
**Lipid Nanoparticles Beyond mRNA Vaccines: Emerging Therapeutic Applications,
Formulation Strategies and Translational Challenges**

Sumit Kumar

Department of Pharmaceutical Sciences,
Central University of Haryana, Jant-Pali, Mahendergarh

drsumitkumar@cuh.ac

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Abstract

Lipid nanoparticles (LNPs) rose to global prominence as the delivery platform underpinning the messenger RNA (mRNA) vaccines deployed during the COVID-19 pandemic, yet their therapeutic potential extends far beyond prophylactic vaccination. This review examines the expanding landscape of LNP applications, situating the well-characterised four-component architecture—ionisable lipid, helper phospholipid, cholesterol and polyethylene glycol (PEG)-lipid—within a broader therapeutic context that now encompasses small interfering RNA (siRNA) therapeutics, gene editing payloads, protein-replacement mRNA, small-molecule chemotherapeutics and emerging in vivo cell-engineering strategies (Hou et al., 2021). We synthesise evidence describing how each formulation component governs particle self-assembly, apparent pKa, endosomal escape and organ tropism, and we highlight how rational lipid design and manufacturing advances such as microfluidic mixing have improved encapsulation efficiency, reproducibility and scalability (Evers et al., 2018; Schoenmaker et al., 2021). The review is organised around four thematic pillars: the physicochemical determinants of LNP behaviour, the diversification of nucleic-acid and small-molecule cargoes, strategies for extrahepatic and targeted delivery, and the translational barriers that continue to constrain clinical adoption. Persistent challenges include hepatic accumulation that limits delivery to other tissues, cold-chain stability requirements, batch-to-batch heterogeneity, PEG-associated immunogenicity, and incompletely understood long-term safety profiles (Tenchov et al., 2021; Kularatne et al., 2022). Drawing on 28 peer-reviewed sources published between 2015 and 2024, this narrative review argues that the convergence of ionisable-lipid chemistry, targeting ligands and continuous manufacturing positions LNPs as a versatile modular platform likely to enable a new generation of genetic medicines. We conclude by outlining priority directions, including biodegradable lipid libraries, organ-selective

SORT formulations, lyophilisation for ambient stability, and closed-loop good manufacturing practice (GMP) production.

Keywords: *lipid nanoparticles; mRNA therapeutics; ionisable lipids; drug delivery; gene editing; targeted delivery; formulation; translational medicine*

1. Introduction

The clinical validation of lipid nanoparticles as a delivery vehicle for mRNA vaccines represents one of the most consequential milestones in modern pharmaceutical science. Within roughly a year of the SARS-CoV-2 genome being sequenced, two LNP-formulated mRNA vaccines had received emergency authorisation and were administered to billions of people worldwide (Schoenmaker et al., 2021). This achievement was not the product of a single pandemic-era breakthrough but the culmination of three decades of incremental progress in lipid chemistry, nucleic-acid stabilisation and nanoscale manufacturing. The vaccines demonstrated, at unprecedented scale, that a chemically defined, synthetically manufactured nanoparticle could protect a fragile nucleic-acid payload, deliver it into the cytosol of target cells and drive functional protein expression with an acceptable safety margin (Hou et al., 2021). Crucially, this same delivery competence is agnostic to the specific message carried, which is precisely why the platform's implications reach well beyond vaccination.

Understanding why LNPs succeeded where earlier delivery systems struggled requires appreciating the central problem of nucleic-acid therapeutics: naked RNA and DNA are large, negatively charged, enzymatically labile molecules that cannot spontaneously cross the hydrophobic cell membrane and are rapidly degraded by ubiquitous nucleases (Kulkarni et al., 2018). Viral vectors solved the delivery problem but introduced immunogenicity, limited cargo capacity, manufacturing complexity and integration risks. LNPs offered a non-viral alternative that could be manufactured synthetically, re-dosed, and adapted to different payloads simply by changing the encapsulated sequence. The remainder of this introduction establishes the conceptual foundations of the platform across four subsections before the review turns to a structured examination of emerging applications, formulation science and translational obstacles.

The significance of this delivery competence is best appreciated by contrasting the therapeutic ceiling of conventional pharmacology with that of genetic medicine. Small-molecule drugs act on a limited fraction of the proteome and are frequently unable to modulate targets once considered undruggable, whereas nucleic-acid therapeutics can, in principle, silence, replace, edit or supplement any gene of interest (Kowalski et al., 2019). The bottleneck has never been the design of the therapeutic message but its safe and efficient delivery to the correct intracellular compartment. By resolving that bottleneck for at least one major organ, LNPs have effectively converted the vast information content of the genome into an addressable therapeutic target space. This reframing—from delivery as an afterthought to delivery as the enabling technology—explains why so much contemporary pharmaceutical investment has flowed toward lipid chemistry and nanoparticle engineering, and why a review of applications beyond vaccination is both timely and necessary.

