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Lipid nanoparticles for mRNA delivery: from rational design and manufacturing to clinical translation

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Abstract

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The clinical success of COVID-19 mRNA vaccines established lipid nanoparticles (LNPs) as a leading platform for *in vivo* RNA delivery, but their therapeutic potential extends far beyond vaccination. This review provides an integrated perspective on LNP-RNA medicines by linking five interconnected dimensions: historical development, RNA payload engineering, LNP design, manufacturing, and clinical translation. We summarize how nucleoside modification, RNA structural optimization, and emerging formats such as circular RNA and self-amplifying RNA have expanded the functional scope of therapeutic payloads. We then examine the design principles of LNPs, with emphasis on ionizable lipids, formulation composition, and microfluidic manufacturing as the basis for reproducible large-scale production. We further distinguish clinically validated applications, including hepatic RNA delivery and intramuscular mRNA vaccination, from newer but less mature directions such as protein replacement, *in vivo* gene editing, and *in vivo* CAR-T generation. Importantly, this review highlights the major barriers that now limit broader translation, including extrahepatic targeting, incomplete understanding of protein corona-mediated delivery, weak cross-species predictability, repeat-dosing challenges, and evolving regulatory requirements. Overall, this review defines the key principles and translational challenges that will shape next-generation LNP-RNA therapeutics.

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1. Introduction

RNA therapeutics have become an important drug modality because they allow transient and programmable control of protein expression or gene regulation. In principle, the therapeutic product can be changed by altering the RNA sequence, which gives this class of medicines a common development logic across different indications. However, the clinical translation of RNA has long been limited by several barriers: RNA is chemically unstable, is rapidly degraded in biological fluids, shows poor membrane permeability, and can activate innate immune sensing pathways.

Progress in the field depended on advances in both payload design and delivery technology. On the payload side, nucleoside modification, control of RNA impurities, and optimization of cap structure, untranslated regions, coding sequence, and poly(A) tail improved translation, reduced immunostimulation, and made *in vitro*-transcribed RNA more suitable for therapeutic use. On the delivery side, lipid-based systems evolved from early liposomes and permanently cationic transfection reagents to ionizable lipid nanoparticles (LNPs), which support RNA encapsulation, systemic administration, cellular uptake, and endosomal escape with improved tolerability. These developments established the design principles that now define clinically useful RNA delivery systems.

Clinical translation has validated this platform. The approval of patisiran showed that systemically delivered RNA formulated in LNPs can meet regulatory requirements for quality, safety, and efficacy. The subsequent success of COVID-19 mRNA vaccines demonstrated that LNP-mRNA products can also be developed and manufactured at global scale. At the same time, current applications are moving beyond hepatic siRNA delivery and intramuscular vaccination toward protein replacement, cancer immunotherapy, *in vivo* gene editing, and *in vivo* immune-cell programming. These newer applications impose stricter requirements on delivery specificity, repeat dosing, safety,

and manufacturability. Important limitations therefore remain, including liver-dominant biodistribution after intravenous administration, toxicity and immunogenicity associated with some lipid components or repeated dosing, incomplete understanding of structure-function relationships, and weak translation of preclinical findings across species.

Several reviews have addressed individual aspects of this field, such as mRNA chemistry, LNP composition, or selected therapeutic applications, but these topics are typically discussed in isolation. This review provides an integrated framework linking historical development, RNA payload engineering, LNP design and manufacturing, and clinical translation. By connecting these dimensions, we aim to clarify both the key principles underlying current LNP-RNA medicines and the major translational barriers, including extrahepatic targeting, repeat-dosing challenges, weak cross-species predictability, and evolving regulatory requirements, that will shape next-generation therapeutics.

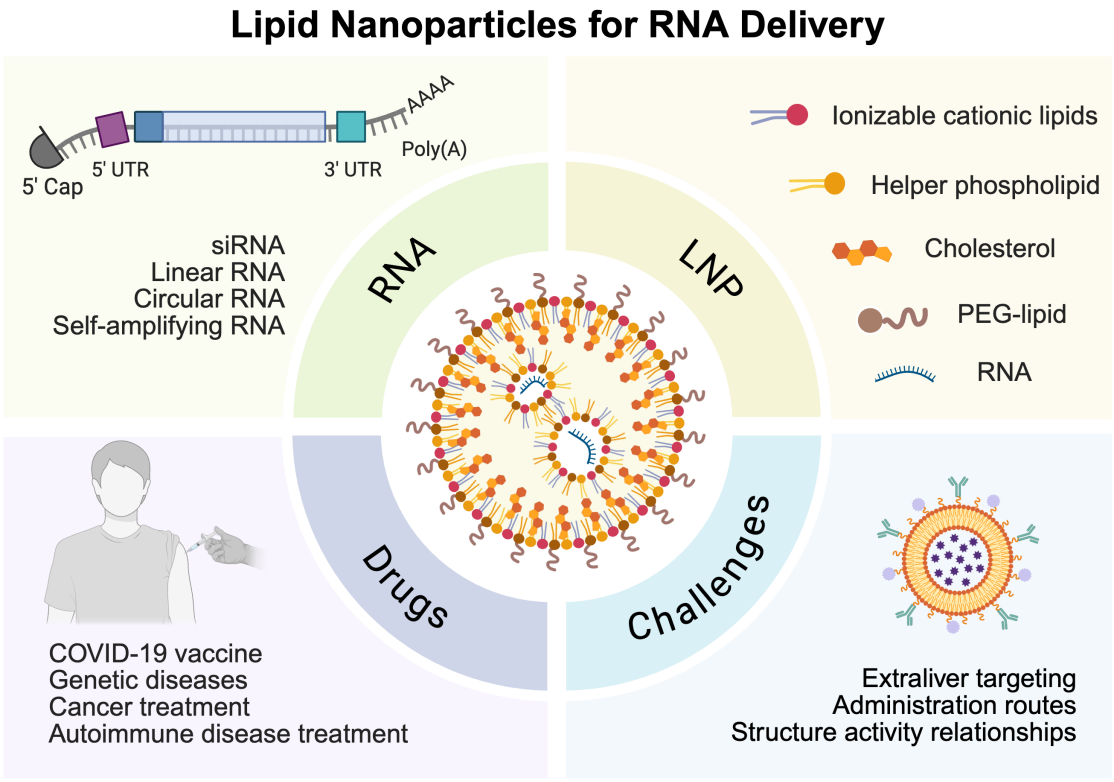

Fig. 1 Lipid nanoparticles for RNA delivery

2. Literature Search Methodology

We searched PubMed, Web of Science, and Google Scholar for articles published from 1961 to 2026, using key words including lipid nanoparticles, mRNA therapeutics, ionizable lipids, RNA delivery, and mRNA vaccines. We prioritized peer-reviewed original research articles, clinical trial reports, regulatory documents, and seminal reviews that established foundational concepts or reported clinical milestones. Studies were included if they contributed to understanding LNP design principles, RNA payload engineering, manufacturing processes, or clinical translation.

