ≤ ~8–10 kb)
Biodistribution & Tissue Access	Restricted; often requires invasive delivery for certain tissues
Immunogenicity / Safety	High; immune responses to viral capsids; toxicity risks at high doses
Redosability	Very limited; prior exposure triggers neutralizing antibodies preventing re-administration
Durability of Expression	Long-term expression possible with AAVs or LVs, but risk of insertional mutagenesis
Manufacturing	Complex, expensive, low yields; major supply bottlenecks
Regulatory Complexity	Long history but associated with severe adverse events and strict monitoring
Market Accessibility	Very high cost of goods; therapies often >$1–2 million per patient

Viral-based delivery systems, particularly including AAV vectors, are powerful but constrained by immune reactions, dose-related toxicities, limited payloads, non-redosability, high manufacturing costs, and safety risks. These limitations are precisely what is driving interest in non-viral alternatives (like LNPs, EVs, and MEVs).

2. Lipid Nanoparticles (LNPs) as a Delivery System [14-26]

Lipid nanoparticles (LNPs) have rapidly emerged as the most widely adopted non-viral delivery platform for nucleic acids. Structurally, they are nanoscale carriers composed of ionizable lipids, phospholipids, cholesterol, and polyethylene glycol (PEG)-lipids. These components self-assemble into spherical particles that encapsulate nucleic acids—such as mRNA, siRNA, or DNA—shielding them from enzymatic degradation in circulation and facilitating uptake into cells.

The mechanism by which LNPs function is now well understood. First, the nucleic acid payload is packaged within the particle, stabilized by the ionizable lipids. Once administered, LNPs protect their cargo from degradation in the bloodstream. They are internalized by target cells through endocytosis. Inside the acidic environment of the endosome, the ionizable lipids acquire a positive charge, which destabilizes the endosomal membrane and enables escape of the payload into the cytoplasm. Once released, the nucleic acid can exert its therapeutic effect—for example, an mRNA transcript is translated into a functional protein.

In summary

LNPs	
Biodistribution & Tissue Access	LNPs show strong tropism for the liver, but poor penetration into extrahepatic tissues such as the retina, brain, muscle, or tumors.
Immunogenicity / Safety	Risk of hypersensitivity, inflammation, and liver toxicity at higher doses.
Redosability	Limited. Immune reactions to lipid and PEG components reduce efficacy and safety upon repeated administration.
Stability constraints	Require ultra-cold storage, complicating logistics and access.
Footprint	Require ultra-cold storage, complicating logistics and access.
Manufacturing	Complex, expensive, low yields; major supply bottlenecks

As a result, while LNPs have revolutionized mRNA vaccines, their utility as a universal delivery system for broader therapeutic applications is fundamentally limited.

3. Extracellular Vesicles: From Mammalian Origin to Microalgae-Derived Platforms [27-31]

Extracellular vesicles (EVs) from mammalian sources have long been recognized as natural mediators of intercellular communication. Secreted by virtually all cell types, mammalian EVs—including exosomes and microvesicles—carry proteins, lipids, and nucleic acids that reflect the physiological state of their parent cells. Their innate ability to interact with specific recipient cells, cross certain biological barriers, and modulate immune responses has positioned them as promising candidates for therapeutic delivery.

Research over the past decade has demonstrated that mammalian EVs can deliver a variety of payloads, such as mRNA, siRNA, proteins, and small molecules, to targeted tissues with minimal toxicity. In particular, EVs derived from stem cells or immune cells have shown potential in regenerative medicine, immunotherapy, and oncology. However, despite their biological advantages, mammalian EVs face significant challenges for large-scale clinical translation. Production is often limited by the complexity of mammalian cell cultures, batch-to-batch variability, and the high costs of scalable biomanufacturing.

Additionally, mammalian EVs carry surface markers and bioactive molecules inherent to their parental cells, which can complicate their biodistribution and immunogenicity. While these features may provide natural targeting in certain contexts, they can also trigger unintended immune responses or restrict the versatility of payload delivery. These limitations have motivated the exploration of alternative sources of EVs, leading to the development of microalgae-derived extracellular vesicles (MEVs) as a next-generation, non-viral delivery platform.

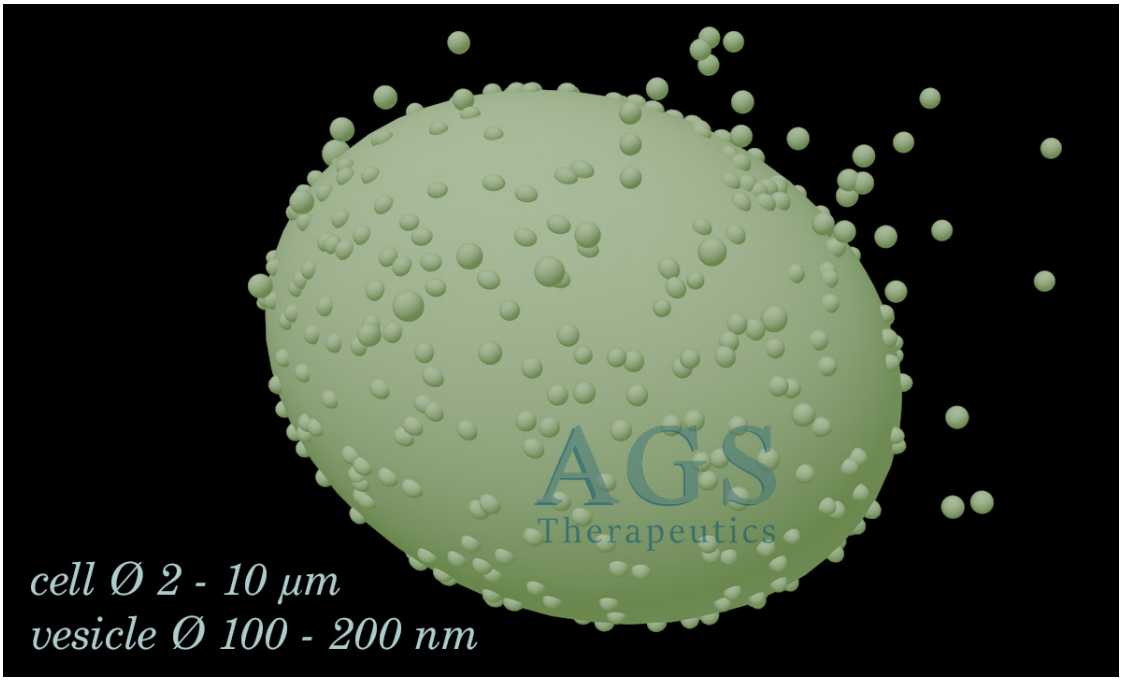
4. Microalgae Extracellular Vesicles (MEVs): A Next-Generation Non-Viral Delivery Platform [32-39]

EVs have emerged as versatile, biocompatible carriers for therapeutic delivery, offering a unique combination of flexibility, safety, and natural targeting. Among EVs, microalgae-derived extracellular vesicles (MEVs) represent a particularly promising innovation, combining, among other the advantages of biogenic nanoparticles with scalable and sustainable production. Particularly remarkable are MEVs derived from the microalgae *Chlorella*. This freshwater microalga has existed on Earth for approximately 2–3 billion years, long before the emergence of any plants or animals, whether extinct or extant.

MEVs are capable of encapsulating a wide variety of payloads, including mRNA, siRNA, DNA/plasmids, proteins, and peptides. Their architecture allows delivery of larger or multiple payloads simultaneously, creating opportunities for complex therapies that are difficult to achieve with viral or synthetic carriers.

One of the defining features of MEVs is their natural ability to cross stringent biological barriers. Unlike other delivery systems, which often require chemical modification (LNPs) or invasive administration (AAVs) to reach specific tissues, MEVs can traverse the gastro-intestinal, olfactory, and retinal barriers with ease. Oral administration allows MEVs to deliver mRNA to intestinal epithelial cells; intranasal administration enables entry into olfactory neurons and delivery to targeted regions of the brain; and ocular drops allow MEVs to reach retinal cells non-invasively, avoiding intravitreal injections. This biodistribution, which is at the same time broad (several non-invasive routes of administration (RoA) are admitted), and highly targeted (specific target tissues are reached by each RoA) expands therapeutic potential across tissues that are otherwise challenging to access.