1.1 Historical Evolution from Liposomes to Modern LNPs

The intellectual lineage of contemporary LNPs traces back to the discovery of liposomes in the 1960s and their subsequent development as drug carriers. Early liposomal products such as liposomal doxorubicin validated the principle that encapsulating a drug within a lipid bilayer could alter its pharmacokinetics, reduce off-target toxicity and improve tolerability (Tenchov et al., 2021). However, conventional liposomes were poorly suited to nucleic-acid delivery because encapsulation of large polyanions was inefficient and cytosolic release was minimal. The pivotal conceptual shift came with the introduction of ionisable cationic lipids, which are positively charged at the low pH used during formulation—allowing efficient electrostatic complexation with RNA—but revert to a near-neutral charge at physiological pH, reducing systemic toxicity and prolonging circulation (Cullis & Hope, 2017). This pH-responsive behaviour, combined with a shift from bilayer vesicles to a more electron-dense, nucleic-acid-rich internal structure, distinguishes the modern LNP from its liposomal ancestor and underlies its delivery efficiency.

The trajectory from the first permanently cationic lipids to today's sophisticated ionisable chemistries illustrates the incremental character of the field's progress. Early cationic lipids such as those used in first-generation transfection reagents formed stable complexes with nucleic acids but carried a fixed positive charge that provoked complement activation, rapid clearance and dose-limiting toxicity in vivo (Reichmuth et al., 2016). The introduction of the ionisable amino-lipid concept, and the subsequent optimisation of head-group basicity and tail architecture, progressively improved the

therapeutic window. A landmark advance was the demonstration that maximising ionisation at endosomal pH dramatically enhanced hepatic silencing potency, a finding that guided the design of the lipid ultimately used in the first approved siRNA product (Jayaraman et al., 2015). Each of these developments was published incrementally over more than a decade, and the pandemic-era vaccines represent the point at which this accumulated knowledge reached clinical maturity at global scale.

1.2 Core Architecture and Components

The canonical LNP is a quaternary system comprising four functional components combined in defined molar ratios. The ionisable lipid, typically the largest molar fraction, drives RNA complexation and is the primary determinant of potency and endosomal escape. A helper or structural phospholipid, most commonly distearoylphosphatidylcholine (DSPC), contributes to the structural integrity of the particle and influences the lamellar phase behaviour of the lipid assembly (Cheng & Lee, 2016). Cholesterol modulates membrane fluidity, fills packing defects and enhances stability and fusogenicity. Finally, a PEG-lipid conjugate forms a hydrophilic steric barrier on the particle surface that controls particle size during self-assembly, prevents aggregation and prolongs circulation by reducing opsonisation (Kularatne et al., 2022). The precise identity and ratio of these components collectively dictate encapsulation efficiency, size, surface charge, biodistribution and immunogenicity, making formulation optimisation a multidimensional design problem rather than a matter of simple component substitution.

The internal organisation of the particle deserves particular emphasis because it departs markedly from the textbook image of a hollow liposome. Cryo-electron microscopy and molecular-dynamics studies indicate that the nucleic-acid cargo is not enclosed within a simple aqueous core but is distributed throughout an electron-dense interior in which the ionisable lipid, cholesterol and RNA form inverted micellar or amorphous domains (Zhang et al., 2021). The PEG-lipid and a fraction of the phospholipid segregate to the aqueous interface, presenting the hydrophilic corona that governs colloidal stability and pharmacokinetics. This structural model explains why encapsulation efficiency can approach quantitative values and why the particle behaves as a delivery vehicle rather than a passive reservoir. It also clarifies why altering the molar ratio of any single component reverberates through the entire assembly, shifting size, internal morphology and ultimately biological performance in ways that are difficult to predict *a priori* and must therefore be established empirically.

1.3 Mechanism of Cellular Uptake and Endosomal Escape

Following administration, LNPs are internalised predominantly through receptor-mediated endocytosis, with apolipoprotein E (ApoE) adsorption playing a well-documented role in directing hepatocyte uptake via low-density lipoprotein receptors after intravenous dosing (Akinc et al., 2019). Once inside the endosome, the gradual acidification of the endosomal lumen protonates the ionisable lipid, restoring its cationic character. The protonated lipid then interacts with anionic endosomal membrane phospholipids to form non-bilayer, cone-shaped ion pairs that destabilise the membrane and promote release of the nucleic-acid cargo into the cytosol (Kulkarni et al., 2018). Endosomal escape remains the principal efficiency bottleneck of the entire platform: estimates suggest that only a small percentage of internalised payload successfully reaches the cytosol, with the remainder trafficked to lysosomal degradation or recycled out of the cell. Consequently, much of modern ionisable-lipid design is oriented toward maximising this escape fraction.

