Research Progress in mRNA Cancer Vaccines: Immunogenicity Regulation, Optimization of Lipid Nanoparticle Delivery Systems, and Challenges in Non-Hepatic Targeting

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Abstract. In 2022, approximately 20 million new cancer cases and 9.7 million cancer-related deaths were reported globally, with lung cancer representing the most prevalent form and the leading cause of cancer-related mortality. Traditional chemotherapy has limitations like poor targeting and severe toxic side effects, while mRNA cancer vaccines are a research hotspot for high safety and rapid antigen translation, though facing suboptimal targeting. This review summarizes recent progress and future directions in mRNA cancer vaccine development. It first introduces the definition and mechanism of mRNA cancer vaccines, analyzes the "double-edged sword" immunogenicity of in vitro transcribed (IVT) mRNA (activation boosts efficacy, but excessive activation risks harm) and its regulatory strategies (e.g., nucleoside modification, UTR optimization). Focusing on lipid nanoparticle (LNP) delivery to address naked mRNA degradation, it discusses optimization of targeting and efficiency via novel lipid screening, ratio adjustment, surface modification, and administration routes. It also explores mechanisms, strategies, and challenges of non-hepatic targeting (e.g., pulmonary mucus barrier). Despite progress, breakthroughs are needed to overcome physiological barriers and LNP toxicity for advancing clinical translation.

Keywords. mRNA cancer vaccines, Immunogenicity regulation, Lipid Nanoparticles (LNPs), Targeted delivery, Administration routes

1 Introduction

Globally in 2022, around 20 million new cancer cases and 9.7 million cancer-related deaths were reported. The lifetime cancer risk stands at roughly 1 in 5, with men facing a 1 in 9 lifetime risk of cancer death and women a 1 in 12. Lung cancer remains the most prevalent and top fatal cancer worldwide[1]. Cancer treatment depends on the stage of the disease and includes surgery, chemotherapy, radiotherapy, and/or targeted therapy. For example, in lung cancer, the management of non-small cell lung cancer (NSCLC) is critically determined by its stage. In early-stage (I-II) disease, the front-line approach typically involves surgical resection integrated with adjuvant therapy. In contrast, for advanced-stage (III-IV) NSCLC, the therapeutic paradigm shifts, with chemotherapy and/or radiotherapy constituting the cornerstone of treatment. Nevertheless, the efficacy of conventional chemotherapy is universally hampered by a

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constellation of inherent drawbacks, including poor cellular specificity, suboptimal bioavailability, considerable off-target toxicity, and the eventual emergence of drug resistance[2]. Over the past few decades, a paradigm shift in cancer therapy has been driven by immunological breakthroughs that enable the precise targeting of tumor cells by the immune system. This has positioned immunotherapies, including a new generation of cancer vaccines (e.g., immune cell, viral vector, and RNA/DNA platforms), at the forefront of oncology. mRNA vaccines, in particular, have demonstrated a compelling profile of unique advantages over other vaccine technologies[3].

mRNA vaccines are a new type of vaccine that deliver mRNA sequences encoding protein antigens into the body. These mRNA sequences guide the body to express the corresponding proteins and induce an immune response against these proteins, thereby achieving disease treatment and prevention. mRNA vaccines show significant advantages in cancer treatment, including high safety, rapid antigen translation, ability to overcome vaccine resistance, activation of broad immune responses, and no risk of mutation. These advantages make them a research hotspot in cancer immunotherapy[4]. However, mRNA vaccines have limitations in targeting, such as limited accuracy, suboptimal organ specificity, and poor cell type specificity—issues that affect their efficacy in cancer treatment and may cause adverse reactions. Resolving these problems is crucial for improving the performance of mRNA vaccines and expanding their application scope. Targeting is the key to enhancing the efficacy and safety of mRNA vaccines. Current research mainly focuses on optimizing delivery systems, modifying structures, and developing combination strategies to achieve precise targeting of tumor tissues. Studies on the targeting of mRNA vaccines revolve around LNP carrier optimization, administration route design, and tumor microenvironment (TME) responsiveness. Through chemical modification, high-throughput screening, and clinical translation, mRNA vaccines are gradually advancing from organ targeting to precise cell type targeting[5]. This review outlines the fundamental principles of mRNA cancer vaccines, examining strategies to modulate the immunogenicity of in vitro-transcribed (IVT) mRNA — through nucleotide modification, optimized purification, and advanced delivery systems — to balance efficacy and toxicity. A significant focus is placed on the development of lipid nanoparticles (LNPs), encompassing the rational design of components, internal ratio adjustment, surface engineering, and administration route optimization, with the goal of providing a comprehensive overview and accelerating their clinical translation.

To elaborate on these core aspects, we first focus on the biological basis of mRNA cancer vaccines, including their functional mechanisms and the dual role of IVT mRNA immunogenicity.

2 mRNA-Based Cancer Vaccines

Functioning as a crucial intermediary in the central dogma, mRNA conveys genetic instructions from DNA to ribosomes for protein synthesis. In vaccinology, two primary RNA formats are explored: non-replicating mRNA and self-amplifying RNA. Both are commonly synthesized via in vitro transcription (IVT), a cell-free method that employs bacteriophage polymerases (e.g., T3, T7, SP6) and linearized DNA templates encoding the antigen. This platform circumvents the need for complex cellular systems, offering a more streamlined, rapid, and pure production process compared to traditional recombinant protein methods[6].

The immunogenicity of IVT mRNA stems from its identification by the innate immune system as exogenous. Key to this are pathogen-associated molecular patterns (PAMPs) present in IVT mRNA, such as dsRNA impurities and 5' triphosphate groups. These PAMPs engage specific pattern recognition receptors (PRRs). For instance, endosomal TLR3 binds dsRNA, and TLR7/8 binds single-stranded RNA (ssRNA). Cytosolic PRRs also play a role: RIG-I recognizes short, 5'-triphosphorylated dsRNA; MDA5 recognizes long dsRNA; NOD2 senses ssRNA; and PKR binds to dsRNA. These recognition events activate downstream signaling pathways, inducing the release of type I interferons (IFN-α/β), interleukin-6 (IL-6), and other inflammatory factors. Moderate activation can promote the maturation of antigen-presenting cells (APCs) and initiate adaptive immunity; however, excessive

activation inhibits mRNA translation via the IFN pathway—for example, IFIT proteins bind to uncapped mRNA, PKR phosphorylates translation initiation factors, and the OAS/RNase L system degrades mRNA. Ultimately, this reduces translation efficiency and mRNA stability, impairing vaccine efficacy. Additionally, the degree of immune activation is related to mRNA sequences (e.g., uridine content), modifications (e.g., nucleoside modification), and purification levels[7].

Nevertheless, IVT mRNA's immunogenicity acts as a dual-purpose agent. Its beneficial role lies in driving protective immunity: innate activation and type I interferons (e.g., IFN-α) release cause dendritic cell (DCs) maturation, boosting antigen presentation and priming a potent adaptive response. This results in robust antigen-specific T cell immunity, unleashing effective anti-tumor and anti-infective vaccine actions. This inherent immune-stimulating property eliminates the need for additional adjuvants, simplifying vaccine design, and can simultaneously activate innate and adaptive immunity to form a synergistic defense mechanism.