3. Historical Evolution of RNA Therapeutics and LNPs

RNA medicine combines biological insights into messenger RNA with engineering advances that protect these fragile molecules in the body [1]. In 1961, two studies in *Nature* identified an "unstable intermediate" carrying genetic information to ribosomes [2, 3]. This molecule, later named mRNA, formed the basis of the Central Dogma and decades of biotechnology research.

From "unstable intermediate" to a drug modality

Early attempts to use RNA as a therapeutic drug faced major obstacles: exogenous RNA is rapidly degraded by widespread RNases and triggers strong immune reactions. Experiments in 1980s demonstrated that *in vitro*-transcribed RNA could drive protein expression in mammalian systems, providing proof-of-principle that RNA could function as an *in vivo* expression cassette [4]. In parallel, the discovery of RNA interference (RNAi) in 1998 established it as a gene silencing method, further expanded RNA-based medicines beyond protein replacement and vaccination [5] (Table 1).

By the 2000s, the main challenges for RNA drug development were physicochemical and immunological: RNA needed protection from degradation, efficient cellular

entry, cytosolic access, and a means to avoid excessive innate immune activation. Two key studies provided solutions for mRNA drugs. First, Karikó and Weissman showed that incorporating naturally occurring nucleoside modifications can markedly suppress RNA recognition by Toll-like receptors (TLRs), directly addressing inflammatory reactogenicity [6]. Second, follow-up work demonstrated that pseudouridine incorporation can further enhance translational capacity and biological stability [7]. Collectively, these advances fundamentally solved the druggability issue of mRNA, a breakthrough eventually recognized with the 2023 Nobel Prize in Physiology or Medicine.

The parallel history of lipid carriers: from liposomes to LNPs

In drug delivery, the origin of LNPs can be traced to liposomes. Bangham and colleagues' early electron microscopy work in the mid-1960s established that phospholipids can self-assemble into closed bilayer structures [8, 9], creating a versatile platform for encapsulation and controlled release. While classical liposomes were initially used for small molecules, a turning point for nucleic acids occurred in the 1980s with synthetic cationic lipids. Felgner and colleagues introduced a highly efficient lipid-mediated DNA transfection method (lipofection), and shortly thereafter similar cationic lipid systems were shown to enable RNA transfection across diverse cell types [10, 11]. These studies established a practical principle that still underlies LNP engineering: electrostatic complexation can package nucleic acids into nanoscale assemblies that cells will internalize. However, permanently cationic lipids often carry narrow therapeutic windows *in vivo* (toxicity, complement activation, nonspecific interactions), motivating the later transition toward ionizable lipids that are near-neutral at physiological pH but become protonated under acidic conditions, such as during endocytosis.

Ionizable lipids and clinically viable LNP

RNAi breakthroughs in the 2000s catalyzed a shift from local to systemic nucleic acid delivery at therapeutically relevant doses. Researchers prioritized overcoming the physiological barriers, including serum instability, RES clearance, and endosomal entrapment [12], while rigorously refining performance metrics for clinical utility. Within this framework, rational engineering of ionizable lipids yielded LNP formulations that enabled potent *in vivo* delivery with improved tolerability. In particular, Semple and colleagues (2010) optimized ionizable lipid structure, helping to establish the modern LNP paradigm: an ionizable lipid for encapsulation and endosomal escape, helper phospholipid for structure, cholesterol for membrane integrity, and a PEG-lipid for colloidal stability and controlled pharmacokinetics [13].

In parallel, manufacturing evolved from variable batch mixing to reproducible, scalable microfluidic processes. By enabling millisecond-scale mixing, microfluidics yielded LNPs with high encapsulation efficiency, precise size control, and superior batch consistency, prerequisites for industrial scale-up and rapid formulation iteration [14]. Structural work on microfluidic-made LNPs further supported that these are not simple hollow vesicles but nanostructured assemblies whose internal organization depends on lipid ionization state and mixing kinetics [15].

Clinical applications of LNP-RNA medicine

Clinical translation provided the validation of LNP-enabled RNA delivery. In 2018, the U.S. FDA approved patisiran (Onpattro), widely recognized as the first approved RNAi therapeutic and a major milestone for systemically delivered RNA formulated in lipid nanoparticles [16]. This approval was not merely a single-product success; it signaled that LNP composition, characterization, and manufacturing controls could satisfy regulatory standards for a complex nanomedicine.

The COVID-19 pandemic then accelerated the field dramatically. Phase 3 trials reported that two LNP‑mRNA vaccines, BNT162b2 and mRNA-1273, achieved high efficacy with acceptable safety profiles, demonstrating at a large scale that the mRNA‑LNP platform can be rapidly designed, manufactured, and deployed in humans [17 - 19]. Importantly, these outcomes reflected the cumulative maturation of the technology: optimized mRNA (sequence, untranslated regions, and nucleoside chemistry) and highly engineered LNPs (ionizable lipid systems plus scalable manufacturing). Thereafter, research into LNP-RNA drugs accelerated rapidly, focusing on optimizing RNA constructs and refining targeted delivery. These technologies were extensively explored for treating a wide array of conditions, such as influenza, tumors, and autoimmune diseases.

Table 1 Milestones of RNA Therapeutics

Year	Key Milestones
1961	mRNA experimentally established as an "unstable intermediate" linking genes to ribosomes.
1964–1965	Liposomes described and characterized as closed phospholipid bilayers.
1987–1989	Cationic lipid lipofection enables DNA and RNA transfection.
1990	*In vivo* expression after direct administration of nucleic acids provides early *in vivo* feasibility signals.

Table 1 Milestones of RNA Therapeutics (Continue)

Year	Key Milestones
1998	RNAi discovered, expanding RNA therapeutics to gene silencing.
2005–2008	Nucleoside modifications suppress innate recognition and improve mRNA translation/stability.
2010–2012	Ionizable lipid LNP design and microfluidic manufacturing enable potent, reproducible *in vivo* delivery.
2018	FDA approval of patisiran validates systemically delivered RNA–LNP medicines.
2020–2021	Phase 3 data for LNP–mRNA vaccines establish broad clinical proof for the platform.