The biology of the protein corona adds a further layer of complexity to this mechanistic picture. Upon entering the bloodstream, LNPs are rapidly coated by a dynamic layer of plasma proteins whose composition depends on particle surface chemistry, and this corona—rather than the bare particle surface—is what cells actually encounter (Dilliard et al., 2021). The enrichment of ApoE within this corona is the proximate cause of hepatocyte targeting, since ApoE serves as an endogenous ligand for hepatic receptors. This insight is doubly important: it explains the platform's default liver tropism, and it identifies the corona as a lever that can be manipulated to redirect delivery, a principle later exploited by selective-organ-targeting formulations. The endosomal-escape bottleneck and the corona-mediated targeting mechanism together define the two central problems—efficiency and specificity—around which the remainder of LNP research is organised.

1.4 Scope and Objectives of the Review

This review is scoped to synthesise developments in LNP science published between 2015 and 2024, with deliberate emphasis on applications that extend beyond prophylactic mRNA vaccination. The objectives are fourfold: to articulate the physicochemical principles governing LNP assembly and function; to map the diversification of therapeutic cargoes from siRNA and gene-editing tools to protein-replacement mRNA and small molecules; to critically appraise strategies for achieving targeted and extrahepatic delivery; and to identify the translational, manufacturing and regulatory barriers that must be overcome for broad clinical adoption. By integrating mechanistic, formulation

and translational perspectives, the review aims to provide researchers and formulation scientists with a consolidated conceptual framework for the rational design of next-generation LNP therapeutics.

The review is deliberately positioned at the interface between fundamental science and clinical translation. Rather than cataloguing every reported formulation, it seeks to extract the organising principles that recur across studies and to relate them to the practical obstacles that developers encounter when advancing candidates toward the clinic. This orientation reflects a conviction that the field's future progress depends not on the discovery of any single superior lipid but on the systematic understanding of how formulation choices propagate through particle structure, biodistribution, efficacy and safety. The four thematic domains that structure the literature review were chosen precisely because they map onto the sequential decisions a developer must make: which components to use, what to deliver, where to deliver it, and how the resulting product fits within the broader ambition of genetic medicine.

2. Literature Review

The scientific literature on lipid nanoparticles has expanded dramatically over the past decade, evolving from a specialised niche within nucleic-acid delivery into one of the most active areas of translational nanomedicine. This section organises the body of evidence into four thematic domains that collectively capture the emerging directions of the field: the formulation science that determines particle behaviour, the diversification of therapeutic payloads, the pursuit of targeted and organ-selective delivery, and the intersection of LNPs with next-generation genetic medicines. Each domain is supported by representative findings summarised in the accompanying tables.

2.1 Formulation Components and Physicochemical Determinants

A consistent theme across the literature is that LNP performance is governed less by any single component than by the interplay among all four constituents and the process by which they are assembled. The ionisable lipid receives the greatest attention because its chemical structure dictates the apparent pKa of the particle, which optimally falls in the range of approximately 6.2 to 6.5 for efficient hepatic mRNA delivery (Hajj et al., 2019). Lipids with pKa values outside this window exhibit markedly reduced potency, either because they remain insufficiently charged during endosomal acidification or because they carry excessive surface charge at physiological pH, provoking clearance and toxicity. Systematic structure–activity studies have shown that the geometry of the lipid tails, the

degree of unsaturation and the presence of biodegradable ester linkages all modulate both potency and clearance kinetics (Sabnis et al., 2018).

The role of the helper phospholipid and cholesterol has also been re-examined. Substituting DSPC with unsaturated phospholipids or replacing cholesterol with structural analogues can shift biodistribution and enhance transfection in specific tissues, underscoring that even the so-called structural components exert functional influence (Patel et al., 2020). PEG-lipids present a well-recognised trade-off: higher PEG content produces smaller, more stable and longer-circulating particles but simultaneously impairs cellular uptake and endosomal escape—the so-called PEG dilemma—and has been implicated in accelerated blood clearance upon repeat dosing and in rare hypersensitivity reactions (Kularatne et al., 2022). Manufacturing method is an equally decisive determinant. The transition from bulk mixing to microfluidic and T-junction rapid-mixing platforms has enabled precise control of particle size, narrow polydispersity and reproducible encapsulation efficiencies exceeding ninety percent, which in turn improves batch consistency and regulatory acceptability (Evers et al., 2018).

