Nano Research

ISSN 1998-0124 CN 11-5974/O4
https://doi.org/10.26599/NR.2026.94909290

Review Article

Recent advances in the use of lipid nanoparticles (LNPs) as delivery vectors for mRNA

jianmei Li[1,2,§], wenlin Wu[1,2,§], jie Dai[3,§], xingchen Peng[1,2](✉)

[1] Department of Biotherapy, Cancer Center, West China Hospital, Sichuan University, Chengdu 610041, China

[2] Nuclear Physics and Medical Research Key Laboratory of Sichuan Province, Sichuan University, Chengdu 610041, China

[3] Department of High Altitude Medicine, Center for High Altitude Medicine, West China Hospital, Sichuan University, Chengdu 610041, China

[§] jianmei Li, wenlin Wu and jie Dai contributed equally to this work.

Nano Res., **Just Accepted Manuscript** • https://doi.org/10.26599/NR.2026.94909290

https://www.sciopen.com/journal/1998-0124 on Oct. 26, 2026

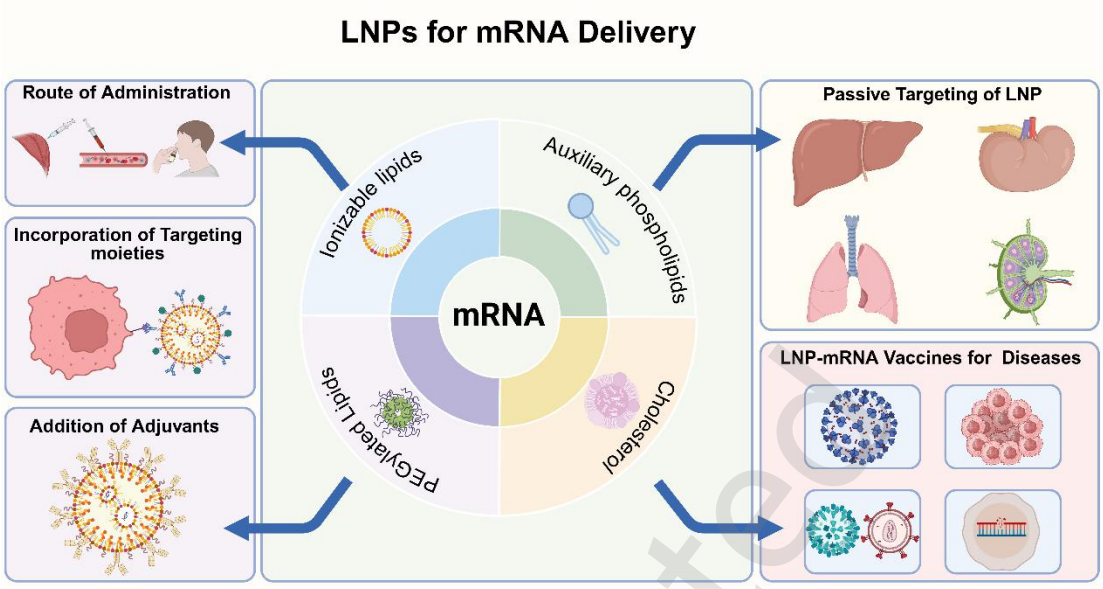
© The Authors(s)

Just Accepted

This is a "Just Accepted" manuscript, which has been examined by the peer-review process and has been accepted for publication. A "Just Accepted" manuscript is published online shortly after its acceptance,which is prior to technical editing and formatting and author proofing. Tsinghua University Press (TUP) provides "Just Accepted" as an optional and free service which allows authors to make their results available to the research community as soon as possible after acceptance. After a manuscript has been technically edited and formatted, and the page proofs have been corrected, it will be removed from the "Just Accepted" web site and published officially with volume and article number (e.g., *Nano Research*, **2025**, *18*, 94906990). Please note that technical editing may introduce minor changes to the manuscript text and/or graphics which may affect the content, and all legal disclaimers that apply to the journal pertain. In no event shall TUP be held responsible for errors or consequences arising from the use of any information contained in these "Just Accepted" manuscripts. To cite this anuscript please use its Digital Object Identifier (DOI®),which is identical for all formats of publication.

Just Accepted

TABLE OF CONTENTS (TOC)



This review systematically summarizes mRNA delivery mediated by lipid nanoparticles (LNPs), including their composition, key influencing factors, and major challenges, and looks ahead to achieving safe, efficient, and precise delivery through rational design and engineering innovations.

Just Accepted

Nano Research

Open Access

Review Article

Recent advances in the use of lipid nanoparticles (LNPs) as delivery vectors for mRNA

jianmei Li [1,2§], wenlin Wu [1,2§], jie Dai [3§], xingchen Peng[1,2]

1 Department of Biotherapy, Cancer Center, West China Hospital, Sichuan University, Chengdu 610041, China
2 Nuclear Physics and Medical Research Key Laboratory of Sichuan Province, Sichuan University, Chengdu 610041, China
3 Department of High Altitude Medicine, Center for High Altitude Medicine, West China Hospital, Sichuan University, Chengdu 610041, China
§ jianmei Li, wenlin Wu and jie Dai contributed equally to this work.

Received: 22 July 2026; **Revised:** 14 October 2026; **Accepted:** 26 October 2026

Address correspondence to pxx2014@163.com, pxx2014@scu.edu.cn

Cite this article: *Nano Research*, 2026, 19, 94909290 https://doi.org/10.26599/NR.2026.94909290

ABSTRACT: Therapeutic mRNA has demonstrated great promise in infectious disease vaccines, cancer immunotherapy, and protein replacement therapies. However, its clinical translation is hampered by intrinsic limitations, including rapid degradation, poor membrane permeability, extremely low endosomal escape, lack of tissue specificity, and potential immunogenicity. Naked mRNA alone is insufficient to overcome these challenges, which has driven the emergence of lipid nanoparticles (LNPs) as the leading clinical-grade delivery platform. LNPs employ a four-component system: ionizable lipids for encapsulation and pH-responsive fusion; helper phospholipids and cholesterol for structural integrity and membrane fluidity; and PEGylated lipids for colloidal stability and prolonged circulation. This review systematically addresses LNP composition, structure–activity relationships, and the interplay with extrinsic factors—including administration routes, targeting ligands, immunostimulant co-delivery, and organ tropism (liver, spleen, lungs, lymph nodes)—that collectively govern mRNA biodistribution and therapeutic efficacy. Nonetheless, several major bottlenecks persist, such as unclear endosomal escape mechanisms, PEG-related immune interference, obstacles to extrahepatic delivery, manufacturing complexities, and challenges in AI-driven lipid design. Future progress will rely on integrating rational design, high-throughput screening, and engineering innovations to develop precision delivery systems that are safe, efficient, and amenable to repeated administration—thereby fully unlocking the clinical potential of mRNA-based therapies.

KEYWORDS: mRNA delivery system, lipid nanoparticles (LNPs), passive targeting, ionizable lipids, liposomes

Just Accepted

1. Introduction

Messenger RNA (mRNA) therapy has made remarkable advances in recent years. Following the emergency authorization of two mRNA vaccines during the COVID-19 pandemic and the subsequent launch of a vaccine against respiratory syncytial virus (RSV), mRNA technology has rapidly expanded into cutting-edge fields such as cancer immunotherapy, protein replacement therapy, gene editing, and treatments for rare diseases. As a result, mRNA has transitioned from a proof of concept to commercialization in an exceptionally short period of time[1,2]. However, for this technology to fully realize its clinical potential, it must overcome a fundamental challenge: the inherent physicochemical and biological limitations of the mRNA molecule itself. These limitations result in multiple delivery barriers within the body—from the extracellular to the intracellular environments, and from endosomes to lysosomes—which collectively represent the primary obstacle to the widespread clinical application of mRNA therapeutics.

These obstacles arise from the inherent properties of mRNA itself. As a single-stranded polynucleotide comprising hundreds to thousands of nucleotides, mRNA has a molecular weight of 10^4–10^6 Da and carries a dense negative charge. Consequently, it encounters challenges at nearly every stage within the physiological environment, The first major hurdle is stability: exposed mRNA is highly susceptible to degradation by extracellular ribonucleases (RNases)[3,4]. It is also rapidly cleared through phagocytosis by macrophages and renal filtration, resulting in an extremely short half-life. Furthermore, mRNA demonstrates poor stability throughout all stages of storage, transportation, and in vivo delivery[5,6]. Second, even if mRNA temporarily escapes degradation, its large size and negative charge cause strong electrostatic repulsion with the negatively charged phospholipid bilayer of the cell membrane. This significantly hinders its ability to cross the cell membrane and enter the cytoplasm. In fact, studies have reported that only about 0.01% of administered mRNA penetrates the blood vessel wall, reaches target cells, and exerts its intended function[7]. The situation is further complicated by the fate of mRNA that does enter the cell via endocytosis: it is typically sequestered within endosomal vesicles. Unless it can promptly escape, mRNA is trafficked to lysosomes and fully degraded by acidic hydrolases—a phenomenon known as “endosomal trapping”[8]. This process is considered the rate-limiting step in mRNA delivery, with less than 1% to 4% of the internalized mRNA managing to escape into the cytoplasm and be successfully translated into proteins[9]. Beyond these intracellular delivery barriers, mRNA also faces substantial challenges in targeting and safety at the systemic level. On one hand, mRNA lacks a natural affinity for specific tissues or cells, leading to non-

specific accumulation in off-target organs—most notably the liver—after systemic administration. This decreases bioavailability and raises the risk of off-target toxicity. On the other hand, exogenous mRNA can be detected by the body's pattern recognition receptors, such as Toll-like receptors 3, 7, and 8, triggering unintended innate immune responses. In addition, the delivery vehicles and their metabolites may induce cytotoxicity and long-term toxic side effects[10]. These multiple and interwoven barriers—from the molecular and cellular scales to the tissue and systemic levels—collectively highlight the significant challenges that must be overcome for the clinical translation of mRNA therapies. They also underscore the strategic importance of developing efficient delivery systems as the key to surmounting these obstacles.

In summary, although mRNA therapy has made groundbreaking advances, its clinical translation remains limited by several bottlenecks, including delivery efficiency, targeting accuracy, and safety concerns. Addressing these challenges requires a two-pronged approach. On the one hand, it is essential to enhance the stability and translational efficiency of mRNA itself while reducing immunogenicity—accomplished through sequence engineering strategies such as 5' cap structure optimization, nucleoside modification, untranslated region (UTR) design, and self-amplifying RNA (saRNA) technology. On the other—and arguably the more critical aspect—it is necessary to develop carrier systems capable of delivering mRNA to target sites efficiently, safely, and precisely. Among currently available delivery vectors, lipid nanoparticles (LNPs) have emerged as the most clinically advanced mRNA delivery platform, owing to their highly efficient nucleic acid encapsulation mediated by ionizable lipids, pH-responsive endosome escape mechanisms, and formulation versatility provided by four key components. LNPs have enabled the successful deployment of several approved products[11]. However, they still face significant challenges regarding targeting specificity, endosomal escape efficiency, immunogenicity control, and large-scale manufacturing. These limitations have prompted researchers to pursue the rational design and precise engineering of next-generation LNPs. This paper systematically reviews the basic composition and functional principles of LNPs, along with the latest research advances in mRNA delivery, with the aim of providing design insights for the development of more efficient, safer, and more targeted mRNA delivery systems.

2. mRNA Delivery System

Among the various mRNA delivery strategies, vector systems are primarily divided into two major categories: viral vectors and non-viral vectors [12,13]. Viral vectors (such as adeno-associated viruses and lentiviruses), leveraging their natural ability to infect cells, can efficiently mediate the delivery of therapeutic RNA after modification[14][15]; however, their inherent drawbacks-including potential carcinogenic risks, high immunogenicity, and complex production processes-severely limit their widespread clinical application. In contrast, non-viral vectors demonstrate significant advantages in terms of structural flexibility, low immunogenicity, and high RNA loading capacity, encompassing various types such as lipids, polymers, inorganic materials, and exosomes. Among these, lipid-based lipid nanoparticles (LNPs) stand out in particular[16][17]. With their well-defined structure, ease of process scale-up, potential for functionalization and targeted modification, low toxicity, and high delivery efficiency, they have surpassed other non-viral vectors to become the most extensively studied and clinically successful platform in the field of mRNA delivery[18–21]. Based on these advantages, the successful application of LNP in several commercially available mRNA vaccines has fully demonstrated its feasibility; however, further breakthroughs are still needed in areas such as targeting specificity, endosomal escape efficiency, and safety-which are the key points to be discussed in the following sections. Figure 1 illustrates the different types of delivery systems for mRNA vaccines.

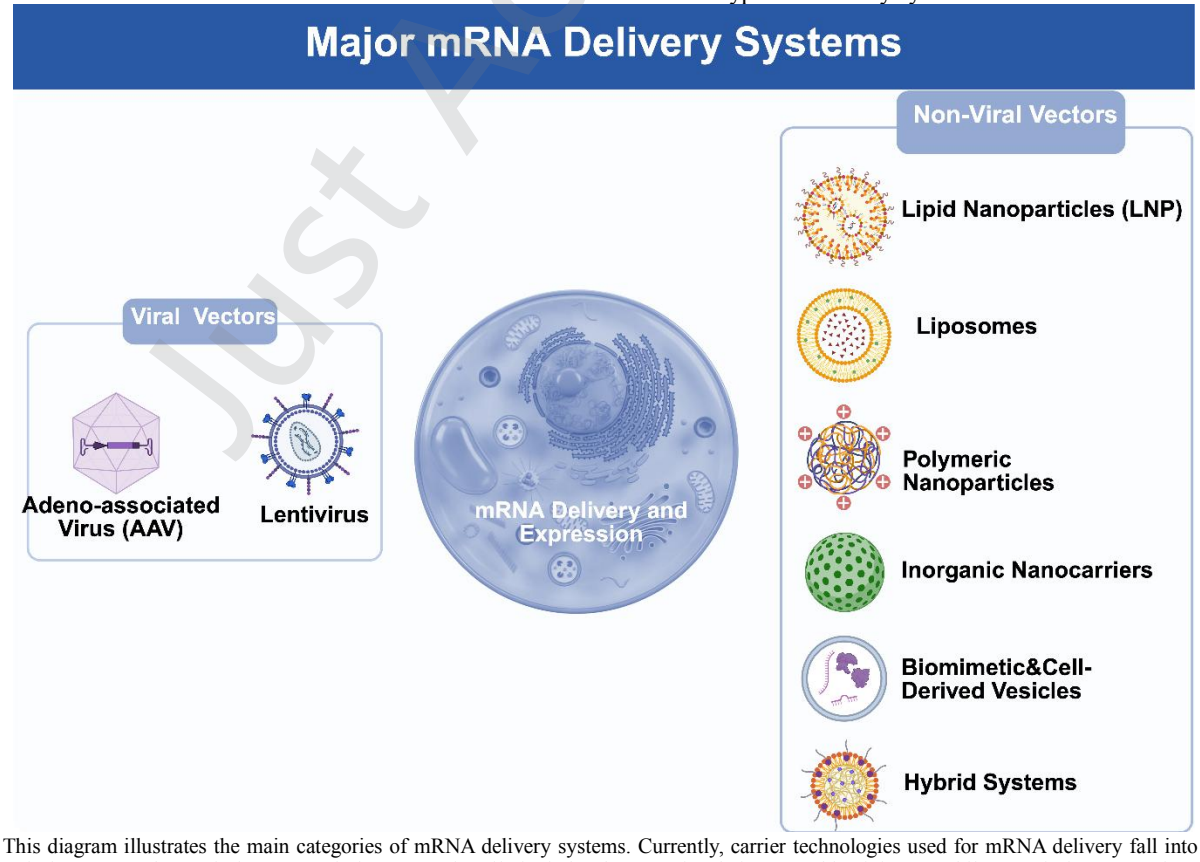


Figure 1. This diagram illustrates the main categories of mRNA delivery systems. Currently, carrier technologies used for mRNA delivery fall into two broad categories: viral vectors and non-viral vectors. Viral vectors primarily include aden-associated viruses and lentiviruses, while non-viral vectors primarily include lipid nanoparticles, liposomes, polymeric nanoparticles, inorganic nanocarriers, biomimetic and cell-derived vesicles, and hybrid systems. Created in

Just Accepted

2.1 Other Nanocarrier Platforms

Polymer nanoparticles (PNPs), formulated from natural or synthetic polymers, serve as important complementary platforms for mRNA delivery [22,23]. Common materials include PLGA, PCL, PEG, PEI, and chitosan [24–27]. Optimized PLGA lipid-polymer hybrid nanoparticles can confine protein expression to the injection site, locally outperforming conventional LNPs in hamster models [28]. Replacing PLGA with medium-chain-length polyhydroxyalkanoate (mcl-PHA) yields nanoparticles with high stability during lyophilization [29] While PEI efficiently complexes mRNA, it exhibits high cytotoxicity [30]; in contrast, ionizable lipopolymers demonstrate up to 300-fold higher delivery efficiency than PEI derivatives, enabling targeted lung delivery and CRISPR editing [31], Surface modification also plays a critical role: PEGylation prolongs circulation but may trigger anti-PEG antibodies and the accelerated blood clearance (ABC) effect, complicating repeated dosing regimens [32]. Furthermore, chitosan- and mannose-modified mRNA LNPs delivered via the lungs have been shown to persist for up to 72 hours, induce mucosal immunity and tissue-resident memory T cells (TRMs), and provide protection against SARS-CoV-2 pseudoviruses [33]. Thus, while PNPs offer diverse therapeutic prospects, challenges regarding toxicity, immunogenicity, and targeting accuracy remain. Future work should focus on optimizing materials, hybrid designs, and multifunctional modifications to facilitate clinical translation.

Inorganic nanocarriers—such as AuNPs, MSNs, and CaP—possess rigid frameworks, controllable sizes, and modifiable surfaces, facilitating the attachment of targeting ligands and imaging probes for integrated theranostic applications. AuNPs can anchor mRNA prior to LNP encapsulation, enabling synergistic multi-mRNA delivery and overcoming issues of uneven encapsulation [34,35]. MSNs, characterized by their high surface areas and tunable pores, are effective for delivering mRNA, siRNA, and CRISPR-Cas systems[36–38]. CaP is highly biocompatible and acid-degradable; Khalifeh et al. incorporated ionizable lipids into the outer layer of lipid-coated CaP nanoparticles (LCPs), significantly enhancing endosomal mRNA release[39]. Additionally, Mn-mRNA nanoparticles, formed via Mn^{2+} coordination, combine delivery capabilities with auto-adjuvant activity to enhance antigen-specific immunity[40–43]. Nevertheless, gold and silica degrade slowly, posing a risk of chronic toxicity[44]; and cationic modifications can increase nonspecific protein adsorption and immunogenicity. Consequently, inorganic carriers remain largely limited to in vitro transfection or local injection, highlighting the need for biodegradable or readily cleared systems for systemic translation.