4. Payload Engineering: How to Design a Therapeutic mRNA

As the central payload delivered by lipid nanoparticles, mRNA performance is determined largely by precise molecular design. An ideal therapeutic mRNA must meet three core requirements: it should remain structurally stable *in vivo*, minimize recognition and clearance by the immune system, and efficiently initiate translation to drive robust protein production. Achieving these goals relies on integrated engineering including chemical modification strategies, optimized sequence and structure, and continued exploration of alternative mRNA architectures. Comprehensive molecular optimization of mRNA is therefore foundational for the clinical translation of LNP-mRNA therapeutics.

The production of clinical-grade mRNA generally comprises three tightly linked modules: *in vitro* transcription (IVT), downstream purification, and analytical quality control/release testing [20]. In IVT, a linear DNA template containing the coding region together with engineered 5′ and 3′ UTRs and a defined poly(A) sequence is transcribed, most commonly by T7 RNA polymerase; modified nucleosides such as N1-methylpseudouridine (m1Ψ) are often incorporated to improve expression and reduce innate immunogenicity [21]. Co-transcriptional capping with trinucleotide cap analogs such as CleanCap is widely used to generate Cap-1 structures in a one-pot reaction. Following IVT, purification is required to remove template DNA, abortive or truncated RNA species, enzymes, unincorporated nucleotides, and immunostimulatory dsRNA byproducts; HPLC-based purification remains a widely used high-purity benchmark [22], while cellulose chromatography provides a scalable alternative for dsRNA depletion. Final QC relies on orthogonal assays, including LC-MS- or RNase H-based methods for capping efficiency, J2-based immunoassays for dsRNA, capillary electrophoresis or LC-MS-based methods for poly(A)-tail characterization, and microfluidic or capillary electrophoresis platforms for overall RNA integrity.

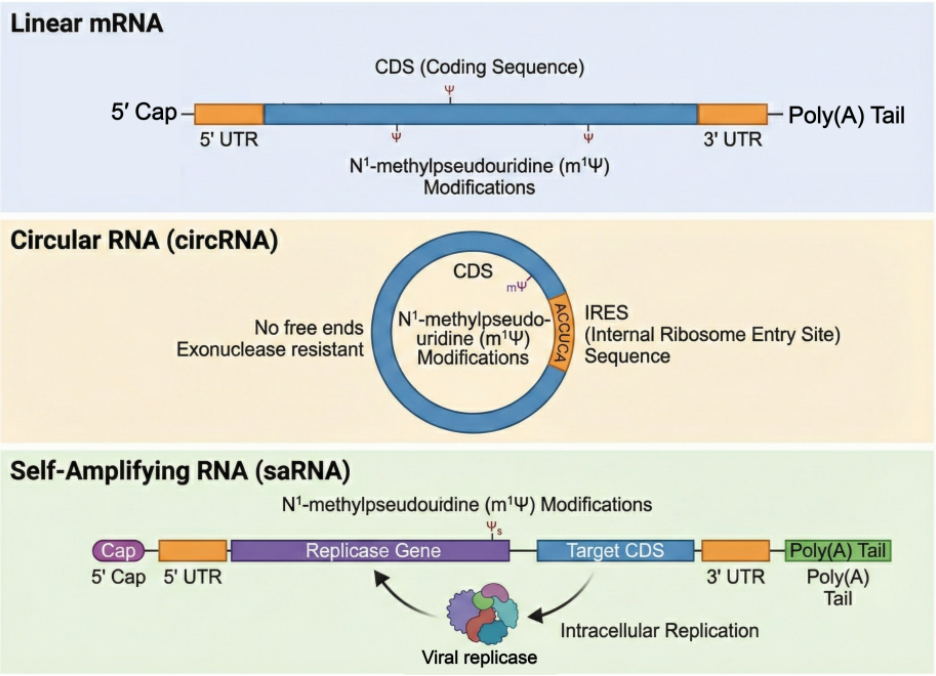

Fig. 2 Structure and types of therapeutic RNA

Chemical modification strategies

A central challenge for IVT RNA is that it can be recognized as "non-self," triggering TLR and cytosolic RNA-sensor pathways that increase inflammatory signaling and reduce translation. Early work demonstrated that incorporating naturally occurring modified nucleosides markedly reduces TLR-driven activation, establishing nucleoside chemistry as a core design axis [6]. Among these, pseudouridine (Ψ) improves translational capacity and biological stability, and reduces innate activation compared with unmodified uridine [7]. Subsequent studies highlighted N1-methylpseudouridine (m1Ψ) as a frequently superior option for maximizing protein output while minimizing immune response—contributing to its adoption in multiple clinical mRNA products [23 - 25].

Manufacturing-related impurities are equally important. IVT reactions can generate dsRNA species and other byproducts that strongly stimulate innate sensors and suppress translation. HPLC purification can remove these contaminants and has been shown to reduce immune activation and improve translation of nucleoside-modified mRNAs [22]. Because these effects depend on both sequence, chemistry and process, payload development typically integrates nucleoside selection with downstream purification and analytical quality control, such as detection of capping and dsRNA contaminants [26].

mRNA structure and sequence design (Cap–UTRs–CDS–poly(A))

Beyond chemical modification, the sequence composition and molecular architecture of mRNA profoundly influence its stability and translational efficiency. Targeted design of the key structural domains—5′ cap, untranslated regions (UTRs), coding sequence (CDS), and poly(A) tail—can further optimize function, enable efficient translation while maintaining stability *in vivo*.

5′ cap selection and capping method. The 5′ cap protects RNA from degradation and supports translation initiation. Cap structure also affects innate restriction: interferon-induced proteins such as IFIT family members can preferentially bind cap 0 RNAs and inhibit their translation, which motivates use of cap 1 structures in many therapeutic designs [27]. For manufacturing, co-transcriptional capping has become widely used because it reduces steps and can improve product uniformity; CleanCap-based methods have been reported to produce high fractions of cap 1 RNA and are used in common workflows for IVT. Cap analog innovations, including anti-reverse cap analogs (ARCA), also contributed to improved capping orientation and translation efficiency in earlier generations of co-transcriptional capping [27, 28].

UTR engineering. The 5′ and 3′ untranslated regions influence translation efficiency and RNA stability through effects on initiation, ribosome scanning, and interactions with RNA-binding proteins. High-performing UTRs are often derived from highly expressed human transcripts (for example, α-globin genes), whose sequence features have been selected through evolution to support efficient translation and favorable mRNA stability. High-throughput screening and rational design have shown that UTR selection alone can produce multi-fold differences in protein output for an identical coding sequence, and that application-tailored synthetic UTRs can outperform commonly used standard UTR sets in specific contexts [29]. More recently, model-guided approaches have enabled prediction and generation of higher-performing UTRs, shifting the field from screening toward data-driven optimization [30, 31].