Safety and redosability are additional advantages of MEVs. Naturally derived and non-pathogenic, MEVs enable repeated dosing—a significant limitation of both viral vectors and many synthetic carriers. In addition, MEVs are flexible and versatile, capable of delivering multiple payload modalities safely and repeatedly. As for other delivery systems, the durability of expression of the payload can be enhanced through optimized payload design, and redosability may compensate for any transient expression, providing sustained therapeutic benefit.



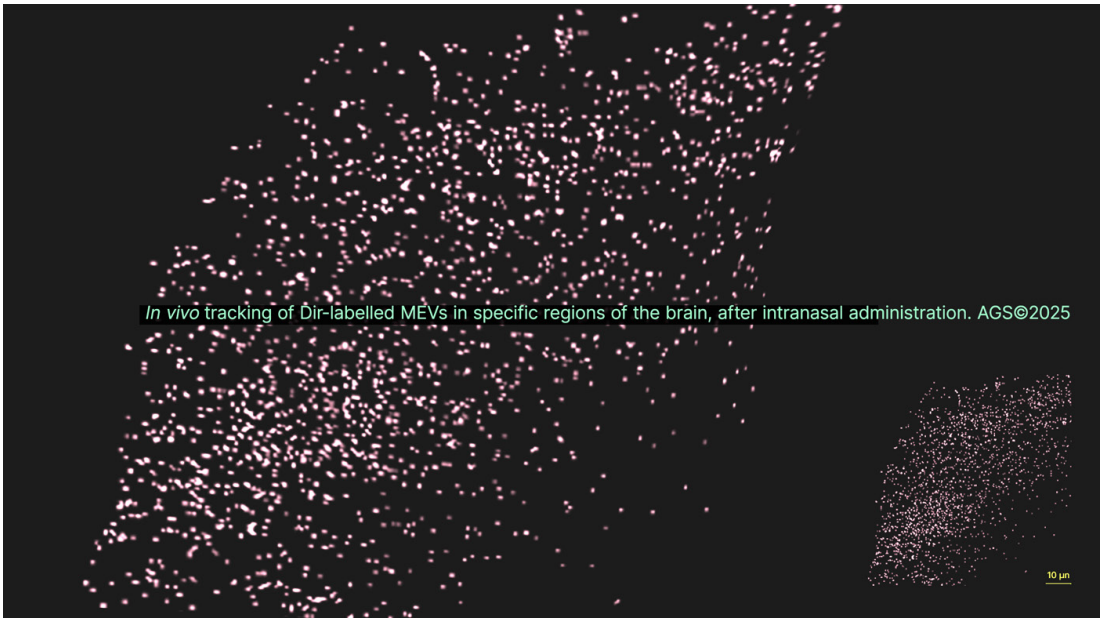
MEVs budding on the surface of Chlorella cells.

Scalable and sustainable production is another hallmark of MEVs. Derived from microalgae such as *Chlorella*, they can be manufactured in large quantities using simple, eco-friendly bioprocesses with minimal natural resources—water, light, and salts. Unlike mammalian cell-derived systems, which require complex culture conditions and expensive bioreactors, MEV production is cost-effective, safe, and globally accessible. Furthermore, MEVs' minimalist surface markers, compared to mammalian EVs, may offer enhanced versatility in biodistribution and tissue targeting.

Compared to viral vectors, MEVs offer a safer, smarter alternative. They avoid risks of insertional mutagenesis and viral toxicity while supporting repeated administration. Unlike AAVs, MEVs are scalable and cost-effective, circumventing the bottlenecks of viral vector manufacturing. Compared to LNPs, MEVs extend delivery beyond the liver, enabling access to hard-to-reach tissues, and support sustainable, green production without reliance on organic solvents.

MEVs also enable a modular approach to drug delivery. The development of the MEV platform has been structured into biologically relevant verticals — such as Ophthalmology, Bowel Diseases, Vaccines, CNS Disorders, and Respiratory Diseases — each corresponding to specific tissues, barriers, and administration routes. For instance, ophthalmic applications can be addressed via eye drops; bowel diseases through oral administration; vaccines through oral-intestinal or intramuscular delivery; CNS disorders via intranasal administration; and respiratory diseases via intratracheal administration or nebulization. This framework allows MEVs to deliver targeted payloads effectively while minimizing systemic exposure and side effects.

Two lead candidates are undergoing early preclinical development: AGS-1010 intended for the treatment of wet age-related macular degeneration (wAMD) by ocular topical administration of MEVs loaded with antiangiogenic factors, and AGS-2010, intended for the treatment of inflammatory bowel disease (IBD) by oral administration of MEVs loaded with an oligo DNA that modulates TLR-9 in the intestine.



In vivo tracking of Dir-labelled MEVs in specific regions of the brain, after intranasal administration. From nose to CNS : example of non-invasive RoA of MEVs

With distinct biochemical and behavioral properties shaped by their microalgal origins, MEVs overcome many of the limitations of traditional viral and synthetic systems, opening pathways for next-generation therapeutics. By combining natural tissue targeting, safety, payload flexibility, and sustainable production, MEVs unlock the full potential of non-viral delivery, offering the possibility of accessible, patient-friendly biomedicines across multiple disease areas.

MEVs stand at the forefront of a new era in therapeutic delivery, seamlessly combining the elegance of mammalian EV biology with the clinical reliability and scalability of LNPs—while leaving behind the risks and constraints of viral vectors. They represent a universal, patient-centric, and sustainable platform with the power to revolutionize medicine, enabling transformative therapies across gene therapy, vaccines, and beyond. With MEVs, the full potential of non-viral delivery is no longer theoretical—it is ready to be realized.

Acknowledgments:

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SMART DELIVERY: DESIGNING AND DECODING TARGETED LIPID NANOPARTICLES

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Lipid nanoparticles (LNPs) carrying mRNA have reshaped medicine, first through vaccines, now expanding into cancer immunotherapy and gene therapy.[1,2] Because mRNA harnesses cellular machinery to transiently produce therapeutic proteins, its clinical impact ultimately hinges on delivery systems that are both efficient and selective.

The central challenge is precise targeting of defined organs, tissues, and cell types to maximize efficacy while minimizing off-target effects. Non-specific accumulation, especially in the liver, can blunt potency and increase adverse events, motivating efforts to deliberately retune biodistribution. In response, researchers are coordinating engineering of LNP composition, architecture, and surface chemistry to guide trafficking and sharpen cell-type selectivity across applications from prophylactic vaccines to in-situ genome editing. Effective targeting is inherently multifactorial, integrating optimized lipids and particle structure with rigorous characterization, purpose-built ligands, and even payload-level engineering. Addressed systematically, these elements form the foundation for next-generation non-viral delivery systems with improved efficacy and safety across diverse diseases.