On the other hand, excessive immune activation poses potential risks: excessive release of type I interferons and pro-inflammatory factors may trigger systemic inflammatory responses (e.g., fever, flu-like symptoms) and even immune-related toxicity; some individuals may experience tolerance issues due to overactive immune responses, limiting dose usage. Furthermore, mRNA modifications (e.g., uridine replacement) and delivery systems can affect immunogenicity, requiring a balance between stimulation intensity and safety—which increases the complexity of vaccine optimization[8].

Strategies for regulating the immunogenicity of IVT mRNA need to balance its immune-stimulating effects and potential toxicity, and are mainly implemented from multiple dimensions, including mRNA modification, purification processes, and delivery system optimization.

2.1 Nucleoside-Modified mRNA

Employing modified nucleosides (e.g., substituting uridine with m1ψ or ψ) suppresses the recognition of IVT mRNA by innate immune sensors like TLR7/8 and MDA5, thereby reducing type I interferon responses. Research demonstrates that mRNA modified with N^1-methylpseudouridine (m1Ψ), alone or combined with 5-methylcytidine (m5C), outperforms pseudouridine (Ψ)-based platforms, boosting reporter gene expression by ~13-fold and up to 44-fold, respectively, with longer persistence in various models. These optimized mRNAs also show lower cytotoxicity, reduced cytokine secretion (e.g., IFN-β, CCL5), and diminished immunogenicity, potentially through enhanced evasion of endosomal TLR3 and its downstream signaling[9]. For example, Pardi et al. reported that after intradermal injection, m1Ψ-modified mRNA encapsulated in lipid nanoparticles (m1Ψ-mRNA-LNPs) showed approximately 20-fold higher protein expression than unmodified mRNA (U-mRNA-LNPs), with longer expression duration (up to 13 days). The kinetics of expression were comparable to those of live virus delivery systems, providing sufficient antigen stimulation for immune responses[10].

2.2 Optimization of Untranslated Regions

The optimization of messenger RNA's (mRNA) untranslated regions (UTRs) is a pivotal strategy to enhance its stability and translational efficiency by modulating interactions with RNA-binding proteins. Key principles for 5'UTR engineering involve employing shorter sequences, eliminating upstream start codons (AUG/CUG) to prevent aberrant translation initiation, minimizing stable secondary structures that hinder ribosomal recruitment and scanning, and utilizing bioinformatic tools for efficiency prediction. For 3'UTR, sequences with translation and stability regulatory elements are typically chosen—e.g., α-globin and β-globin 3'UTRs from Xenopus laevis or humans—and AU-rich/GU-rich sequences can be added to further boost RNA stability[11]. Additionally, tandem addition of two 3'UTR sequences may enhance transcription efficiency[12]. It should be noted that the performance of UTRs is influenced by species, cell type, and cell state; thus, their design should consider the pharmacological characteristics of target cells.

2.3 5' Cap Modification

5' cap modification is a key strategy for overcoming the inherent immunogenicity of mRNA. Its core principle is to optimize the 5' end structure of mRNA to avoid recognition by pattern recognition receptors (PRRs), while enhancing translation efficiency and reducing innate immune activation. The 5′ cap, a defining feature at the 5′ terminus of eukaryotic mRNA, is characterized by a 7-methylguanosine (m7G) linked via a 5′-5′ triphosphate bridge (the Cap 0 structure). It facilitates ribosome binding and initiates translation by being recognized by the eukaryotic translation initiation factor 4E (eIF4E). Uncapped mRNA (e.g., with 5'ppp or 5'pp) or improperly modified mRNA is recognized as exogenous material by PRRs (e.g., TLR7/8, RIG-I, IFIT), activating innate immune pathways and leading to the release of type I interferons and pro-inflammatory factors—conversely inhibiting antigen expression and accelerating mRNA degradation.

To address this issue, 5' cap modification strategies include: 1. Using technologies such as CleanCap™ to generate natural Cap 1 structures (adding 2'-O-methylation to the Cap 0 structure) to reduce the probability of PRR recognition; 2. Using anti-reverse cap analogs (ARCA) to avoid reverse integration of the cap structure, reducing the production of unmodified mRNA; 3. Ensuring 100% capping efficiency through enzymatic reactions and removing free phosphate groups (via phosphatase treatment) to prevent PRR activation.

These modifications not only protect mRNA from RNase degradation but also enhance translation efficiency by mimicking the structure of natural mRNA. Thus, while reducing inherent immunogenicity, they ensure effective antigen expression—laying the foundation for in vivo delivery of mRNA vaccines and activation of immune responses[4].

2.4 Codon Optimization of the Open Reading Frame

The inherent immunogenicity can be overcome through codon optimization of the open reading frame (ORF). This strategy requires two main actions: reducing the recognition by pattern recognition receptors (PRRs) and improving translation efficiency. Firstly, it is crucial to reduce uridine (U) usage. The reason is that uridine-rich sequences are easily recognized by PRRs like RIG-I, an event that triggers the type I interferon pathway. Replacing uridine-containing synonymous codons can reduce the probability of PRR recognition, while increasing GC content to enhance mRNA stability. Second, avoid abnormal secondary structures: stable structures such as stem-loops are recognized by PRRs like MDA-5 as pathogen-associated molecular patterns. Selecting specific codons to adjust GC content and avoid complementary pairing can reduce the formation of such structures. Third, match the codon preference of target cells: using codons corresponding to highly abundant tRNAs improves translation efficiency, reduces ribosomal stalling, and prevents mRNA from exposing unfolded regions that activate PRRs. Fourth, balance translation rate: incorporating an appropriate number of low-frequency codons to slow translation rate ensures correct antigen folding and reduces immune responses triggered by abnormal fragments[2]. To achieve a balanced translation rate and high fidelity, the codon usage within the open reading frame (ORF) must be precisely tuned.

2.5 Poly(A) Tail Modification

Overcoming inherent immunogenicity via poly(A) tail modification requires addressing three aspects: length regulation, structural design, and functional synergy. First, optimize length to reduce abnormal activation: a 120-unit poly(A) tail enhances mRNA stability and translation efficiency in dendritic cells, avoiding the formation of abnormal structures (recognized by PRRs) due to excessively long tails; in primary T cells, although a poly(A) tail longer than 300 nucleotides is required, it can be processed into a stable 30-unit form via a "trimming model," reducing the risk of recognition by cytoplasmic PRRs (e.g., RIG-I). Second, introduce double-stranded structures to achieve controlled immune stimulation: adding poly(U) to form double strands with poly(A) can specifically activate TLR-3 and RIG-I, moderately inducing cytokines to promote APC maturation while avoiding excessive activation of MDA-5—balancing adjuvant effects and translation efficiency. Additionally, the poly(A) tail binds to poly(A)-binding protein (PABP) to form a closed-loop structure, which protects mRNA by interacting with the

5' cap, reducing nuclease degradation and PRR recognition—indirectly lowering immunogenicity and improving translation persistence[2]. Evidence suggests that optimizing poly(A) tail length is crucial for efficient IVT-mRNA antigen expression, as shorter tails enhance translation by promoting a closed-loop conformation[13]. Future work should further elucidate the dynamics of this relationship.