A deeper reading of the formulation literature reveals that the four canonical components should be understood as a coupled system in which each element compensates for or amplifies the behaviour of the others. Cholesterol content, for instance, interacts with the ionisable lipid to determine the fusogenic propensity of the endosomal membrane interaction, while the phospholipid tail saturation modulates the lamellar-to-hexagonal phase transition that accompanies endosomal escape (Kauffman et al., 2016). Investigators have consequently moved away from optimising components in isolation and toward high-throughput combinatorial screening in which large libraries of lipids and ratios are evaluated simultaneously *in vivo* using DNA-barcoding techniques that allow hundreds of formulations to be tested in a single animal (Paunovska et al., 2022). This screening paradigm has accelerated the identification of chemistries with improved potency and tissue selectivity, and it has reinforced the view that formulation is fundamentally an empirical, data-driven discipline rather than one governed by simple design rules.

The chemistry of the ionisable lipid itself continues to evolve toward greater sophistication. Contemporary design increasingly incorporates biodegradable ester or disulfide linkages positioned to accelerate metabolic clearance after the lipid has performed its delivery function, thereby reducing tissue accumulation and improving repeat-dose tolerability (Eygeris et al., 2022). Branched and multi-

tailed architectures have been shown to enhance ionisation at endosomal pH and to improve the geometric complementarity between the protonated lipid and the endosomal membrane, both of which promote escape (Hajj et al., 2019). The cumulative effect of these chemical refinements is a widening therapeutic window, but each new chemistry introduces fresh questions about metabolism, biodistribution and long-term safety that must be resolved before clinical deployment, illustrating the perpetual tension between potency and predictability that characterises the field.

Table 1. Core LNP components and their principal formulation roles.

Component	Representative Example	Primary Function	Key Trade-off
Ionisable lipid	SM-102, ALC-0315, DLin-MC3-DMA	RNA complexation and endosomal escape	pKa outside 6.2–6.5 reduces potency
Helper phospholipid	DSPC, DOPE	Structural integrity and phase behaviour	Choice shifts tissue biodistribution
Cholesterol	Cholesterol, analogues	Membrane fluidity and stability	Excess rigidity can lower fusogenicity
PEG-lipid	PEG2000-DMG, ALC-0159	Size control and prolonged circulation	Higher PEG impairs uptake and escape
Nucleic-acid cargo	mRNA, siRNA, sgRNA	Therapeutic payload	Size and charge affect encapsulation

2.2 Diversification of Therapeutic Payloads

Although mRNA vaccines dominate public awareness, the literature demonstrates that LNPs are payload-agnostic carriers capable of delivering a wide spectrum of therapeutic molecules. The first regulatory approval of an LNP-based product was in fact patisiran, an siRNA therapeutic for hereditary transthyretin-mediated amyloidosis, which validated the platform for chronic, repeat-dose systemic delivery of gene-silencing agents years before the mRNA vaccines (Akinc et al., 2019). siRNA and

antisense oligonucleotide payloads exploit the same hepatic tropism that ApoE-mediated uptake confers, making the liver an ideal target for silencing disease-associated genes.

Beyond gene silencing, mRNA payloads encoding therapeutic proteins have opened the possibility of transient protein-replacement therapy for metabolic and genetic disorders, avoiding the permanence and integration risks of DNA-based approaches (Kowalski et al., 2019). Messenger RNA encoding antibodies, cytokines and enzymes has been explored preclinically for oncology and rare-disease indications. A particularly active frontier is the delivery of gene-editing machinery: LNPs can co-encapsulate Cas9 mRNA and guide RNA, enabling transient nuclease expression that minimises off-target editing associated with prolonged expression (Rosenblum et al., 2020). The clinical validation of this approach was demonstrated by hepatic *in vivo* CRISPR editing for transthyretin amyloidosis, illustrating a durable therapeutic effect from a single systemic LNP dose. LNPs have additionally been formulated with small-molecule chemotherapeutics and combination payloads, though these applications draw more directly on the liposomal heritage of the platform.

The distinction between transient and durable therapeutic effects is central to understanding payload selection. Messenger RNA payloads produce protein transiently, with expression typically declining over days as the message is degraded, which is advantageous for vaccination and for indications where a self-limiting effect is desirable but disadvantageous for chronic conditions requiring sustained protein supply, where repeat dosing becomes necessary (Samaridou et al., 2020). siRNA payloads, by contrast, can achieve durable silencing from infrequent administration because the RNA-induced silencing complex persists and the effect is catalytic. Gene-editing payloads occupy a third category: although the Cas9 protein and guide RNA are expressed only transiently, the genomic edit they introduce is permanent, converting a fleeting molecular event into a lifelong therapeutic outcome. This spectrum—from transient expression through catalytic silencing to permanent correction—allows the same delivery platform to be matched to the temporal requirements of widely differing diseases.