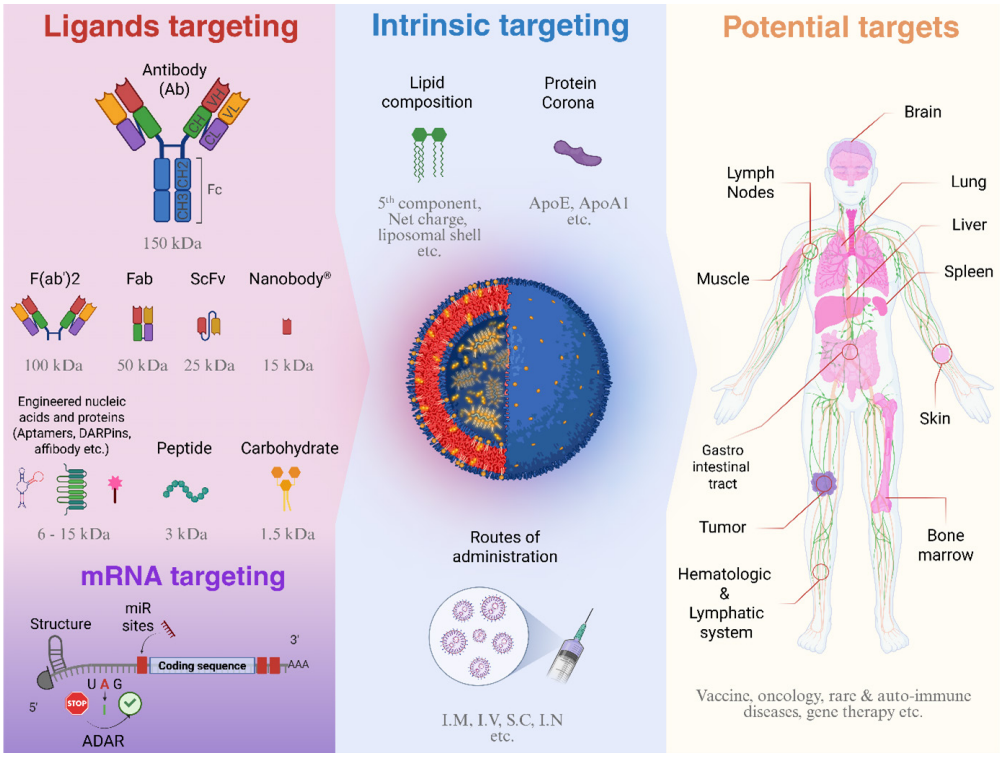

Figure 1. The art of precision: programming LNPs for a therapeutic odyssey.

Modulating Intrinsic LNP Properties for Targeted Delivery

LNP biodistribution can be controlled intrinsically by composition, size, charge and routes of administration. Ionizable lipids drive mRNA encapsulation and endosomal escape; helper phospholipids and sterols stabilize membranes and tune fusion/fluidity; PEG-lipids reduce opsonization and prolong circulation. Together these features set organ tropism, and small ratio changes can markedly shift targeting.

A landmark real-world case now anchors the field: a personalized LNP–CRISPR therapy edited hepatocytes in an infant with CPS1 deficiency, proving in situ correction via systemic delivery.[3] Building on this, the liver remains the predominant sink for LNPs, enabling a "protein-factory" paradigm and supporting passive, liver-directed applications across metabolic and cardiovascular disease (e.g., PCSK9).[4,5] The spleen is likewise leveraged for cancer-vaccine strategies, while inhaled LNPs localize to the lung.[6,8] Intramuscular dosing can traffics to draining lymph nodes or remains local for vaccines and myopathic targets.[9,10] Moreover, composition is a powerful lever: for example, higher DSPC reshapes the protein corona, and can in a formulation-dependent manner reroute delivery from liver to spleen with greater B-cell uptake and lower hepatotoxicity, while enriching bilayer-forming lipids yields liposomal morphologies with longer circulation and extrahepatic transfection.[11,13] The SORT concept adds small fractions of cationic or anionic lipids to redirect otherwise liver-tropic LNPs (e.g., to spleen or lung) by further tuning protein corona composition.[14,15] Multiple pipelines (Omega, Beam, Intellia, CRISPR Therapeutics; ReCode's SORT) are applying these principles across oncology, metabolic, and pulmonary diseases.

Ligand-Mediated Targeting

Ligand-mediated targeting involves conjugating specific ligands to the LNP surface to promote receptor-mediated uptake by the specific target cells and tissues, making it paramount for maximizing therapeutic efficacy and minimizing off-target effects, especially for gene editing applications.

Antibodies are widely used as LNP ligands because of their high specificity to cell-surface antigens.16 Although full IgGs provide excellent selectivity, their bulky size can overcrowd nanoparticles and hinder tissue penetration. Smaller fragments (Fab, scFv, $F(ab')_2$, nanobodies®) retain this specificity while improving tissue penetration and allowing higher ligand density, thereby supporting selective delivery to immune cells such as T cells and dendritic cells, even though they display shorter serum half-lives than IgGs.[17,21] These fragments have already enabled in vivo T-cell programming, for example through CAR expression.22 However, as protein ligands, antibodies and their fragments can induce anti-drug antibodies, hence it is necessary to consider de-immunization strategies during their engineering.[23] In addition, site-specific conjugation (e.g., engineered cysteines or click chemistry) and controlled ligand density can help maintain orientation, reduce steric hindrance, and balance internalization against off-target binding.

Peptides (≈1–5 kDa) are versatile LNP ligands: they are easily modified, conjugate efficiently, penetrate tissue well, and are typically less immunogenic than antibodies.[24] Their robustness broadens formulation options. Practically, they enable VLA-4–mediated delivery to immature hematopoietic progenitors, dendritic-cell targeting to amplify vaccine responses, and CD47-derived motifs that engage SIRPα on phagocytes to dampen clearance and favor immune-cell uptake.[25,27] The main constraints, modest affinity and rapid systemic clearance, are often mitigated by multivalent display and careful control of surface density to sustain potent, durable targeting.

Small-molecule ligands, especially carbohydrates, add synthetic versatility, precise structural control, and much simpler formulation/purification than protein ligands. GalNAc targeting of hepatocyte ASGPR is the leading paradigm for liver delivery: GalNAc–siRNA conjugates enable approved knockdown therapies such as givosiran (Givlaari) for acute hepatic porphyria and inclisiran (Leqvio) for hypercholesterolemia, and analogous targeting is under clinical evaluation in LNP formats (e.g., Verve-102).[4,5]

Mannosylated lipids address dendritic cells and macrophages via the mannose receptor (CD206), which binds branched and linear oligosaccharides; multivalent mannose further boosts uptake. As with any ligand system, however, balance is crucial: high surface mannose density can, in some systems, bias uptake to highly acidifying pathways and lower productive mRNA translation; thus density should be tuned carefully.[28,29]

LNP field is turning into the use of relatively new and disruptive modalities. Aptamers, short single-stranded RNA or DNA ligands (20–100 nt; ~5–30 kDa), fold into defined 3D structures that confer high specificity and affinity, with advantages of compact size, stability, and straightforward expression.[30,32] Aptamer-decorated nanoparticles can target monocytes and dendritic cells to enhance antitumor immunity and typically show lower intrinsic immunogenicity than protein ligands.[33,34] In parallel, engineered protein scaffolds such as affibodies (~6.5 kDa) and DARPins (~15 kDa) combine small size, robustness, and efficient bacterial production with precise binding: affibodies have been applied to HER2-directed therapy and mRNA delivery to T cells, while DARPins deliver payloads to HER2-positive tumors.[35,39] Despite these strengths, aptamer- and scaffold-based targeting remain comparatively underexplored in nanomedicine and warrant further investigation.

Beyond ligand selection, therapeutic efficacy relies heavily on how ligands are presented, their orientation and surface density. Random couplings (e.g., carbodiimide) can bury receptor-binding sites against the nanoparticle, blunting targeting, whereas site-specific maleimide–thiol conjugation preserves orientation and has for example yielded stronger T-cell activation at lower ligand densities than randomly immobilized controls.[40,41] Density, in turn, governs receptor clustering, avidity, and uptake: moderate loading promotes productive clustering and sensitivity; excessive loading introduces steric hindrance, enlarges hydrodynamic diameter, slows diffusion, and can saturate receptors, while too little fails to engage targets. In practice, each system has an "optimal window" set by the ligand's biophysics, particle size/shape, and the biological context.[42,43] A major in vivo constraint is stability. Antibody ligands can for example dissociate from liposomes rapidly (study showing 50% loss within hours) eroding effective targeting.[44] Mitigations include site-selective conjugation to control orientation; linker architectures that balance reach with hydrolytic and serum stability; and stronger anchoring into the LNP surface (e.g., longer or multivalent lipid tails) to resist exchange.