CDS optimization. The coding sequence can be optimized without changing the protein sequence by adjusting synonymous codons, GC content, and predicted RNA secondary structure [32]. A major goal is to improve translation efficiency and increase functional mRNA stability while avoiding sequence features that increase innate sensing or translation errors [33]. An algorithmic approach (LinearDesign) demonstrated that simultaneous optimization of codon usage and RNA structure can improve mRNA stability and expression [34], illustrating that CDS design needs to optimize both structural stability and codon usage. Machine learning and deep learning tools are now widely applied to CDS optimization, enabling higher-dimensional exploration of sequence–expression relationships and accelerating data-driven design [31, 35, 36].

Poly(A) tail design. Poly(A) tails support mRNA stability and translation through interactions with poly(A)-binding proteins and translation initiation factors. In clinical products, poly(A) length is commonly set in the ~100–150 nt range: tails that are too short can accelerate exonuclease-driven decay and truncate the expression window, whereas excessively long tails may increase steric bulk [37], potentially affecting LNP encapsulation efficiency and complicating manufacturing and purification. Reviews in RNA biology and mRNA vaccine design emphasize that poly(A) length and composition influence half-life and protein output, and that the tail must be tightly controlled to ensure batch-to-batch consistency [38, 39].

Alternative RNA formats: linear, circular, and self-amplifying

Although chemically modified and sequence-optimized linear mRNA has achieved clinical success, it still has practical limitations, including a limited duration of protein expression, and in some settings, relatively high dose requirements. To broaden the scope of mRNA therapeutics, researchers have developed alternative RNA architectures, including circular RNA (circRNA) and self-amplifying RNA (saRNA), that seek to overcome inherent shortcomings of linear mRNA (Table 2) [40, 41].

CircRNA: a stable carrier for extended expression. Circular RNA is a covalently closed RNA molecule generated through engineered circularization reactions. Its key advantage is the absence of free ends, which removes

the typical entry points for exonuclease attack. In linear mRNA, degradation is often initiated from terminal structures at the 5′ and 3′ ends, whereas circRNA has no exposed termini, making it intrinsically more resistant to exonuclease-mediated decay. As a result, circRNA can exhibit a markedly prolonged *in vivo* half-life, and the protein-expression window may extend from several days (typical of linear mRNA systems) to weeks, or in some contexts even months [41 - 43]. In addition, circRNA can support translation without relying on the canonical 5′ cap and 3′ poly(A) tail, and its circular topology may reduce ribosome drop-off during elongation, further improving translational output. These properties make circRNA particularly attractive for indications that benefit from sustained protein production, such as protein replacement therapy for chronic diseases, where longer expression could reduce dosing frequency and improve patient adherence.

SaRNA: a high-efficiency format for low-dose, high-level expression. Self-amplifying RNA is inspired by viral genomes. Beyond encoding the target protein coding sequence, saRNA carries a virus-derived replicase gene. Once delivered into cells, saRNA can serve as a template for immediate translation of the encoded protein; simultaneously, the replicase initiates intracellular RNA replication, leading to an exponential increase in saRNA copy number [44]. This enables a low input dose, high output expression profile [40]. Compared with conventional linear mRNA, saRNA doses can often be reduced by one to two orders of magnitude. Dose sparing can lower the amount of LNP material required, potentially reducing formulation-related toxicity risk, and can also substantially decrease manufacturing costs. These advantages position saRNA as a strong candidate for vaccines [44, 45]. saRNA may also offer new options for therapeutic areas in which achieving sufficient protein exposure with standard mRNA would otherwise require high doses, including certain protein replacement applications.

Table 2 Comparison of RNA Architectures

Feature	Linear mRNA	circRNA	saRNA
Structure	Linear, 5′ cap–UTR–CDS–UTR–poly(A)	Covalently closed loop, no free ends	Linear, contains replicase gene + GOI
Typical size	1–5 kb	1–5 kb	9–12 kb
Stability	Moderate (exonuclease-sensitive)	High (exonuclease-resistant)	Moderate (replication extends functional window)
Expression duration	Hours to days	Days to weeks (potentially months)	Days to weeks (self-amplification)
Dose requirement	Standard	Potentially lower (prolonged expression)	10–100-fold lower than linear mRNA
Immune activation	Low with nucleoside modifications (m1Ψ)	Variable; circularization byproducts may trigger innate sensing	Higher; dsRNA replication intermediates activate innate sensors
Manufacturing complexity	Established (IVT + capping)	Moderate (requires circularization + purification of linear precursors)	Moderate (larger construct; IVT established)
Key advantages	Clinically validated; tunable; well-characterized	Extended protein expression; no cap/poly(A) needed; reduced dosing frequency	Dose sparing; high protein output; cost-effective manufacturing
Key limitations	Short expression window; higher dose for sustained effect	Circularization efficiency variable; limited clinical data; IRES-dependent translation	Large RNA size complicates LNP encapsulation; innate immune activation; limited clinical validation
Best-suited applications	Vaccines, protein replacement, gene editing	Chronic protein replacement, sustained expression therapies	Vaccines (dose sparing), pandemic preparedness

5. LNP as a leading platform for in vivo RNA delivery

Naked RNA is not a practical systemic drug because it is rapidly degraded by ubiquitous nucleases, it has limited ability to cross biological membranes due to its size and polyanionic charge, and it can activate innate immune sensors. Delivery systems are therefore required to protect RNA *in vivo*, enable cellular uptake, and facilitate endosomal escape to the cytosol, where siRNA and mRNA function. LNPs have become the most clinically mature non-viral platform for these tasks.

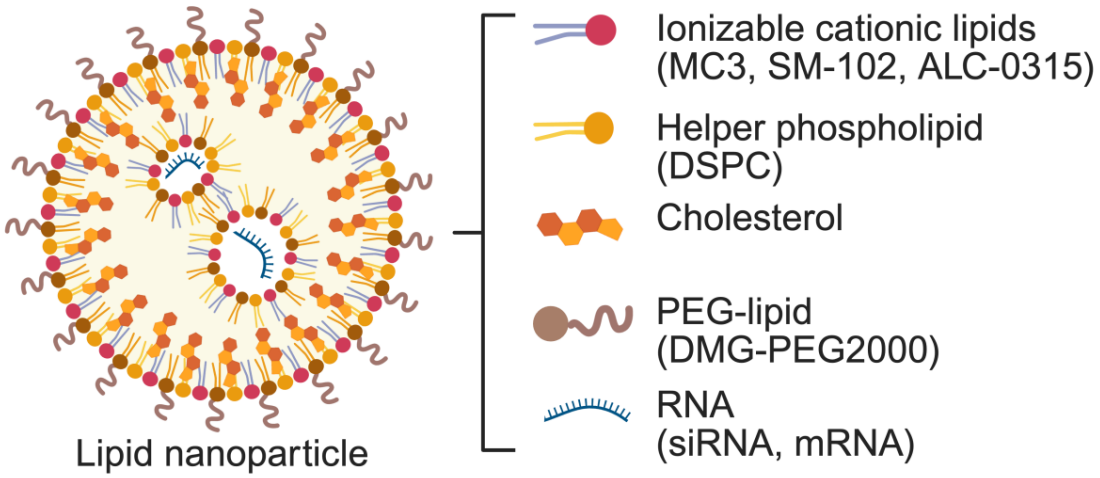

Fig. 3 Components of lipid nanoparticles

The four core LNP components and their functions

Most clinically used LNPs comprise four lipid classes: ionizable lipid, helper phospholipid, cholesterol, and a PEG-lipid (Fig. 3).