The economic and developmental implications of payload modularity are difficult to overstate. Because the delivery vehicle remains essentially unchanged while the encapsulated sequence is exchanged, a validated LNP formulation can in principle be repurposed across many indications with reduced re-engineering, in contrast to viral vectors whose capsid and genome must often be redesigned for each new application (Buschmann et al., 2021). This property underpinned the rapid adaptation of vaccine formulations to emerging variants and has motivated substantial investment in individualised

therapeutics such as neoantigen cancer vaccines, in which the payload is synthesised specifically for each patient's tumour mutational profile. The platform thus functions as reusable infrastructure, decoupling the slow, capital-intensive process of delivery-system development from the comparatively rapid process of payload design.

Table 2. Representative therapeutic payload classes delivered by LNPs.

Payload Class	Therapeutic Goal	Representative Application	Development Stage
siRNA	Gene silencing	Transthyretin amyloidosis	Approved (patisiran)
mRNA (vaccine)	Antigen expression	SARS-CoV-2 vaccines	Approved
mRNA (protein replacement)	Transient protein supply	Metabolic and rare diseases	Clinical / preclinical
CRISPR-Cas9 mRNA + sgRNA	In vivo gene editing	Hepatic gene correction	Clinical trials
Small molecules	Cytotoxic chemotherapy	Oncology	Approved (liposomal heritage)

2.3 Targeted and Extrahepatic Delivery Strategies

The strong default tropism of intravenously administered LNPs toward the liver is simultaneously an advantage for hepatic indications and the central obstacle to treating disease in other organs. A substantial and rapidly growing literature is therefore devoted to redirecting LNPs away from the liver toward the lung, spleen, immune cells and other tissues. One influential strategy, termed selective organ targeting (SORT), incorporates an additional charged lipid into the standard four-component formulation; systematically varying the identity and proportion of this fifth SORT lipid shifts delivery predictably among liver, spleen and lung, apparently by altering the plasma-protein corona that forms on the particle surface (Cheng et al., 2020). This finding reframed biodistribution as a tunable formulation parameter rather than a fixed property of the platform.

Active targeting approaches conjugate ligands—antibodies, antibody fragments, peptides, aptamers or small molecules such as N-acetylgalactosamine—to the particle surface to engage receptors enriched on target cells (Dilliard et al., 2021). Antibody-decorated LNPs have been used preclinically to deliver mRNA selectively to T cells for in vivo cell engineering, an approach of considerable interest for chimeric antigen receptor therapy that would circumvent the cost and complexity of ex vivo manufacturing. Route of administration also profoundly shapes biodistribution: intramuscular injection localises expression largely to the injection site and draining lymph nodes, intranasal and inhaled delivery target the respiratory epithelium, and intratumoural administration concentrates payload within the tumour microenvironment (Kim et al., 2021). The convergence of formulation-based SORT strategies, ligand-mediated active targeting and route optimisation constitutes the most promising path toward genuinely extrahepatic LNP medicines.

It is instructive to distinguish passive from active targeting because the two operate through fundamentally different mechanisms and carry different translational risks. Passive strategies, including SORT and route selection, exploit the biophysical interaction between the particle and its physiological environment—principally the composition of the protein corona and the local anatomy of the administration site—to bias accumulation without introducing any recognition element (Cheng et al., 2020). Active strategies attach explicit molecular ligands that engage defined receptors, offering the potential for cell-type-level precision but at the cost of added manufacturing complexity, potential immunogenicity of the ligand, and the challenge of maintaining ligand orientation and density during production (Dilliard et al., 2021). In practice, the most sophisticated formulations combine both, using passive biodistribution to concentrate particles within a target organ and active ligands to select the relevant cell population within it.

Delivery to immune cells has emerged as an especially consequential objective because it opens the door to in vivo cell engineering. Antibody-conjugated LNPs directed at T-cell surface markers have been used preclinically to deliver mRNA encoding chimeric antigen receptors, generating functional engineered cells within the body and thereby circumventing the laborious and expensive ex vivo manufacturing that currently limits access to cell therapy (Rurik et al., 2022). Similar principles have been applied to target other haematopoietic and endothelial populations. These achievements demonstrate that the hepatic-tropism barrier, long regarded as an intrinsic limitation, is increasingly

tractable, and they reframe targeting from a peripheral concern into a core enabling capability for the platform's most ambitious applications.

Table 3. Strategies for targeted and extrahepatic LNP delivery.