Biomimetic strategies, including the use of exosomes and cell membrane-coated nanoparticles, provide natural vehicles for mRNA delivery. Exosomes (30–150 nm) naturally display CD47, which confers low immunogenicity, prolonged circulation, organ tropism, and the ability to cross the blood-brain barrier [45–47] . Small extracellular vesicles delivering CAR-mRNA via inhalation have successfully generated lung CAR macrophages and inhibited lung cancer metastasis [48]. However, the clinical translation of exosomes is hindered by challenges in high-yield purification, batch consistency, and mRNA loading efficiency; current automated platforms, such as FACTORY, remain insufficient for clinical-grade standardization [49]. Alternatively, cell membrane coating transfers parent-cell identity signals to nanocarriers, promoting active targeting and immune evasion [50]. Macrophage membranes can guide nanoparticles to arthritic or tumor microenvironments [41,51]; platelet membranes can target damaged vessels and circulating tumor cells [52], and red blood cell (RBC)-coated LNPs prolong systemic circulation while reducing liver uptake [53–55]. Key bottlenecks for this technology include membrane heterogeneity, coating integrity, and in vivo stability. Future advances in automated isolation, non-destructive loading techniques, and engineered cell lines are essential to translate biomimetic systems into viable clinical therapeutics for cancer, neurological disorders, and genetic diseases.

2.2 Liposomes

As one of the earliest nanocarriers employed for nucleic acid delivery, liposomes are composed of a closed lipid bilayer formed by the spontaneous assembly of phospholipid molecules in an aqueous phase. The aqueous core can encapsulate hydrophilic nucleic acid drugs such as mRNA, while the lipid bilayer can incorporate functional lipids[56–58]. However, traditional liposomes—especially cationic liposomes—have long faced multiple insurmountable challenges during in vivo mRNA delivery, including poor stability, strong serum protein adsorption, low endosomal escape efficiency, and significant cytotoxicity[59]. A major breakthrough in overcoming these obstacles was achieved with the introduction of ionizable lipids. These lipids remain nearly electrically neutral at physiological pH, which significantly reduces serum protein adsorption and clearance by the mononuclear phagocyte system. Once inside the cell, they become protonated in the acidic endosomal environment, triggering membrane fusion and facilitating the release of mRNA into the cytoplasm. This innovation in molecular design was pivotal for the emergence of modern lipid nanoparticles (LNPs), ultimately enabling the clinical translation of mRNA vaccines[19,60]. Figure 2 illustrates the history of the LNP delivery system. The profound transformation in the fate of liposomes stems from precise regulation of their chemical structure. Studies have shown that the rigidity of the lipid bilayer, the surface charge state, and the degree of polyethylene glycol (PEG) conjugation collectively determine the interaction patterns between liposomes and serum proteins, thereby influencing their circulatory clearance rates and tissue distribution[61,62]. Traditional cationic liposomes are rapidly cleared because their positive charge promotes adsorption of negatively charged serum proteins, whereas ionizable liposomes maintain electrical neutrality under physiological conditions, resulting in a significantly prolonged circulation half-life[63]. This key advantage was clinically validated in Onpattro, the first approved siRNA drug, which utilizes the ionizable lipid MC3[64]. Subsequently, mRNA vaccines targeting COVID-19 further demonstrated the versatility of this platform. Although Moderna's SM-102 and BioNTech/Pfizer's ALC-0315 differ markedly in structure—the former incorporates ester bonds to enhance biodegradability, while the latter features highly branched tails to optimize endosomal escape kinetics—both achieved high levels of antigen expression and robust immunoprotection [65,66] , These success stories clearly illustrate that even subtle modifications to the chemical structure of lipids can have a decisive impact on delivery efficiency.

With the delivery capacity of the LNP platform established, current research is now expanding from "high-efficiency delivery" to "precision delivery." By adjusting lipid composition and preparation parameters, researchers have achieved selective targeting of organs such as the liver, spleen, and even the lungs. Additionally, the development of responsive liposomes (such as pH-sensitive and enzyme-sensitive types) enables spatiotemporally controlled release of mRNA in specific physiological or pathological microenvironments[67]. Nevertheless, the LNP platform still faces unavoidable

Under Review

challenges: immunogenicity associated with repeated PEGylated dosing, dosing intervals inadequate for the long-term treatment of chronic diseases, and batch-to-batch variability in large-scale production all continue to limit its broader application. Overcoming these bottlenecks will depend on the development of novel biodegradable lipids, innovations in non-PEG-based surface modification strategies, and the widespread adoption of advanced manufacturing processes such as microfluidics.

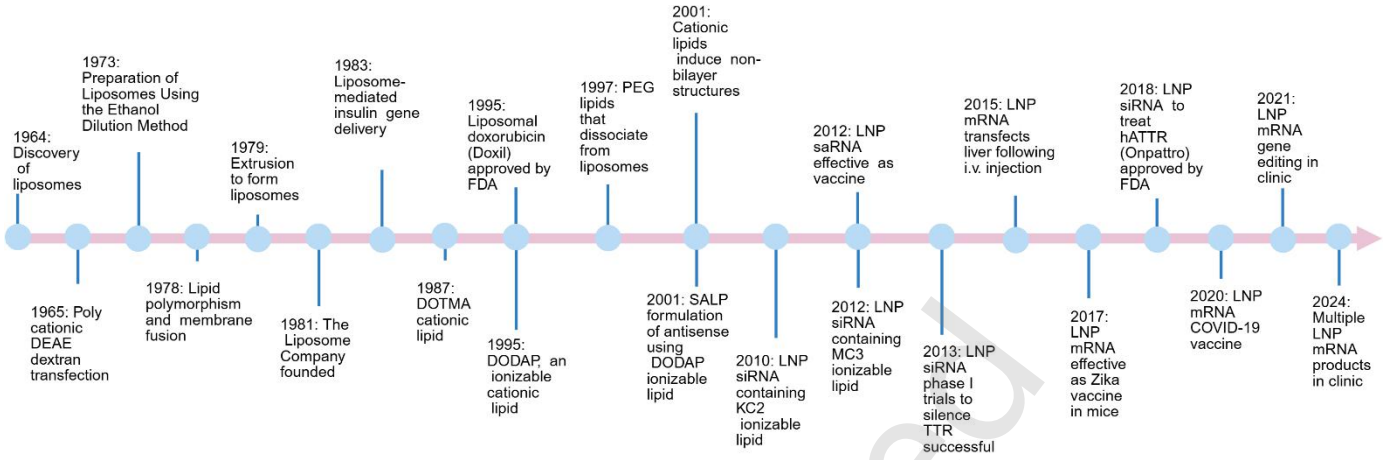


Figure 2. This diagram illustrates the timeline of LNP's progress[68]. DEAE, diethyaminoethyl; i.v., intravenous; LNP, lipid nanoparticle; PEG, polyethylene glycol; siRNA, small interfering RNA. Created in BioRender.com.

3. Basic Structure and Functional Principles of LNP Delivery Vectors

Lipid nanoparticles (LNPs) are a well-established and clinically validated system for mRNA delivery. Their effectiveness arises from the synergistic interaction of four key components: ionizable lipids, auxiliary phospholipids, cholesterol, and PEG-conjugated lipids. Ionizable lipids facilitate endosomal escape, while auxiliary phospholipids and cholesterol maintain structural stability. PEG-conjugated lipids extend circulation time but may reduce cellular uptake. The interplay—both cooperative and antagonistic—among these components ultimately determines delivery performance. The structure-activity relationships of each component will be analyzed in the following sections. Figure 3 shows the main components of the LNP delivery system.

3.1 Ionizable lipids

Ionizable lipids serve as the functional core of lipid nanoparticle (LNP) delivery systems; their sophisticated pH-responsive mechanisms directly determine whether mRNA therapeutics can reach their targets efficiently and safely. The key to this mechanism lies in the precise design of their acid dissociation constant (pKa): at physiological pH (approximately 7.4), ionizable lipids remain electrically neutral or only weakly cationic, which effectively reduces nonspecific protein adsorption and systemic toxicity. However, when LNPs are internalized by cells into endosomes, the acidic microenvironment triggers protonation, rendering the lipids positively charged. This protonation enables electrostatic interactions with the anionic endosomal membrane, thereby disrupting membrane structure and facilitating the escape of mRNA into the cytoplasm, where it can exert its therapeutic effect[69–71].It is precisely this "neutral in extracellular space, acid-activated" intelligent response that makes the pKa value a key parameter in determining delivery efficiency. Early cationic lipids, due to their excessively high pKa values, remained positively charged under physiological conditions, which resulted in strong hemolytic activity and toxic reactions such as damage to red blood cell membranes and hemoglobin leakage[72]. To address this limitation, researchers developed ionizable lipids with pKa values ranging from 6.0 to 7.0[73].These lipids are designed to achieve a triple functional balance: they become fully protonated in acidic environments (e.g., pH 4.0), efficiently encapsulating nucleic acid drugs[74]; they maintain near-neutrality at physiological pH, thus reducing circulatory toxicity[75]; and they re-protonate upon entering endosomes or lysosomes (where the pH is lower than the apparent pKa), promoting membrane destabilization and mRNA release[76]. It is worth noting that the optimal pKa range varies depending on the route of administration. Studies have shown that intravenously administered LNPs exhibit optimal delivery efficiency at a pKa of 6.2–6.6, while intramuscularly administered mRNA vaccines perform best within the 6.6–6.9 range[64].This finding provides quantitative evidence for the rational design of lipid components. Currently, the three most prominent clinical systems—MC3, SM-102, and ALC-0315—are classic examples embodying these design principles[77].Studies indicate that LNPs formulated with SM-102 and ALC-0315 significantly outperform MC3 in terms of in vivo protein expression, with no significant differences observed between the former two, thereby demonstrating the delivery advantages of this new generation of lipids[78,79]. Recent studies, employing a systematic "subtraction" strategy to sequentially remove individual lipid components from LNPs, have revealed the central role of ionizable lipids in mRNA encapsulation, membrane fusion, and endosomal escape, while also quantifying their contributions to in vivo homing and immune activation[17]. In addition, using a cheminformatics-guided synthetic strategy, Su et al. developed aromatic ionizable lipids that enhance mRNA delivery by coordinating multiple mechanisms[7]. Looking ahead, innovation in ionizable lipids is advancing toward more refined pH responsiveness, lower toxicity, and higher efficiency. Novel scaffold designs—such as molecular structures based on amino acid or polyamine derivatives—together with systematic optimization of hydrophobic chain length and branching degree, have all been shown to effectively regulate LNP stability, cellular uptake, and endosomal escape. These ongoing advancements will continue to propel LNP delivery technology from "effective" to "precise and controllable," providing a stronger material foundation for the clinical translation of mRNA therapies.

Just Accepted

3.2 Auxiliary phospholipids

Ionizable lipids determine whether LNPs can achieve "precise release," while auxiliary phospholipids regulate "how LNPs reach their target, how they enter cells, and how they respond." As key structural components of LNP formulations, auxiliary phospholipids (primarily DSPC, DOPE, and DOPC) do not participate in pH-responsive charge switching. Instead, they form an inert lipid bilayer backbone that profoundly influences the stability, cellular uptake, organotropism, and immunogenicity of the delivery system by modulating membrane rigidity, fluidity, and endosomal fusion properties[80].Structural differences among auxiliary phospholipids are mainly reflected in their membrane physical properties, which in turn impact functional performance at the cellular level[81]. For example, Zhang et al. systematically summarized the effects of different types of phospholipids on the efficacy, targeting, and safety of LNPs[82]. DSPC, which contains saturated alkyl chains (C18:0), imparts high membrane rigidity and chemical stability to LNPs, making it the most widely used auxiliary phospholipid in FDA-approved clinical mRNA-LNP formulations[83].However, this rigid structure also results in functional trade-offs: lower membrane fluidity inhibits cellular uptake efficiency. In contrast, unsaturated phospholipids (such as DOPE, DOPC, and POPC) significantly enhance membrane fluidity, thereby promoting uptake by immune cells, including macrophages and dendritic cells. Among these, DOPE—owing to its conical molecular geometry—is particularly effective at facilitating endosomal escape[84].These physicochemical differences lay the structural foundation for all subsequent functional variations. Differences in membrane fluidity further translate into organ-specific biodistribution, positioning the auxiliary phospholipid as a key regulatory node for LNP distribution in vivo[85].Studies have shown that DOPE-containing LNPs exhibit a higher affinity for the liver, a mechanism attributed to DOPE's ability to enhance LNP binding to apolipoprotein E (ApoE), which mediates hepatic uptake of lipoproteins. However, when DOPE is replaced with DSPC, LNP spleen-targeting efficiency is significantly improved: the delivery efficiency of siRNA and mRNA to the spleen increases by 2-fold and 5-fold, respectively[86].This suggests that simply changing the type of auxiliary phospholipid enables fundamental regulation of LNP organ distribution without the need to modify targeting ligands.

3.3 PEGylated Lipids

Although PEG-modified lipids comprise only 1.0%–2.5% of the molar composition in LNPs, their functional impact is profound. Their molecular design follows a "lipophilic anchoring + hydrophilic extension" block structure: the hydrophobic acyl chains are embedded in the lipid bilayer to anchor the particles, while the hydrophilic PEG chains extend outward to form an "invisible" coating. This effectively reduces serum protein adsorption, prevents immune system clearance, and inhibits particle aggregation, thereby significantly prolonging the in vivo circulation half-life[87–89].All commercial mRNA vaccines adopt this design paradigm—Moderna uses PEG2000-DMG (C14 chain), while BioNTech/Pfizer uses ALC-0159; both employ a C14 acyl chain paired with PEG2000 as the standard configuration[90].Notably, the structural parameters of PEGylated lipids do not simply determine the "stealth effect," but rather regulate the "timing of detachment" through the length of the chains, thereby influencing the entire subsequent delivery process. Studies have shown that acyl chain length is a decisive factor: short chains (C14) dissociate more rapidly in vivo, enabling timely detachment of the PEG coating and exposing the LNP surface, which facilitates cellular uptake and endosomal escape. Consequently, their transfection efficiency is significantly higher than that of long-chain (C18) systems[87,91–93],Further screening has revealed that DMG-PEG5000 can perform even better after optimization, suggesting room for synergistic optimization between PEG chain length and lipid tail length[87]. However, while PEGylated lipids provide the advantage of extended circulation, they also present an unavoidable immunogenicity bottleneck—the "PEG dilemma." Anti-PEG antibodies are prevalent in the general population, and repeated dosing can induce an accelerated blood clearance (ABC) effect, leading to rapid clearance of subsequent doses and severely compromising the efficacy of multidose regimens[94].This contradiction is particularly challenging in the treatment of chronic diseases or in scenarios requiring repeated administration, prompting researchers to explore alternative non-PEG polymers to overcome this issue. Current alternative platforms have demonstrated clear advantages. Both POEGMA (with a bristle-like structure) and PDMA can avoid the ABC effect: among these, POEGMA(+) is suitable for individuals with low pre-existing antibody levels, while PDMA is more appropriate for patients with existing anti-PEG antibodies[32];Poly(2-ethyl-2-oxazoline) (PEtOx/PEOZ), which has a distinct cellular uptake mechanism from PEG and exhibits lower immunogenicity, has—in some formulations—even outperformed the PEG lipids used in Comirnaty in animal studies[95,96]; Polycarboxybetaine (PCB) LNPs maintain transfection efficiency during repeated dosing and have been successfully applied to gene editing in primary human T cells[97]. In the future, the mRNA delivery landscape will no longer be dominated by a single PEG-LNP platform. Instead, it will evolve toward a model in which classic PEG-LNPs and novel PEG-free platforms coexist, enabling on-demand selection and offering more targeted, differentiated options for various indications and dosing regimens.

3.4 Cholesterol

Among the four major lipid components of LNPs, cholesterol—although it does not directly participate in pH-responsive charge switching nor play a dominant role in regulating membrane fluidity—serves as a "binder" and "regulator" that bridges the physical stability and biological activity of LNPs, owing to its unique rigid steroidal ring structure and hydrophobic tail chains. Its role has evolved from that of a mere structural filler to a multifunctional module actively regulating delivery efficiency, tissue targeting, and immune responses. Cholesterol's functional contribution lies primarily in stabilizing the physical properties of the membrane[86,98,99].Its steroidal ring and lipophilic tail can insert into the lipid bilayer, synergizing with auxiliary phospholipids (such as DSPC) to form "liporaft"-like microdomains. These structures significantly enhance membrane rigidity and compactness, thereby improving both the structural stability of LNPs and the encapsulation efficiency of mRNA[100,101].This increased rigidity not only prevents premature leakage of nucleic acid drugs during circulation but also provides the necessary membrane tension to facilitate subsequent endosomal escape. However, the effect of cholesterol on LNP performance is not simply "the more, the better"; rather, there exists a precise "optimal window." Studies have shown that excessively high cholesterol content can cause LNPs to become trapped in early endosomes, hindering the effective release of mRNA into the cytoplasm. Conversely, a 20 mol% cholesterol incorporation has been shown to yield optimal transfection efficiency in pulmonary macrophages[102].This dose-response relationship suggests that cholesterol modulation requires a delicate balance between "maintaining integrity" and "facilitating escape"—an excess is inhibitory, while insufficiency leads to instability.To

Just Accepted

overcome the functional limitations of native cholesterol, researchers have expanded its application through three main approaches: substitution, modification, and simplification. Regarding substitution, phytosterols (such as β-sitosterol) have been shown to alter LNP morphology and enhance endosomal escape capacity. However, excessive amounts can also inhibit escape efficiency, indicating that substitutes must also be carefully optimized[100].In terms of functionalization, the cholesterol molecule itself can be engineered to serve as a carrier for targeting ligands: for example, mannose-conjugated cholesterol enables active targeting of CD206-positive cells[103];histidamide-conjugated cholesterol (Hchol) exploits the protonation of its imidazole group in acidic environments to exacerbate endosomal membrane destabilization[104]; and ionizable cholesterol lipids (iChol), by weakening their interaction with apolipoprotein E (ApoE), can successfully redirect LNP delivery from the liver to the spleen[105].Furthermore, cholesterol-modified cationic peptides (such as DP7) can be combined with liposomes to form co-delivery systems that possess both transmembrane capabilities and immunostimulatory functions, further enhancing dendritic cell activation by personalized neoantigen mRNA vaccines[106].