2.6 Purification of In Vitro Transcribed mRNA

During in vitro transcription (IVT), bacteriophage polymerases often generate byproducts such as short RNA fragments and double-stranded RNA (dsRNA). These impurities are potent activators of intracellular pattern recognition receptors (PRRs) like PKR, MDA-5, and OAS, which subsequently suppress mRNA translation and provoke innate immune responses. To address this, Kariko et al. demonstrated that HPLC purification employing an alkylated non-porous polystyrene-divinylbenzene matrix effectively removes these contaminants. The purified, nucleoside-modified mRNA exhibited a complete abrogation of immune activation in dendritic cells, with no induction of IFN-α, TNF-α, or immune-related genes. Furthermore, this method markedly enhanced translational efficiency, yielding 2–20-fold and 10–1000-fold increases in 293T cells and primary cells, respectively, without eliciting toxicity upon repeated administration[14]. However, owing to the drawbacks of HPLC purification—namely high cost and low yield—alternative methods have been explored. One such method, reported by Baiersdorfer et al., utilizes cellulose to selectively capture dsRNA impurities in an ethanol-containing buffer. This method can remove at least 90% of dsRNA with an mRNA recovery rate exceeding 65%, and is applicable to mRNA of different lengths, sequences, and nucleoside compositions. It can be scaled up from microgram to milligram levels, achieving effects comparable to HPLC but with greater simplicity and no toxicity. In vivo experiments showed that purified mRNA had improved translation efficiency, and 1-methylpseudouridine-modified mRNA no longer induced interferon-α—providing a reliable solution for in vivo applications of IVT mRNA[15].

Having discussed strategies to optimize the mRNA molecule itself, we now turn to the critical role of delivery systems in ensuring its effective transport and function in vivo.

3 Ionizable Lipid Nanoparticle-Based mRNA Delivery Systems

The inherent instability and poor cellular uptake of naked mRNA, attributable to its large size, negative charge, and susceptibility to degradation, preclude its direct therapeutic application. To overcome these limitations, researchers have designed delivery systems. An ideal mRNA delivery platform must efficiently encapsulate and protect the nucleic acid, facilitate cellular uptake, and mediate endosomal escape. Lipid nanoparticles (LNPs) have emerged as the leading non-viral platform fulfilling these criteria, a status validated by the clinical success of the COVID-19 vaccines BNT162b2 (Comirnaty®) and mRNA-1273 (Spikevax®)[16]. This review will focus on LNP delivery systems for mRNA.

The canonical architecture of lipid nanoparticles (LNPs) comprises four key constituents: ionizable cationic lipids, phospholipids, cholesterol, and PEGylated lipids (Figure 1), each contributing critically to the system's stability, transfection efficiency, and safety. Consequently, the pursuit of enhanced mRNA vaccine targeting and broader clinical utility focuses on four principal development avenues: the design of novel lipid molecules, optimization of internal lipid ratios, surface engineering, and the strategic selection of administration routes.

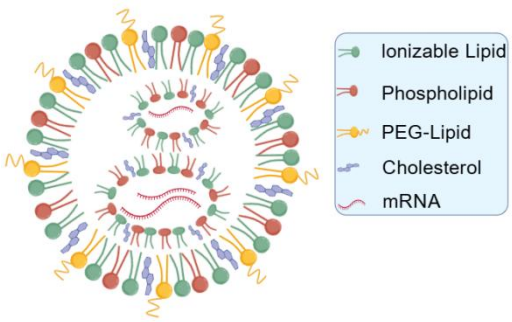

Fig. 1. Composition of Lipid Nanoparticles. Lipid nanoparticles consist of four components: ionizable cationic lipids, phospholipids, cholesterol, and PEGylated lipids. Their core function is to encapsulate mRNA to protect it from degradation and assist its entry into target cells. The formation

of their structure is related to the ratio of lipid components and the ratio of mRNA to lipids; different ratios may form different structures, thereby affecting mRNA release efficiency and cellular uptake.

3.1 The Design of Novel Lipid Molecules

3.1.1 Ionizable Cationic Lipids

Ionizable cationic lipids are the core of RNA delivery, with charge states that change with pH. They form nanoparticles with negatively charged RNA to prevent degradation; their low charge at physiological pH facilitates cellular uptake; in the acidic endosomal environment, they become protonated due to an appropriate pKa (6–7), triggering endosomal escape and RNA release. Regulating pKa can balance efficiency and toxicity, adapting to different RNA delivery needs.Ionizable lipid-based LNPs exploit pH-dependent properties: they encapsulate nucleic acids efficiently at low pH while maintaining a neutral charge for reduced toxicity during systemic circulation. Upon endocytosis, the acidic lysosomal environment reprotonates them, triggering endosomal escape and cytoplasmic release of the cargo. Empirical evidence indicates that this process is optimized when the LNP pKa matches the target tissue, with values of 6.2–6.5 favoring hepatic siRNA delivery and 6.6–6.9 enhancing intramuscular mRNA vaccination[17].

High-throughput screening (HTS) is commonly used to screen the optimal ionizable lipids from hundreds of candidates in vitro and in vivo. Dahlman et al. exemplified the power of HTS by designing 7C1 nanoparticles from low-molecular-weight polyamines and lipids. This LNP variant demonstrates a marked ability to deliver siRNA to endothelial cells in multiple organs, including the lungs, thereby overcoming the typical hepatic tropism[18]. This method is also applicable to the screening of ionizable cationic lipids for mRNA vaccines. For example, Li et al. synthesized 144 lipids using CALB enzyme, screened out AA3-DLin, optimized the LNP formulation, and achieved higher efficiency than FDA-approved counterparts with easier storage—providing a high-efficiency and green platform for mRNA delivery[19].

The rational design of LNPs can be advanced not only by HTS but also by studying the structure-activity relationship (SAR) of ionizable cationic lipids and by employing targeting moiety conjugation strategies. Specific lipid structures can achieve liver and lung targeting; researchers developed N-series lipid nanoparticles, which can selectively deliver mRNA to the lungs of mice (while O-series targets the liver), and differences in their surface protein corona may be the key factor. Adjusting the head structure of N-series lipids can target different lung cells. In a model of pulmonary lymphangioleiomyomatosis, delivery of Tsc2 mRNA via these nanoparticles reduced tumor burden—providing a new strategy for mRNA therapy of pulmonary diseases. This demonstrates that in-depth study of structure-activity relationships is expected to accelerate the development of functional ionizable lipids.

The high cationic charge of ionizable cationic lipids is the core cause of toxicity during mRNA delivery, mainly inducing cytotoxicity and inflammatory responses by disrupting membrane structures, interfering with organelle functions, and activating immune pathways. Optimization strategies need to balance charge properties (e.g., adjusting pKa to 6.2–7 to reduce charge at physiological pH) and degradability (e.g., using enzyme-degradable ester bonds and linear tails) to reduce toxicity risks[5].