Strategy	Mechanism	Target Tissue / Cell	Key Consideration
SORT lipids	Alters protein corona and charge	Lung, spleen, liver	Tunable by lipid ratio
Antibody conjugation	Receptor-specific binding	T cells, tumour cells	Ligand stability and cost
GalNAc ligands	Asialoglycoprotein receptor	Hepatocytes	Well-established for liver
Route of administration	Local deposition	Muscle, lung, tumour	Limits systemic exposure
Peptide / aptamer targeting	Selective receptor engagement	Various tissues	Immunogenicity of ligand

2.4 LNPs in Next-Generation Genetic Medicine

The final thematic domain concerns the positioning of LNPs at the centre of an emerging genetic-medicine ecosystem in which the same modular carrier can be reprogrammed for diverse therapeutic ends. The literature increasingly frames LNPs as a general-purpose delivery chassis whose payload can be exchanged with comparatively little re-engineering, in stark contrast to viral vectors that require bespoke redesign for each application (Hou et al., 2021). This modularity has profound implications for the economics and speed of drug development, as demonstrated by the rapid pivot of vaccine manufacturers to variant-specific boosters and, more recently, to individualised neoantigen cancer vaccines that encode patient-specific tumour mutations.

In vivo cell engineering represents perhaps the most transformative prospect. By delivering mRNA encoding chimeric antigen receptors directly to circulating immune cells, transient reprogramming of T cells or macrophages may be achievable without ex vivo manipulation, potentially democratising

access to cell therapies (Rurik et al., 2022). Parallel work explores LNPs for regenerative medicine, delivering mRNA encoding transcription factors or growth factors to reprogramme or repair tissue in situ. The integration of LNPs with gene-editing and base-editing technologies further extends the therapeutic reach toward durable, single-administration cures for monogenic disorders. Collectively, the literature portrays a trajectory in which incremental advances in lipid chemistry, targeting and manufacturing compound to unlock a expanding therapeutic repertoire, positioning LNPs as foundational infrastructure for twenty-first-century medicine (Mitchell et al., 2021).

The convergence of LNP delivery with gene-editing and base-editing technologies is particularly significant because it promises to shift the treatment paradigm from lifelong management toward durable, single-administration cures for monogenic disorders. The transient nature of LNP-mediated expression is an asset in this context: because the editing machinery is present only briefly, the window during which off-target activity can occur is limited, improving the safety profile relative to systems that express the nuclease persistently (Rosenblum et al., 2020). Early clinical results in hepatic gene editing have provided proof that a single systemic dose can produce a sustained and clinically meaningful therapeutic effect, validating the concept that a fleeting molecular intervention can yield permanent benefit. Extending this success beyond the liver, however, depends critically on the extrahepatic targeting advances discussed in the preceding section.

Beyond editing, the literature envisions LNPs as enablers of regenerative and reprogramming medicine, in which mRNA encoding lineage-specifying transcription factors is delivered to coax cells toward repair or regeneration in situ (Gómez-Aguado et al., 2020). Such approaches would sidestep the difficulties of cell transplantation while harnessing the body's own tissues as the therapeutic factory. The unifying thread across these diverse applications is the conception of the LNP as a programmable interface between synthetic genetic information and living cells. As lipid chemistry, targeting biology and manufacturing technology continue to mature in parallel, the range of diseases addressable by this single modular platform is expected to broaden substantially, reinforcing the argument that LNPs constitute foundational infrastructure rather than a single-purpose vaccine technology (Zhang et al., 2021).

Table 4. Emerging LNP applications in next-generation genetic medicine.

Application	Payload	Therapeutic Rationale	Status
In vivo CAR-T generation	CAR-encoding mRNA	Avoids ex vivo cell manufacturing	Preclinical
Personalised cancer vaccines	Neoantigen mRNA	Patient-specific tumour targeting	Clinical trials
In vivo gene editing	Cas9 mRNA + sgRNA	Durable single-dose correction	Clinical trials
Protein-replacement therapy	Enzyme-encoding mRNA	Transient, re-dosable supply	Preclinical / clinical
Regenerative medicine	Transcription-factor mRNA	In situ tissue reprogramming	Preclinical

Taken together, the four domains reviewed above reveal a field in rapid transition. Formulation science has matured to the point where particle properties can be engineered with considerable precision; payload diversity has expanded from a single siRNA product to a broad therapeutic portfolio; targeting strategies are beginning to overcome the hepatic-tropism barrier; and the platform is increasingly conceived as modular infrastructure for genetic medicine. The comparative synthesis in Table 5 distils the principal advantages and limitations that recur across the literature and frames the translational discussion that follows.

Table 5. Comparative summary of LNP advantages and limitations from the reviewed literature.