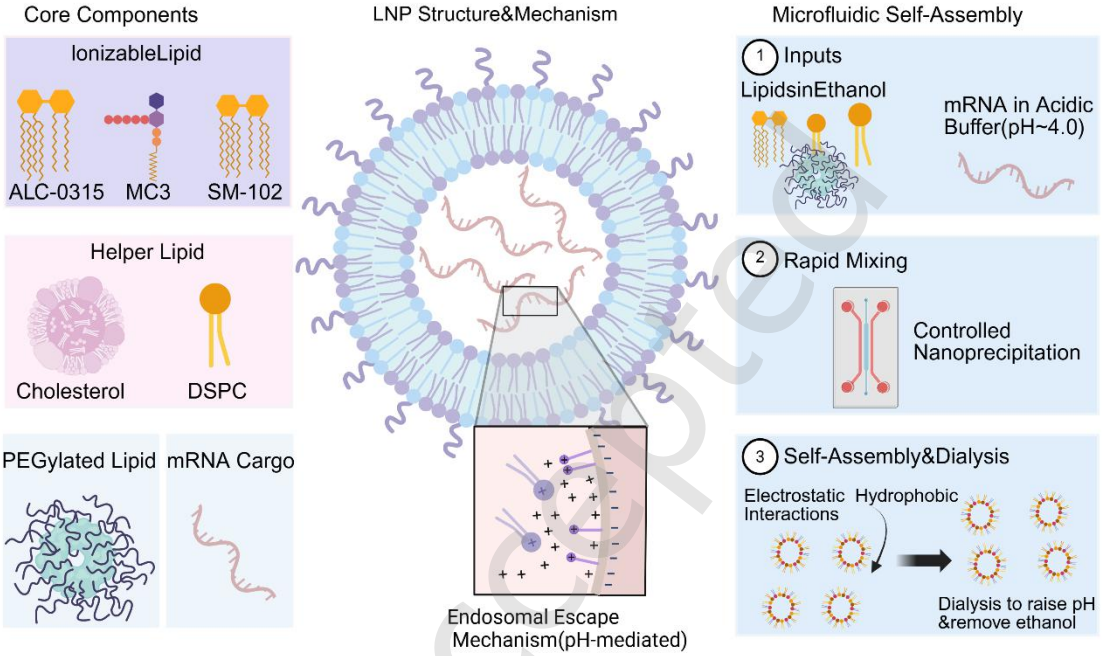


Figure 3. The schematic diagram illustrates the structure and components of LNP, which consists primarily of four parts: ionizable lipids, auxiliary phospholipids, polyethylene glycol-modified lipids, and cholesterol. Created in BioRender.com.

4.Factors Affecting the In Vivo Delivery and Uptake of RNA/LNP Vaccines

Although intrinsic optimization of the LNP formulation and mRNA sequence forms the foundation for enhancing vaccine performance, actual in vivo efficacy is often limited by both macroscopic delivery pathways and microscopic targeting precision. The route of administration determines the first barrier the LNP encounters-such as retention in muscle tissue or the first-pass effect in the liver-while the incorporation of targeting ligands is essential for enabling the LNP to actively navigate and overcome nonspecific distribution. At the same time, the judicious inclusion of adjuvants provides the intrinsic stimulus necessary for eliciting a robust immune response. The synergistic design of these three elements serves as a crucial bridge, linking the physicochemical properties of the drug to clinical immunological outcomes. Figure 4 illustrates the key factors affecting the in vivo efficacy of LNP-delivered mRNA therapies.

4.1 Route of Administration

The four major lipid components of LNPs collectively determine their physicochemical properties and in vivo behavior. However, whether they ultimately exert therapeutic effects at the target site also depends on an equally critical variable: the route of administration. Different routes present distinct biological barriers, target tissue microenvironments, and immunological requirements. Therefore, the selection of an administration route is not simply a matter of "injection method," but rather a fundamental aspect of LNP delivery strategy design. It must be synergistically optimized with lipid composition to maximize efficacy and minimize toxicity[107].Intramuscular injection is the preferred route for currently commercialized mRNA vaccines, with its primary advantage being the natural coupling of "local sustained release and lymphatic drainage"[108–110].LNPs form a drug reservoir in muscle tissue and are gradually drained via lymphatic vessels to the draining lymph nodes, where antigens are presented on antigen-presenting cells such as dendritic cells and macrophages. This process efficiently stimulates adaptive immunity[111,112].For this route, LNPs must exhibit "moderate retention." They should neither diffuse into the bloodstream too rapidly—a scenario that could reduce local efficacy—nor persist in the tissue for too long, which would delay clearance. Studies have shown that LNPs formulated with the YK series of ionizable lipids can strictly limit protein expression to the injection site. When loaded with RSV preF-mRNA, these LNPs not only elicit a superior immune response compared to SM-102, but also significantly reduce lung injury[113].Similarly, DOG-IM4 LNPs achieve local retention, whereas MC3 LNPs induce higher levels of inflammatory cytokines due to their rapid migration to draining lymph nodes[114].However, when systemic drug distribution is required, intravenous injection is employed to deliver LNPs directly into the bloodstream. This route, however, introduces a new challenge: "first-pass" capture by the liver. In the bloodstream, apolipoprotein E (ApoE) preferentially binds to the LNP surface, mediating uptake by hepatocytes and resulting in highly concentrated mRNA expression in the liver[79].To overcome this hepatic bias, researchers have developed various

First Accepted

redirection strategies. For example, a barcoded mRNA platform enables the simultaneous tracking of different LNPs' distribution in the liver, lungs, and spleen[115].BA-LNPs (such as CA-100), constructed by replacing cholesterol with bile acids, significantly enhance splenotropism; moreover, this strategy enables transfer from the liver to the spleen across various ionizable lipid systems[116].These examples demonstrate that intravenous administration urgently requires lipid engineering to overcome natural barriers and redirect LNPs to extrahepatic target organs.

In terms of local immune protection, intranasal or inhaled administration offers a unique "frontline defense" mechanism. This approach directly stimulates respiratory secretory IgA and tissue-resident memory T cells (TRM), establishing a barrier at the initial site of pathogen entry—an effect that is particularly important for respiratory viral infections[117,118].When RSV mRNA was encapsulated in an optimized intranasal F4-LNP, protein expression was significantly higher than that observed with MC3-based LNPs, and high titers of mucosal IgA were locally induced in both the nose and lungs[119].More recent studies have reported the development of the first class of sLNPs utilizing osmium ions as charged groups. After intranasal administration, these particles efficiently delivered mRNA to pulmonary club cells, ciliated cells, and macrophages, achieving successful CRISPR gene editing and immune regulation in lung tissue without causing significant local or systemic damage[120].These findings suggest that inhalation-based delivery is not limited to vaccines but can also be extended to gene therapy for lung diseases.

Furthermore, intradermal or transdermal administration—as needle-free alternatives—offer both high convenience and strong immune-stimulating capabilities[121,122].Lyophilized saRNA-LNP delivered intramuscularly or intradermally can induce potent immune responses. When administered via a microfluidic microneedle array patch, it effectively stimulates both humoral and cellular immunity[123].Powder-coated microneedle platforms can even directly deliver the lyophilized powder from Moderna's clinically approved formulation, achieving robust immune activation and complete viral clearance while preserving the structural integrity of the LNP and significantly reducing off-target distribution to the liver[124],This approach also enables self-administration by non-professionals. Based on a comprehensive review of the functional studies of these components, the design of LNPs must balance four key principles: efficacy, targeting, safety, and stability[82]. Additionally, researchers have provided a systematic framework encompassing preparation, characterization, and in vitro and in vivo evaluation, offering methodological support for the rational design of LNPs [125].

4.2 Incorporation of Targeting moieties

In vivo, passive-targeting lipid nanoparticles (LNPs) depend primarily on their protein corona composition and hemodynamic distribution; however, their ultimate fate is heavily biased toward accumulation in the liver. This tendency poses significant challenges for delivering therapeutic payloads to extrahepatic tissues or specific cell types[126].To address this "liver accumulation" bottleneck, active targeting strategies have been developed that involve introducing specific ligands—such as antibodies, adaptors, peptides, or glycans—onto the LNP surface. These modifications enable precise molecular recognition of target cell-surface receptors, thereby substantially enhancing delivery specificity and transfection efficiency. This approach has become a core technological solution for overcoming the limitations of passive targeting[83,127–129].Among protein-based ligands, antibodies and their derivatives exhibit exceptionally high affinity and selectivity. Recent studies have shown that presenting optimally oriented anti-Fc nanobodies on the LNP surface allows for the capture of target antibodies without additional chemical modifications. This strategy enables highly efficient targeting of T cells and achieves protein expression levels over 1,000 times higher than non-targeted LNPs[129].Another approach utilizes an apolipoprotein A1-fused antibody Fc domain to facilitate the spontaneous presentation of anti-HER2 antibodies on the LNP surface, successfully delivering p53 mRNA to tumor tissues[130].These examples highlight that the spatial orientation and anchoring methods of antibody-based ligands are decisive factors in targeting efficiency. Aptamers, as a class of oligonucleotide ligands, combine high affinity, small molecular size, and low immunogenicity. Modification of LNPs with CD4-targeting aptamers has been shown to increase mRNA accumulation in the spleen by 70-fold compared to controls[131].Similarly, RNA aptamers targeting the transferrin receptor, when conjugated to DSPE lipids, can guide LNPs across the blood-brain barrier, resulting in a 6.5-fold increase in brain accumulation[132].The flexible design of aptamers offers unique potential for crossing physiological barriers and targeting hard-to-reach tissues. Peptide ligands provide a programmable "amino acid sequence–targeting" regulatory platform. CD47-modified LNPs reduce phagocytic clearance, and when combined with antibodies, they increase targeting efficiency for hematopoietic stem cells threefold[133].The PILOT platform systematically fuses functional peptides directly with ionizable lipids, enabling organ tropism to be regulated by altering the amino acid sequence: lysine or arginine preferentially targets the lungs, while glutamic acid, aspartic acid, or proline direct LNPs to the spleen. A single dose achieves editing efficiencies of 13.3% in the liver and 7.4% in the lungs[134],demonstrating the high flexibility of peptide-based platforms for organ-level targeting. Carbohydrate ligands, in contrast, fully exploit the recognition mechanisms of endogenous receptors. Mannosylated LNPs target the CD206 receptor, enhancing delivery to dendritic cells and activating antitumor immunity [135];A mannose-conjugated cholesterol scaffold also enables precise targeting of sinusoidal endothelial and Kupffer cells in the liver[103],providing an effective and gentle strategy for immune modulation and localized liver therapy.

4.3 Addition of Adjuvants

The immunogenicity of mRNA-LNPs depends not only on the level of antigen expression but also heavily on their ability to activate the innate immune system. LNPs inherently possess "intrinsic adjuvant activity"; for example, ionizable lipids can directly activate the NF-κB and IRF pathways via the TLR4 signaling axis, and even unloaded LNPs can elicit innate immune stimulation comparable to that of mRNA-loaded LNPs[136].Regarding TLR pathways, researchers have attempted to co-encapsulate different TLR agonists in distinct compartments within the same LNP to achieve synergistic enhancement. For instance, the TLR9 agonist CpG-ODN (loaded into the aqueous phase) and the TLR4 agonist MPLA (embedded in the lipid phase) were co-encapsulated within an ALC-0315 LNP, significantly increasing the secretion of cytokines such as IFN-γ and TNF-α by dendritic cells[137].Incorporating a TLR4/TLR9 dual agonist into self-amplifying RNA LNPs has also been shown to induce a stronger Th1-type immune response[138].Furthermore, partial replacement of the ionizable lipids with a TLR7/8 agonist (AD7/8) can directly confer significant TLR7/8 agonistic activity to SARS-CoV-2 mRNA-LNPs[139],This "substitution-based incorporation" strategy not only simplifies formulation complexity but also enhances immunogenicity. In addition to TLR pathways, the STING pathway—another key component of the innate immune

Under Review

system—has been integrated into LNP adjuvant design. Xu et al. co-encapsulated the STING agonist cGAMP with mutant KRAS mRNA in MC3-LNPs, effectively inhibiting liver metastasis in pancreatic cancer[140].Even more innovatively, Qiao et al. partially replaced cholesterol with SitoC7A—a covalent conjugate of the STING agonist C7A and sitosterol—achieving a dual-function design in which "the same lipid serves as both carrier and adjuvant." This enables selective activation of STING signaling in dendritic cells while avoiding a systemic interferon response[141].Furthermore, manganese ions (Mn2+), as natural STING activators, have been identified as key drivers of the cGAS-STING pathway in vaccine-induced immune responses [142].Overall, the intrinsic adjuvant activity of LNPs provides a natural foundation for immune regulation, while the rational incorporation of exogenous TLR or STING agonists enables the engineering and fine-tuning of both the type and intensity of the immune response.

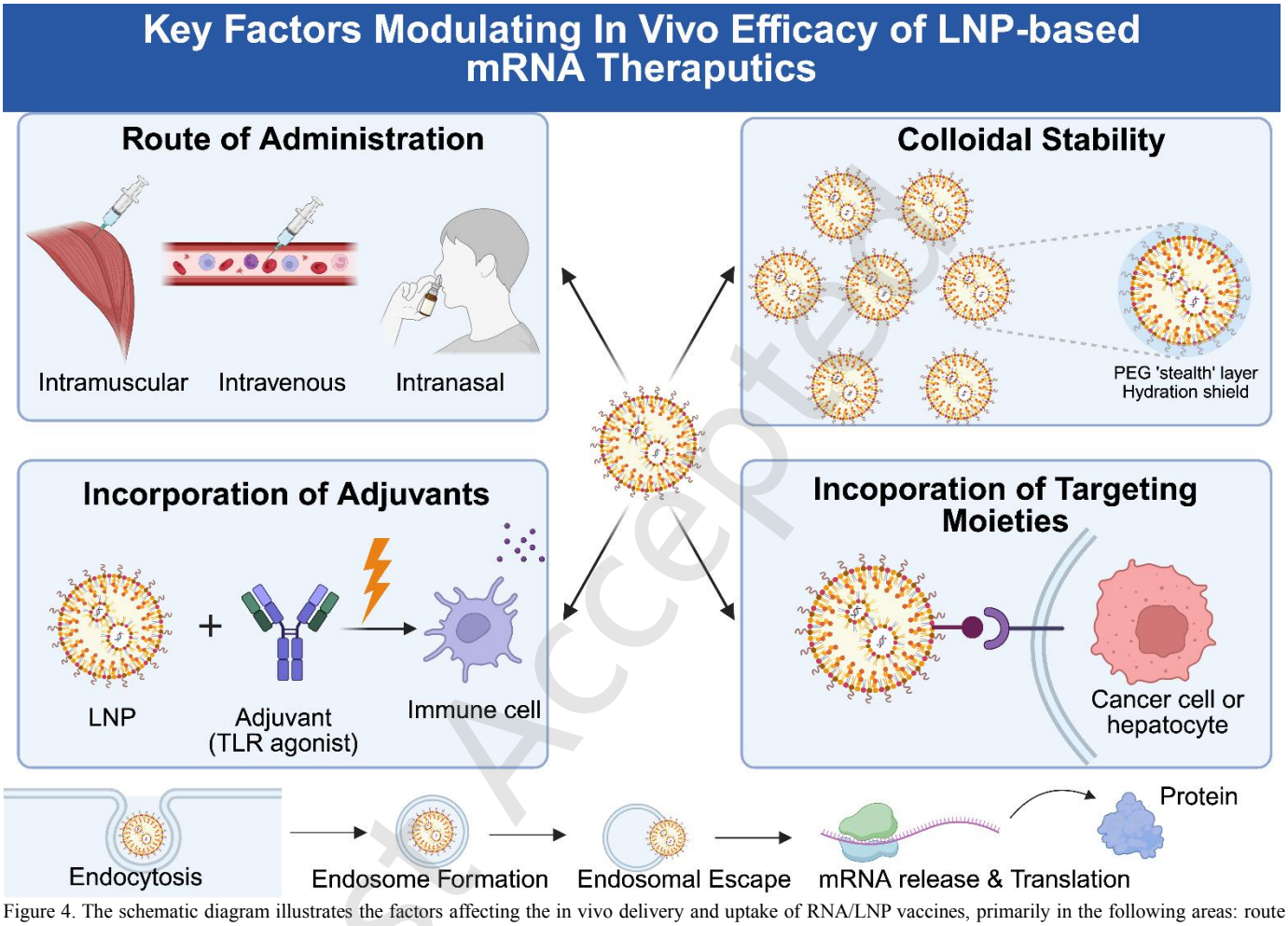


Figure 4. The schematic diagram illustrates the factors affecting the in vivo delivery and uptake of RNA/LNP vaccines, primarily in the following areas: route of administration; integration of targeting elements; and the addition of adjuvants. Created in BioRender.com.

5. Passive Targeting of LNP

Passive targeting refers to the ability of LNPs to accumulate in specific organs solely through their physicochemical properties—such as particle size, surface charge, lipid composition, and degree of surface PEGylation—without the need for specific targeting ligands on their surface. This "pre-designed, natural homing" delivery mode is primarily driven by interactions between LNPs and plasma proteins in vivo, resulting in the formation of a protein crown. Compared to active targeting, passive targeting offers significantly greater manufacturing feasibility, as it avoids the additional purification steps and heterogeneity associated with ligand conjugation. Consequently, it remains the most well-established strategy for the industrialization and clinical scaling of LNP delivery systems. Figure 5 shows the passive targeting of mRNA vaccines delivered via LNP to different organs.

5.1 Liver-Targeted

Following intravenous injection, lipid nanoparticles (LNPs) naturally accumulate in the liver. This inherent property is advantageous for treating liver diseases, yet it also represents a major bottleneck limiting the extrahepatic applications of LNPs [143].This targeted behavior is not coincidental; rather, it arises from precise molecular interactions between LNPs and endogenous protein coronas. Upon entering the bloodstream, the polyethylene glycol (PEG) lipids on LNPs gradually detach, allowing serum apolipoprotein E (ApoE) to adsorb onto the LNP surface. Subsequently, the LNPs are internalized by hepatocytes via low-density lipoprotein receptor (LDLR)-mediated endocytosis [144];The direct interaction between cholesterol and ApoE is the primary driver of this pathway, although studies also indicate the existence of alternative uptake mechanisms independent of LDLR[145].This phenomenon of "physiological homing" is not uniform: the chemical structure of the ionizable lipids can fine-tune the selectivity of LNPs for specific hepatic cell subsets, building upon the general tendency for liver accumulation. Although LNPs formulated with lipids such as MC3 and SM-102 predominantly target the liver, their pharmacokinetics and patterns of cellular distribution differ significantly [79];Notably, the delivery efficiency of ALC-0315 to

Just Accepted

hepatic stellate cells far surpasses that of MC3, and this uptake is partially independent of ApoE, suggesting that the specific lipid structure can modulate the tropism for different hepatic cell types [146–148].This implies that LNPs can be directed not only to the liver at the "organ level," but may also enable precise delivery at the "cellular level."An in-depth understanding of these mechanisms has allowed researchers to develop various strategies for modulating the degree of liver targeting as needed. For example, ionizable cholesterol (iChol) lipids reduce ApoE adsorption, resulting in lower hepatic uptake and enhanced splenic accumulation [149,150];"Flower-shaped" LNPs evade hepatic capture by increasing surface curvature, thus hindering effective ApoE adsorption [151];Alternatively, SORT LNPs redirect LNP distribution from the liver to other organs by incorporating permanently cationic lipids [115,152,153].The underlying logic of these strategies is that, without changing the targeting ligand, the in vivo biodistribution of LNPs can be rationally reprogrammed simply by adjusting the lipid composition. In summary, the passive targeting of LNPs to the liver results from a physiological recognition mechanism mediated by ApoE. However, through lipid engineering, it is now possible to achieve both "adjustable targeting intensity" and "selective hepatic subpopulation delivery," while still maintaining the general propensity for liver accumulation.

5.2 Lymph Node Targeting

Lymph nodes serve as central hubs for initiating adaptive immune responses. However, traditional LNPs administered via intravenous injection frequently accumulate in the liver, which limits the effective expression of mRNA within lymph nodes and directly compromises the efficacy of vaccines and cancer immunotherapies[112,154].To address this challenge, researchers in recent years have developed several highly efficient lymph node-targeting LNP platforms through sophisticated lipid chemistry engineering and by harnessing biological transport mechanisms. These design strategies can be categorized into three main approaches: innovation in lipid molecular scaffolds, leveraging endogenous lymphatic transport, and synergistic enhancement using surface ligands.