Single-Particle Characterization of Targeted Lipid Nanoparticles: An Essential Approach for Advancing Nanomedicines

A significant challenge is the inherent heterogeneity of the formulations, particularly concerning the precise quantification of ligand density on the nanoparticle surface.[45] Conventional bulk-averaged analytical techniques only provide mean values, thereby masking critical particle-to-particle variations in ligand density and cargo distribution. This limitation makes it difficult to fully understand how ligand density affects cellular uptake, optimize formulation protocols, and carry out rigorous quality control. Consequently, single-particle characterization is fundamentally important to reveal these variations, establish structure-property-function relationships, and accelerate the clinical translation of advanced targeted nanomedicines.

Among the cutting-edge techniques, nanoflow cytometry (nFCM) has emerged as a powerful, high-throughput method that provides unprecedented resolution and analytical depth for targeted LNPs.[46,47] It overcomes the limitations of conventional flow cytometers, which struggle to detect nanoparticles smaller than 200 nm without fluorescence triggering. By adopting strategies like prolonged transit time, reduced detection volume, and efficient background scattering blockage, nFCM can analyze nanoparticles as small as 40 nm. At the single-particle level, nFCM leverages multi-laser, multi-channel (commonly SSC + 1–2 fluorescence channels) for multiparameter characterization. This allows for detailed insights into LNP homogeneity by detecting labelled payloads, labelled lipids, or stained RNA. Furthermore, nFCM facilitates LNP phenotyping through detection with fluorescent probes, such as labelled antibodies or receptors (figure 2)

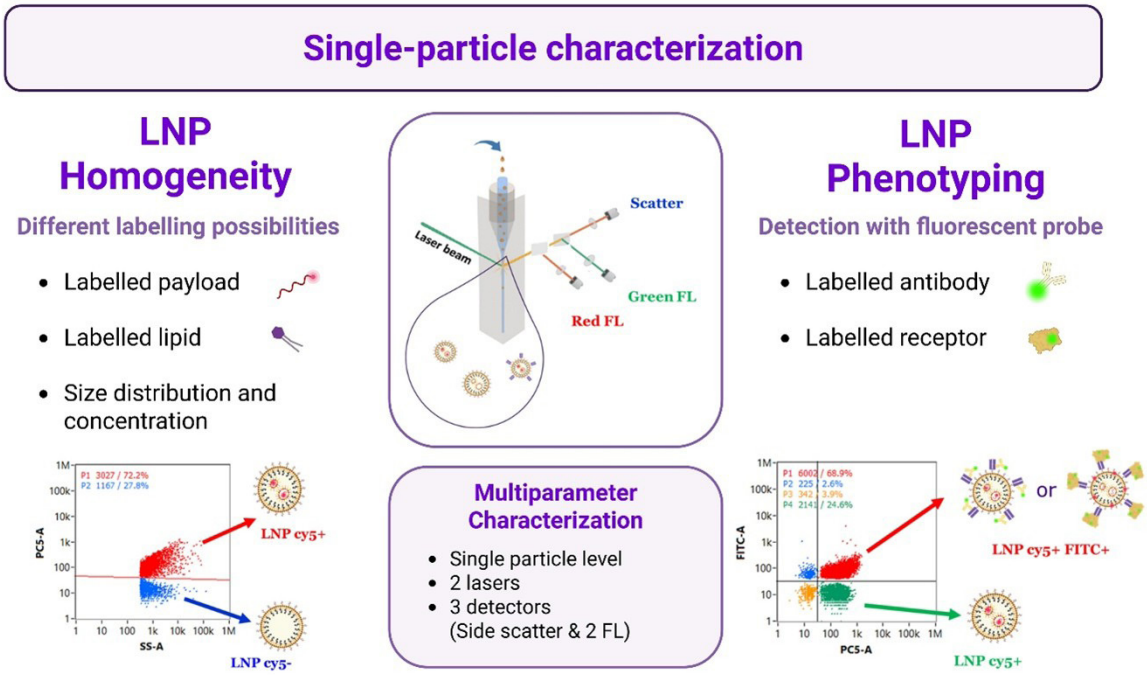

Figure 2. Enabling Single-Particle Characterization for LNP Homogeneity and Phenotyping

Figure 3 exemplifies nFCM's capacity to compare different conjugation strategies (e.g., LNP-N3, LNP-Mal, LNP-PI) across varying Fab concentrations. This is achieved by precisely quantifying critical metrics: First, the percentage (%) of Functionalized LNPs quantifies the proportion of particles successfully carrying the ligand, offering insights into the efficiency and heterogeneity of the functionalization process. Then, the Mean Fluorescence Intensity (MFI) of Targeted LNPs provides an average measure of ligand content, supporting LNP phenotyping. Altogether, Fab density per LNP and Fab density per 100 nm² directly reveal how different conjugation approaches influence the amount of targeting ligand on individual nanoparticles.

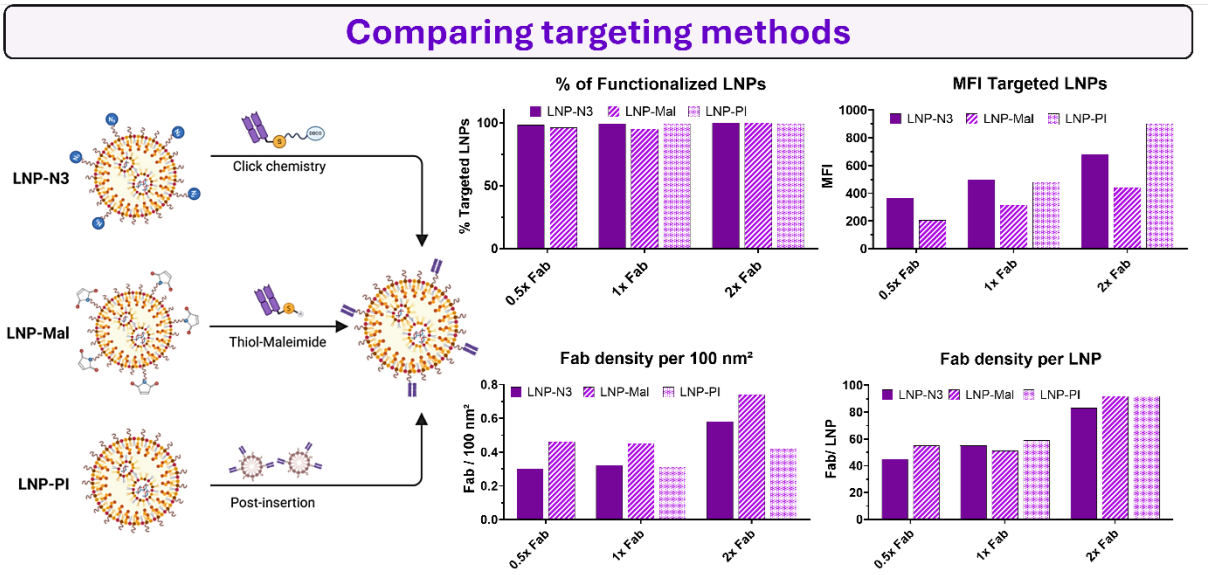

Figure 3. Comparative Analysis of LNP Conjugation Strategies Using Advanced Single-Particle Metrics

Nano-flow cytometry (nFCM) is an indispensable single-particle tool: it quantifies accessible ligand density and function, reveals effects of conjugation chemistry, and profiles cargo distribution across individual particles. By exposing heterogeneity in targeted LNPs, nFCM anchors structure–property–function mapping and strengthens quality control. Another single particle technology, known as Single Particle Automated Raman Trapping Analysis, combining optical trapping and Raman spectroscopy, provides rapid, label-free, and composition-specific measurements of individual lipid nanoparticles. This technic enables the detailed compositional analysis of single LNPs and allows for the determination of formulation heterogeneity and facilitates the quantification of subtle compositional changes.[48,49] To extend these single-particle readouts, bulk orthogonal techniques can give supplementary information. Size-exclusion chromatography (SEC) enables analysis and purification without deformulation.[41,50] In turn, surface plasmon resonance (SPR) offers label-free, real-time kinetics for ligand–receptor interactions, delivering association/dissociation rates and apparent KD values.[51] Together, nFCM, Raman, SEC, and SPR provide a coherent analytical toolkit, linking particle composition and surface presentation to biological performance, and accelerating development of next-generation targeted nanomedicines.