In summary, the choice of ionizable lipid fundamentally shapes the biodistribution and delivery efficiency of LNPs in vivo. Thus, discovering optimal ionizable lipids for specific applications, such as improving anti-tumor mRNA therapies, remains a key goal for ongoing research, building upon the considerable optimization progress to date.

3.1.2 Phospholipids

Phospholipids are one of the core components of lipid nanoparticles (LNPs); they can bind to mRNA through hydrophobic interactions and electrostatic interactions, encapsulating it to form stable nanoparticle structures. This encapsulation protects mRNA from nuclease degradation, maintaining its integrity during in vivo transport and laying the foundation for subsequent cellular entry and function. Traditional phospholipids

(e.g., DSPC, DOPE) have limitations such as lack of pH responsiveness, poor structural flexibility, limited selectivity, and insufficient efficacy—failing to meet the requirements of modern nucleic acid therapies for efficient and precise delivery systems. Liu et al. synthesized 572 multi-tailed ionizable phospholipids (iPhos), which contain pH-responsive zwitterionic heads and three hydrophobic tails. Under the acidic conditions of endosomes, they adopt a conical shape, promoting membranous hexagonal phase transition to facilitate cargo release. Structure-activity relationship studies showed that their structure regulates in vivo efficacy and organ selectivity. LNPs based on iPhos can achieve targeted delivery to the liver, spleen, and lungs, enabling selective mRNA delivery and CRISPR-Cas gene editing. The most effective 9A1P9 exhibited 40–965-fold higher efficacy than traditional phospholipids, with low toxicity and suitability for repeated administration—providing a powerful tool for gene therapy[20]. Systematic in vitro screening by Benedicto et al. identified distinct roles for phospholipids in LNP function. Phosphoethanolamine (PE) headgroups enhanced endosomal escape and mRNA delivery efficiency through their fusogenic properties, while the phospholipid charge dictated organ tropism: zwitterionic types favored liver delivery, whereas negatively charged phospholipids redirected targeting to the spleen[21]. These examples illustrate the important role of phospholipids in mRNA-LNP therapy.

3.1.3 Cholesterol

The structure of cholesterol is a critical determinant of LNP performance in vivo. Paunovska et al. systematically evaluated this by screening 141 LNP formulations with six cholesterol variants. Their analysis of extensive in vivo data revealed that esterified cholesterol significantly boosts nucleic acid delivery efficiency. A standout candidate, cholesterol oleate, enabled a threefold higher delivery efficiency to liver endothelial cells over hepatocytes and effectively delivered both siRNA and sgRNA, underscoring the rational design of cholesterol variants as a viable strategy for optimizing LNP targeting[22].

Patel et al. screened a variety of natural cholesterol analogs and found that enhanced lipid nanoparticles (eLNPs) containing C-24 alkyl phytosterols improved mRNA delivery efficiency. eLNPs have a polyhedral shape; compared with traditional spherical LNPs, they exhibit stronger cellular uptake and retention, and more effective endosomal escape. They are applicable to various cell types and nucleic acid types—providing a basis for optimizing RNA delivery systems[23]. Patel et al. also constructed an LNP library containing 6 hydroxycholesterols and found that replacing 25% or 50% of cholesterol with 7α-hydroxycholesterol increased delivery efficiency by 1.8–2.0-fold. The mechanism was related to increased late endosomes and reduced recycling endosomes—providing a new platform for T cell immunotherapy[24].

3.1.4 PEGylated Lipids

Adding PEGylated lipids can prolong in vivo circulation, maintain stability, assist in drug encapsulation, and enhance tumor targeting. Mui et al. studied the effect of desorption rates of PEG-lipids with different carbon chain lengths (14, 16, 18 carbons) on siRNA-loaded lipid nanoparticles (LNPs). The results showed that the desorption rate of PEG-lipids increased with decreasing carbon chain length. 1.5 mol% was the threshold concentration: below this concentration, carbon chain length had little effect on liver gene silencing but altered the pharmacokinetics and distribution of LNPs; when the concentration increased to 2.5 and 3.5 mol%, liver gene silencing by 18-carbon chain PEG-lipids was significantly impaired, while the 14-carbon chain was less affected. Therefore, the delivery efficiency of LNPs can be optimized by adjusting the chain length and concentration of PEG-lipids, and short chains can reduce negative impacts on efficacy[25].

The induction of anti-PEG antibodies by PEG can trigger the accelerated blood clearance (ABC) phenomenon, thereby leading to a shortened circulation time of drugs; anti-PEG IgE can cause type I hypersensitivity reactions such as anaphylactic shock; PEG can also activate the complement system, inducing pseudo-allergic reactions (e.g., cardiopulmonary abnormalities); furthermore, anti-PEG antibodies may cross-react with other polymers containing C-C-O structures[26]. To address these issues, researchers have

developed various PEG alternatives, such as polyoxazolines (e.g., poly(2-ethyl-2-oxazoline) (PEOz), which has high tunability and low immunogenicity, with some entering commercial development), polyvinylpyrrolidone (PVP, which has a long safety record and can inhibit complement activation), and zwitterionic polymers (which have strong anti-protein adsorption capacity and low immunogenicity)[27]. These alternatives have respective advantages in biodegradability and immune safety, but further studies are needed to verify their long-term safety and stability.

3.2 Optimization of Internal Lipid Ratios

Selecting appropriate components and balancing their internal ratios is crucial for LNP-mediated mRNA delivery. Cheng et al. systematically optimized dendrimer-based lipid nanoparticles (DLNPs) of 5A2-SC8 for the treatment of type I hepatorenal tyrosinemia (HT-1). Initial screening showed that reducing the proportion of the cationic lipid 5A2-SC8 and increasing the proportion of the zwitterionic phospholipid DOPE was beneficial for mRNA delivery. Further optimization revealed that DMG-PEG was crucial for carrier stability—without it, activity decreased. The optimal formulation C10 (i.e., mDLNPs) was finally determined, with a 5A2-SC8/mRNA weight ratio of 20/1, a DMG-PEG molar ratio of 4.76%, a diameter of approximately 100 nm, and good stability. The optimized mDLNPs could transfect >44% of hepatocytes, and an effective dose of 0.05 mg/kg normalized liver function indicators in FAH-knockout mice and prolonged their survival[28]. This method provides a rational strategy for optimizing existing LNPs to achieve liver-targeted mRNA delivery.