Innovations in the lipid molecular backbone have fundamentally reshaped the organ distribution patterns of lipid nanoparticles (LNPs). The incorporation of aromatic ring structures in aroLNPs reduces liver delivery by a factor of 10 and increases the lymph node-to-liver delivery ratio by 5–10-fold[154];Cyclic amino alcohol lipids (such as AMG1541) significantly decrease hepatic expression following intramuscular injection, achieving equivalent protective efficacy at only one-hundredth the dose required for SM-102[155];t The cycloalkene-based lipid OE -K further enhances uptake by dendritic cells in lymph nodes and activates antitumor immunity[156].All three designs modify the surface curvature and protein corona composition of LNPs by introducing rigid ring structures, thereby weakening ApoE-mediated hepatic recognition and enhancing lymphatic uptake. Drawing inspiration from endogenous transport mechanisms offers a clever strategy of "leveraging existing pathways." Albumin-binding EB-LNPs utilize the naturally high concentration of albumin in lymph fluid; through surface engineering, they actively bind albumin, directing drainage to lymph nodes via albumin's lymphatic return pathway. In various animal models, this platform has significantly reduced hepatic distribution, elicited stronger immune responses, and avoided the induction of anti-PEG antibodies, thus circumventing the immune barriers associated with repeated dosing[112].Furthermore, the FLNP system reduces hepatic accumulation by 94.6% and, for the first time, elucidates the immune-initiated process by which antigen-presenting cells migrate to lymph nodes[157],providing dynamic insights into LNP–immune cell interactions. The synergistic enhancement of surface ligands further refines the precision of lymph node delivery at the cellular level. LLNs modified with dual ligands (CD47 peptide and mannose) significantly increase mRNA expression within lymph nodes by combining "don't eat me" signals with dendritic cell phagocytosis signals[133];Additionally, engineered extracellular vesicles bearing viral envelope proteins facilitate efficient transport of LNPs to dendritic cells and lymph nodes[158].Collectively, these strategies demonstrate that combining precise cellular interaction regulation with organ targeting can greatly amplify immune activation.

5.3 Targeting the spleen and lungs

The intrinsic tendency of LNPs to accumulate in the liver following intravenous injection has historically served as a natural bottleneck, limiting their application in immunotherapy and the treatment of respiratory diseases. The spleen, which is rich in antigen-presenting cells, is a key target organ for mRNA immunotherapy[159–162];meanwhile, the lungs are critical sites for intervention in respiratory infections, tumors, and genetic diseases. In recent years, through systematic regulation of lipid composition, researchers have successfully "redirected" LNPs from the liver to the spleen and lungs, marking the dawn of a programmable era for precise extrahepatic delivery. The SORT (Selective Organ Targeting) strategy pioneered a universal methodology for extrahepatic redirection. Developed by the Siegwart team, the SORT platform achieves lung- and spleen-specific targeting by incorporating a fifth lipid with a permanent positive or negative charge into the traditional four-component LNP formulation[163].Zeta potential has emerged as a core predictive indicator: spleen-targeted LNPs typically exhibit a weak negative charge, while lung-targeted LNPs carry a positive charge[164].As a result, organ selectivity can now be rationally designed based on surface charge, rather than relying on serendipitous discovery. In recent years, multiple parallel pathways of breakthrough have emerged in the field of spleen targeting, each with its own distinct advantages. One research team designed a library of fluorinated ionizable lipids based on the clinical SM-102 scaffold and identified SSC6F5 from multiple candidates, achieving spleen-targeting specificity via intravenous, intramuscular, and subcutaneous administration. Further studies revealed the enrichment of apolipoprotein D in the spleen's protein corona, uncovering a novel endogenous spleen-targeting pathway[165].Another groundbreaking study took a different approach, replacing cholesterol with glucocorticoids: LNPs incorporating corticosterone and progesterone exhibited splenic selectivity as high as 97% and 99%, respectively. This mechanism was closely related to the adsorption of β2-glycoprotein I on the LNP surface[166], demonstrating that the cholesterol backbone is not irreplaceable. A more streamlined three-component ThrCo LNP, which uses the zwitterionic pyridinecarboxylic betaine to replace both cholesterol and PEG lipids, increased mRNA translation in the spleen by 4.5-fold compared to the BNT162b2 LNP and reduced hepatic accumulation by approximately 70%[167].Wu et al. further expanded the application boundaries of cholesterol-free LNPs by establishing a CAIL library containing161 types of carbonate lipids. This enabled efficient delivery of mRNA to splenic NK cells, achieving transfection in 21% of splenic NK cells in Ai9 mice [168], and providing a new tool for tumor immunotherapy. Lung targeting has also been efficiently advanced within this charge-driven framework. A high-throughput four-component reaction platform was used to synthesize 91 ionizable lipids, among which a permanently cationic lipid formulation achieved a 32-fold increase in protein expression in the lungs—significantly outperforming existing

Not Accepted

endogenous targeting strategies [169].Another finding with even greater translational value is that the ionizable lipid A3T2C7 (CP-LC-1495), when used in a conventional four-component formulation without the addition of cationic lipids, achieved 97% lung selectivity and protein expression levels in lung tissue as high as 1.21×10^{8} p/s [164], greatly reducing formulation complexity and facilitating clinical-scale production.

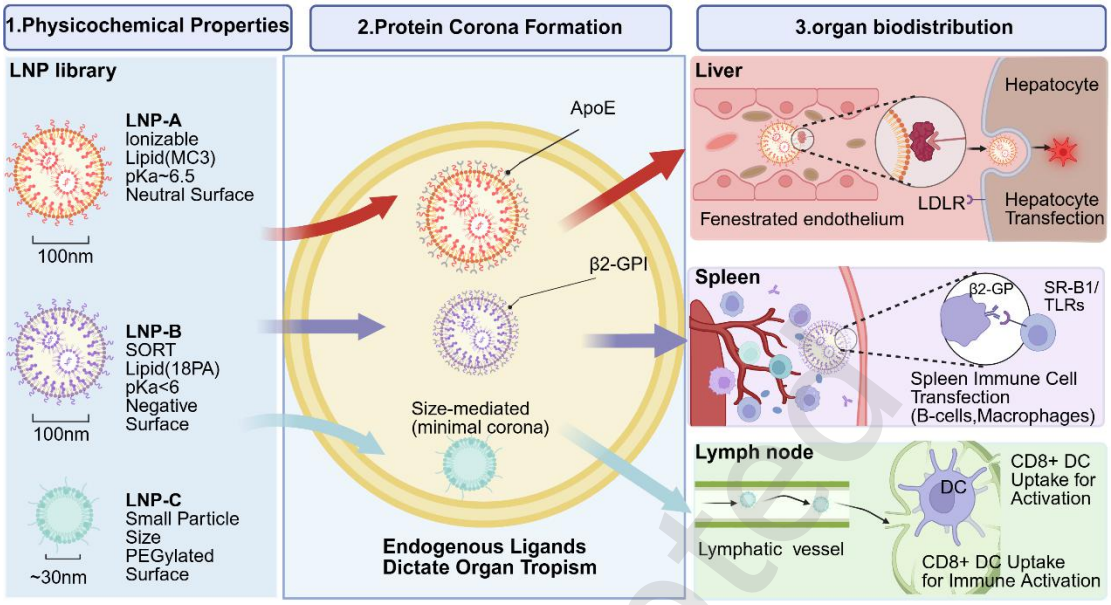


Figure 5. The diagram illustrates the passive targeting of LNP, which primarily targets the liver, spleen, lymph nodes, and other organs. Created in BioRender.com.

6. Case Studies of LNP Technology in mRNA Vaccine Development

6.1 Lessons Learned from the Success of COVID-19 mRNA Vaccines

The development and deployment of COVID-19 mRNA vaccines represent not only a milestone in the history of vaccinology, but also a landmark validation of the LNP (lipid nanoparticle) delivery system's transition from laboratory research to large-scale clinical use. It is estimated that, within just one year of their rollout, these vaccines prevented more than 14 million deaths worldwide[170].Pfizer-BioNTech's BNT162b2 and Moderna's mRNA-1273 received Emergency Use Authorization in December 2020 and January 2021, respectively, demonstrating Phase III efficacy rates of 95.0% and 94.1%[170,171],This clinical success was not incidental, but rather the result of a synergistic combination between the technological advantages of the mRNA platform and the maturity of LNP delivery technology. The former provides the capability for rapid development and scalable production, while the latter enables the effective delivery of nucleic acid molecules, facilitating their translation into functional proteins within the body.Both vaccines utilize N1-methylpseudouridine-modified mRNA encoding the spike protein, encapsulated within a four-component LNP; the similarities in their LNP designs far outweigh any differences, with both adhering to the core principles of lipid engineering described earlier[136].Regarding their formulation details, the molar ratio for BNT162b2 is 46.3:9.4:42.7:1.5, while for mRNA-1273 it is 50:10:38.5:1.5. Differences in dosage may partially account for the stronger antibody response observed with mRNA-1273.The success of the COVID-19 vaccines has established a reusable design paradigm for subsequent mRNA therapies: nucleoside modifications mitigate the inherent immunogenicity of mRNA; the pH responsiveness and biodegradability of ionizable lipids balance efficient delivery with safe clearance; and the inherent adjuvant activity of LNPs synergistically amplifies antigen-specific immune responses[172].More importantly, these clinical data have, for the first time at a human scale, validated the entire design logic of LNP lipid engineering—from the selection of ionizable lipids with optimal pKa values to the precise pairing of helper phospholipids, from cholesterol-mediated membrane regulation to the shedding kinetics of PEG chain lengths. The functional hypotheses underlying each component have been empirically validated through vaccination campaigns involving hundreds of millions of people. This paradigm not only lays the foundation for next-generation vaccines against infectious diseases but also charts a viable technical path for broader mRNA applications, such as cancer immunotherapy, treatments for rare diseases, and in vivo gene editing.

6.2 Progress in the Development of LNP-mRNA Vaccines for Other Disease Areas

The success of COVID-19 mRNA vaccines has paved the way for the application of LNP-mRNA technology in other disease areas. Currently, more than 150 RNA-LNP formulations are in clinical trials worldwide, with over 80% targeting cancer and infectious diseases. The use of LNP-mRNA vaccines has rapidly expanded beyond COVID-19, now encompassing cancer immunotherapy, influenza, HIV, and rare diseases.

6.2.1 Cancer Vaccines

Tumor vaccines activate cytotoxic T lymphocytes to recognize and eliminate tumor cells by delivering mRNA encoding tumor-associated antigens or patient-specific neoantigens[173–177].The clinical validation of lipid nanoparticles (LNPs) has provided strong impetus for this strategy[178].Among the clinical developments of mRNA-LNP cancer vaccines, the most notable advancement is the KEYNOTE-942 study, which evaluates mRNA-4157 (V940) in combination with pembrolizumab. This Phase IIb trial assessed the efficacy of a personalized neoantigen vaccine combined with PD-1 blockade in patients with resected high-risk melanoma. Five-year follow-up data showed that the combination therapy group demonstrated a sustained improvement in recurrence-free

Just Accepted

survival compared to the pembrolizumab monotherapy group[179].This result marks the first time an mRNA-LNP cancer vaccine has demonstrated statistically significant evidence of a survival benefit in a randomized controlled clinical trial, setting a milestone for the clinical translation of personalized neoantigen vaccines.

In the field of hepatitis B virus (HBV)-associated hepatocellular carcinoma, WGc-0201 Injection—developed under the leadership of West China Hospital, Sichuan University—is the world's first mRNA therapeutic vaccine for HBV-related liver cancer. Utilizing proprietary mRNA sequence design and an advanced, targeted LNP delivery system, it activates a dual immune response against both HBV and associated tumors, thereby overcoming immune tolerance in virus-driven cancers. The product has received Investigational New Drug (IND) approval from the U.S. FDA.

Several breakthroughs have also been achieved in the innovation of LNP formulations. An albumin-recruiting LNP (EB-LNP) replaces traditional PEG lipids with Evans Blue-modified lipids, endowing the nanoparticles with the ability to bind to albumin. Following intramuscular injection, EB-LNPs are efficiently drained via lymphatic vessels to lymph nodes, thereby avoiding accumulation in the liver. mRNA vaccines based on this platform have demonstrated potent cellular and humoral immune responses in models of cancer and infectious diseases[112].LiNx (mRNA LNP-nanofiber-hydrogel composite) integrates mRNA LNPs into an injectable microgel matrix, achieving an immune response equivalent to that of a conventional three-dose LNP immunization with a single administration. It also induces a Type 17 T helper cell response, which is critical for efficacy against solid tumors[180].Regarding splenic immune cell targeting, researchers have achieved specific delivery of mRNA to splenic immune cells following intravenous administration by modulating the lipid composition of the LNP. This approach inhibited tumor growth by approximately 75% in tumor-bearing mice—a therapeutic effect superior to that of clinically relevant control formulations[159].

6.2.2 Vaccines for infectious diseases (such as influenza and HIV)

Regarding influenza vaccines, the LNP-mRNA platform has now entered clinical trials. In terms of lipid chemistry, the biodegradable cyclic amino alcohol lipid AMG1541 achieved comparable immunogenicity to SM-102 in an H3 influenza model while using only 1% of the SM-102 dose, and also reduced hepatotoxicity[155].In vaccine design, chimeric hemagglutinin (cHA) and M2 mRNA vaccines provide broad-spectrum protection against at least six influenza subtypes[181];The bivalent Fluvid vaccine achieved 100% protection against both influenza and SARS-CoV-2 challenges[182];HA mRNA-LNP induced a strong immune response in neonatal non-human primates[183];and M2e mRNA combined with seasonal vaccines enhanced cross-protection[184].

A clinical proof of concept has been achieved in the field of HIV vaccines. The IAVI G002/G003 Phase I trials confirmed that the eOD-GT8 and core-g28v2 clonally targeted immunogens, delivered via mRNA-LNP, successfully drove the early maturation of broadly neutralizing antibody precursors in humans for the first time and validated the feasibility of a primary vaccine in Africa[185].In preclinical studies, the mRNA-LNP-encoded Env NFL trimer induced autologous Tier 2 neutralizing antibodies in rabbits, and the multi-epitope clonal-targeting strategy simultaneously activated multiple bnAb precursor lineages in mice[186].

6.2.3 Treatment of Rare Diseases: Applications of LNP in Gene Therapy

The application of LNP-mRNA technology in the field of rare diseases is progressing from preclinical to clinical stages. Its advantages stem from the ability of mRNA to provide transient, programmable protein expression, while LNPs, as non-viral vectors, avoid the risks of immunogenicity and genomic integration. Significant breakthroughs have been achieved in inherited metabolic liver diseases: for example, in phenylketonuria, mRNA-LNP delivery of the Prime Editor enzyme has improved gene-editing efficiency, reducing blood phenylalanine levels below the therapeutic threshold[187];In citrullinemia type I, a single dose resulted in highly efficient and sustained gene correction in newborn mice [188].In the field of rare respiratory diseases, for primary ciliary dyskinesia (PCD) caused by DNAI1 mutations, inhaled SORT-LNP delivery of mRNA has restored ciliary function; this approach has advanced to clinical trials and can potentially be extended to other mutations [189].Additionally, Intellia Therapeutics' two major LNP-CRISPR pipelines, NTLA-2001 and NTLA-2002, have achieved significant clinical progress.

7. Conclusion and Future Outlook

After more than a decade of development, LNP-mRNA technology has progressed from a laboratory concept to full-scale commercialization. The approval of Onpattro (2018) and the emergency use authorization of COVID-19 mRNA vaccines have firmly established LNPs as the most advanced mRNA delivery system available today. These three clinical milestones are underpinned by three key pillars: chemical innovations in ionizable lipids, the synergistic design of four formulation components, and breakthroughs in microfluidic-based large-scale manufacturing. Currently, the research paradigm is shifting from "passive adaptation" to "active design." At the lipid chemistry level, platforms such as HETAIL and POST have enabled selective delivery to multiple organs—including the liver, lungs, spleen, and lymph nodes—by modulating tail chain heterogeneity or incorporating peptide fusions. Additionally, protein-capsid-mediated, ligand-free targeting strategies have extended tissue specificity to organs such as the brain, tumors, and placenta. A deeper understanding of safety mechanisms has led to more precise design principles. In 2025, studies revealed that membrane damage associated with endosomal escape can be recognized by galectin, triggering inflammation; however, by engineering novel lipids with reparable pores, it is possible to achieve both highly efficient delivery and low levels of inflammation[190];At the same time, researchers discovered that the spatial decoupling of LNP components within endosomes is the primary reason for escape efficiencies of only 1%–4%[191], providing a clear target for optimizing lipid structures. Regarding alternative components, replacing PEG with POx lipids increased transfection efficiency in mice pre-treated with anti-PEG antibodies by 200-fold compared to PEG-LNPs[192];The zwitterionic PyCB LNP reduced liver accumulation by 70% and increased splenic translation by 4.5-fold[167];cholesterol-free G-LNP achieved highly efficient splenic targeting. Clinical applications are now expanding beyond vaccines to address chronic diseases. IN026, a liver-targeted uric acid oxidase mRNA therapy, received FDA IND approval, becoming the first-in-class mRNA protein replacement therapy to enter clinical trials. ReCode Therapeutics' SORT LNP inhalation therapy demonstrated restoration of airway function in patients with ciliary dyskinesia, representing the first evidence of biological activity in humans for an inhaled mRNA-LNP product. Collectively, these advancements demonstrate that LNP-mRNA technology is transitioning from a "proven vaccine platform" to a "programmable, multi-indication therapeutic platform." Its future will depend on the continued synergy between innovations in lipid chemistry, a deeper understanding

Under Review

of mechanisms of action, and clinical demand.

Currently, LNP-mRNA technology has progressed from proof of concept to commercialization, but it still has a considerable way to go before it can be widely implemented in a manner that is precise, safe, and accessible. The core challenges for future development will unfold across the following four dimensions. First, overcoming the liver's "natural trap" to achieve programmable, tissue-specific delivery. Although intravenous injection is the default route for liver targeting, recent studies have shown that hepatocytes can also take up LNPs via an ApoE-mediated pathway that is independent of LDLR. This finding suggests that the mechanisms underlying recognition within the liver are more complex than previously understood[145].Future efforts must synergistically integrate the structural design of lipid tail chains, the regulation of protein coat components, and ligand modification to expand the scope of mRNA delivery beyond the liver to include the lungs, spleen, lymph nodes, and even the central nervous system, thereby truly enabling "on-demand homing." Additionally, it is essential to balance efficiency and safety to broaden the therapeutic window. Endosomal escape efficiency—which remains below 1–4%—continues to be a major bottleneck. Furthermore, a 2025 study revealed that the membrane damage associated with endosomal escape can be recognized by galectins, subsequently triggering inflammation[190].This requires balancing "high-efficiency release" with "membrane-friendliness" in lipid design, such as developing novel ionizable lipids that generate only reparable pores or replacing existing PEG-modified lipids with non-PEG hydrophilic polymers (such as POx) to reduce immunogenicity and support repeated dosing. Third, it is essential to break the reliance on cold chain logistics and promote technology dissemination. Current cold-chain storage requirements for LNPs significantly limit accessibility in low- and middle-income regions. Optimizing lipid chemistry to enhance the long-term stability of lyophilized formulations—combined with continuous production processes, such as vertically stacked microfluidics—will make LNP preparation more reliable and automated, ultimately enabling room-temperature distribution and immediate use. This is a critical step toward widespread industrial adoption. Fourth, introducing AI-driven design can significantly shorten the R&D cycle. Traditional lipid screening depends on high-throughput experiments, with development phases often lasting several years. By leveraging artificial intelligence to predict the pKa of ionizable lipids, degradation kinetics, protein corona composition, and to optimize the ratios of the four main component formulations, it is expected that the development cycle can be reduced to just a few months. This acceleration is vital for facilitating rapid responses of the LNP-mRNA platform to emerging infectious diseases and for advancing personalized cancer vaccines[193].In summary, with the deep integration of innovations in lipid chemistry, advancements in formulation engineering, and AI-assisted design, LNP–mRNA technology is expected to open entirely new therapeutic avenues in fields such as tumor immunotherapy, genetic disease correction, and replacement therapies for rare diseases, ultimately emerging as a cornerstone of precision medicine.