mRNA Sequence-Level Targeting

Beyond LNP surface modifications, mRNA design itself can contribute to cell-specific expression, providing an additional layer of specificity. This strategy ensures that even if the LNP payload is delivered off-target, the mRNA is only translated in the desired cell types. One strategy is de-targeting using MicroRNA (miRNA) target sites. By incorporating specific miRNA target sites into therapeutic mRNAs, translation can be suppressed in healthy tissues where those miRNAs are abundant, while allowing expression in diseased cells where these miRNAs are downregulated.[52] For instance, miR-122 target sites can prevent mRNA translation in healthy hepatocytes (liver de-targeting), and miR-142 sites can restrict expression in immune cells. Furthermore, "Multiorgan Protection" (MOP) sequences can confine mRNA vaccine expression to the injection site, reducing off-target expression in various organs.[53] An emerging approach for active translation involves structure-based strategies, such as toehold switches embedded in the 5' untranslated region (UTR) of the mRNA, which form hairpin structures that block ribosomal access, only unfolding and allowing translation in the presence of a specific "trigger RNA" expressed by the target cell.[54,55] Other approaches involve ADAR enzymes (Adenosine Deaminases Acting on RNA) that enable RNA-level recoding by catalyzing the deamination of adenosine to inosine within double-stranded RNA, which is subsequently translated as guanosine.[56] This natural process is leveraged in engineered platforms like CellREADR and RADAR, which employ specific RNA sensor domains to conditionally translate therapeutic payloads in target cells, offering a highly selective and programmable strategy based on endogenous RNA signatures.[57,58] These mRNA-level strategies are particularly crucial for gene editing applications to minimize unintended genomic changes in off-target cells.

Target Organs, Cell Types, and Therapeutic Domains

LNPs span many organs and cell types, enabling broad therapeutic reach. The liver remains the hub, with hepatic tropism enabling a “production-factory” model for systemic protein secretion. The spleen, lungs (asthma, COPD, cystic fibrosis), and lymph nodes (vaccines) are likewise important. Crossing the blood–brain barrier is challenging, but ligand strategies are advancing (e.g TfR). Other relevant tissues include heart, kidney, smooth muscle, and endothelium, particularly for rare diseases (e.g., muscular dystrophies). In oncology, intratumoral dosing can drive local uptake, while systemically delivered LNPs are being tuned to recruit and program immune cells within the tumor microenvironment.[4,14,59,62]

mRNA–LNPs deliver complex, transient programs across immune and somatic targets, enabling vaccination, gene editing, and in vivo cell programming. In cancer, LNPs reprogram immunity, inducing transient CAR-T, driving BiTE secretion, or through NK cell manipulation, with targeted LNP formulations standing as a landmark achievement for in vivo CAR-T cell reprogramming for hematologic malignancies.[63,65] Outside oncology, LNPs deliver CRISPR components for in situ correction or protein expression; notably, a personalized CRISPR therapy edited hepatocytes in an infant with CPS1 deficiency.[3,66,67] Vaccination remains the most validated use case (e.g., COVID-19), while antibody-encoding mRNAs extend antiviral options.[68] Finally, regenerative payloads, growth factors or cytokines such as BMP-2, promote tissue repair and regeneration.[69]

Challenges and Emerging Trends

Despite significant advancements, several key hurdles remain, notably achieving reproducible, robust characterization, and large-scale manufacturing for clinical translation. The "PEG dilemma", involving anti-PEG antibody formation and accelerated blood clearance upon repeated administration, also represents an ongoing challenge especially for repeated therapeutic use of LNPs.70 Furthermore, the success of mRNA delivery significantly depends on both the design of the lipid-based nanoparticles and the biological state of the target cell, necessitating continuous refinement of lipid composition and formulation methods, alongside an understanding of the cell's activation status, metabolic profile, and differentiation stage. Mechanistic studies, e.g., nano-flow cytometry, RNA-interaction mapping, protein corona profiling, clarify structure–function and biodistribution. The EMA's evolving quality guidance for mRNA vaccines underscores how critical such assays are. With targeted LNPs, complexity increases further, making standardized, benchmarked characterization essential for safety, consistency, and clinical reliability. The field of targeted LNP delivery for mRNA therapeutics is rapidly evolving, driven by technological advancements and deeper biological understanding. Artificial Intelligence (AI) and computational methods are becoming instrumental in the rational design and optimization of LNPs, enabling a shift from traditional trial-and-error to data-driven "fit-for-purpose" LNP design.[71] AI models not only predict LNP behavior, but also accelerate the identification of novel lipid components and guide the optimization of formulation parameters, thereby streamlining the development process.[72,75] Complementing these approaches, computational methods such as molecular dynamics simulations offer atomistic insights into LNP organization, self-assembly, and lipid–cargo interactions, providing a level of mechanistic understanding that often remains inaccessible through experimental techniques alone.[76]

Targeted LNPs are moving from concept to clinical infrastructure. They have already transformed vaccines and now enable systemic gene editing and in situ immune programming across organs. Recent consolidation in 2024-2025 reflects this trajectory, as AbbVie acquired Capstan potentially securing in vivo CAR T capabilities, Lilly agreed to acquire Verve, Novo Nordisk entered a multi-target partnership with NanoVation to pursue extrahepatic long-circulating LNP delivery, BioNTech's acquisition of CureVac, Moderna's collaborations with Life Edit and Generation Bio, and ReCode's inhaled SORT LNP programs, and many others. In 2025, a personalized LNP-CRISPR therapy edited hepatocytes in an infant with CPS1 deficiency, offering real-world proof of feasibility. The future of therapeutic nanomedicines lies in Smart Delivery: designing and decoding targeted lipid nanoparticles that unite organ specificity, repeat-dose performance, rigorous analytics, and GMP scalability. Statements: All authors are Sanofi employees and shareholders. The authors declare that generative AI was used to refine the text for grammar, spelling, and clarity. All figures were created with BioRender.com.

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FROM DISCOVERY TO CLINIC: BRIDGING SCALES IN RNA-LNP MANUFACTURING

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1 The rise of RNA-LNPs

The past decade has witnessed RNA-LNP technologies move from concept to clinic, with mRNA vaccines against COVID-19 marking their most transformative milestone. The success of these vaccines has validated LNPs as the leading delivery platform for nucleic acids, offering biocompatibility, versatility, rapid development timelines, and scalability. [1] Beyond infectious diseases, LNP-mediated drug delivery also holds great promises for immunotherapy, oncology, rare diseases, and gene therapy. [2,3] Industry pipelines are expanding at an unprecedented pace, with LNP platforms progressing through all stages of R&D and clinical testing. In this context, achieving consistent particle properties and robust scale-up is a key factor in translating LNP technology from the laboratory to patients.

Within this landscape, this review examines current RNA–LNP manufacturing strategies, outlining their strengths and limitations, highlights the scaling challenges that impede translation, and introduces two platforms from Inside Therapeutics—TAMARA for R&D and NanoPulse for seamless production across all scales.

2 Manufacturing LNPs

2.1 Impact of manufacturing techniques on LNP quality

The final properties of LNPs depend not only on the choice of lipids, RNA, and solvents but critically on the manufacturing approach. Process parameters influence nearly all critical quality attributes (CQAs), including particle size, polydispersity, surface charge, morphology, encapsulation efficiency and yield. These attributes, in turn, determine pharmacokinetics, cellular uptake, biodistribution, toxicity, and ultimately therapeutic efficacy. Even subtle changes in processing might lead to significant variations in these attributes, emphasizing the need for well-controlled and reproducible production. Selecting the optimal formulation strategy, tailored to the intended route of administration and target tissue, is therefore essential for efficient and reliable LNP drug development. [4]

2.2 Overview of LNP manufacturing approaches

LNP manufacturing landscape has evolved from early bulk methods, such as thin film hydration and ethanol injection, toward more sophisticated approaches, including microfluidics and impingement jet mixing (Figure 1). While bulk approaches offer a rapid and easy way to start formulating, microfluidic platforms enable screening at small scale, and IJMs deliver the throughput required for clinical supply. Each approach has trade-offs in scalability, reproducibility, and accessibility. Therefore, understanding these techniques and their limitations provides a useful framework for guiding both process development and regulatory strategy.