Ionizable lipid (functional core). Ionizable cationic lipids are the functional core of LNPs. During formulation, ionizable lipids are positively charged under acidic conditions (typically ~pH 4), enabling electrostatic complexation with RNA and efficient encapsulation into LNPs[13]. After dosing, they are largely neutral at physiological pH (~7.4), which reduces nonspecific plasma protein adsorption, limits RES-mediated clearance, and mitigates systemic toxicity. Following endocytosis, endosomal acidification (pH < ~6.5) protonates the headgroup, increasing positive charge and promoting interactions with anionic endosomal lipids, membrane destabilization, and cytosolic release of mRNA. The ionizable lipids moved from DLin-MC3-DMA used for siRNA to COVID-19 vaccine lipids such as SM-102 and ALC-0315, alongside a broader shift toward biodegradable ionizable lipids [46, 47]. Incorporation of hydrolysable motifs (for example, ester linkages) accelerates metabolic clearance and reduces the risk of accumulation-associated toxicity [48].

Helper phospholipid (structural support and membrane interaction). Phospholipids contribute to particle integrity and can influence membrane fusion behavior and intracellular trafficking. DSPC-like "stabilizing" phospholipids and more fusogenic lipids (e.g., DOPE-like) are choices depending on the application and route of administration [49].

Cholesterol (packing and stability). Cholesterol improves particle stability by modulating lipid packing and membrane fluidity, and it can influence fusion and endosomal escape efficiency [50]. Cholesterol analogs and substitutions are an active area of optimization as a structural and functional component.

PEG-lipid (surface stabilizer). PEG-lipids are amphiphiles that insert into the LNP membrane via a lipid anchor while presenting a PEG chain at the surface. They form a hydrated steric barrier that reduces aggregation and nonspecific serum protein adsorption, thereby improving colloidal stability and prolonging circulation. Excessive PEG density can reduce cellular association and uptake, whereas insufficient PEG can compromise stability and accelerate clearance [51].

Recent advances in LNP composition

Although the canonical four-component LNP formulation remains the benchmark and clinical standard for mRNA delivery, recent studies have increasingly explored compositionally simplified or functionally reprogrammed systems that combine multiple roles within fewer lipid species. Three-component LNPs are currently the most mature example of this trend. In zwitterionic amino lipid (ZAL) systems, the ZAL molecule integrates features of ionizable and helper lipids, enabling extrahepatic delivery in formulations composed only of ZAL, cholesterol, and a PEG-lipid [52]. Ionizable cholesteryl (iChol) lipids have further advanced this concept by chemically merging cholesterol-like structural functionality with ionizable behavior; these cholesterol-free three-component nanoparticles reduce ApoE adsorption, attenuate hepatic accumulation, and enhance extrahepatic mRNA delivery [53]. Simplified three-component formulations can also impart organ selectivity. For example, the cholesterol-free guanidine-based GL5-3 formulation preferentially delivers mRNA to the spleen, shows strong transfection of antigen-presenting cells, and improves antitumour immune responses [54]. In parallel, the SELECT platform demonstrated that simplified targeted LNPs can be combined with miRNA-responsive mRNA engineering to achieve tissue- and cell-type-selective expression, enabling targeted delivery to the lung, liver, or spleen and more precise tumour-cell-directed activity in a lung metastasis model [55]. Extending this logic, a 2026 Nature Communications study reported a PEG-free three-component targeted LNP (tLNP) strategy together with sequential selective organ-to-cell targeting (SSOCT), in

which mRNA expression was first directed to the spleen and then to splenic dendritic cells, thereby improving glioma vaccine efficacy while reducing hepatotoxicity and PEG-associated hypersensitivity [56]. By contrast, five-component formulations remain highly influential for programmable biodistribution: the addition of a supplementary SORT lipid to the conventional four-component backbone can redirect delivery from the liver toward the spleen, lung, and other extrahepatic tissues [57]. Overall, these advances suggest that the field is moving beyond simple lipid minimization toward role integration, programmable organ tropism, and multilevel selectivity; however, most simplified formulations are still preclinical and will require broader validation of manufacturability, robustness, and safety before clinical translation.

Formulation and manufacturing processes

Manufacturing quality controls particle size uniformity, encapsulation efficiency, and batch-to-batch consistency, which are core critical qualities for LNP drug products. Conventional methods (for example, thin-film hydration and sonication/emulsification) often produce broad size distributions, lower encapsulation, and poor scalability, which has limited clinical translation. Microfluidic mixing has addressed these constraints and is now the dominant process for clinical-grade LNP production [14]. Microfluidics leverages controlled fluid dynamics in microscale channels to mix an ethanol phase containing lipids (organic phase) with an aqueous phase containing mRNA. Using high-pressure pumps, both streams are introduced into a microchannel at precisely defined flow rates. Within the channel, rapid mixing, often on the millisecond timescale, drives lipid self-assembly in the aqueous environment while simultaneously encapsulating mRNA to form LNPs. This approach enables narrow size distributions, high encapsulation efficiency, straightforward GMP scale-up, and robust process reproducibility with low inter-batch variability, and is widely regarded as a standard method for LNP manufacturing [58, 59].

6. Clinical Application Landscape for LNP-RNA Medicines

LNP delivery has enabled RNA drugs to clinical use and expanded the range of indications. Clinically validated LNP-RNA products currently focus on systemic hepatic delivery of siRNA, and intramuscular vaccination with LNP-mRNA, while late-stage pipelines extend to therapeutic vaccination, protein replacement (enzyme or protein expression), *in vivo* genome editing, and *in vivo* immune-cell programming (Table 3).