Data availability

Not applicable

Acknowledgements

The work was supported by the Regional Innovation and Development Joint Fund Key Project of the National Natural Science Foundation of China (U24A20735), the Noncommunicable Chronic Diseases-National Science and Technology Major Project (2023ZD0503000, 2023ZD0503004), Fundamental and Interdisciplinary Disciplines Breakthrough Plan of the Ministry of Education of China (JYB2025XDXM607), Tianfu Jincheng Laboratory (Future Medical City, No. TFJC-2023-JB004), the Institutional Joint Innovation Fund from Sichuan University and Nuclear Power Institute of China (HG2024220), Sichuan Science and Technology Program (2025YFHZ0087), Science and Technology Project of Sichuan Provincial Health Commission (Clinical Research Special Project JH2023082), the International Science and Technology Cooperation Program of Chengdu Science and Technology Bureau (2024-YF06-00011-HZ and 2022-GH03-00004-HZ), the Health Research Project of Chengdu Eastern New Area Management Committee (202304), 1.3.5 project for disciplines of excellence from West China Hospital of Sichuan University (ZYYC23006), Clinical Research Incubation Project of West China Hospital (23HXFH001), Clinical Research Youth Fund of West China Hospital (2025HXFH028), West China Hospital "Qimingxing" Research Fund for Young Talents (HXQMX0184), Yunnan Province Key Laboratory of Precision Diagnosis and Treatment for Thoracic Diseases (202449CE340026), National Key R&D·Program of China (2023YFC3403200, 2023YFC3403204) and the Ministry of Education University-Industry Collaborative Education Program (230720523707281), the National Natural Sciences Foundation of China (82473434). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Declaration of competing interest

All the contributing authors reports no conflict of interests in this work.

Author contribution statement

Jianmei Li: investigation, visualization, Data curation, Writing—original draft. Wenlin Wu：investigation, visualization, Data curation, Writing—original draft. Jie Dai: investigation, visualization, Data curation, Writing—original draft. Xingchen Peng: Conceptualization, Supervision, Funding acquisition, Writing—review and editing. All authors discussed the results and commented on the manuscript.

Informed consent

Not applicable

Ethics statement

Not applicable

Use of AI statement

None

References

[1] Zhong, L.; Tao, L.; Zhai, Z.; Liao, L.; Shi, C.; Yao, S. The stability landscape of mRNA vaccines : challenges and mitigation strategies. Vaccine **2026**, *79*, 128515.

[2] Wang, J.; Cai, L.; Li, N.; Luo, Z.; Ren, H.; Zhang, B. Developing mRNA Nanomedicines with Advanced Targeting Functions. Nano-Micro Letters **2025**, *17*(1), 155.

[3] Camilla Hald Albertsen, Jayesh Kulkarni, Dominik Witzigmann, Marianne Lind, Karsten Petersson, J.B.S. The role of lipid components in lipid nanoparticles for vaccines and gene therapy. Advanced drug delivery

Under Peer Review

reviews **2022**, *188*, 114416.
[4] Le, T.; Sun, C.; Chang, J.; Zhang, G.; Yin, X. MRNA Vaccine Development for Emerging Animal and Zoonotic Diseases. Viruses **2022**, *14*(2), 401.
[5] Zhang, Z.; Ong, H.; Yang, B.; Fan, B.; Yang, Y. Chemical engineering strategies to enhance mRNA–LNP stability for therapeutic applications Ze. Biomaterials Science **2026**, *14*(6), 1370–1392.
[6] Wang, Y.; Wang, X.; Lu, Y. MRNA Stability: An Unresolved Challenge for Broad Therapeutic Applications. Biotechnology journal **2025**, *20*(10), e70146.
[7] Lin, L.; Zhang, X.; Yan, X.; Shi, L.; Su, K.; He, S.; Yu, M.; Fu, X.; Sheng, X.; Kong, N.; Liu, S. Aromatic Ionizable Lipids Enhance mRNA Delivery via Coordinating Multiple Mechanisms. Journal of the American Chemical Society **2026**, *148*(7), 6960–6969.
[8] Thuy, C.; Nguyen, G.; Meng, F. Unleashing the power of nucleic acid therapeutics through efficient cytosolic delivery. Journal of Controlled Release **2025**, *383*, 113774.
[9] Bai, Y.; Cui, L.; Liu, T.; Wang, Z.; Cao, H.; He, S.; Qiu, J.; Li, Y.; Zhou, Y.; Shi, J.; Wang, J. Recent Advances in mRNA-LNP Delivery Systems for Extrahepatic Organs : A Review. Molecular Pharmaceutics **2025**, *22*(8), 4474–4493.
[10] Bitounis, D.; Jacquinet, E.; Rogers, M.A.; Amiji, M.M. Strategies to reduce the risks of mRNA drug and vaccine toxicity. Nature Reviews Drug Discovery **2024**, *23*(4), 281–300.
[11] Shinya Sato,Syusuke Sano,Hiroki Muto,Kenji Kubara, Keita Kondo,Takayuki Miyazaki,Yuta Suzuki, Yoshifumi Uemoto, and K.U. Understanding the Manufacturing Process of Lipid Nanoparticles for mRNA Delivery Using Machine Learning. Chemical & pharmaceutical bulletin **2024**, *72*(6), 529–539.
[12] Geng, G.; Xu, Y.; Hu, Z.; Wang, H.; Chen, X.; Yuan, W.; Shu, Y. Viral and non-viral vectors in gene therapy: current state and clinical perspectives Guannan. eBioMedicine **2025**, *118*, 105834.
[13] Cring, M.R.; Shef, V.C. Gene therapy and gene correction : targets , progress , and challenges for treating human diseases. Gene Therapy **2022**, *29*(1–2), 3–12.
[14] Nourhan A. Abou Madawi · Zeinab E. Darwish · Enas M. Omar. Targeted gene therapy for cancer: the impact of microRNA multipotentiality. 214. 2024.
[15] Ma, Y.; Dong, S.; Wu, A.; Jeong, S.D.; Lee, A.S.; Jiang, W.; Kim, B.Y.S. Engineering challenges and translational opportunities in emerging gene delivery platforms. Nature Biomedical Engineering **2026**, *10*(6), 1060–1083.
[16] Alshorman, J.; Mehran, M.J.; Tembo, K.M.; Bolideei, M. Applications of genome editing technologies in the treatment of human diseases. Gene Therapy **2026**, *46*(2), 521–534.
[17] Su, K.; Wang, Z.; Shi, L.; Fu, X.; Sheng, X.; Kong, N.; Xu, T.; Liu, S. Decoding the Functional Roles of Individual Components in mRNA Lipid Nanoparticles. ACS Nano **2026**, *20*(31), 22008–22020.
[18] Buck, J.; Grossen, P.; Cullis, P.R.; Huwyler, J.; Witzigmann, D. Lipid-based DNA therapeutics: Hallmarks of non-viral gene delivery. ACS Nano **2019**, *13*(4), 3754–3782.
[19] Akinc, A.; Maier, M.A.; Manoharan, M.; Fitzgerald, K.; Jayaraman, M.; Barros, S.; Ansell, S.; Du, X.; Hope, M.J.; Madden, T.D.; Mui, B.L.; Semple, S.C.; Tam, Y.K.; Ciufolini, M.; Witzigmann, D.; Kulkarni, J.A.; Meel, R. Van Der; Cullis, P.R. The Onpattro story and the clinical translation of nanomedicines containing nucleic acid-based drugs. Nature Nanotechnology **2019**, *14*(12), 1084–1087.
[20] Witzigmann, D.; Kulkarni, J.A.; Leung, J.; Chen, S.; Cullis, P.R.; Meel, R. Van Der. Lipid nanoparticle technology for therapeutic gene regulation in the liver. Advanced drug delivery reviews **2020**, *159*, 344–363.
[21] Wu, W.; Wei, Z.; Peng, X. From LNPs to hybrid nanocarriers: development, challenges and redesign of non-viral gene delivery. Journal of nanobiotechnology **2026**, *24*(1), 727.
[22] Kauffman, K.J.; Webber, M.J.; Anderson, D.G. Materials for non-viral intracellular delivery of messenger RNA therapeutics. Journal of Controlled Release **2016**, *240*, 227–234.
[23] Piotrowski-daspit, A.S.; Kauffman, A.C.; Laura, G.; Saltzman, W.M. Polymeric vehicles for nucleic acid delivery Alexandra. Advanced Drug Delivery Reviews **2020**, *335*, 465–480.
[24] Fang, Y.; Yan, C.; Yue, W.; Li, Y.; Zi, L.; Lu, D.; Jiao, X.; Cong, D.; Xu, F. Optimized Cationic Lipid - assisted Nanoparticle for Delivering CpG Oligodeoxynucleotides to Treat Hepatitis B Virus Infection. Pharmaceutical Research **2022**, *40*(1), 145–156.
[25] Luo, Y.; Liang, L.; Gan, Y.; Liu, J.; Zhang, Y.; Fan, Y.; Zhao, G.; Czarna, A.; Lu, Z.; Du, X.; Shen, S.; Xu, C.; Lian, Z.; Wang, J. An All-in-One Nanomedicine Consisting of CRISPR-Cas9 and an Autoantigen Peptide for Restoring Specific Immune Tolerance. ACS Appl Mater Interfaces **2020**, *12*(43), 48259–48271.
[26] Shen, S.; Mao, C.; Yang, X.; Du, X.; Liu, Y.; Zhu, Y. Cationic Lipid-Assisted Polymeric Nanoparticle Mediated GATA2 siRNA Delivery for Synthetic Lethal Therapy of KRAS Mutant Non- Small-Cell Lung Carcinoma. Molecular Pharmaceutics **2014**, *11*(8), 2612–2622.
[27] Fan, Y.N.; Li, M.; Luo, Y.L.; Chen, Q.; Wang, L.; Zhang, H.B.; Shen, S.; Gu, Z.; Wang, J. Cationic lipid-assisted nanoparticles for delivery of mRNA cancer vaccine. Biomaterials Science **2018**, *6*(11), 3009–3018.
[28] Saiding, Q.; Xiao, F.; Khan, M.M.; Kong, N.; Huang, X.; Tao, W. Lipid − Polymer Hybrid Nanoparticles (LPHNPs) for RNA Delivery. Accounts of Chemical Research **2026**, *59*(5), 762–775.
[29] Mazrad, Z.A.I.; Leng, C.; Zhang, T.; Warne, N.M.; Thao, N.; Nur-a-tomal, S.; Cameron, N.R.; Van, L.; Pouton, C.W.; Kempe, K. European Journal of Pharmaceutics and Biopharmaceutics Lipid-Polyhydroxyalkanoate Hybrid Nanoparticles as Sustainable Platform for mRNA delivery. European Journal of Pharmaceutics and Biopharmaceutics **2025**, *213*, 114755.
[30] Pardi, N.; Hogan, M.J.; Porter, F.W.; Weissman, D. MRNA vaccines-a new era in vaccinology. Nature Reviews Drug Discovery **2018**, *17*(4), 261–279.
[31] Vlasova, K.Y.; Kerr, A.; Pennock, N.D.; Jozic, A.; Sahel, D.K.; Walker, J.M.; Luxenhofer, R.; Sahay, G. Synthesis of ionizable lipopolymers using split-Ugi reaction for pulmonary delivery of various size RNAs and gene editing. Nature Communications **2025**, *16*(1), 4021.
[32] Algarni, A.; Lee, L.; Pilkington, E.H.; Kent, S.J.; Pouton, C.W. Influence of hydrophilic polymers on the accelerated blood clearance of mRNA lipid nanoparticles upon repeated administration. Nanoscale horizons **2025**, *10*(12), 3396–3404.
[33] Yi, J.; Lu, Y.; Liu, N.; Wang, Z.; Zhang, H.; Xing, H.; Li, M.; Jin, L.; Zheng, A. Chitosan and mannose-modified dual-functional mRNA-LNP vaccines for robust systemic and mucosal immune responses. Journal of Controlled Release **2025**, *384*, 113891.
[34] Hwang, D.; Park, S.A.; Kim, J.H.; Lee, S.; Lee, J.; Kim, H.S.; Kim, K.; Lee, S.; Oh, T.J.; Lee, J.; An, S. Gold Nanoparticle−mRNA Conjugates Encapsulated in Lipid Nanoparticles for Coordinated Codelivery of Multiple mRNAs. ACS Omega **2025**, *10*(30), 32998–33007.
[35] Seaberg, J.; Gulla, S.K.; Labombarde, J.; Roy, R.; Kumar, S.; Dwivedi, D.; Xu, C.; Kovats, S. Enhancing mRNA therapy through iterative delivery. Science Advances **2025**, *11*(40), eadx0916.
[36] Huq, T.B.; Anil, P.; Jeeja, K.; Dam, S.K.; Das, S.S.; Vivero-escoto, J.L. Recent Applications ofMesoporous Silica Nanoparticles in Gene Therapy. Advanced healthcare materials **2025**, *14*(26), e2404781.
[37] Suwalski, A.; Dabboue, H.; Delalande, A.; Bensamoun, S.F.; Canon, F.;

Just Accepted

Midoux, P.; Saillant, G.; Klatzmann, D.; Salvetat, J.; Pichon, C. Biomaterials Accelerated Achilles tendon healing by PDGF gene delivery with mesoporous silica nanoparticles. Biomaterials **2010**, *31*(19), 5237–5245.

[38] Zheng, G.; Zhao, R.; Xu, A.; Shen, Z.; Chen, X.; Shao, J. Co-delivery of sorafenib and siVEGF based on mesoporous silica nanoparticles for ASGPR mediated targeted HCC therapy. European Journal of Pharmaceutical Sciences **2018**, *111*, 492–502.

[39] Khalifeh, M.; Oude, R.; Roverts, R.; Brock, R. Incorporation of ionizable lipids into the outer shell of lipid-coated calcium phosphate nanoparticles boosts cellular mRNA delivery. International Journal of Pharmaceutics **2025**, *670*, 125109.

[40] Ma, X.; Liu, S.; Zhang, S.; Liu, Z.; Wang, H.; Luo, W.; Qin, H.; Li, Z.; Zhong, J.; Li, J.; Chen, Q.; Lin, J.; Liu, A.; Zhang, X.; Li, H.; Lu, X.; Shi, X.; Li, L.; Gu, Z.; Nie, G.; Ji, T. Engineering ofmRNA vaccine platform with reduced lipids and enhanced efficacy. Nature Communications **2025**, *16*(1), 8913.

[41] Guo, M.; Guo, J.; Yao, H.; Gao, F.; Zhao, L.; Cai, L.; Fan, X.; Bao, L.; Li, X.; Zhang, X.; Hu, M.; Mei, J.; Huang, Y.; Wang, L.; Mao, J.; Wang, H. Adjuvant-metal-ion-chelating PTEN mRNA with cell-membrane-coating augments the immune sensitivity for precise cancer immunotherapy. Science bulletin **2026**, *71*(3), 609–622.

[42] He, C.; Shi, C.; Fang, M.; Chang, P.; Hou, P. Amplifying STING activation by biomimetic manganese mRNA nanovaccines for local and systemic cancer immunotherapy. Journal of Controlled Release **2025**, *383*, 113788.

[43] Gu, Y.; Chen, J.; Wang, Z.; Liu, C.; Wang, T.; Kim, C.; Durikova, H.; Fernandes, S.; Johnson, D.N.; Rose, R. De; Cortez-jugo, C.; Caruso, F. MRNA delivery enabled by metal – organic nanoparticles. Nature Communications **2024**, *15*(1), 9664.

[44] Mitchell, M.J.; Billingsley, M.M.; Haley, R.M.; Langer, R.; Wechsler, M.E.; Peppas, N.A. Engineering precision nanoparticles for drug delivery. Nature Reviews Drug Discovery **2021**, *20*(2), 101–124.

[45] Chen, Y.F.; Luh, F.; Ho, Y.S.; Yen, Y. Exosomes : a review of biologic function , diagnostic and targeted therapy applications , and clinical trials. Journal of Biomedical Science **2024**, *31*(1), 67.

[46] Wang, J.S.; Schellenberg, S.J.; Demeros, A.; Lin, A.Y. Exosomes in review: A new frontier in CAR-T cell therapies. Neoplasia **2025**, *62*, 101147.

[47] Chung, J.; Hwa, K.; Hyun, S.; Bae, D.; Kyeong, H.; Choi, Y.; Hwan, J.; Kwon, K.; Kim, S. A single extracellular vesicle-based platform supporting both RBD protein and mRNA vaccination against SARS-CoV-2. Journal of Controlled Release **2026**, *390*, 114515.

[48] Chen, Z. Lung metastasis and recurrence is mitigated byCARmacrophages, in-situ-generated from mRNA delivered by small extracellular vesicles. Nature Communications **2025**, *16*(1), 7166.

[49] Liu, X.; Tian, J. Extracellular vesicle manufacture via FACTORY: fully automated collection technology and optimum machinery for clinical translational applications. Trends in Biotechnology **2025**, *43*(11), 2947–2969.

[50] Han, W.; He, Z.; Xing, J.; Zheng, B.; Liu, S.; Liu, H.; Liu, Y.; Xiao, Y.; Luo, E. Cell membrane-coating nanotechnology: An integrated strategy against the multifactorial pathogenesis of periodontitis. Biomaterials **2026**, *332*, 124139.

[51] Eun, H.; Han, J.; Shin, S.; Bae, G.; Park, W. M1-polarized macrophage-derived cellular nanovesicle-coated lipid nanoparticles for enhanced cancer treatment through hybridization of gene therapy and cancer immunotherapy. Acta Pharmaceutica Sinica B **2024**, *14*(7), 3169–3183.