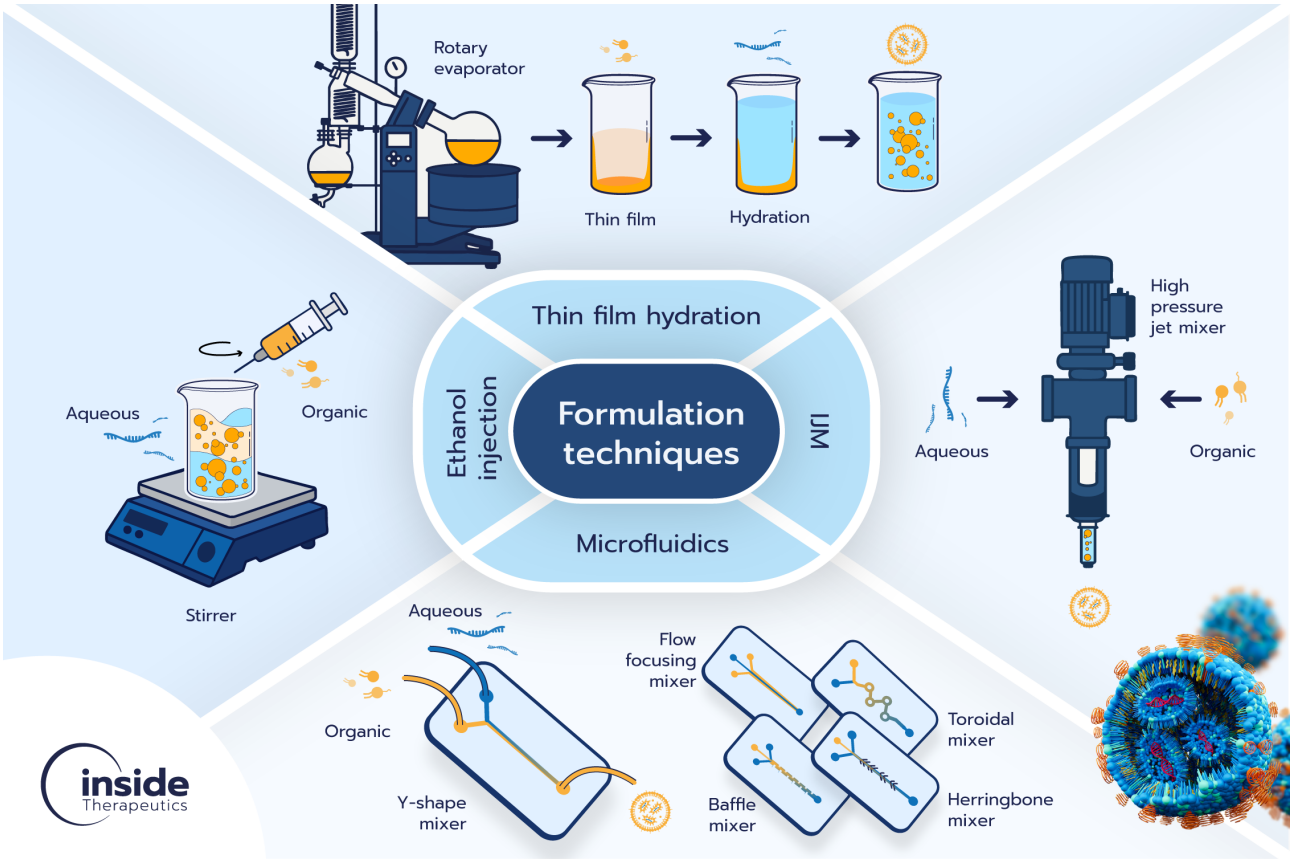

Figure 1. LNP manufacturing methods.

2.2.1 Traditional bulk methods

Early methods for LNP preparation, often referred to as bulk or batch techniques, remain widely used in academic settings. These include thin film hydration and ethanol injection, which provide relatively simple routes for generating nanoparticles.

Thin film hydration is considered the historical starting point for lipid-based nanoparticle production. In this process, lipids are first dissolved in an organic solvent, which is then evaporated to create a thin lipid film on the flask's wall. Upon hydration with an aqueous phase, the lipids self-assemble into multilamellar vesicles. The resulting particles are usually large (>100 nm) and polydisperse, necessitating additional size reduction steps such as extrusion, high-pressure homogenization (HPH), or sonication. Although practical at small volumes, this method is challenging to scale up and suffers from low encapsulation efficiency and poor reproducibility. [4–6]

On the other hand, ethanol injection involves dissolving lipids in ethanol and injecting the solution into an aqueous phase under stirring, where the sudden dilution of ethanol drives spontaneous formation of vesicles. This approach is conceptually simple and reproducible at small scales, but low encapsulation efficiency, batch variability, and poor scalability limit its use beyond early-stage formulation. [6]

2.2.2 Impingement jet mixers

Impingement jet mixers (IJMs) drive two opposing high-velocity fluid streams into direct collision in a confined mixing zone, producing fast and homogeneous mixing. This near-instantaneous turbulent mixing leads to flash nanoprecipitation, enabling the formation of LNPs in a highly controllable and reproducible manner that is essential for clinical applications. [4] Particle characteristics can be finely tuned by adjusting flow rates and mixing chamber geometries. [4,7] Most importantly, IJMs offer a clear route to scale-up through parallelization, enabling continuous, high-throughput production, and translation from bench to industry. As such, IJMs became the method of choice for large-scale mRNA vaccine production during the COVID-19 pandemic, where companies such as Pfizer implemented parallel IJM systems to achieve continuous, GMP-compliant synthesis. [4,6] Commercially, companies such as Knauer provide IJM platforms at multiple scales. [8] Despite these benefits at industrial volumes, relatively large minimum working volumes can limit their utility in early-stage screening studies.

2.2.3 Microfluidic approaches

Microfluidics has revolutionized RNA–LNP production by enabling tightly controlled nanoprecipitation with far greater reproducibility than bulk methods. In microfluidic devices, LNPs are generated at the aqueous–ethanol interface through diffusion-driven solvent exchange. When lipid-dissolving ethanol is diluted below a critical concentration by the RNA-containing aqueous buffer, spontaneous self-assembly yields small nanoparticles. [9] The microscale dimensions promote rapid mixing, as well as efficient mass and heat transfer, collectively resulting in homogeneous LNP populations. By finely tuning process parameters such as aqueous-to-organic flow rate ratio (FRR) and total flow rate (TFR), researchers can systematically modulate particle size, polydispersity, and encapsulation efficiency [4,10]. This level of reproducibility and control is essential for RNA-LNPs, where subtle physicochemical shifts can dramatically alter biological performance. Microfluidic platforms support high-throughput screening and optimization, accelerating discovery-to-preclinical translation. These advantages position microfluidics as a critical enabler of next-generation LNP therapeutics. In the following subsections, we review the principal micromixer architectures employed for RNA–LNP production—including T- and Y-junctions, hydrodynamic flow focusing, staggered herringbone, and toroidal mixers—highlighting their respective advantages and limitations.