Approved LNP-RNA medicines

Onpattro (patisiran) was the first FDA-approved LNP-formulated siRNA drug (2018) for hereditary transthyretin-mediated amyloidosis with polyneuropathy, establishing that LNP-enabled systemic RNA delivery can meet regulatory standards for quality, safety, and efficacy [60]. From 2020, the COVID-19 pandemic validated mRNA-LNP vaccination at global scale. Comirnaty (BNT162b2) and Spikevax (mRNA-1273) received full FDA approvals (2021 and 2022, respectively) and are key examples of nucleoside-modified mRNA formulated in LNPs for intramuscular immunization [17, 18]. Beyond COVID-19, mRESVIA (mRNA-1345) represents a major milestone as an FDA-approved, non-COVID-19 mRNA-LNP vaccine (2024) encoding RSV prefusion F (preF), with subsequent regulatory expansion to younger at-risk adults reported in 2025 [61].

Table 3 LNP-RNA Medicines and Development Status

Product	RNA type	Indication	Status (US)	Developer
Onpattro (patisiran)	siRNA	hATTR amyloidosis polyneuropathy	Approved (2018)	Alnylam
Comirnaty (BNT162b2)	mRNA	COVID-19	Approved (2021)	Pfizer/Bio NTech
Spikevax (mRNA-1273)	mRNA	COVID-19	Approved (2022)	Moderna
mRESVIA (mRNA-1345)	mRNA	RSV disease (older adults; expanded risk groups)	Approved (2024;expanded 2025)	Moderna
mRNA-4157/V940	mRNA	Personalized cancer vaccine + anti–PD-1	Phase 3	Moderna/ Merck
mRNA-3927	mRNA	Propionic acidemia	Phase 1/2	Moderna
mRNA-3705	mRNA	Methylmalonic acidemia	Clinical (early)	Moderna
NTLA-2001	Cas9 mRNA + sgRNA	ATTR amyloidosis (gene knockout)	Clinical	Intellia
NTLA-2002	Cas9 mRNA + sgRNA	Hereditary angioedema (KLKB1)	Phase 3	Intellia
VERVE-101	base editor mRNA + gRNA	HeFH / ASCVD risk (PCSK9)	Clinical (early; safety management)	Verve
CPTX2309	CAR mRNA	Autoimmune disease	Phase 1	Capstan/ AbbVie

LNP－RNA pipelines in clinical development

Vaccines. The most advanced expansion beyond infectious disease prevention is therapeutic and personalized cancer vaccination. Moderna/Merck's individualized neoantigen therapy mRNA-4157/V940 (LNP-formulated) has shown improved recurrence-free survival in resected high-risk melanoma in a randomized trial and has progressed into Phase 3 evaluation in combination with pembrolizumab [62].

Tumor immunotherapy. LNP-mRNA has also been evaluated to express therapeutic proteins *in vivo*, including mRNA-encoded monoclonal antibodies (passive immunization concept). BNT142 is an mRNA-LNP candidate encoding the anti-CLDN6/CD3 bispecific antibody RiboMab02.1. It was evaluated in patients with CLDN6-positive advanced solid tumors in a Phase I/IIa clinical trial (NCT05262530), providing early clinical proof-of-concept for mRNA-encoded bispecific antibodies in cancer immunotherapy [63].

Protein replacement and enzyme restoration. Inherited metabolic diseases remain a hot development area since hepatocyte uptake of LNP has been clinically established. mRNA-3927 is an LNP-encapsulated dual-mRNA therapy (PCCA/PCCB) for propionic acidemia, with first-in-human Phase 1/2 analyses reported in 2024 [64]. Additional enzyme-expression programs for methylmalonic acidemia such as Moderna's mRNA-3705 is currently under clinical-stage evaluation [65].

Genome editing (CRISPR and base editing). LNPs are also enabling *in vivo* delivery of gene-editing payloads (typically an editor mRNA plus guide RNA) with the liver as the leading initial target tissue. Intellia's NTLA-2001 reported *in vivo* CRISPR–Cas9 editing in transthyretin amyloidosis, providing a clinical proof-of-concept for LNP-enabled systemic editing [66]. Intellia's NTLA-2002 applies a similar approach to hereditary angioedema, with clinical development progressing to later-stage trials [67, 68]. For base editing, adenine base editor mRNA plus guide RNA in an LNP was used to target PCSK9 [69, 70]. Recently, a potent epigenetic editor targeting human PCSK9 was used to reduce low-density lipoprotein cholesterol levels [71].

In vivo CAR-T and immune-cell programming. Targeted LNPs enable direct *in vivo* "cell programming" by delivering CAR-encoding mRNA to immune cells, aiming to replace *ex vivo* CAR-T manufacturing. LNPs offer transient, non-integrating CAR expression that can be tuned by dose and formulation [72, 73]. Clinically, Capstan's CPTX2309 exemplifies this strategy, using a CD8-targeted LNP (tLNP) to preferentially deliver anti-CD19 CAR mRNA to cytotoxic T cells; first-in-human Phase 1 evaluation initiated in 2025, supported by non-human primate and translational datasets showing efficient *in vivo* CAR induction and profound B-cell depletion without *ex vivo* manipulation [74]. Importantly, early clinical validation has also emerged from China. Shenzhen MagicRNA reported first-in-human clinical data for HN2301 (a CD8-targeted LNP delivering CD19 CAR mRNA) in refractory systemic lupus erythematosus, published in September 2025 [75]. In that report, *in vivo* generated CAR T cells were detectable within hours after intravenous dosing, accompanied by rapid peripheral B-cell depletion and encouraging short-term clinical activity with manageable acute toxicity in a small cohort. Overall, *in vivo* generation of CAR T cells by LNP-mRNA represents a promising direction to balance cost, safety, and efficacy, and provides a potential pathway toward more scalable cell programming therapies.

Challenges in LNP clinical advancement.

Translating emerging innovations into the clinic remains uneven. Biodegradable ionizable lipids are designed to improve clearance and reduce prolonged tissue exposure, whereas circular RNA (circRNA) may support longer protein expression and self-amplifying RNA (saRNA) can enable dose sparing through intracellular RNA amplification. However, the translational maturity of these platforms differs substantially. SaRNA has already entered clinical practice in vaccines while CircRNA therapeutics remain largely preclinical [76, 77]. Across these modalities, key barriers to clinical translation include robust large-scale manufacturing, analytical comparability, cross-species reproducibility, and the establishment of long-term safety profiles for novel lipid chemistries and RNA architectures.

Repeat-dosing challenges and immunogenicity. Repeated administration remains a major obstacle for chronic LNP-RNA therapies. PEGylated LNPs can induce or boost anti-PEG antibodies, which in turn may accelerate blood clearance and reduce the efficacy of subsequent systemic doses, particularly after intravenous administration [78]. By contrast, current evidence suggests that the impact of anti-PEG antibodies on intramuscular vaccine immunogenicity is less pronounced than their effect on systemic delivery performance. Accordingly, alternative stealth chemistries, including poly(carboxybetaine)-based lipids and other PEG substitutes [79], are being actively developed to mitigate anti-PEG binding and improve repeat-dose consistency; however, evidence in humans remains limited and most data are still preclinical.