[52] Strong, C.; Columbia, B.; Leung, J.; Columbia, B.; Kang, E.; Columbia, B.; Badior, K.; States, U.; Robertson, M.; Columbia, B.; Pereyra, N.; Columbia, B.; Rowe, E.; Columbia, B.; Wietzrny, A.; States, U.; Columbia, B.; Noronha, Z.; Columbia, B.; Arnold, D.; Therapeutics, N.; Ciufolini, M.; Therapeutics, N.; Devine, D.; Columbia, B.; Jan, E.; Columbia, B.; Cullis, P.; Columbia, B.; Kastrup, C.; Columbia, B.; Lnp, R.-. Genetic Engineering of Transfusable Platelets with mRNA-Lipid Nanoparticles is Compatible with Blood Banking Practices. Blood **2024**, *144*(21), 2223–2236.

[53] Kwon, J.; Sohn, H.S.; Yoon, J.S.; Jeon, S.W.; Ko, H.G.; Kang, M.; Lee, J.; Nam, J.; Merriam, L.T.; Kim, E.Y.; Kim, H.Y. Spleen-Targeting Biomimetic Hybrid Nanocarriers for Systemic Immune Reprogramming in Colitis : RBC Membrane Vesicle-Fused Lipid Nanoparticles. Advanced Functional Materials **2025**, *16976*, 1–16.

[54] Nie, X.; Liu, Y.; Song, Y.; Yao, X.; Chen, Y.; Lee, H.-Y.; Gao, X. In vivo generation of CAR myeloid cells through mediated mRNA delivery for cancer immunotherapy. Science Translational Medicine **2026**, *18*(842), eady6730.

[55] Rodríguez, D.A.; Lefebvre, G.P.R.; Yang, Q.; Barendrecht, A.D.; Seinen, C.W.; Schiffelers, R.M.; Vader, P. Incorporation of cellular membrane protein extracts into lipid nanoparticles enhances their cellular uptake and mRNA delivery efficiency. Journal of Controlled Release **2025**, *382*, 113676.

[56] Kranz, L.M.; Diken, M.; Haas, H.; Kreiter, S.; Loquai, C.; Reuter, K.C.; Meng, M.; Fritz, D.; Vascotto, F.; Hefesha, H.; Grunwitz, C.; Vormehr, M.; Hüsemann, Y.; Selmi, A.; Kuhn, A.N.; Buck, J.; Derhovanessian, E.; Rae, R.; Attig, S.; Diekmann, J.; Jabulowsky, R.A.; Heesch, S.; Hassel, J.; Langguth, P.; Grabbe, S.; Huber, C. Systemic RNA delivery to dendritic cells exploits antiviral defence for cancer immunotherapy. Nature **2016**, *534*(7607), 396–401.

[57] Christina Krienke, Laura Kolb, Elif Diken, Michael Streuber, Sarah Kirchhoff, Thomas Bukur, Özlem Akilli-Öztürk, Lena M. Kranz, Hendrik Berger, Jutta Petschenka, Mustafa Diken, Sebastian Kreiter, Nir Yogev, Ari Waisman, Katalin Karikó, Özlem Türeci, U.S. A noninflammatory mRNA vaccine for treatment of experimental autoimmune encephalomyelitis. Science **2021**, *371*(6525), 145–153.

[58] Ngoc-bich-dao, V.; Ngoc-duy, P.; Thi-ngoc-mai, T.; Xuan-hai, P.; Dai-nghiep, N.; Minh-hiep, N. Possibility of nanostructured lipid carriers encapsulating astaxanthin from Haematococcus pluvialis to alleviate skin injury in radiotherapy. International Journal of Radiation Biology **2024**, *100*(2), 209–219.

[59] Hu, M.; Li, X.; You, Z.; Cai, R.; Chen, C. Physiological Barriers and Strategies of Lipid-Based Nanoparticles for Nucleic Acid Drug Delivery. Advanced Materials **2024**, *36*(33), e2303266.

[60] Kulkarni, J.A.; Witzigmann, D.; Thomson, S.B.; Chen, S.; Leavitt, B.R.; Cullis, P.R.; Meel, R. Van Der. The current landscape of nucleic acid therapeutics. Nature Nanotechnology **2021**, *16*(6), 630–643.

[61] Miao, L.; Li, L.; Huang, Y.; Delcassian, D.; Chahal, J.; Han, J.; Shi, Y.; Sadtler, K.; Gao, W.; Lin, J.; Doloff, J.C.; Langer, R.; Anderson, D.G. Delivery of mRNA vaccines with heterocyclic lipids increases anti-tumor efficacy by STING-mediated immune cell activation. Nature Biotechnology **2019**, *37*(10), 1174–1185.

[62] Patel, S.; Ashwanikumar, N.; Robinson, E.; Duross, A.; Sun, C.; Murphy-benenato, K.E.; Mihai, C.; Almarsson, O.; Sahay, G. Boosting Intracellular Delivery of Lipid Nanoparticle-Encapsulated mRNA. Nano Letters **2017**, *17*(9), 5711–5718.

[63] Maier, M.A.; Jayaraman, M.; Matsuda, S.; Liu, J.; Barros, S.; Querbes, W.; Tam, Y.K.; Ansell, S.M.; Kumar, V.; Qin, J.; Zhang, X.; Wang, Q.; Panesar, S.; Hutabarat, R.; Carioto, M.; Hettinger, J.; Kandasamy, P.; Butler, D.; Rajeev, K.G.; Pang, B.; Charisse, K.; Fitzgerald, K.; Mui, B.L.; Du, X.; Cullis, P.; Madden, T.D.; Hope, M.J.; Manoharan, M.; Akinc, A. Biodegradable Lipids Enabling Rapidly Eliminated Lipid Nanoparticles for Systemic Delivery of RNAi Therapeutics. Molecular therapy : the journal of the American Society of Gene Therapy **2013**, *21*(8), 1570–1578.

[64] Hassett, K.J.; Benenato, K.E.; Jacquinet, E.; Lee, A.; Woods, A.; Yuzhakov, O.; Himansu, S.; Deterling, J.; Geilich, B.M.; Ketova, T.; Mihai,

Just Accepted

C.; Lynn, A.; Mcfadyen, I.; Moore, M.J.; Senn, J.J.; Stanton, M.G.; Almarsson, Ö.; Ciaramella, G.; Brito, L.A. Optimization of Lipid Nanoparticles for Intramuscular Administration of mRNA Vaccines. Molecular Therapy: Nucleic Acid **2019**, *15*, 1–11.

[65] Sabnis, S.; Kumarasinghe, E.S.; Salerno, T.; Mihai, C.; Senn, J.J.; Lynn, A.; Bulychev, A.; Mcfadyen, I.; Chan, J.; Stanton, M.G.; Benenato, K.E. A novel amino lipid series for mRNA delivery: improved endosomal escape and sustained pharmacology and safety in non-human primates. Molecular Therapy **2018**, *26*(6), 1509–1519.

[66] Lokugamage, M.P.; Vanover, D.; Beyersdorf, J.; Hatit, M.Z.C.; Rotolo, L.; Echeverri, E.S.; Peck, H.E.; Ni, H.; Yoon, J.; Kim, Y.; Santangelo, P.J.; Dahlman, J.E. Optimization of lipid nanoparticles for the delivery of nebulized therapeutic mRNA to the lungs. Nature Biomedical Engineering **2021**, *5*(9), 1059–1068.

[67] Zhou, Q.; Liu, W.; Ge, J.; Liu, X.; Wang, H.; Fu, W.; Deng, D. Lipid Nanoparticles Enable Efficient EGF mRNA Delivery for Wound Healing. Pharmaceutics **2026**, *18*(2), 215.

[68] Felgner, P.L. The 60-year evolution of lipid nanoparticles for nucleic acid delivery. Nature Reviews Drug Discovery **2024**, *23*(9), 709–722.

[69] Gilleron, J.; Querbes, W.; Zeigerer, A.; Borodovsky, A.; Marsico, G.; Schubert, U.; Manygoats, K.; Seifert, S.; Andree, C.; Stöter, M.; Epstein-barash, H.; Zhang, L.; Koteliansky, V.; Fitzgerald, K.; Fava, E.; Bickle, M.; Kalaidzidis, Y.; Akinc, A.; Maier, M.; Zerial, M. Image-based analysis of lipid nanoparticle – mediated siRNA delivery , intracellular trafficking and endosomal escape. Nature Biotechnology **2013**, *31*(7), 638–646.

[70] Wittrup, A.; Ai, A.; Liu, X.; Hamar, P.; Trifonova, R.; Charisse, K.; Manoharan, M.; Kirchhausen, T.; Lieberman, J. Visualizing lipid-formulated siRNA release from endosomes and target gene knockdown. Nature Biotechnology **2015**, *33*(8), 870–876.

[71] Paramasivam, P.; Franke, C.; St, M.; Bartesaghi, S.; Sabirsh, A.; Lindfors, L.; Arteta, M.Y.; Dahl, A. Endosomal escape of delivered mRNA from endosomal recycling tubules visualized at the nanoscale. The Journal of cell biology **2021**, *221*(2), e202110137.

[72] Vhora, I.; Lalani, R.; Bhatt, P.; Patil, S.; Misra, A. Lipid-nucleic acid nanoparticles of novel ionizable lipids for systemic BMP-9 gene delivery to bone-marrow mesenchymal stem cells for osteoinduction. International Journal of Pharmaceutics **2019**, *563*, 324–336.

[73] Kumar, V.; Qin, J.; Jiang, Y.; Duncan, R.G.; Brigham, B.; Fishman, S.; Nair, J.K.; Akinc, A.; Barros, S.A.; Kasperkovitz, P. V. Shielding of Lipid Nanoparticles for siRNA Delivery : Impact on Physicochemical Properties , Cytokine Induction , and Efficacy. Nucleic acids **2014**, *3*(11), e210.

[74] Zong, Y.; Lin, Y.; Wei, T.; Cheng, Q. Lipid Nanoparticle (LNP) Enables mRNA Delivery for Cancer Therapy. Advanced Materials **2023**, *35*(51), 0–2.

[75] Zhao, F.; Luppi, B.; Chao, P.; Yang, J.; Zhang, Y.; Feng, R.; Chan, V.; Kannan, R.; Dong, S.; Gogoulis, A.; Wang, L.; Lee, A.; Hudson, Z.; Li, S. Biodegradable polymers with tertiary amines enhance mRNA delivery of lipid nanoparticles via improved endosomal escape. Biomaterials **2026**, *324*, 123541.

[76] Li, T., Zhang, J., Guo, J., Sun, B., Han, Y., Xu, H., Weng, Y., Cao, Q., Li, M., Zhao, G., Liu, L., Gao, X., Dai, L., W. Engineered internal architecture of core-shell lipid nanoparticles promotes efficient mRNA endosomal release. Nature Communications **2026**, *17*(1), 2187.

[77] Girgis, M.; Saed, A.; Alishetty, S.; Carrasco, M.; Dillon, O.; Baby, N.; Petruncio, G.; Hoemann, C.; Albano, M.; Peng, X.; Wang, B.; Forsberg, E. Gauging lipid vulnerabilities in mRNA-LNPs under various environmental stressors through TIMS-TOF analysis. RSC Advances **2026**, *16*(21), 18984–18994.

[78] Binici, B.; Rattray, Z.; Zinger, A.; Perrie, Y. Exploring the impact of commonly used ionizable and pegylated lipids on mRNA-LNPs : A combined in vitro and preclinical perspective. Journal of Controlled Release **2025**, *377*, 162–173.

[79] Ren, Y.; Lin, L.; Abdallah, M.; Zhu, X.; Liu, H.; Fabb, S.A.; Payne, T.J.; Pouton, C.W.; Johnston, A.P.R.; Trevaskis, N.L. Impact of ionizable lipid type on the pharmacokinetics and biodistribution of mRNA-lipid nanoparticles after intravenous and subcutaneous injection. Journal of controlled release : official journal of the Controlled Release Society **2025**, *384*, 113945.

[80] Chen, R.; Xu, L.; Zhang, N.; Yu, H.; Wang, X.; Zhai, J.; Whelan, F.; Elliott, P.; Zhao, C. Replacing Helper Lipids With Cationic Lipids Enhances mRNA Lipid Nanoparticles Stability in Solution. Advanced Science **2026**, *13*(3), e11637.

[81] Iwakawa, K.; Sato, R.; Konaka, M.; Yamada, Y.; Harashima, H. Cubic Phase-Inducible Zwitterionic Phospholipids Improve the Functional Delivery of mRNA. Advanced science (Weinheim, Baden-Wurttemberg, Germany) **2025**, *12*(17), e2413016.

[82] Zhang, Y.; Zhang, X.; Gao, Y.; Liu, S. Principles of lipid nanoparticle design for mRNA delivery. **2024**, *3*, e12116.

[83] Li, X. Design Strategies for Novel Lipid Nanoparticle for mRNA Vaccine and Therapeutics : Current Understandings and Future Perspectives. MedComm **2025**, *6*(10), e70414.

[84] Ji, W.; Zhu, K.; Zhou, Y.; Yan, H.; Wei, J.; Zhu, Y.; Li, S.; Dai, J.; Dong, S.; Hu, H.; You, J.; Qiu, Y.; Lou, Y. Covalent conjugation of TLR9 agonists to phospholipids enhances mRNA-LNP delivery efficiency and dendritic cell cross-priming. Journal of nanobiotechnology **2026**, *24*(1), 585.

[85] Guerrero, E.D.; Vaidya, A.; Santelli, J.; Tian, Z.; Pazzi, G.A.; Siegwart, D.J. Investigating the functional contributions of phospholipids in selective organ targeting lipid nanoparticles. Biomaterials **2026**, *326*, 123671.

[86] Zhang, R.; El-mayta, R.; Murdoch, T.J.; Warzecha, C.C.; Billingsley, M.M.; Shepherd, S.J.; Gong, N.; Wang, L.; Wilson, J.M.; Mitchell, M.J. Helper lipid structure influences protein adsorption and delivery of lipid nanoparticles to spleen and liver. Biomaterials Science **2021**, *9*(4), 1449–1463.

[87] Karve, S.; Liu, L.; Tang, J.; Kim, J.; Li, Z.; Sun, M.; Northen, T.; Mcintosh, E.; Karve, S.; Derosa, F. PEGylated lipid screening, composition optimization, and structure–activity relationship determination for lipid nanoparticle-mediated mRNA delivery. Nanoscale **2025**, *17*(18), 11329–11344.

[88] Chander, N.; Basha, G.; Hok, M.; Cheng, Y.; Witzigmann, D.; Cullis, P.R. Lipid nanoparticle mRNA systems containing high levels of sphingomyelin engender higher protein expression in hepatic and extra-hepatic tissues. Molecular Therapy: Methods & Clinical Development **2023**, *30*, 235–245.

[89] Lopresti, S.T.; Arral, M.L.; Chaudhary, N.; Whitehead, A. The replacement of helper lipids with charged alternatives in lipid nanoparticles facilities targeted mRNA delivery to the spleen and lungs. Journal of Controlled Release **2022**, *345*, 819–831.

[90] Quan, Y.; Yang, H.; Li, W.; Li, L. MRNA vaccines: immunogenicity and quality characteristics. Journal of nanobiotechnology **2025**, *24*(1), 6.

[91] Engineering, B.; Campus, S.K. Stability Modelling of mRNA Vaccine Quality Based on Temperature Monitoring throughout the Distribution Chain. Pharmaceutics **2022**, *14*(2), 430.

[92] Turjeman, K.; Barenholz, Y.; Crommelin, D.J.A.; Dobrovolskaia, M.A. Applying lessons learned from nanomedicines to understand rare hypersensitivity reactions to mRNA-based SARS-CoV-2 vaccines. Nature nanotechnology **2022**, *17*(4), 337–346.

[93] Borah, A.; Giacobbo, V.; Binici, B.; Baillie, R.; Perrie, Y. From in vitro to in vivo: The Dominant role of PEG-Lipids in LNP performance. European Journal of Pharmaceutics and Biopharmaceutics **2025**, *212*, 114726.

[94] Moreadith, R.W.; Kim, W.; Smith, K.; Yoon, K.; Weimer, R.

Just Accepted

Overcoming the PEG dilemma with Poly (2-ethyl-2-oxazoline) lipids in lipid nanoparticle formulations. European Polymer Journal **2025**, *241*, 114392.

[95] Holick, C.T.; Klein, T.; Mehnert, C.; Adermann, F.; Anufriev, I.; Streiber, M.; Harder, L.; Traeger, A.; Hoeppener, S.; Franke, C.; Nischang, I.; Schubert, S.; Schubert, U.S. Poly(2-ethyl-2-oxazoline) (POx) as Poly(ethylene glycol) (PEG)-Lipid Substitute for Lipid Nanoparticle Formulations. Small **2025**, *21*(16), e2411354.

[96] Da, A.J.; Sanchez, S.; Loughrey, D.; Echeverri, E.S.; Huayamares, S.G.; Radmand, A.; Paunovska, K.; Hatit, M.; Tiegreen, K.E.; Santangelo, P.J.; Dahlman, J.E. Substituting Poly (ethylene glycol) Lipids with Poly (2-ethyl-2-oxazoline) Lipids Improves Lipid Nanoparticle Repeat Dosing. Advanced healthcare materials **2024**, *13*(17), e2304033.

[97] PEG alternatives for RNA therapeutics. Nature Materials **2025**, *24*(11), 1665.

[98] Cheng, Q.; Wei, T.; Farbiak, L.; Johnson, L.T.; Dilliard, S.A.; Siegwart, D.J. Selective organ targeting (SORT) nanoparticles for tissue-specific mRNA delivery and CRISPR–Cas gene editing. Nature Nanotechnology **2020**, *15*(4), 313–320.

[99] Sago, C.D.; Lokugamage, M.P.; Paunovska, K.; Vanover, D.A. High-throughput in vivo screen of functional mRNA delivery identifies nanoparticles for endothelial cell gene editing. Proceedings of the National Academy of Sciences of the United States of America **2018**, *115*(42), E9944–E9952.

[100] Patel, S.; Ashwanikumar, N.; Robinson, E.; Xia, Y.; Mihai, C.; Grif, J.P.; Hou, S.; Esposito, A.A.; Ketova, T.; Welsher, K.; Joyal, J.L.; Almarsson, Ö.; Sahay, G. Naturally-occurring cholesterol analogues in lipid nanoparticles induce polymorphic shape and enhance intracellular delivery of mRNA. Nature Communications **2020**, *11*(1), 983.

[101] Wang, X.; Shi, X.; Wang, R. Regulating mRNA endosomal escape through lipid rafts: A review. International Journal of Pharmaceutics **2025**, *675*, 125571.

[102] Luo, C.; Li, Y.; Liu, H.; He, J.; Yang, X.; Zhao, E.; Xu, Y.; Peng, B. Intracellular trafficking of lipid nanoparticles is hindered by cholesterol. International Journal of Pharmaceutics **2025**, *671*, 125240.

[103] Kim, Y.; Chatterjee, S.; Robertson, A.L.; Guerrero, E.D.; Vaidya, A.; Wang, X.; Lee, S.M.; Wei, J.; Miller, W.E.; Farbiak, L.; Siegwart, D.J. Mannose-Conjugated Cholesterol Containing Lipid Nanoparticles for Active Targeted mRNA Delivery to Liver Sinusoidal Endothelial and Kupffer Cells. Bioconjugate chemistry **2025**, *36*(10), 2181–2196.