2.2.3.1 T- or Y-mixer

T- and Y-mixers represent some of the earliest microfluidic platforms developed for LNPs. In these flat microchannel devices, organic and aqueous streams converge at an orthogonal or angled junction, and nanoparticle formation occurs at the interface through solvent diffusion and dilution. Operating typically at high flow rates (40–60 mL/min), these mixers enable production of larger LNP batches but require relatively high material inputs, making it less suited to discovery studies. Their use is hindered by limited control over particle size and throughput volume. These constraints have motivated the development of more advanced microfluidic geometries that enhance size control and accommodate lower-volume applications. [4]

2.2.3.2 Hydrodynamic flow focusing

By directing an organic lipid solution into a central channel and sheathing it with aqueous buffer streams, hydrodynamic flow focusing (HFF) generates a narrow laminar jet that undergoes rapid diffusion-driven mixing, yielding small (<150 nm) and narrowly distributed nanoparticles. Despite producing uniform RNA–LNPs with tunable sizes, the low throughput of typical chip-based 2D HFF (<10 mL/h) restricts its use beyond small-scale applications, and channel clogging remains a challenge. [4,6] Capillary-based 3D HFF systems have been developed [11], achieving several-fold increases in production capacity, though at the expense of higher device complexity, operational cost, and dilution of formulations. As a result of these limitations, HFF has not achieved the widespread adoption seen with other microfluidic platforms. [4,6]

2.2.3.3 Staggered herringbone micromixer

Among microfluidic designs, the staggered herringbone micromixer (SHM) has proven particularly powerful for RNA–LNP production. The asymmetric herringbone grooves embedded into the channel floor generate transverse flows that induce chaotic advection, enabling near-instantaneous mixing (<10 ms). This rapid diffusion at the organic–aqueous interface produces highly homogeneous RNA–LNPs with tunable sizes (as small as 30 nm) and excellent reproducibility [4,6], offering greater control than T- or Y-junction mixers at comparable operating flow rates. [9] SHM technology has been widely adopted, including in commercial platforms such as those developed by Precision NanoSystems (now part of Cytiva) [12], or Inside Therapeutics, and has become a standard for preclinical RNA–LNP pipelines. Nevertheless, the relatively low throughput of SHM devices (<100 mL/h) constrains their application to early development, creating a bottleneck when moving toward GMP-scale manufacturing. Recent innovations in parallelization [4,6], alternative geometries (e.g., toroidal mixers) [13], and antifouling surface treatments (e.g., perfluorodecalin coating) [14], aim to overcome this limitation, enabling microfluidic mixing technologies to support higher-throughput manufacturing.

2.2.3.4 Toroidal mixers

Toroidal (also known as bifurcating) mixers utilize a series of toroidal channels that repeatedly split and recombine fluid streams to generate rapid and chaotic mixing, producing highly uniform LNPs with tight control over size, polydispersity, and encapsulation efficiency. Compared with traditional SHM mixers, toroidal designs support substantially higher total flow rates (up to 200 mL/min). [4,15] This approach preserves compatibility with early-stage and preclinical production and simplifies the transition to larger-scale manufacturing. [5] The NxGen™ by Precision Nanosystems (now part of Cytiva) platform exemplifies the practical use of this design. [4,15]

2.2.3.5 Baffle mixers

Baffle mixers represent an alternative microfluidic platform for the controlled synthesis of lipid-based nanoparticles. By incorporating a series of perpendicular turns within the microfluidic channels [16], these mixers induce secondary flows, backflow, and recirculation, which enhance the rapid mixing of aqueous and organic streams. This architecture allows precise tuning of LNP size, exemplified by the iLiNP system. [17] Compared with the complex three-dimensional grooved structures of SHM devices, the simpler two-dimensional design of baffle mixers could improve robustness and reduce the risk of channel clogging and flow stagnation. [17]

3 Case study: Comparing different microfluidic LNP formulation approaches

In this section, we evaluate the performance of the TAMARA formulation system (Inside Therapeutics) relative to toroidal mixers, highlighting its capabilities and limitations for RNA–LNP production.

The TAMARA system enables RNA-LNP synthesis from screening volumes (~0.2 mL) up to in vivo scales (~30 mL). It integrates a dual mixer architecture combining a hydrodynamic flow-focusing junction with either staggered herringbone or baffle structures in a single reusable chip. This hybrid configuration enhances mixing efficiency compared with conventional herringbone-only designs. [18] By controlling critical process parameters, including TFR and FRR, TAMARA allows fine-tuning of nanoparticle size (~50–200 nm), polydispersity index (<0.2), and encapsulation efficiency (>98%), with high batch-to-batch reproducibility (Figure 2).

Head-to-head comparisons between TAMARA and toroidal mixers show comparable encapsulation efficiency, with TAMARA achieving higher encapsulation yield at low working volumes (e.g., >90% at 700 µL versus ~75% for toroidal mixers). In vitro evaluation demonstrated similar transfection efficiency and cell viability across systems, while TAMARA-formulated LNPs exhibited up to 30% higher protein expression (Figure 3).

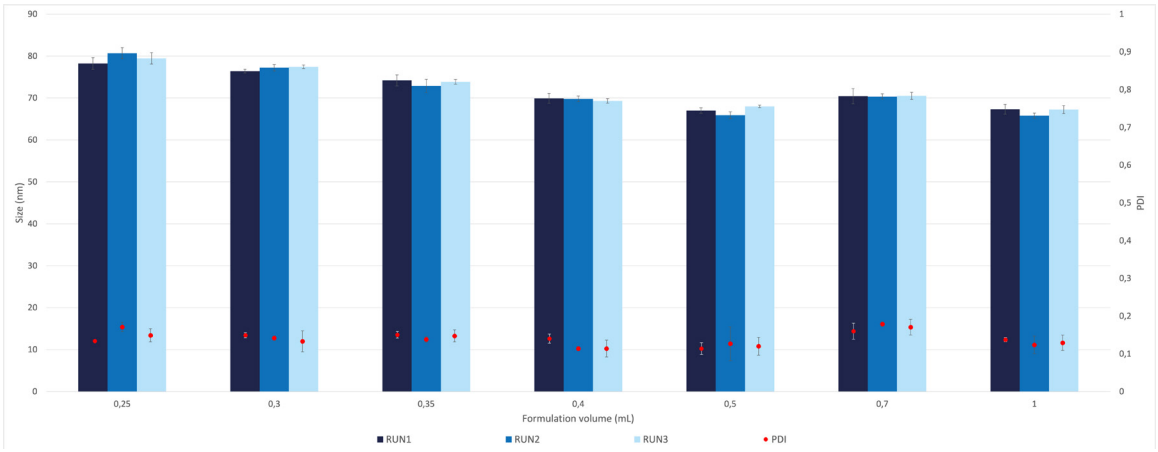

Figure 2. Batch-to-batch uniformity in RNA-LNP synthesis with TAMARA at sub-milliliter scales

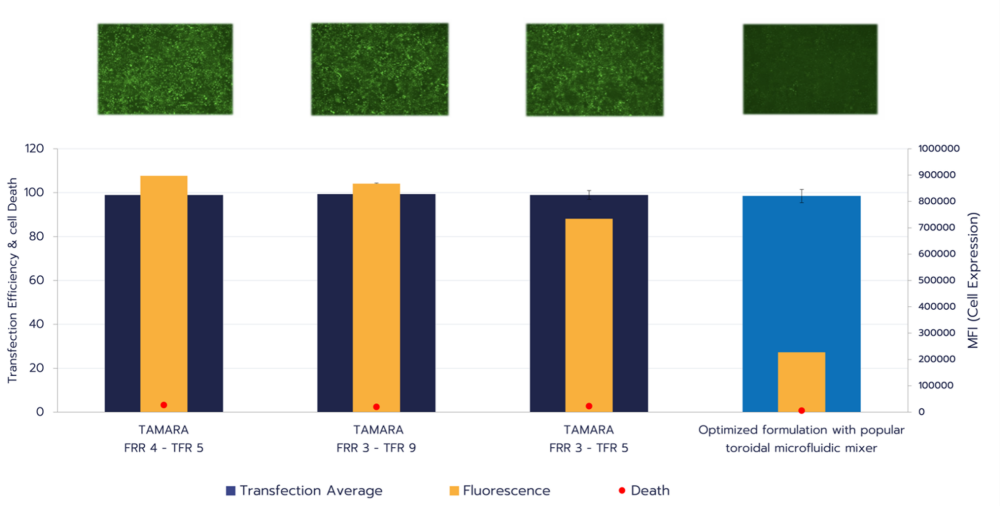

Figure 3. TAMARA vs. toroidal mixer: Fluorescence microscopy images (top) and quantitative analysis of transfection efficiency, fluorescence-based protein expression, and cell death (bottom).