Dimension	Advantage	Limitation
Manufacturing	Synthetic, scalable, cell-free production	Batch-to-batch variability persists
Payload flexibility	Payload-agnostic modular platform	Cargo size affects encapsulation

Dimension	Advantage	Limitation
Safety	Non-integrating, transient expression	PEG immunogenicity, reactogenicity
Biodistribution	SORT enables organ tuning	Default hepatic accumulation
Stability	Protects labile nucleic acids	Cold-chain and storage constraints

3. Research Methodology

This paper adopts a structured narrative review methodology designed to synthesise heterogeneous evidence on lipid nanoparticle science published between 2015 and 2024. A narrative approach was selected over a systematic meta-analysis because the objective is conceptual integration across mechanistic, formulation and translational domains rather than quantitative pooling of comparable experimental outcomes. The methodology comprised four sequential phases: literature identification, screening and selection, thematic categorisation, and synthesis.

In the identification phase, peer-reviewed literature was surveyed using established scientific databases and citation indices, employing combinations of search terms including "lipid nanoparticles", "ionisable lipids", "mRNA delivery", "siRNA therapeutics", "targeted delivery", "endosomal escape", "selective organ targeting" and "LNP formulation". The search was deliberately restricted to sources published between 2015 and 2024 to ensure that the review reflects the contemporary state of the field, particularly the rapid advances that accompanied and followed the clinical validation of mRNA vaccines. Foundational concepts predating this window are acknowledged where necessary but were not included as primary citations.

During screening and selection, retrieved records were prioritised according to relevance, scientific rigour, citation impact and representativeness across the four thematic domains defined in the literature review. Preference was given to primary research articles reporting formulation–function relationships, authoritative review articles synthesising mechanistic understanding, and reports describing clinical or advanced preclinical translation. Sources were excluded if they lacked peer review, fell outside the temporal window, or addressed lipid-based systems unrelated to the

nanoparticle architectures of interest. This selective process yielded a curated evidence base of twenty-eight sources judged to collectively represent the principal advances and debates in the field.

In the categorisation phase, selected sources were mapped to the four thematic pillars—physicochemical determinants, payload diversification, targeted delivery, and next-generation applications—with many sources contributing to more than one theme. Finally, in the synthesis phase, findings were integrated into a coherent narrative supported by five summary tables that distil component roles, payload classes, targeting strategies, emerging applications and comparative trade-offs. This methodology, while not exhaustive, provides a transparent and reproducible framework for organising a large and rapidly evolving body of evidence, and its principal limitation—susceptibility to selection subjectivity inherent in narrative review—is acknowledged and mitigated through explicit thematic structuring.

4. Translational Challenges and Discussion

Despite the platform's demonstrated clinical success, several interconnected barriers continue to constrain the broader translation of LNP therapeutics. The most persistent is the pronounced hepatic tropism of systemically administered particles, which, while advantageous for liver-directed indications, necessitates the targeting innovations discussed above for any disease residing elsewhere. Even with SORT and ligand-based strategies, achieving robust, reproducible and clinically meaningful extrahepatic delivery at tolerable doses remains an unsolved problem for many tissues (Cheng et al., 2020).

Manufacturing and stability constraints form a second major barrier. Although microfluidic mixing has substantially improved reproducibility, batch-to-batch heterogeneity, scale-up from laboratory to commercial volumes, and the analytical characterisation of complex multi-component particles remain non-trivial (Evers et al., 2018). The stringent cold-chain requirements of current mRNA-LNP products—arising from the chemical lability of both the RNA and the lipids—impose considerable logistical and economic burdens, particularly for global distribution to resource-limited settings. Lyophilisation and the development of thermostable formulations are active areas of investigation aimed at enabling ambient-temperature storage (Schoenmaker et al., 2021).

Immunogenicity and safety considerations constitute a third domain of concern. The PEG-lipid component, while essential for stability, has been associated with the generation of anti-PEG antibodies, accelerated blood clearance on repeat dosing and, in rare cases, hypersensitivity reactions, prompting interest in PEG alternatives and cleavable PEG-lipids (Kularatne et al., 2022). Reactogenicity attributable to the innate immune sensing of both the lipid and the nucleic-acid components must be carefully balanced against therapeutic benefit, especially for chronic, repeat-dose indications where cumulative immune activation is undesirable. The long-term safety profile of repeated LNP administration in non-vaccine contexts remains incompletely characterised and will require extended clinical follow-up.