In addition to liver targeting, optimizing LNP components can also achieve mRNA delivery to non-hepatic tissues. To overcome the limitation of lipid nanoparticles (LNPs) targeting only the liver, Cheng et al. developed a selective organ targeting (SORT) strategy. In this process, SORT molecules are added to traditional LNPs, and their targeting is altered by adjusting the ratio and biophysical properties (e.g., charge) of SORT molecules: increasing the proportion of cationic molecules (e.g., DOTAP) redirects targeting from the liver to the spleen and lungs; anionic molecules (e.g., 18PA) achieve spleen targeting; ionizable molecules (e.g., DODAP) enhance liver targeting[29]. The SORT technology constitutes a versatile platform, offering a broadly applicable strategy for achieving tissue-specific delivery across diverse LNP systems. Cui et al. constructed lung-targeted LNPs via SORT technology to encapsulate CRISPR-Cas13d, targeting the mRNA of the lung protease Ctsl for blocking SARS-CoV-2 infection. This system could efficiently, specifically, and safely reduce Ctsl expression in mouse lungs, prolong the survival of mice with lethal infections, and reduce pulmonary viral load, pro-inflammatory factors, and pulmonary interstitial inflammation[30]. This universal targeting strategy opens new avenues for research on mRNA-LNP therapy for extrahepatic tissues. Researchers found that the chemical properties of SORT molecules regulate the biodistribution, apparent pKa, and serum protein interactions of nanoparticles. The targeting mechanism involves three steps: PEG lipid desorption exposes SORT molecules, specific serum proteins bind to form a protein corona, and these proteins then interact with receptors in target tissues. Experiments showed that liver targeting depends on serum apolipoprotein E (ApoE), spleen targeting depends on β2-glycoprotein I (β2-GPI), and lung targeting depends on vitronectin (Vtn)—with the latter two independent of ApoE. This study provides a new paradigm for overcoming liver accumulation limitations and designing delivery systems for non-hepatic gene drugs. Challenging conventional LNP design, Su et al. demonstrated that cholesterol and phospholipids are not essential components. By constructing a library of degradable core ionizable lipids (nAcx-Cm) and optimizing the formulation, they found that omitting cholesterol prevented hepatic accumulation. Superior lung targeting was achieved with a streamlined, three-component LNP formulation (comprising an ionizable lipid, a permanent cationic lipid, and a PEG-lipid). This advancement paves the way for novel strategies in precise mRNA therapy[31].

3.3 Surface Engineering

Surface modification of lipid nanoparticles (LNPs) refers to the functional modification of LNP surfaces via

chemical, physical, or other means to optimize their performance. In particular, conjugating antibodies or other molecules to LNP surfaces can improve their targeting ability.

Achieving lung-selective delivery remains a significant hurdle for treating related diseases. To this end, specific lung cell surface receptors have been exploited for targeted delivery. Parhiz et al. developed an anti-PECAM-1 antibody-conjugated LNP system for nucleoside-modified mRNA. Following intravenous injection, this strategy suppressed hepatic uptake by over 200-fold and increased pulmonary protein production by approximately 25-fold. This ApoE-independent mechanism produced rapid, transient expression within 4–24 hours, presenting a promising strategy for extrahepatic mRNA therapy[32]. Li et al. covalently bound αPV1 antibodies to LNP surfaces to target PV1 protein in caveolae of lung endothelial cells. Results showed that compared with control LNPs, PV1-targeted LNPs significantly increased mRNA delivery to the lungs, with a 40-fold increase in protein expression. Targeted LNPs with a size of 160 nm exhibited stronger mRNA expression in the lungs due to their combined elasticity and targeting ability, and the optimal mRNA:lipid weight ratio was 3. This achievement provides an effective platform for RNA therapy of pulmonary diseases[33]. Collectively, these findings underscore the considerable utility of antibody-conjugated LNPs for targeted mRNA delivery.

In addition to antibodies, peptides—owing to their considerably lower molecular weights—serve as another ideal class of ligands for LNP modification, capable of imparting specific targeting properties. Herrera-Barrera et al. developed such peptide-guided LNPs for mRNA delivery to the neural retina. They identified the MH42 peptide from an M13 phage display library, which, when conjugated to LNPs, facilitated mRNA delivery to photoreceptors, retinal pigment epithelium (RPE), and Müller glial cells in mice. This efficacy was further validated in non-human primates, demonstrating robust protein expression and highlighting a breakthrough that expands the scope of LNP-mRNA therapy for hereditary retinal degeneration[34].

DNA modification can also be used to optimize LNPs. The work of Andrew et al. on LNP-SNAs presents a platform for cytoplasmic nucleic acid delivery whose efficacy is governed by LNP core components and surface DNA design. Optimization, guided by cholesterol content and G-rich sequences, yielded a 100-fold improvement in siRNA silencing potency over liposomal SNAs. A key feature of this platform is its ability to alter biodistribution, inducing a marked shift from liver to spleen expression for mRNA, thereby offering a novel design strategy for targeted gene drugs[35].

3.4 The Strategic Selection of Administration Routes

The pharmacokinetic profile and resultant efficacy of an mRNA cancer vaccine is a function of its administration route. Options such as intravenous, intradermal, subcutaneous, intramuscular, intranodal, intratumoral, and nebulized administration are available, each defined by a unique set of characteristics affecting delivery and outcome (Table 1)[36].

Selecting an appropriate administration route can accelerate the development of targeted mRNA-LNPs. The optimal administration method should be determined based on the anatomical structure and function of the target tissue. Intravenous injection (i.v.) is the best choice for liver targeting, as the liver is one of the organs with the richest systemic blood flow—after intravenous injection, drugs can directly enter the systemic circulation and preferentially flow through the liver, increasing the chance of contact with liver cells. Liver sinusoidal endothelial cells have a large number of fenestrae (50–100 nm in diameter), allowing nanoparticles to rapidly extravasate into the liver parenchyma via passive diffusion—an effect difficult to achieve with the continuous endothelial barriers of other organs (e.g., lungs, brain). Liver cells (e.g., hepatocytes) highly express receptors such as LDLR; nanoparticles administered intravenously can form a protein corona by adsorbing ApoE or be modified with GalNAc ligands to specifically bind to liver cell membrane receptors, efficiently initiating endocytosis. Intramuscular injection (i.m.) is an important administration route for mRNA vaccines, but its efficacy is highly dependent on delivery systems. Intramuscular injection of LNP/mRNA

vaccines recruits neutrophils, monocytes, and DCs, activates the type I interferon pathway, promotes monocytes and DCs to take up and translate mRNA, and ultimately initiates a potent and sustained specific immune response in draining lymph nodes. Compared with intramuscular injection (a common route for COVID-19 vaccines), subcutaneous injection (s.c.) is more conducive to LNP accumulation in draining lymph nodes, reducing non-targeted distribution in the liver and thus lowering liver side effects (e.g., reversible liver injury), while enhancing local immune activation efficiency. The novel 113-O12B mRNA-LNP cancer vaccine platform, demonstrated a superior biodistribution profile following subcutaneous administration. Specifically, it achieved significantly enhanced mRNA expression in the lymph nodes compared to the conventional ALC-0315 formulation, while its expression in the liver was markedly reduced. This resulted in a favorable liver-to-lymph node expression ratio of 32% for 113-O12B, which is substantially lower than the 400% ratio observed with ALC-0315. The improved targeting efficiency facilitated higher transfection rates of key antigen-presenting cells (APCs) within the lymphoid tissues, which correlated with a more potent anti-tumor immune response.