Ionizable lipid design trade-offs. Clinically used ionizable lipids such as DLin-MC3-DMA, SM-102 and ALC-0315 illustrate a practical balance among delivery potency, tolerability and scalable manufacture. Future ionizable lipids are increasingly being engineered for improved biodegradability, immune stealth and extrahepatic targeting, but translational success will depend not only on preclinical activity but also on synthetic accessibility, formulation robustness, and analytically tractable chemistry, manufacturing and controls (CMC). In this context, structurally elaborate lipids with marginal functional gains may be less clinically attractive than molecules that offer moderate performance improvements together with simpler synthesis and more reproducible manufacturability.

Regulatory landscape and maturation needs. The regulatory framework for LNP-RNA medicines is

advancing but remains incomplete. In the United States, the FDA's 2024 Platform Technology Designation draft guidance is intended to create efficiencies in development, manufacturing and review for designated platform technologies, but it does not eliminate the need for product-specific evidence. In Europe, the EMA's 2025 draft guideline addresses the quality aspects of mRNA vaccines against infectious diseases and explicitly notes that the applicability of platform or prior-knowledge approaches remains case by case[20]; relevant scientific principles may inform other mRNA products, but the guideline does not directly establish a unified pathway for all LNP-RNA therapeutics. As the field matures, regulatory progress will depend on better standardization of analytical characterization, clearer expectations for novel excipients, and stronger evidence frameworks for repeat-dosing safety.

7. Frontiers and Challenges in LNP-RNA Drug Development

Despite clinical validation, LNP-RNA technologies remain constrained by a limited delivery pattern: notably liver enrichment after intravenous dosing. Current research therefore concentrates on extrahepatic targeting, expanding indication space, and broadening administration routes, while addressing safety and repeat-dosing limitations.

Targeted delivery: strategies for extrahepatic and cell-selective delivery

Systemically administered LNPs often enrich in the liver, which is advantageous for hepatic indications but narrows the broader therapeutic scope. A major frontier is to shift delivery by altering formulation composition. The selective organ targeting (SORT) framework established that introducing a supplemental "SORT" lipid as an additional component can systematically shift delivery away from the liver toward organs such as the lung and spleen [80]. Mechanistic studies further indicate that composition-dependent protein corona formation and downstream intracellular trafficking contribute to organ preference and functional delivery [81, 82]. Recent work has also expanded high-throughput, *in vivo* screening approaches (including DNA-barcoded or otherwise multiplexed LNP libraries) to map extrahepatic biodistribution and identify chemistries with functional activity beyond the liver, especially in the lung, spleen, and kidney [83 - 85]. In parallel, large-scale synthesis and structure–activity screening of ionizable lipids continues to yield headgroup/linker/tail designs that improve delivery to lung endothelium and pulmonary tissues, with growing preclinical validation in lung gene editing and lung-associated disease models (including fibrotic settings), as well as delivery to spleen immune compartments and bone marrow associated cell populations [86 - 90].

Beyond composition-driven passive organ targeting, active targeting via ligand decoration (antibodies, peptides, aptamers, or small-molecule ligands) enables cell-selective delivery within a given organ. Antibody-functionalized LNPs have achieved efficient mRNA delivery to T cells *in vivo*, a hard-to-transfect population, supporting applications such as immune-cell programming and *in vivo* CAR expression [73, 91 - 93]. Peptide-functionalized LNPs have also achieved cell-type targeting effects *in vivo [94]*, and macrophage-targeted LNP designs (using ligand/peptide concepts) have shown therapeutic activity in disease models where myeloid programming is desired [95, 96]. However, this rational, ligand-directed active targeting strategy also faces practical challenges: complex *in vivo* pharmacokinetics and the LNP protein corona can mask or remodel the displayed ligands, potentially diminishing the intended targeting effect [97]. So rigorous *in vivo* mechanistic evaluation is essential to define the true contribution of ligand engagement to delivery outcomes. Overall, extrahepatic targeting strategies represent a critical pathway to broaden the therapeutic scope of LNP-mRNA drugs.

Administration routes: from systemic injection to non-invasive delivery

Intramuscular and intravenous injections currently constitute the primary methods of clinical LNP administration. Intramuscular injection remains the gold standard for vaccination, as it effectively leverages local antigen presentation and lymph node drainage to elicit robust systemic immunity (e.g., COVID-19 mRNA vaccines). Conversely, IV administration is the dominant route for systemic therapies, such as siRNA treatment for amyloidosis (e.g., patisiran) [98]. However, IV delivery inherently results in accumulation in the liver, posing a significant barrier for treating extrahepatic diseases. In recent years, novel non-invasive and site-specific administration routes, including inhalation, *in situ*, and oral delivery, have been explored to expand the therapeutic application of LNPs.

Inhaled Delivery: Inhalation offers a direct, non-invasive route to the respiratory epithelium, making it a high-priority frontier for treating genetic lung disorders, like cystic fibrosis, primary ciliary dyskinesia. The primary advantage of this route is its ability to achieve high local drug concentrations while minimizing systemic off-target effects. Researchers have successfully utilized nebulized mRNA-LNPs to express therapeutic proteins (like CFTR) specifically in lung epithelial cells [99 - 101]. Recent engineering efforts have focused on overcoming the physical shear forces of nebulization and penetrating the mucus barrier. For example, two 2024 *Nature Communications* studies highlighted key advancements: one introduced a charge-assisted stabilization strategy that prevents LNP disintegration during aerosolization, and another reported a high-throughput workflow that screens for formulations with superior mucus-penetrating and pulmonary transfection capabilities [102, 103].

Oral Delivery: The potential applications range from oral vaccination to the local treatment of gastrointestinal disorders, such as Inflammatory Bowel Disease (IBD) and

Colitis. There are harsh physiological barriers for oral LNP, including gastric acid, digestive enzymes, and the intestinal epithelial barrier [104 - 106]. Recent innovations involve the use of enteric coatings to protect LNPs from low pH and the surface functionalization of nanoparticles with transport receptors to facilitate uptake by intestinal M cells or enterocytes [107, 108]. While this route remains less mature than parenteral or inhaled methods, these emerging strategies are enabling new non-invasive therapies.