[104] Deng, Z.; Lu, L.; Xu, T.; Zhao, Z.; Geng, Y.; Liu, L.; Yang, M.; Zheng, Y.; Sun, Y.; Lovell, J.F.; Chen, X.; Ding, J.; Jin, H. *Enhancing antitumour nanovaccine efficacy via integrated cholesterol modulation in situ*. Springer US: , 2026; 21 pp.

[105] Jung, O.; Jung, H.; Thuy, L.T.; Choi, M.; Kim, S.; Yang, J.; Kim, S.; Kim, T.; Lee, E.; Kim, Y.; Sig, J. Modulating Lipid Nanoparticles with Histidinamide-Conjugated Cholesterol for Improved Intracellular Delivery of mRNA. Advanced healthcare materials **2024**, *13*(14), e2303857.

[106] Zhang, R.; Tang, L.; Tian, Y.; Ji, X.; Hu, Q.; Zhou, B.; Ding, Z.; Xu, H.; Yang, L. DP7-C-modified liposomes enhance immune responses and the antitumor effect of a neoantigen-based mRNA vaccine. Journal of Controlled Release **2020**, *328*, 210–221.

[107] Jallow, M.B.; Huang, K.; Qiu, M.; Qiu, M. Versatility of LNPs across different administration routes for targeted RNA delivery. Journal of materials chemistry. B **2025**, *13*(26), 7637–7652.

[108] Meulewaeter, S.; Aernout, I.; Deprez, J.; Engelen, Y.; Velder, M. De; Franceschini, L.; Breckpot, K.; Calenbergh, S. Van; Asselman, C.; Boucher, K.; Impens, F.; Smedt, S.C. De; Verbeke, R. Alpha-galactosylceramide improves the potency of mRNA LNP vaccines against cancer and intracellular bacteria. Journal of Controlled Release **2024**, *370*, 379–391.

[109] Wei, C.; Zhu, Y.; Lu, X.; Goodier, K.D.; Yu, D.; Liu, X.; Choy, J.; Calderón, A.T.; Su, Y.; Ma, J.; Lin, J.; Li, S. Lipid nanoparticle composition directs systemic trafficking and tissue-specific T cell immunity after intramuscular injection. Nature biomedical engineering **2026**, 15–17.

[110] Künzli, M.; Flanagan, S.D.O.; Larue, M.; Talukder, P.; Dileepan, T.; Stolley, J.M.; Soerens, A.G.; Quarnstrom, C.F.; Wijeyesinghe, S.; Ye, Y.; Mcpartlan, J.S.; Mitchell, J.S.; Mandl, C.W.; Vile, R.; Jenkins, M.K.; Ahmed, R.; Vezys, V.; Chahal, J.S.; Masopust, D. Route of self-amplifying mRNA vaccination modulates the establishment of pulmonary resident memory CD8 and CD4 T cells. Science Immunology **2022**, *7*(78), eadd3075.

[111] Buckley, M.; Araínga, M.; Maiorino, L.; Pires, I.S.; Kim, B.J.; Michaels, K.K.; Dye, J.; Qureshi, K.; Zhang, Y.; Mak, H.; Steichen, J.M.; Schief, W.R.; Villinger, F.; Irvine, D.J. Visualizing lipid nanoparticle trafficking for mRNA vaccine delivery in non-human primates. Molecular Therapy **2025**, *33*(3), 1105–1117.

[112] Feng, Y.; Tai, W.; Huang, P.; Qi, S.; Yu, X.; Yang, K.; Bai, B.; Lei, J.; Cheng, M.; Li, Y. Albumin-recruiting lipid nanoparticle potentiates the safety and efficacy of mRNA vaccines by avoiding liver accumulation. Nature Materials **2025**, *24*(11), 1826–1839.

[113] Chen, X.; Zhang, H.; Liu, D.; Ma, J.; Jin, L.; Ma, Y.; Li, J.; Song, G.; Wang, J. Distinct dynamics of mRNA LNPs in mice and nonhuman primates revealed by in vivo imaging. Journal of materials chemistry. B **2025**, *13*(18), 5290–5296.

[114] Marlin, R.; Mayet, C.; Perkov, V.; Pascal, Q.; Ripoll, M.; Relouzat, F.; Dhooge, N.; Bossevot, L.; Dereuddre-bosquet, N.; Dargazanli, G.; Thibaut-duprey, K.; Haensler, J.; Chapon, C.; Prost, C.; Grand, R. Le. Distinct dynamics of mRNA LNPs in mice and nonhuman primates revealed by in vivo imaging. NPJ Vaccines **2024**, *1*, 113.

[115] Communications, N. Multiplexed lipid nanoparticle barcoding reveals tissue-dynamic kinetic insights and enriched cellular tropism in hepatic zones. Nature Communications **2026**, *17*(1), 1345.

[116] Patel, S.K.; Billingsley, M.M.; Mukalel, A.J.; Thatte, A.S.; Hamilton, A.G.; El-mayta, R.; Safford, H.C.; Merolle, M.; Mitchell, M.J. Bile acid-containing lipid nanoparticles enhance extrahepatic mRNA delivery. Theranostics **2024**, *14*(1), 1–16.

[117] Kiyono, H.; Ernst, P.B. Nasal vaccines for respiratory infections. Nature **2025**, *641*(8062), 321–330.

[118] Ren, H.; Yu, X.; Li, M.; Luo, Z.; Lin, Y.; Meng, Y.; Cheng, Q.; Wei, T. Nebulized Lipid Nanoparticles Deliver mRNA to the Liver for Treatment of Metabolic Diseases. Nano Letters **2025**, *25*(37), 13802–13810.

[119] Li, Y.; Long, H.; Wang, C.; Weng, J.; Kuang, Z.; Peng, Y. An optimized intranasal LNP formulation for potent immune responses of mRNA-based RSV vaccines. Nanoscale **2025**, *17*(42), 24541–24553.

[120] Men, Y.; Popoola, D.O.; Cao, Z.; Li, Y.; Wilkens, S.; Teng, Y.; Meng, Q.; Hershenson, M.; Li, Y. Sulfonium lipid nanoparticles for intranasal mRNA delivery to lung epithelial and immune cells. Acta Biomaterialia **2025**, *205*, 616–633.

[121] Song, M.; Ha, M.; Shin, S.; Kim, M.; Son, S.; Lee, J.; Hwang, G.W.; Kim, J.; Duong, V.H.; Park, J.H. A Hierarchical Short Microneedle - Cupping Dual - Amplified Patch Enables Accelerated , Uniform , Pain - Free Transdermal Delivery of Extracellular Vesicles. Nano-Micro Letters **2025**, *18*(1), 11.

[122] Qin, X.; Wei, T.; Zhang, Z.; Qin, H.; Lin, Y.; Song, J. Topical framework nucleic acid transdermal delivery system for reprogramming cutaneous dendritic cells to maintain graft immune homeostasis. Bioactive Materials **2026**, *64*, 394–409.

[123] Driskill, M.M.; Coates, I.A.; Hurst, P.J.; Rajesh, N.U.; Dulay, M.T.; Waymouth, R.M.; Akahata, W.; Matsuda, K.; Smith, J.F.; Jacobson, G.B.; Perry, J.L.; Tian, S.; Desimone, J.M. Lyophilized SARS-CoV-2 self-amplifying RNA vaccines for microneedle array patch delivery. Journal of

Just Accepted

Controlled Release **2025**, *384*, 113944.
[124] Seok, J.; Kim, N.; Choi, J.; Choi, I.; Choi, J.; Kang, A.; Kwon, D.; Lim, M.; Kim, J.; Baek, S.; Song, M.; Song, Y.; Park, J. Powder-attached microneedles for the stable and effective transdermal delivery of clinically validated mRNA-LNP vaccine. European Journal of Pharmaceutics and Biopharmaceutics **2025**, *214*, 114811.
[125] Ma, Y.; Li, S.; Lin, X.; Chen, Y. A perspective of lipid nanoparticles for RNA delivery. Exploration (Beijing) **2024**, *4*(6), 20230147.
[126] Winkeljann, B.; Lapuhs, P.; Alonso, M.J. A multiscale approach to lipid nanoparticle engineering from molecular structure to in vivo performance. Journal of Controlled Release **2026**, *394*, 114911.
[127] Han, E.L.; Tang, S.; Kim, D.; Murray, A.M.; Swingle, K.L.; Hamilton, A.G.; Mrksich, K.; Padilla, M.S.; Palanki, R.; Li, J.J.; Mitchell, M.J. Peptide-Functionalized Lipid Nanoparticles for Targeted Systemic mRNA Delivery to the Brain. Nano Letters **2025**, *25*(2), 800–810.
[128] Kon, E.; Ad-el, N.; Hazan-halevy, I.; Stotsky-oterin, L.; Peer, D. Targeting cancer with mRNA – lipid nanoparticles : key considerations and future prospects. Nature Reviews Clinical Oncology **2023**, *20*(11), 739–754.
[129] Chen, M.Z.; Yuen, D.; Mcleod, V.M.; Yong, K.W.; Smyth, C.H.; Herling, B.R.; Payne, T.J.; Fabb, S.A.; Belousoff, M.J.; Algarni, A.; Sexton, P.M.; Porter, C.J.H.; Pouton, C.W.; Johnston, A.P.R. A versatile antibody capture system drives specific in vivo delivery of mRNA-loaded lipid nanoparticles. Nature Nanotechnology **2025**, *20*(9), 1273–1284.
[130] Park, W.; Choi, J.; Hwang, J.; Kim, S.; Kim, Y.; Shim, M.K.; Park, W.; Yu, S.; Jung, S.; Yang, Y.; Kweon, D. Apolipoprotein Fusion Enables Spontaneous Functionalization of mRNA Lipid Nanoparticles with Antibody for Targeted Cancer Therapy. ACS Nano **2025**, *19*(6), 6412–6425.
[131] Shah, S.; Ranasinghe, M.; Decker, J.; Fraser, K.; Wang, Y.; Kinlaw, S. Lipid nanoparticles with aptamers enable targeted mRNA delivery to $CD4^+$ T cells. Drug Delivery **2026**, *33*(1), 2637266.
[132] Song, M.; Bustos, A.H.; Bastue, L.; Mikutavicius, J.; Swiderski, P.; Clemens, K.J.; Habib, N.; Rossi, J.; Astakhova, K. New Transferrin Receptor-Targeting Conjugate Effectively Delivers DNA to Mouse Brain. Angewandte Chemie (International ed. in English) **2025**, *64*(24), e202500247.
[133] Papp, T.E.; Zeng, J.; Shahnawaz, H.; Akyianu, A.; Breda, L.; Yadegari, A.; Steward, J.; Shi, R.; Li, Q.; Mui, B.L.; Tam, Y.K.; Rivella, S.; Shuvaev, V.; Muzykantov, V.R. CD47 Peptide-Cloaked Lipid Nanoparticles Promote Cell-Specific mRNA Delivery. Molecular Therapy **2025**, *33*(7), 3195–3208.
[134] Lin, Y.; Li, M.; Luo, Z.; Meng, Y.; Zong, Y.; Ren, H.; Yu, X.; Tan, X.; Liu, F.; Wei, T.; Cheng, Q. Tissue-specific mRNA delivery and prime editing with peptide–ionizable lipid nanoparticles. Nature Materials **2026**, *25*(1), 133–145.
[135] Wang, S.; Zheng, J.; Zhou, J.; Jiang, W.; Chen, Z.; Wu, X.; Guo, B.; Wu, Y.; Yang, F. Engineered lipid nanoparticles with synergistic dendritic cell targeting and enhanced endosomal escape for boosted mRNA cancer vaccines. Materials Today Bio **2025**, *34*, 102107.
[136] Zelkoski, A.E.; Lu, Z.; Sukumar, G.; Dalgard, C.; Said, H.; Alameh, M.; Mitre, E.; Malloy, A.M.W. Ionizable lipid nanoparticles of mRNA vaccines elicit NF- κ B and IRF responses through toll-like receptor 4. npj Vaccines **2025**, *10*(1), 73.
[137] Pena, A.; Calderon-ruiz, P.; Gambaro, R.; Rivero-berti, I.; Meyer, C.U.; Cacicedo, M.L.; Hankeln, T.; Si, S.; Lieberwirth, I.; Landfester, K.; Alba, C.; Tekiel, V.; Bros, M.; Gehring, S.; Islan, G.A. International Journal of Biological Macromolecules Incorporation of monophosphoryl lipid A and CpG-oligodeoxynucleotides into lipid nanoparticles activates toll-like receptor signaling pathways while maintaining antigen expression for mRNA-based vaccinati. International Journal of Biological Macromolecules **2025**, *330*(1), 148058.
[138] Keikha, R.; Balali, E.; Khadivi, R.; Jebali, A. The Combination of TLR4 and TLR9 Agonists with Self-Amplifying RNA Lipid Nanoparticles Leads to a More Powerful Immune Response Against SARS-CoV-2. Viral immunology **2025**, *38*(5), 190–202.
[139] Choi, H.; Lee, S.; Kim, H.; Bae, S.; Jo, S.; Kim, J.; Lee, Y.; Ha, D.; Oh, A.; Yoon, S.; Jeon, S.; Lee, Y.; Cho, Y.; Cho, S.; Roh, G.; Lee, S.; Gowda, J.; Park, H.; Lee, J.; Song, D.; Hong, S. Integration of TLR7 / 8 agonists into lipid nanoparticles enhances antigen-specific immune responses to N1-methyl-Ψ-modified mRNA-LNP vaccines. Journal of biological engineering **2025**, *19*(1), 103.
[140] Xu, X.; Wang, X.; Liao, Y.; Luo, L.; Nel, A.E. Reprogramming the Tolerogenic Immune Response Against Pancreatic Cancer Metastases by Lipid Nanoparticles Delivering a STING Agonist Plus Mutant KRAS mRNA. Nature Reviews Drug Discovery **2024**, *23*(4), 281–300.
[141] Song, X. SSitoC7A-modified lipid nanoparticles for integrated mRNA delivery and targeted STING activation. Journal of Controlled Release **2025**, *387*, 114238.
[142] Yang, Q.; Shi, X.; Yang, K.; Gao, Q.; Cao, Y.; Huang, Y.; Chen, L.; Xiao, B. A manganese-based nanoplatform leveraging chemodynamic and adjuvant effects for in situ vaccination against colorectal cancer. Biomaterials **2026**, *331*, 124085.
[143] Hok, M.; Cheng, Y.; Zhang, Y.; Fox, K.; Leung, J.; Strong, C.; Kang, E.; Chen, Y.; Tong, M.; Bommadevara, H.; Jan, E.; Yuk, O.; Ip, L.; Rodríguez-rodríguez, C.; Saatchi, K.; Häfeli, U.O.; Abdolahzadeh, A.; Witzigmann, D.; Cullis, P.R. Liposomal lipid nanoparticles for extrahepatic delivery of mRNA. Nature Communications **2025**, *16*(1), 4135.
[144] Teng, Y.; Guo, Y.; Liu, Z.; Tang, M.; Stewart, W.; Xu, X.; Zhang, X. Computationally Aided Design of Ionizable Cholesteryl Lipids for Lipid Nanoparticles to Modulate Hepatic mRNA Accumulation. Journal of the American Chemical Society **2025**, *147*(42), 38865–38878.
[145] Sarode, A.; Ortiz, C.; Derstepanian, T.; Vargas-montoya, N.; Patel, P.; Khadse, N.; Manna, S.; Landis, R.; Skaleski, J.; Boeglin, L.; Deng, H.; Dias, A.; Wang, H.; Petropolis, D.B.; Yahalom, B.; Karve, S.; Derosa, F. Potent Liver-Tropic mRNA Lipid Nanoparticles : ApoE-Mediated Delivery Through a Low-Density Lipoprotein Receptor Independent Uptake Mechanism. Advanced Materials **2026**, *38*(22), e17893.
[146] Muto, H.; Kubara, K.; Hitaoka, S.; Osada, Y.; Suzuki, Y. Ionizable lipid chemistry in lipid nanoparticles determines delivery efficiency to hepatic stellate cells. Journal of Controlled Release **2025**, *385*, 114055.
[147] Shan, X.; Zhao, Z.; Lai, P.; Liu, Y.; Li, B.; Ke, Y.; Jiang, H.; Zhou, Y.; Li, W.; Wang, Q.; Qin, P.; Xue, Y.; Zhang, Z.; Wei, C.; Ma, B.; Liu, W. RNA nanotherapeutics with fi brosis overexpression and retention for MASH treatment. Nature Communications **2024**, *15*(1), 7263.
[148] Zhang, C.; Teng, Y.; Bai, X.; Tang, M.; Stewart, W.; Chen, J.J. Prevent and Reverse Metabolic Dysfunction-Associated Steatohepatitis and Hepatic Fibrosis via mRNA-Mediated Liver-Specific Antibody Therapy. ACS Nano **2024**, *18*(50), 34375–34390.
[149] Teng, Y.; Guo, Y.; Liu, Z.; Tang, M.; Stewart, W.; Xu, X.; Zhang, X. Computationally Aided Design of Ionizable Cholesteryl Lipids for Lipid Nanoparticles to Modulate Hepatic mRNA Accumulation. **2025**.
[150] Chen, X.; Li, X.; Yan, Y.; Li, Z.; Li, J.; Xue, J.; Bai, C.; Li, J.; Wu, D.; Liu, X.; Zhu, Y.; Wang, J. Splenic dendritic cell-targeting mRNA transfection of H-type ionizable lipid-based LNPs for enhancing tumor immunotherapy. Bioactive Materials **2026**, *61*, 429–447.
[151] Zou, C.; Sun, B.; Zheng, C.; Wang, Z.; Hou, J.; Shi, Y.; Jin, Z. Flower-Shaped Lipid Nanoparticles Evade Apolipoprotein E - Mediated Liver Tropism for Safe and Enhanced Cytokine-Based Cancer. ACS Nano **2025**, *19*(40), 35675–35691.
[152] Kim, M.; Song, E.S.; Chen, J.C.; Chatterjee, S.; Sun, Y.; Lee, S.M.; Wu, S.; Patel, P.; Tian, Z.; Kantor, A.; Wustman, B.A.; Lockhart, D.J.; Siegwart, D.J. Dual SORT LNPs for multi-organ base editing. Nature