Nebulized administration can directly deliver therapeutic mRNA to the lungs, enabling efficient function while reducing systemic exposure and side effects. It can activate respiratory immune responses, with strong targeting for pulmonary diseases. The operation is performed via a nebulizer, making it suitable for delivering drugs such as mRNA, and has shown significant efficacy in the prevention and treatment of respiratory infectious diseases such as influenza. Lokugamage et al. optimized lipid nanoparticles (LNPs) for nebulized delivery of mRNA to the lungs. Through cluster screening, they found that the proportion of PEG was related to the charge of helper lipids: neutral lipids required low PEG, while cationic lipids required high PEG. The optimized NLD1 exhibited higher efficiency than traditional LNPs and could deliver anti-influenza antibody mRNA to protect mice from lethal H1N1 challenge[37]. Beyond nebulization, intratracheal instillation provides another direct route for pulmonary mRNA-LNP delivery. This was exemplified by Massaro et al., who administered Cy5-Luc mRNA-LNPs intratracheally to mice with bleomycin-induced pulmonary fibrosis. The treatment resulted in lung-specific accumulation, with fluorescence detected within 2 hours, bioluminescence peaking at 24 hours, and signal persisting for 48 hours. Importantly, the LNPs localized specifically with alveolar epithelial cells and fibroblasts, demonstrating the feasibility of highly targeted local delivery via this route[38]. The administration routes can be reasonably selected based on the application scenario of mRNA-LNPs.

Table 1. Comparison of Administration Routes for mRNA Cancer Vaccines.

Administration Route	**Characteristics and Advantages**	**Limitations**
Intravenous	Large-volume vaccines can be injected, directly reaching lymphoid organs; conducive to immune cell recruitment	High risk of systemic toxicity
Intradermal	The dermis is rich in antigen-presenting cells (DCs, macrophages), blood vessels, and lymphatic vessels; high immune activation efficiency	Small injection volume (limited by dense connective tissue); may cause local adverse reactions (e.g., swelling, erythema)
Subcutaneous	Larger injection volume (due to loose adipose tissue); fewer local side effects (e.g., pain)	Fewer antigen-presenting cells than the dermis
Intramuscular	Muscles have dense blood vessels, conducive to immune cell recruitment; moderate injection volume; mild local side effects	High dependence on delivery systems; uncertain immune responses
Intranodal	Lymph nodes are rich in antigen-presenting cells, ensuring high delivery efficiency;	Complex operation

	small vaccine volume required	
Intratumoral	Directly acts on the tumor microenvironment, suitable for vaccines containing immunomodulatory molecules; small volume required	Complex operation; mainly used for adjuvant immunotherapy

Building upon the optimization of LNP components and administration, the next critical step is applying these strategies to overcome the inherent hepatic tropism of conventional LNPs and achieve effective delivery to other tissues.

4 Mechanisms, Strategies, and Challenges of Non-Hepatic Targeting

4.1 Core Mechanisms and Key Strategies of Non-Hepatic Targeting

Traditional lipid nanoparticles (LNPs) exhibit a natural propensity for liver targeting, a phenomenon driven by the organ's extensive blood supply, the 50–100 nm fenestrations in sinusoidal endothelial cells, and the high expression of LDLR on hepatocytes. In contrast, extending mRNA therapy to extrahepatic tissues like the lungs, spleen, and retina requires deliberate engineering strategies. This shift necessitates four core approaches—the design of novel lipid molecules, optimization of internal lipid ratios, surface engineering, and the strategic selection of administration routes—which collectively modulate LNP-biological environment interactions to overcome inherent hepatic tropism, as detailed in Table 2.

Table 2. Mechanisms and Strategies of Non-Hepatic Targeting.

Core Approach	Mechanism of Non-Hepatic Targeting	Key Strategies	Target Organ/Effect
The design of novel lipid molecules	Design pH-responsive ionizable lipids; reduce positive charge at physiological pH through pKa regulation to decrease non-specific hepatic adsorption; Optimize the hydrophobic tail/head structure of lipids to enhance interactions with non-hepatic cell membranes; Develop organ-selective lipids to alter biodistribution tendencies and avoid hepatic accumulation.	High-throughput screening (HTS) of ionizable cationic lipids to obtain lipids with non-hepatic targeting properties; Design and synthesize organ-selective lipid series (e.g., N-series, Pi-Lipids); Develop multi-tailed ionizable phospholipids (iPhos); Screen cholesterol analogs (e.g., phytosterols, hydroxycholesterols) to optimize LNPs.	Lungs: Specific ionizable lipids (e.g., 7C1, N-series) achieve lung targeting; Spleen/lungs: Pi-Lipids target splenic immune cells; iPhos (e.g., 9A1P9) achieve spleen and lung targeting; Spleen/lungs: Cholesterol analog-modified LNPs enhance delivery efficiency to the spleen and lungs.
Optimization of internal lipid ratios	Alter lipid ratios to regulate LNP charge, reducing ApoE adsorption (avoiding the ApoE-LDLR pathway for liver targeting); Optimize the ratio of PEGylated lipids and cholesterol content to adjust protein corona composition, guiding binding to non-hepatic organs; Balance component ratios to adapt to the penetration requirements of non-hepatic tissue barriers (e.g., pulmonary air-blood barrier).	Adopt the selective organ targeting (SORT) strategy; add SORT molecules and adjust their ratio to alter targeting; Construct cholesterol-free three-component LNPs to optimize lung targeting effects; Adjust phospholipid type (e.g., PE headgroup phospholipids, negatively charged phospholipids) and ratio to optimize targeting and delivery	Spleen/lungs: SORT technology enables LNP targeting to the spleen and lungs, improving related therapeutic effects; Lungs: Three-component LNPs enhance lung targeting efficiency and reduce hepatic accumulation; Spleen: Negatively charged phospholipids guide LNP accumulation in the spleen; PE headgroup phospholipids enhance mRNA delivery efficiency.

		efficiency.	
Surface engineering	Conjugate specific molecules (antibodies/peptides/DNA) to bind to rare receptors on non-hepatic cell surfaces (active targeting, avoiding hepatocyte recognition); Modify molecules to optimize LNP surface hydrophilicity/hydrophobicity, reducing non-specific adsorption of plasma proteins; Peptide modification facilitates penetration of dense barriers in non-hepatic organs (e.g., the blood-retinal barrier in the retina).	Conjugate specific antibodies (e.g., anti-PECAM-1 antibodies, αPV1 antibodies) to modify LNPs; Screen targeting peptides (e.g., MH42 peptide) to modify LNPs; Modify surface DNA sequences to construct LNP-SNAs.	Lungs: Antibody-modified LNPs significantly improve mRNA delivery and protein expression efficiency in the lungs; Retina: Peptide-modified LNPs achieve targeted delivery to retinal cells; Spleen: LNP-SNAs enable main mRNA expression in the spleen, reducing siRNA usage concentration.
The strategic selection of administration routes	Avoid systemic blood circulation (reducing the probability of LNPs flowing through the liver) and directly deliver to local non-hepatic organs; Guide LNPs into the lymphatic circulation (instead of blood circulation) for accumulation in lymph nodes/spleen; Adapt to the anatomical structure of non-hepatic organs (e.g., pulmonary airways, vitreous cavity of the retina) to improve local uptake efficiency.	Adopt nebulized or intratracheal administration to directly deliver LNPs to the lungs; Adopt subcutaneous administration to guide LNPs into the lymphatic circulation for accumulation in lymph nodes; Adopt intravitreal or subretinal injection to directly deliver LNPs to the local retina.	Lungs: Nebulized/intratracheally administered LNPs are efficiently accumulated in the lungs, achieving related therapeutic effects; Lymph nodes/spleen: Subcutaneously administered LNPs show high expression in lymph nodes, improving APC transfection efficiency; Retina: Local injection combined with peptide modification achieves precise targeting of retinal cells.