Finally, regulatory and characterisation frameworks are still adapting to the complexity of these products. Establishing meaningful critical quality attributes, validated release assays and predictive potency measures for structurally heterogeneous nanoparticles poses ongoing challenges for both developers and regulators (Mitchell et al., 2021). Addressing these interconnected barriers—biodistribution, manufacturing, stability, immunogenicity and regulation—will determine the pace at which the platform's considerable promise is realised across the therapeutic landscape.

A cross-cutting theme uniting these challenges is the tension between the platform's structural complexity and the pharmaceutical demand for reproducibility and characterisation. Unlike a small-molecule drug defined by a single chemical structure, an LNP is a heterogeneous supramolecular assembly whose therapeutic behaviour depends on emergent properties that resist complete analytical description (Tenchov et al., 2021). This complexity complicates every downstream activity, from establishing structure–activity relationships to defining release specifications and demonstrating comparability after manufacturing changes. Progress therefore depends not only on chemical innovation but on the parallel development of advanced characterisation techniques capable of resolving particle heterogeneity, internal morphology and cargo distribution with sufficient precision to satisfy regulatory scrutiny.

It is also worth situating these challenges within the broader context of equitable access. The cold-chain and manufacturing demands of current products have concentrated their benefits in high-income settings, and the pursuit of thermostable, lyophilised formulations and simplified, decentralised manufacturing is therefore not merely a technical goal but a matter of global health equity (Schoenmaker et al., 2021). Realising the platform's potential to address diseases prevalent in resource-

limited regions will require deliberate attention to affordability, distribution and local manufacturing capacity alongside the scientific refinements emphasised elsewhere in this review. The discussion thus reinforces that the translation of LNP science is a socio-technical endeavour in which formulation advances must be matched by commensurate progress in logistics, regulation and health-systems infrastructure.

5. Conclusion

Lipid nanoparticles have transitioned from a specialised nucleic-acid delivery technology into one of the most versatile and clinically validated platforms in modern medicine. This review has traced how the four-component architecture that enabled mRNA vaccines is being adapted to a far broader therapeutic mission encompassing gene silencing, protein replacement, in vivo gene editing and cell engineering. The synthesis across four thematic domains reveals a coherent picture: formulation science now permits precise engineering of particle properties; payload diversity has expanded dramatically from the single approved siRNA product to a rich pipeline of genetic medicines; targeting strategies such as SORT and ligand conjugation are beginning to overcome the platform's default hepatic tropism; and the modular, payload-agnostic nature of LNPs positions them as foundational infrastructure for next-generation therapeutics.

At the same time, the review has been candid about the barriers that remain. Extrahepatic delivery, manufacturing reproducibility, cold-chain dependence, PEG-associated immunogenicity and incompletely defined long-term safety collectively temper the platform's promise and define the agenda for the coming decade. The central insight to emerge from the literature is that these challenges are not intrinsic limits of the concept but engineering problems amenable to rational solution through advances in lipid chemistry, targeting biology and process technology. The convergence of these disciplines suggests that LNPs will continue to expand their therapeutic footprint well beyond the vaccines that brought them to prominence.

6. Future Work

Several priority directions emerge from this synthesis. First, the design of expanded libraries of biodegradable ionisable lipids—incorporating cleavable linkages that accelerate clearance and reduce accumulation—should improve both the safety and repeat-dose tolerability of the platform, and

combinatorial and computationally guided screening offers a route to accelerate discovery of superior chemistries (Sabnis et al., 2018). Second, the refinement of organ-selective formulations, building on SORT principles and integrating them with active ligand-mediated targeting, is essential to unlock robust extrahepatic and cell-specific delivery for oncology, immunology and central-nervous-system indications (Cheng et al., 2020).

Third, formulation-stability research aimed at lyophilised, thermostable products should be prioritised to eliminate cold-chain dependence and enable equitable global access, a goal of particular importance for deployment in resource-limited settings (Schoenmaker et al., 2021). Fourth, the maturation of continuous, closed-loop GMP manufacturing with real-time analytical monitoring will be necessary to ensure the reproducibility and scalability demanded by commercial and personalised-medicine applications alike. Fifth, the field would benefit from systematic long-term safety studies of repeat-dose LNP administration in non-vaccine contexts, alongside the development of PEG alternatives and immunologically inert surface chemistries.

Finally, the most transformative future work lies in advancing in vivo cell-engineering and gene-editing applications from proof-of-concept toward routine clinical practice. Realising the vision of a single injection that reprogrammes immune cells or durably corrects a genetic defect will require the coordinated advancement of targeting precision, editing efficiency, payload co-delivery and safety monitoring. Pursuing these directions in an integrated fashion offers the clearest path to fulfilling the extraordinary therapeutic potential that lipid nanoparticles have only begun to demonstrate (Mitchell et al., 2021; Hou et al., 2021).

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