Just Accepted

Biotechnology **2026**, *44*(4), 578–586.
[153] Song, D.; Zhao, Y.; Wang, Z.; Xu, Q. Tuning Lipid Nanoparticles for RNA Delivery to Extrahepatic Organs. Advanced materials (Deerfield Beach, Fla.) **2024**, *36*(44), e2401445.
[154] Yamagata, H.M.; Padilla, M.S.; Hamilton, A.G.; Swingle, K.L.; Thatte, A.S.; Ricciardi, A.S.; Agrawal, A.; Fitzgerald, E.; Poirier, A.J.; Geisler, H.C.; Joseph, R.A.; Chalom, O.Z.; Du, S.; Li, J.J.; Kim, D.; Whitaker, R.C.; Figueroa-espada, C.G.; Han, E.L.; Murray, A.M.; Alameh, M.; Weissman, D.; Mitchell, M.J. Liver-Detargeted Aromatic Bioreducible mRNA Lipid Nanoparticles Confer Lymph Node Tropism and Robust Antigen-Specific Immunity. Journal of the American Chemical Society **2026**, *148*(13), 14137–14150.
[155] Rudra, A.; Gupta, A.; Reed, K.; Deik, A.; Min, J.; Mansour, H.M.A.; Trung, Q.; Nguyen, C.; Danko, A.; Hu, Y.; Berger, A.; Prado, M.; Beck, A.; Clish, C.B.; Klauda, J.B.; Langer, R.; Anderson, D.G. Degradable cyclic amino alcohol ionizable lipids as vectors for potent influenza mRNA vaccines. Nature Nanotechnology **2025**, *20*(12), 1831–1842.
[156] Zhang, L.; Chen, P.; Tian, X.; Hu, Y.; Wang, R.; Zhang, J. Cyclen-based lipidoids for mRNA delivery and immunotherapy. Biomaterials Science **2025**, *13*(10), 2783–2793.
[157] Xie, K.; Zhu, L.; Duan, M.; Fu, Y.; Wang, H.; Shen, Y. Fluorinated lipid nanoparticles enable real - time tracking of mRNA delivery and uncover spatiotemporal mechanisms of immune activation. Proceedings of the National Academy of Sciences of the United States of America **2026**, *123*(1), e2519823123.
[158] Wang, Y.; Tang, L. An Engineered Extracellular Vesicle With Enhanced Tumor and Lymph Nodes Targeting as siRNA Delivery System for Robust Tumor Immunotherapy. MedComm **2026**, *7*(3), e70673.
[159] Deng, X.; Li, Y.; Zhu, X.; Du, Y.; Chi, H.; Cheng, M.; Wang, Y.; An, K.; Zhang, P. PH-Isomerizable Acylhydrazone-Based Ionizable Lipids for Spleen- Targeted mRNA Vaccines. Journal of the American Chemical Society **2025**, *147*(51), 47428–47442.
[160] Dong, W.; Li, Z.; Hou, T.; Shen, Y.; Guo, Z.; Su, Y.; Chen, Z.; Pan, H.; Jiang, W.; Wang, Y. Multicomponent Synthesis of Imidazole-Based Ionizable Lipids for Highly Efficient and Spleen-Selective Messenger RNA Delivery. Journal of the American Chemical Society **2024**, *146*(22), 15085–15095.
[161] Lin, X.; Chen, G.; Tang, R. Spleen-targeted neoantigen mRNA vaccine induces ISG15 + CD8 + T cell-mediated tertiary lymphoid structure formation in hepatocellular carcinoma. Cell Reports Medicine **2026**, *7*(5), 102754.
[162] Zhang, H.; Ma, J.; Pan, F.; Liu, Y.; Zhang, M.; Li, Y. Prediction of high-performing spleen-targeted lipid nanoparticles using a deep learning model for robust anticancer immunotherapy. Journal of materials chemistry. B **2025**, *13*(37), 11611–11620.
[163] Lian, X.; Chatterjee, S.; Sun, Y.; Dilliard, S.A.; Moore, S.; Xiao, Y.; Bian, X.; Yamada, K.; Sung, Y.; Levine, R.M.; Mayberry, K.; John, S.; Liu, X.; Smith, C.; Johnson, L.T.; Wang, X.; Zhang, C.C.; Liu, D.R.; Newby, G.A.; Weiss, M.J.; Yen, J.S.; Siegwart, D.J. Bone-marrow-homing lipid nanoparticles for genome editing in diseased and malignant haematopoietic stem cells. Nature nanotechnology **2024**, *19*(9), 1409–1417.
[164] Heredero, J.; Peña, Á.; Broset, E.; Blandín, B.; Miguel, D. De; Alejo, T.; Toro, A.; Mata, E.; López-gavín, A.; Gallego-lleyda, A.; Casabona, D.; Lampaya, V.; Larraga, A.; Pérez-herrán, E.; Luna, D.; Orera, I.; Romanos, E.; García, A.; Martínez-oliván, J.; Giménez-warren, J. Predictive Lung- and Spleen-Targeted mRNA Delivery with Biodegradable Ionizable Lipids in Four-Component LNPs. Pharmaceutics **2025**, *17*(4), 459.
[165] Zhang, Y.; Shao, B.; Jiang, K.; Cen, J.; Liu, Y.; Tan, J.; Zhang, L.; Li, K.; You, C.; He, Z.; Zhang, G.; Chen, M.; Hu, J.; Liu, S. Fluorinated Ionizable Lipids for Efficient Spleen-Targeted mRNA Delivery in Cancer Immunotherapy. Journal of the American Chemical Society **2025**, *147*(45), 41756–41767.
[166] Isaac, I.; Patel, L.; Yun, D.; Park, S.; Bhattacharya, C. Endogenous Corticosteroid Derived Lipid Nanoparticles (Cortico LNPs) Enable Selective mRNA Delivery to the Spleen. Small (Weinheim an der Bergstrasse, Germany) **2025**, *21*(36), e03034.
[167] Zhao, Y.; Tian, Z.; Wang, J.; Cui, M.; Cao, Z.; Liu, P.; Li, R.; Cai, S.; Hu, Y.; Ma, Y.; Wagner, E.; Laflin, A.; Gu, W.; Cui, Y.; Tang, C.; Fountain, H.; Wang, R.; Jiang, S. Replacing cholesterol and PEGylated lipids with zwitterionic ionizable lipids in LNPs for spleen- specific mRNA translation. Science Advances **2025**, *11*(41), eady6460.
[168] Wu, S.; Shi, L.; Su, K.; Lin, L.; Yan, X.; Zhang, Y.; Gu, T.; Wang, Z.; Xu, T.; Liu, S. Carbonate-Bearing Ionizable Lipids for mRNA Delivery to Splenic NK Cells. Journal of the American Chemical Society **2025**, *147*(32), 28665–28673.
[169] Peña, Á.; Heredero, J.; Blandín, B.; Mata, E.; Miguel, D. De; Toro, A.; Alejo, T.; Casabona, D.; López, A.; Gallego-lleyda, A.; Pérez-herrán, E.; Martínez-oliván, J.; Giménez-warren, J. Multicomponent thiolactone-based ionizable lipid screening platform for ef fi cient and tunable mRNA delivery to the lungs. Communications Chemistry **2025**, *8*(1), 116.
[170] Prates-syed, W.A.; Leandro, D.; Pour, S.Z.; Lira, A.; Cortes, N.; Dinis, J.; Silva, Q.; Carvalho, E.; Filgueiras, I.S.; Geraldine, T.; Vinces, C.; Schimke, L.F.; Chaves, L.C.S.; Wunderlich, G.; Durães-carvalho, R.; Dias, H.D.; Ochs, H.D.; Câmara, N.O.S.; Nakaya, H.I.; Krieger, J.E. COVID-19 vaccination atlas using an integrative systems vaccinology approach. NPJ Vaccines **2025**, *10*(1), 111.
[171] Laliberte, J.P.; Cai, Y.; Kenny, T.; Zhang, Y.; Ren, K.; Rosenthal, K.; Flores, D.; Roque, R.; Acton, O.; Peano, V.C.; Janney, N.; Puri, V.; Siddiqui, S.; Pack, A.; Padilla, G.; Diallo, S.; Adissu, H.; Choudhary, R.; Pruitt, H.; Cheng, Y.; Thom, G.; Newton, M.; Kurokawa, C.; Guest, J.; Swanson, P.; Loo, Y.; Joseph, R.; Blair, W.; Esser, M. Immunogenicity and efficacy of an mRNA vaccine expressing a virus-like particle spike antigen against SARS-CoV-2. NPJ Vaccines **2025**, *10*(1), 258.
[172] Lu, Z. MRNA-LNP vaccines: rational design, delivery optimization, and clinical translation. Journal of materials chemistry. B **2025**, *13*(48), 15447–15467.
[173] Approach, A.P. Cancer vaccinations: a personalized approach. BioTechniques **2021**, *70*(6), 303–305.
[174] Group, B.; Oncology, D.G.; Cancer, G. Individualized mRNA vaccines evoke durable T cell immunity in adjuvant TNBC. Nature **2026**, *651*(8107), 1088–1096.
[175] Sahin, U.; Derhovanessian, E.; Miller, M.; Kloke, B.P.; Simon, P.; Löwer, M.; Bukur, V.; Tadmor, A.D.; Luxemburger, U.; Schrörs, B.; Omokoko, T.; Vormehr, M.; Albrecht, C.; Paruzynski, A.; Kuhn, A.N.; Buck, J.; Heesch, S.; Schreeb, K.H.; Müller, F.; Ortseifer, I.; Vogler, I.; Godehardt, E.; Attig, S.; Rae, R.; Breitkreuz, A.; Tolliver, C.; Suchan, M.; Martic, G.; Hohberger, A.; Sorn, P.; Diekmann, J.; Ciesla, J.; Waksmann, O.; Brück, A.K.; Witt, M.; Zillgen, M.; Rothermel, A.; Kasemann, B.; Langer, D.; Bolte, S.; Diken, M.; Kreiter, S.; Nemecek, R.; Gebhardt, C.; Grabbe, S.; Höller, C.; Utikal, J.; Huber, C.; Loquai, C.; Türeci, Ö. Personalized RNA mutanome vaccines mobilize poly-specific therapeutic immunity against cancer. Nature **2017**, *547*(7662), 222–226.
[176] Sahin, U.; Oehm, P.; Derhovanessian, E.; Jabulowsky, R.A.; Vormehr, M.; Gold, M.; Maurus, D.; Schwarck-Kokarakis, D.; Kuhn, A.N.; Omokoko, T.; Kranz, L.M.; Diken, M.; Kreiter, S.; Haas, H.; Attig, S.; Rae, R.; Cuk, K.; Kemmer-Brück, A.; Breitkreuz, A.; Tolliver, C.; Caspar, J.; Quinkhardt, J.; Hebich, L.; Stein, M.; Hohberger, A.; Vogler, I.; Liebig, I.; Renken, S.; Sikorski, J.; Leierer, M.; Müller, V.; Mitzel-Rink, H.; Miederer, M.; Huber, C.; Grabbe, S.; Utikal, J.; Pinter, A.; Kaufmann, R.; Hassel, J.C.; Loquai, C.; Türeci, Ö. An RNA vaccine drives immunity in checkpoint-inhibitor-treated

Just Accepted

melanoma. Nature **2020**, *585*(7823), 107–112.

[177] Qiao, N. MRNA vaccines in cancer immunotherapy : current progress and perspectives in solid tumors and hematologic malignancies. MedScience **2026**, *20*(1), 23–58.

[178] Hasan, N.; Aftab, M.; Ikram, S.; Zaenal, A.; Sriwidodo, S.; Shalahudin, H.; Nur, M.; Tockary, T.A.; Uchida, S. Recent advances in lipid nanoparticles for cancer vaccine delivery : Challenges and future perspectives. International Journal of Pharmaceutics: X **2026**, *11*, 100484.

[179] Cao, L.; Min, J.; Yu, M.; Zhang, Z.; Yuan, D.; Chen, D. Key Clinical Frontiers of mRNA Loaded Lipid Nanoparticles in Cancer Vaccines. International Journal of Nanomedicine **2025**, *20*, 14935–14953.

[180] Zhu, Y.; Yao, Z.; Li, S.; Ma, J.; Wei, C.; Yu, D.; Stelzel, J.L.; Ni, B.Y.X.; Miao, Y.; Batavia, K. Van; Lu, X.; Lin, J.; Dai, Y.; Kong, J.; Shen, R.; Goodier, K.D.; Liu, X.; Cheng, L.; Vuong, I.; Howard, G.P.; Livingston, N.K.; Choy, J.; Schneck, J.P.; Doloff, J.C.; Reddy, S.K.; Hickey, J.W. An mRNA lipid nanoparticle-incorporated nano fi ber-hydrogel composite for cancer immunotherapy. Nature communications **2025**, *16*(1), 5707.

[181] Yi, D.; Liu, Q.; Guo, S.; Li, Q.; Zhang, Y.; Li, N.; Zhang, Q.; Lv, K.; Wang, D.; Liao, G.; Huang, C.; Zhang, W.; Dong, Y.; Shu, Y. Chimeric hemagglutinin and M2 mRNA vaccine for broad in fl uenza subtype protection. NPJ Vaccines **2025**, *10*(1), 113.

[182] Felgner, J.; Hernandez-davies, J.E.; Strahsburger, E.; Silzel, E.; Nakajima, R.; Jain, A.; Laster, J.; Chiang, J.; Tsai, Y.; Felgner, P.L.; Davies, D.H.; Liang, L. Lipid Nanoparticle Development for A Fluvid mRNA Vaccine Targeting Seasonal In fl uenza and SARS-CoV-2. NPJ Vaccines **2025**, *10*(1), 123.

[183] Erick Bermúdez-Méndez1, 2; , Klara Lenart1, 2, 4; , Rodrigo Arcoverde Cerveira1, 2; , Olivia Engstrand1, 2; Fredrika Hellgren1, 2, Alberto Cagigi1, 2, 5; , Sebastian Ols1, 2, 6; Jasny3, E.; , Annika Reinhardt1, 2; Benjamin Petsch3, 7& Karin Loré1, 2; Seasonal. SeasonalinfluenzamRNAvaccineinduces stronger innate and comparable or better adaptive responses than licensed inactivated vaccines. NPJ Vaccines **2026**, *11*(1), 108.

[184] Xiong, F.; Zhang, C.; Shang, B.; Zheng, M.; Wang, Q. An mRNA-based broad-spectrum vaccine candidate confers cross-protection against heterosubtypic in fl uenza A viruses. Emerg Microbes Infect **2023**, *12*(2), 2256422.

[185] Willis, J.R.; Willis, J.R.; Prabhakaran, M.; Muthui, M.; Naidoo, A.; Sincomb, T.; Cottrell, C.A.; Landais, E.; Allan, C.; Keshavarzi, N.R.; Lee, J.H.; Murungi, L.M.; Ogonda, W.A.; Yates, N.L.; Martin, M.; Phulera, S.; Musando, J.; Tsai, A.; Lemire, G.; Sein, Y.; Nyabundi, V.; Chirchir, R.; Anzala, O.; Kimotho, J.N.; Kibet, C.; Greene, K.; Gao, H.; Gajjala, S.; Rikhtegaran-tehrani, Z.; Benner, A.; Ramaswami, M.; Lu, D.; Adachi, Y.; Liguori, A.; Flynn, C.; Mckenney, K.; Zhou, X.; Collins, D.; Diedrich, J.; Schiffner, T.; Shields, M.; Matsoso, M.; Santos, J. Vaccination with mRNA-encoded nanoparticles drives early maturation of HIV bnAb precursors in humans. Science **2025**, *389*(6759), eadr8382.

[186] Willis, J.R.; Prabhakaran, M.; Wu, W.; Naidoo, A.; Sincomb, T.; Keshavarzi, N.R.; Cottrell, C.A.; DeCamp, A.C.; Hyun Lee, J.; Yates, N.L.; Tsai, A.; Corcoran, M.M.; Alamuri, P.; Bohl, J.A.; Holman, D.; Himansu, S.; Leav, B.; Reuter, C.; Lin, L.-A.; Ding, B.; Straus, W.L.; MacPhee, K.J.; Green, K.; Gao, H.; Beatman, E.; Laddy, D.; Brown, D.M.; Bronson, R.; Jean-Baptiste, J.; Gajjala, S.; Rikhtegaran-Tehrani, Z.; Benner, A.; Ramaswami, M.; Lu, D.; Alavi, N.; Amirzehni, S.; Kubitz, M.; Tingle, R.; Georgeson, E.; Phelps, N.; Adachi, Y.; Liguori, A.; Flynn, C.; McKenney, K.; Zhou, X.; Duff, M.; Gibson, G.; Ozorowski, G.; Baboo, S.; Diedrich, J.; Shields, M.; Santos, J.; Syvertsen, K.; Kennedy, A.; Schroeter, M.; Vekemans, J.; Yates III, J.R.; Paulson, J.C.; Ward, A.B.; Hyrien, O.; McDermott, A.B.; Maenza, J.; Shapiro, A.E.; Juliana McElrath, M.; Edupuganti, S.; Taylor, B.S.; Diemert, D.; Koup, R.A.; Karlsson Hedestam, G.B.; Montefiori, D.; Tomaras, G.; Sok, D.; Hunt, D.J.; Laufer, D.S.; Andrews, S.F.; Schief, W.R. Vaccination with mRNA-encoded nanoparticles drives early maturation of HIV bnAb precursors in humans. Science **2025**, *389*(6759), eadr8382.

[187] Engineering, N.B. Treatment of a metabolic liver disease in mice with a transient prime editing approach. Nature biomedical engineering **2025**, *9*(10), 1705–1718.

[188] Tálas, A.; Ioannidi, E.I.; Weber, Y.; Haenggi, T.; Kulcsár, P.I.; Zürcher, N.; Faccin, E.; Moon, W.J.; Kissling, L.; Villiger, E.A.; Matsushita, M.; Rothgangl, T.; Janjuha, S.; Lin, P.J.C.; Poms, M.; Muramatsu, H.; Vadovics, M.; Reichmuth, A.; Kopf, M.; Pardi, N.; Tam, Y.K.; Häberle, J.; Schwank, G. RNA- LNP–mediated in vivo prime editing corrects disease phenotypes in a mouse model of citrullinemia type I. Science Translational Medicine **2026**, *18*(849), eaec7274.

[189] Woo, C.J.; Allawzi, A.; Clark, N.; Kaushal, N.; Efthymiou, T.; Thamsen, M.; Nguyen, J.; Wooster, R.; Sullivan, J.C. Inhaled delivery of a lipid nanoparticle encapsulated messenger RNA encoding a ciliary protein for the treatment of primary ciliary dyskinesia. Pulmonary Pharmacology & Therapeutics **2022**, *75*, 102134.

[190] Omo-lamai, S.; Wang, Y.; Patel, M.N.; Milosavljevic, A.; Majumder, A.; Essien, E.; Shen, M.; Channer, B.; Murali, S.; Chase, L.S.; Zamora, M.E.; Arral, M.L.; Oscar, A.; Gaskill, J.; Tsourkas, A.; Muzykantov, V.R.; Brodsky, I.; Shin, S.; Brenner, J.S. Limiting endosomal damage sensing reduces inflammation triggered by lipid nanoparticle endosomal escape. Nature Nanotechnology **2025**, *20*(9), 1285–1297.

[191] Johansson, J.M.; Rietz, H. Du; Hedlund, H.; Eriksson, H.C.; Blenke, E.O.; Pote, A.; Harun, S.; Nordenfelt, P.; Lindfors, L.; Wittrup, A. Cellular and biophysical barriers to lipid nanoparticle mediated delivery of RNA to the cytosol. Nature Communications **2025**, *16*(1), 5354.

[192] Khunsuk, P.; Pongma, C.; Palaga, T.; Hoven, V.P. Zwitterionic Polymer-Decorated Lipid Nanoparticles for mRNA Delivery in Mammalian Cells. Biomacromolecules **2023**, *24*(12), 5654–5665.

[193] Amoako, K.; Mokhammad, A.; Malik, A. Enhancing nucleic acid delivery by the integration of arti fi cial intelligence into lipid nanoparticle formulation. Front Med Technol **2025**, *7*, 1591119.

© The Author(s) 2026. *Nano Research* published by Tsinghua University Press. The articles published in this open access journal are distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

Just Accepted