Equipped with these core strategies to overcome hepatic tropism, it is crucial to recognize the major physiological barriers that still impede efficient non-hepatic delivery.

4.2 Main Challenges of Non-Hepatic Targeting

Physiological barriers of non-hepatic tissues and inherent properties of LNPs limit targeting efficiency. Typical challenges include:

• Barriers to Pulmonary Delivery

Mucociliary clearance: Ciliary beating on the surface of pulmonary mucosa rapidly clears foreign particles, resulting in short LNP retention time;

Phagocytosis by alveolar macrophages: Alveolar macrophages actively phagocytose LNPs, reducing their delivery efficiency to pulmonary parenchymal cells;

Mucus barrier: The high viscosity and negative charge of airway mucus hinder LNP penetration—an issue that is more severe in diseases such as cystic fibrosis.

• Barriers to Retinal Delivery

The blood-retinal barrier (BRB), which is primarily formed by retinal capillary endothelial cells and retinal pigment epithelial (RPE) cells, functions to tightly regulate molecular passage through tight junctions. Thus, the restrictive nature of this barrier, particularly against macromolecules and particles, presents a significant challenge for traditional lipid nanoparticles (LNPs) to reach critical photoreceptor cells essential for visual function;

Retinal immune microenvironment: The retina is an "immune-privileged site"; foreign LNPs may trigger local inflammation, affecting delivery safety.

• Challenges in Other Tissues

Spleen/lymphoid tissues: Prone to clearance by the reticuloendothelial system; non-specific phagocytosis by macrophages needs to be avoided;

Brain delivery: The highly selective blood-brain barrier (BBB) hinders LNP penetration; the complexity of brain cell types (e.g., neurons, astrocytes) makes it difficult to ensure targeting specificity[39].

5 Conclusion and Future Outlook

This article has reviewed strategic approaches—including novel lipid design, LNP compositional tuning, surface engineering, and route selection—to advance the extrahepatic delivery of mRNA-LNPs to tissues such as the lungs, retina, and brain, thereby overcoming limitations in efficiency and specificity. The translation of these strategies is, however, constrained by formidable physiological barriers including the pulmonary mucociliary system, blood-retinal barrier, and blood-brain barrier, necessitating further refinement of LNP physicochemical properties. Concurrently, the rational selection of administration routes remains critical for directing mRNA distribution and vaccine efficacy. Beyond targeting and delivery, considerations of LNP immunogenicity and toxicity must be addressed through biodegradable materials, dose optimization, and novel lipid substitutes. Resolving these multifaceted challenges is paramount to deepening our understanding and accelerating the successful clinical application of mRNA cancer vaccines.

Challenges in the LNP-mRNA delivery system mainly focus on targeting efficiency, safety, and production barriers; however, solutions to these challenges have shown promise in basic research and clinical trials. In the future, breakthroughs in targeted delivery technology (e.g., overcoming pulmonary mucus barriers or blood-retinal barriers) and the development of low-toxicity biodegradable LNPs will not only accelerate the clinical translation of mRNA cancer vaccines but also guide the design of next-generation personalized vaccines—enabling 'one drug per patient' precision therapy, breaking the limitations of existing therapies for rare diseases, and expanding their application to more cancer types and extrahepatic disease areas.

References

[1] Bray F, Laversanne M, Sung H, et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2024;74(3):229-263. doi:10.3322/caac.21834

[2] Wathoni N, Puluhulawa LE, Joni IM, et al. Monoclonal antibody as a targeting mediator for nanoparticle targeted delivery system for lung cancer. Drug Deliv. 2022;29(1):2959-2970. doi:10.1080/10717544.2022.2120566

[3] Duan LJ, Wang Q, Zhang C, Yang DX, Zhang XY. Potentialities and Challenges of mRNA Vaccine in Cancer Immunotherapy. Front Immunol. 2022;13:923647. Published 2022 May 26. doi:10.3389/fimmu.2022.923647

[4] Miao L, Zhang Y, Huang L. mRNA vaccine for cancer immunotherapy. Mol Cancer. 2021;20(1):41. Published 2021 Feb 25. doi:10.1186/s12943-021-01335-5

[5] Zong Y, Lin Y, Wei T, Cheng Q. Lipid Nanoparticle (LNP) Enables mRNA Delivery for Cancer Therapy. Adv Mater. 2023;35(51):e2303261. doi:10.1002/adma.202303261

[6] Pardi N, Hogan MJ, Porter FW, Weissman D. mRNA vaccines - a new era in vaccinology. Nat Rev Drug Discov. 2018;17(4):261-279. doi:10.1038/nrd.2017.243

[7] Linares-Fernández S, Lacroix C, Exposito JY, Verrier B. Tailoring mRNA Vaccine to Balance Innate/Adaptive Immune Response. Trends Mol Med. 2020;26(3):311-323.

[8] Kranz LM, Diken M, Haas H, et al. Systemic RNA delivery to dendritic cells exploits antiviral defence for cancer immunotherapy. Nature. 2016;534(7607):396-401. doi:10.1038/nature18300

[9] Andries O, Mc Cafferty S, De Smedt SC, Weiss R, Sanders NN, Kitada T. N(1)-methylpseudouridine-incorporated mRNA outperforms pseudouridine-incorporated mRNA by providing enhanced protein expression and reduced immunogenicity in mammalian cell lines and mice. J Control Release. 2015;217:337-344. doi:10.1016/j.jconrel.2015.08.051

[10] Pardi N, Hogan MJ, Naradikian MS, et al. Nucleoside-modified mRNA vaccines induce potent T follicular helper and germinal center B cell responses. J Exp Med. 2018;215(6):1571-1588. doi:10.1084/jem.20171450

[11] Weissman D. mRNA transcript therapy. Expert Rev Vaccines. 2015;14(2):265-281. doi:10.1586/14760584.2015.973859

[12] Orlandini von Niessen AG, Poleganov MA, Rechner C, et al. Improving mRNA-Based Therapeutic Gene Delivery by Expression-Augmenting 3' UTRs Identified by Cellular Library Screening. Mol Ther. 2019;27(4):824-836. doi:10.1016/j.ymthe.2018.12.011

[13] Lima SA, Chipman LB, Nicholson AL, et al. Short poly(A) tails are a conserved feature of highly expressed genes. Nat Struct Mol Biol. 2017;24(12):1057-1063. doi:10.1038/nsmb.3499

[14] Karikó K, Muramatsu H, Ludwig J, Weissman D. Generating the optimal mRNA for therapy: HPLC purification eliminates immune activation and improves translation of nucleoside-modified, protein-encoding mRNA. Nucleic Acids Res. 2011;39(21):e142. doi:10.1093/nar/gkr695