Taken together, these findings illustrate how microfluidic design impacts formulation outcomes. However, throughput limitations remain a key constraint for translation to GMP manufacturing. To place this in context, the next section will discuss scaling challenges across different RNA–LNP manufacturing technologies.

4 Scaling challenges across manufacturing techniques

The development of RNA-LNP therapeutics is critically constrained by the absence of a single formulation technology that spans the entire pipeline—from microliter-scale discovery to large-scale clinical manufacturing. Microfluidic devices dominate early discovery, yet these systems face throughput and scalability limitations, making them unsuitable for clinical production. Conversely, IJMs are well established for high-volume manufacturing, but they struggle to handle the low-volume formulations needed for early-stage screening. [4,6] Current workflows typically require transitioning between different mixing platforms as volumes increase, which can alter CQAs of nanoparticles. Each transition necessitates re-optimization and re-validation, slowing down therapeutic development and increasing the risk of failure. A single, scalable technology capable of spanning all scales of production is therefore essential to overcome operational and regulatory challenges. In the next section, we introduce NanoPULSE (Inside Therapeutics), a technology designed to address this gap.

5 NanoPULSE: A new platform bridging early development and clinical manufacturing workflows

NanoPULSE, a patented micromixing technology developed by Inside Therapeutics, provides a unified platform for RNA-LNP formulation across all scales. It employs high-frequency valve actuation to sequentially inject aqueous and organic solvent fringes into a T-junction. The fringes quickly diffuse into one another through Taylor–Aris dispersion (Figure 4a). This pulse-driven, self-cleaning design enables precise control of mixing dynamics while avoiding aggregation, ensuring reproducible nanoparticle synthesis from microliter-scale screening to continuous tens-of-liters production. Lagrangian simulations, implemented in Python, were used to model how input parameters influence nanoparticle formation and to refine channel geometry. These studies also introduced a novel index to quantitatively assess mixing efficiency, providing a predictive framework for nanoparticle synthesis with NanoPULSE.

The key advantage of NanoPULSE lies in its scalability. Proof-of-concept studies demonstrated continuous liposome manufacturing up to ~40 L/day, with dynamic light scattering (DLS) confirming consistent particle size and polydispersity (<5% variation) from 500 µL to 4 L (Figure 4b). Moreover, tuning the pulse frequency enables modulation of RNA-LNP size (Figure 4c), highlighting platform's flexibility for diverse therapeutic applications. Encapsulation efficiency and structure of these RNA-LNPs were comparable to those synthesized using TAMARA.

By bridging discovery-scale experimentation with clinical-scale manufacturing, NanoPULSE has the potential to overcome two major bottlenecks in RNA-LNP development: variability induced during scale-up and aggregation during continuous production. Its scalability, reproducibility, and efficiency accelerate RNA-LNP therapeutic development while reducing regulatory complexity during scale-up.

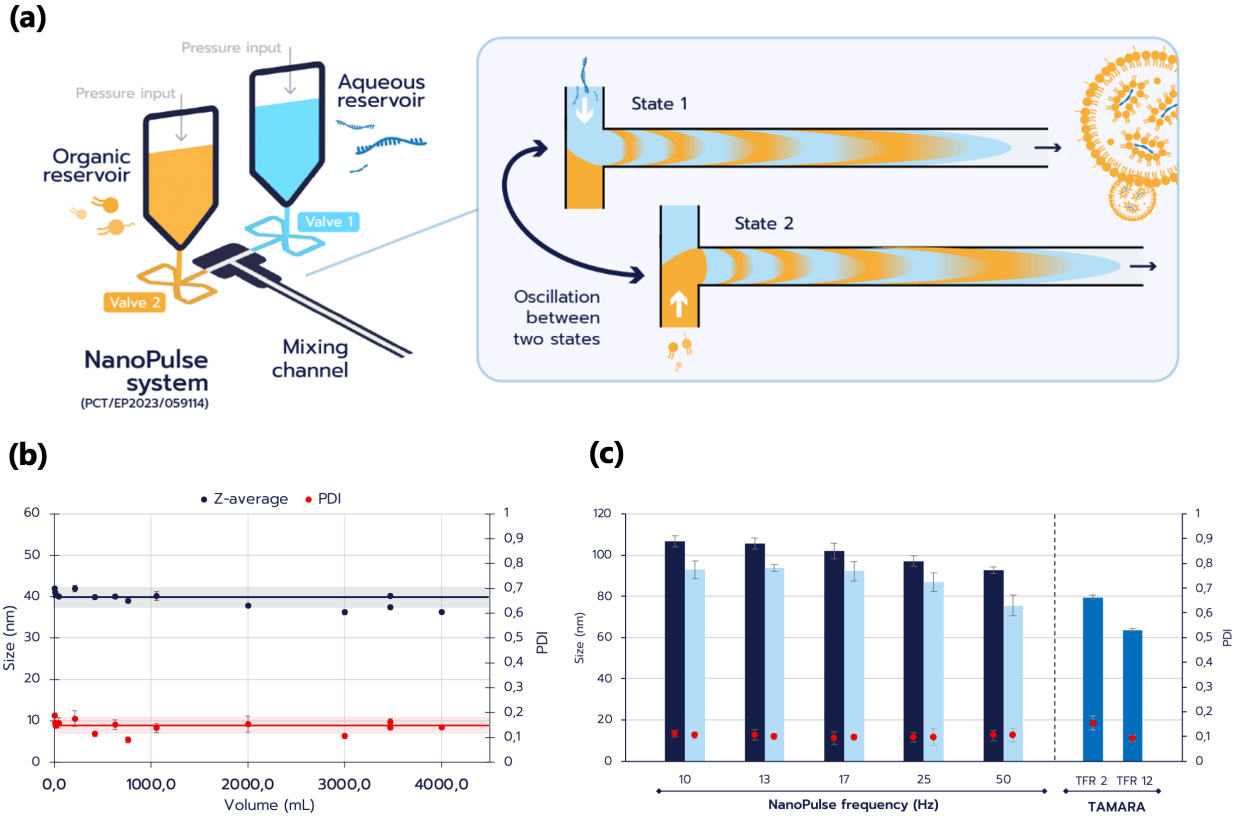

Figure 4. NanoPULSE technology for scalable RNA–LNP production. (a) NanoPULSE mixing principle based on alternating solvent pulses and Taylor–Aris dispersion. (b) Consistent liposome size and PDI during continuous production. (c) Effect of pulse frequency on RNA-LNP particle size and PDI and comparison with standard microfluidics.

6 Conclusion

The LNP landscape continues to evolve rapidly, driven by the expanding application of RNA-based therapeutics beyond vaccines. Despite remarkable progress, several challenges remain in the industrial development of RNA-LNPs, including formulation stability, long-term storage, and compliance with regulatory and quality standards. [4] These hurdles are further complicated by the growing demand for continuous and reproducible manufacturing processes capable of maintaining CQAs across scales. Current technologies are lacking in this respect, fragmenting the development process and slowing down the translation process. Consequently, there is an increasing emphasis on robust, flexible, and scalable platforms that can streamline the transition from early discovery to clinical production while satisfying regulatory expectations.

Within this context, microfluidic-based methods represent a reliable preclinical platform, enabling low-volume screening with reproducible nanoparticle characteristics, minimal reagent consumption, and rapid optimization of formulation parameters. In parallel, NanoPULSE seamlessly translates formulation principles from microliter-scale discovery to multi-liter clinical production, supporting the full continuum of RNA-LNP development.

By enabling scalable and high-quality production of nanoparticles, NanoPULSE paves the way for the next-generation of RNA therapeutics—including cancer immunotherapies, gene-editing applications, and protein replacement strategies. By reducing the barriers between bench-scale discovery and clinical translation, this technology has the potential to accelerate therapeutic development, streamline regulatory pathways, and ultimately expand the accessibility and impact of RNA-based medicines.

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