Local administration: Direct local administration, such as intratumoral and intra-articular injection, offers a strategic advantage by delivering LNPs precisely to the site of disease, effectively decoupling therapeutic efficacy from systemic toxicity. In immuno-oncology, intratumoral injection enables the safe delivery of potent cytokines (e.g., IL-12) directly into the tumor microenvironment, turning "cold" tumors "hot" without triggering the severe side effects associated with systemic administration [109, 110]. Similarly, in orthopedics, intra-articular injection overcomes the challenge of avascular cartilage, allowing regenerative mRNA to bypass the blood-joint barrier and directly reach damaged tissues in osteoarthritis [111]. By focusing the drug payload exactly where it is needed, local delivery significantly widens the therapeutic index for diseases that are either too toxic or too inaccessible to treat systemically.

The translational gap: challenges in cross-species extrapolation

Unlike small molecules or protein-based drugs, which follow relatively predictable pharmacokinetic (PK) profiles, LNPs are complex supramolecular assemblies whose biological fate is governed by many interacting factors. The *in vivo* biodistribution and metabolism of LNPs are dictated by a combination of lipid chemistry, formulation composition, particle size, and surface charge[49]. Unfortunately, the precise structure-activity relationships (SAR) governing these interactions remain elusive. A major bottleneck is the poor *in vitro-in vivo* correlation [112]; high transfection efficiency in cell culture frequently fails to translate to animal models, and currently, there is no standardized evaluation framework to predict *in vivo* metabolic fate based on physicochemical parameters.

This lack of SAR study creates a significant barrier in translating findings from preclinical models to human clinical applications. Significant interspecies heterogeneity exists in serum protein composition (e.g., ApoE levels) and immune system clearance responses. Consequently, LNP formulations that achieve robust delivery in rodents often show diminished efficacy or altered biodistribution in non-human primates (NHPs) and humans [113]. This species gap is particularly pronounced for systemic administration and targeted delivery strategies, where physiological differences in organ perfusion and capillary fenestration further complicate the extrapolation of dosing regimens and safety profiles from bench to bedside.

To bridge this translational divide, future research must prioritize the understanding how structure affects function and the establishment of comprehensive pharmacokinetic models that account for species-specific physiological differences. Developing predictive frameworks that correlate physicochemical properties with *in vivo* biological fate will be essential for rationalizing LNP design and improving the success rate of clinical translation.

8. Conclusion and Future Perspectives

The strongest clinical validation of LNP-RNA medicines has come from two settings: systemic hepatic delivery of siRNA and intramuscular administration of mRNA vaccines. These achievements established that RNA payload design, LNP formulation, and scalable manufacturing can be integrated into drug products that meet both regulatory and clinical requirements. However, many of the newer applications discussed in this review, including protein replacement, *in vivo* gene editing, and *in vivo* CAR-T generation, remain at an early stage of clinical development. The next phase of LNP-RNA medicine will therefore be defined less by whether RNA can be delivered at all, and more by whether it can be delivered selectively, safely, and reproducibly in clinically relevant settings.

Mechanism of extrahepatic and cell-selective delivery remains unclear. In recent years, extrahepatic targeting to organs such as the spleen and lung has been achieved in mice through adjustments in LNP composition or screening of new ionizable lipids. This targeting behavior is widely thought to be mediated by protein corona formation and subsequent receptor-dependent uptake. However, this mechanism remains insufficiently understood and presents clear translational limitations. Current methods for protein corona characterization typically rely on *ex vivo* incubation of LNPs with peripheral serum followed by purification and analysis. These procedures are technically complex and may introduce experimental artifacts. Furthermore, *in vitro* or *ex vivo* assays cannot reproduce the hemodynamic conditions, vascular architecture, and tissue microenvironments present *in vivo.* As a result, they may not accurately reflect the actual biological identity and targeting behavior of LNPs after administration. For this reason, *in situ* analysis of LNP distribution and functional expression in living tissues will be increasingly important. Addressing this problem will require closer integration of nanomedicine, biology, and pharmacology to clarify how LNP targeting is generated *in vivo.*

The translational gap between preclinical models and humans remains another major obstacle. Species differences in serum protein composition, immune clearance, vascular structure, and tissue microenvironment likely contribute to this problem. Better *in vitro-in vivo* correlation, improved structure-function analysis, and more predictive cross-species evaluation frameworks are therefore needed. This issue is especially relevant to the rapidly growing field of *in vivo* CAR-T. Although targeted LNPs have shown encouraging results in mice, there is still little evidence that the same targeting behavior can be reproduced in non-

human primates. Because LNPs are complex supramolecular systems, their pharmacokinetics and pharmacodynamics are more difficult to extrapolate across species than those of many small-molecule or protein drugs. Therefore, as *in vivo* CAR-T programs move forward rapidly, greater attention should be paid to species-dependent differences in the biological fate of targeted LNPs. Progress in this area will require mechanistic studies that connect chemical structure, nanoparticle organization, protein adsorption, and functional delivery outcomes across species.

Safety and repeat-dosing compatibility will determine whether LNP-RNA drugs can move from episodic use to chronic treatment. For vaccines, transient exposure may be sufficient. For protein replacement and some gene-editing applications, however, repeated administration or prolonged expression may be required. Biodegradable ionizable lipids, alternative stealth coatings, and new RNA formats such as circRNA or saRNA may help address some of these issues, but they do not remove the need for careful evaluation of cumulative exposure, dosing interval, and long-term tolerability in relevant animal models and human studies. The development of novel PEG alternatives or biomimetic delivery strategies is expected to reduce immunogenicity and improve the feasibility of repeated dosing regimens for chronic disease treatment.

Clinical translation will also depend on whether platform innovation can be matched by advances in manufacturing and regulatory science. Microfluidic production has improved reproducibility and scale-up, but newer lipids, more complex RNA architectures, and targeted formulations will place greater demands on characterization, quality control, and comparability assessment. At the same time, the regulatory pathway for LNP-RNA drugs is still evolving, especially for repeated dosing, novel excipients, and platform-based product adaptation. Regulatory agencies should accelerate the establishment of clearer standards for product characterization, immunogenicity monitoring, pharmacokinetic and biodistribution assessment, and clinically meaningful endpoints across different therapeutic settings.

Taken together, the future of LNP-RNA medicines will depend on coordinated advances in materials design, fundamental biology, pharmacology, clinical investigation, and regulatory science. Continued interdisciplinary collaboration will be essential for deepening our understanding of LNP behavior in vivo and for accelerating the translation of RNA therapeutics into clinically effective medicines.

9. Declaration of generative AI in scientific writing

The authors drafted the initial manuscript and used Gemini 3 pro for language polishing to improve readability.

10. CRediT author statement

Jinshi Yu: Conceptualization, Investigation, Writing - Original Draft.

Xingxue Yan: Investigation, Visualization, Writing - Original Draft.

Yingying Yu: Supervision, Writing - Review & Editing.

11. Declaration of Competing Interest

The authors declare no competing interests.

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