[15] Baiersdörfer M, Boros G, Muramatsu H, et al. A Facile Method for the Removal of dsRNA Contaminant from In Vitro-Transcribed mRNA. Mol Ther Nucleic Acids. 2019;15:26-35. doi:10.1016/j.omtn.2019.02.018

[16] Hald Albertsen C, Kulkarni JA, Witzigmann D, Lind M, Petersson K, Simonsen JB. The role of lipid components in lipid nanoparticles for vaccines and gene therapy. Adv Drug Deliv Rev. 2022;188:114416. doi:10.1016/j.addr.2022.114416

[17] Patel P, Ibrahim NM, Cheng K. The Importance of Apparent pKa in the Development of Nanoparticles Encapsulating siRNA and mRNA. Trends Pharmacol Sci. 2021;42(6):448-460. doi:10.1016/j.tips.2021.03.002

[18] Dahlman JE, Barnes C, Khan O, et al. In vivo endothelial siRNA delivery using polymeric nanoparticles with low molecular weight. Nat Nanotechnol. 2014;9(8):648-655. doi:10.1038/nnano.2014.84

[19] Li Z, Zhang XQ, Ho W, et al. Enzyme-Catalyzed One-Step Synthesis of Ionizable Cationic Lipids for Lipid Nanoparticle-Based mRNA COVID-19 Vaccines. ACS Nano. 2022;16(11):18936-18950. doi:10.1021/acsnano.2c07822

[20] Liu S, Cheng Q, Wei T, et al. Membrane-destabilizing ionizable phospholipids for organ-selective mRNA delivery and CRISPR-Cas gene editing. Nat Mater. 2021;20(5):701-710. doi:10.1038/s41563-020-00886-0

[21] Álvarez-Benedicto E, Farbiak L, Márquez Ramírez M, et al. Optimization of phospholipid chemistry for improved lipid nanoparticle (LNP) delivery of messenger RNA (mRNA). Biomater Sci. 2022;10(2):549-559. Published 2022 Jan 18. doi:10.1039/d1bm01454d

[22] Paunovska K, Gil CJ, Lokugamage MP, et al. Analyzing 2000 in Vivo Drug Delivery Data Points Reveals Cholesterol Structure Impacts Nanoparticle Delivery. ACS Nano. 2018;12(8):8341-8349. doi:10.1021/acsnano.8b03640

[23] Patel S, Ashwanikumar N, Robinson E, et al. Naturally-occurring cholesterol analogues in lipid nanoparticles induce polymorphic shape and enhance intracellular delivery of mRNA. Nat Commun. 2020;11(1):983. Published 2020 Feb 20. doi:10.1038/s41467-020-14527-2

[24] Patel SK, Billingsley MM, Frazee C, et al. Hydroxycholesterol substitution in ionizable lipid nanoparticles for mRNA delivery to T cells. J Control Release. 2022;347:521-532. doi:10.1016/j.jconrel.2022.05.020

[25] Mui BL, Tam YK, Jayaraman M, et al. Influence of Polyethylene Glycol Lipid Desorption Rates on Pharmacokinetics and Pharmacodynamics of siRNA Lipid Nanoparticles. Mol Ther Nucleic Acids. 2013;2(12):e139. Published 2013 Dec 17. doi:10.1038/mtna.2013.66

[26] Shi D, Beasock D, Fessler A, et al. To PEGylate or not to PEGylate: Immunological properties of nanomedicine's most popular component, polyethylene glycol and its alternatives. Adv Drug

Deliv Rev. 2022;180:114079. doi:10.1016/j.addr.2021.114079

[27] Simberg D, Barenholz Y, Roffler SR, Landfester K, Kabanov AV, Moghimi SM. PEGylation technology: addressing concerns, moving forward. Drug Deliv. 2025;32(1):2494775. doi:10.1080/10717544.2025.2494775

[28] Cheng Q, Wei T, Jia Y, et al. Dendrimer-Based Lipid Nanoparticles Deliver Therapeutic FAH mRNA to Normalize Liver Function and Extend Survival in a Mouse Model of Hepatorenal Tyrosinemia Type I. Adv Mater. 2018;30(52):e1805308. doi:10.1002/adma.201805308

[29] Cheng Q, Wei T, Farbiak L, Johnson LT, Dilliard SA, Siegwart DJ. Selective organ targeting (SORT) nanoparticles for tissue-specific mRNA delivery and CRISPR-Cas gene editing. Nat Nanotechnol. 2020;15(4):313-320. doi:10.1038/s41565-020-0669-6

[30] Cui Z, Zeng C, Huang F, et al. Cas13d knockdown of lung protease Ctsl prevents and treats SARS-CoV-2 infection. Nat Chem Biol. 2022;18(10):1056-1064. doi:10.1038/s41589-022-01094-4

[31] Su K, Shi L, Sheng T, et al. Reformulating lipid nanoparticles for organ-targeted mRNA accumulation and translation. Nat Commun. 2024;15(1):5659. Published 2024 Jul 5. doi:10.1038/s41467-024-50093-7

[32] Parhiz H, Shuvaev VV, Pardi N, et al. PECAM-1 directed re-targeting of exogenous mRNA providing two orders of magnitude enhancement of vascular delivery and expression in lungs independent of apolipoprotein E-mediated uptake. J Control Release. 2018;291:106-115. doi:10.1016/j.jconrel.2018.10.015

[33] Li Q, Chan C, Peterson N, et al. Engineering Caveolae-Targeted Lipid Nanoparticles To Deliver mRNA to the Lungs. ACS Chem Biol. 2020;15(4):830-836. doi:10.1021/acschembio.0c00003

[34] Herrera-Barrera M, Ryals RC, Gautam M, et al. Peptide-guided lipid nanoparticles deliver mRNA to the neural retina of rodents and nonhuman primates. Sci Adv. 2023;9(2):eadd4623. doi:10.1126/sciadv.add4623

[35] Sinegra AJ, Evangelopoulos M, Park J, Huang Z, Mirkin CA. Lipid Nanoparticle Spherical Nucleic Acids for Intracellular DNA and RNA Delivery. Nano Lett. 2021;21(15):6584-6591. doi:10.1021/acs.nanolett.1c01973

[36] He Q, Gao H, Tan D, Zhang H, Wang JZ. mRNA cancer vaccines: Advances, trends and challenges. Acta Pharm Sin B. 2022;12(7):2969-2989. doi:10.1016/j.apsb.2022.03.011

[37] Lokugamage MP, Vanover D, Beyersdorf J, et al. Optimization of lipid nanoparticles for the delivery of nebulized therapeutic mRNA to the lungs. Nat Biomed Eng. 2021;5(9):1059-1068. doi:10.1038/s41551-021-00786-x

[38] Massaro M, Wu S, Baudo G, et al. Lipid nanoparticle-mediated mRNA delivery in lung fibrosis. Eur J Pharm Sci. 2023;183:106370. doi:10.1016/j.ejps.2023.106370

[39] Liu Y, Huang Y, He G, Guo C, Dong J, Wu L. Development of mRNA Lipid Nanoparticles: Targeting and Therapeutic Aspects. Int J Mol Sci. 2024;25(18):10166. Published 2024 Sep 22. doi:10.3390/ijms25181016