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Review Article

The evolution of lipid nanoparticles: Paving the way for next-generation nucleic acid medicines

Xuan Lin[a,#], *Hongguang Xiang*[a,#], *Jiong Wu*[a,#], *Ruixi Liao*[a], *Yuhao Li*[a], *Bohui Xu*[b], *Ying Xu*[c], *Yan Shen*[a], *Qian Li*[d,*], *Yu Tian*[e,*]

[a] *Department of Pharmaceutics, School of Pharmacy, China Pharmaceutical University, Nanjing 210009, China*
[b] *School of Pharmacy, Nantong University, Nantong 226019, China*
[c] *College of Pharmacy, Jiangsu University, Zhenjiang 212013, China*
[d] *School of Life Science & Technology, China Pharmaceutical University, Nanjing 210009, China*
[e] *School of Medicine, Shanghai University, Shanghai 200444, China*

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ABSTRACT

Nucleic acid-based therapies have emerged as promising strategies for the regulation of gene expression and the production of therapeutic antigens or proteins for a series of diseases, including cancers, rare diseases, and infectious diseases. However, their clinical application faces challenges. These include high molecular weight, limited cellular uptake, and susceptibility to enzymatic degradation by nucleases *in vivo*. Both viral and non-viral delivery vectors have been developed as a means of addressing these limitations, including lipid nanoparticles (LNPs), exosomes, polymers, and inorganic nanoparticles. Among these, LNPs have garnered significant attention due to their superior biocompatibility, high delivery efficiency and customizable design potential, as demonstrated by the clinical success of the FDA-approved siRNA drug Onpattro®. The critical role of nucleic acid drug carriers is discussed in this review. It also outlines the major types of carriers under development and examines the advancements and applications in LNP-based systems for nucleic acid delivery. By conducting a review of recent advancements in LNP design, delivery mechanisms, and clinical applications, this article aims to clarify the ways in which LNPs overcome delivery barriers, compare LNPs with other carriers, and identify key trends that can inform the development of next-generation LNP platforms for nucleic acid therapeutics.

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* Corresponding authors.
E-mail addresses: leqean@163.com (Q. Li), shu_yutian@shu.edu.cn (Y. Tian).
# These authors are contributed equally.
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1. Introduction

For almost 50 years, nucleic acid medicines have attracted a great deal of attention due to their promising applications in the diagnosis and treatment of various diseases [1], as can be seen in Fig. 1. In 1978, Zamecnik demonstrated the first instance of antisense oligonucleotide (ASO)-mediated gene silencing by inhibiting viral gene expression in chicken embryo fibroblasts infected with Rous sarcoma virus. Over the following decades, the potential of nucleic acid fragments to regulate gene expression and treat diseases has gained increasing recognition [1]. Researchers have continued their exploration of the clinical potential of nucleic acid medicines in infectious diseases, tumors, and numerous genetic disorders [2–4]. In 1998, the FDA approved vitravene, which was the first ASO drug [5], for the therapy of cytomegalovirus retinitis. This marked the start of the era of nucleic acid drug therapy. In addition, the global application of mRNA-based COVID-19 vaccines has served to highlight the immense potential of nucleic acid drugs [6,7]. These vaccines, developed by BioNTech/Pfizer and Moderna, marked a pivotal moment in lipid nanoparticle (LNP)-enabled mRNA therapeutics, highlighting the effectiveness of nanocarrier systems in clinical deployment [8]. It should be noted that nucleic acid therapeutics are not limited to vaccines for infectious diseases, and they also show potential in cancer immunization, protein substitution treatments and gene-editing strategies that aim to address rare genetic conditions [8]. In comparison to traditional small-molecule drugs or antibody-based therapies, nucleic acid drugs have distinct advantages. Firstly, through the delivery of exogenous nucleic acids, they enable the precise modulation of gene expression that is associated with diseases, which effectively compensates for defective genes. Secondly, synthesizing them is relatively straightforward and they exhibit broad therapeutic versatility. These characteristics serve to position nucleic acid drugs as unique tools for gene-targeted therapies and establish them as a pivotal focus for drug development in the future [9,10].

Despite these advantages, there are still several limitations to the widespread application of nucleic acid therapy. Unmodified nucleic acids have low cellular uptake due to electrostatic repulsion, which hinders their membrane passage. In addition, endogenous nucleases and immune responses can rapidly degrade nucleic acid drugs, which further constrains their clinical application [11]. Researchers have explored biocompatible and more efficient delivery strategies, including chemical modifications and advanced delivery platforms, in order to overcome these limitations [12–13]. Among these, LNPs have gained significant attention, particularly in 2018, upon the FDA approval of Alnylam's first siRNA drug, Onpattro® [14]. This encapsulation technique has demonstrated the ability to effectively protect nucleic acid drugs from nuclease-mediated degradation while also enhancing their intracellular delivery efficiency. Recent studies have further explored LNP structural engineering, PEGylation strategies, and organ-specific delivery capabilities to expand therapeutic indications beyond the liver [15]. Researchers have made significant efforts to tackle the new challenges that are related to LNP-based nucleic acid delivery, focusing in particular on improving targeting accuracy, facilitating endosomal release, and reducing unintended liver accumulation during delivery [16]. Therefore, this paper will review the delivery systems that have managed to successfully advance nucleic acid therapeutics into clinical use, providing an evaluation of their advantages and shortcomings. Of the various delivery systems, the focus of this paper will be on LNPs as a delivery carrier for nucleic acid drugs. Their mechanisms of action will be explained and key technological advancements that can optimize delivery will be highlighted. In addition, the current clinical

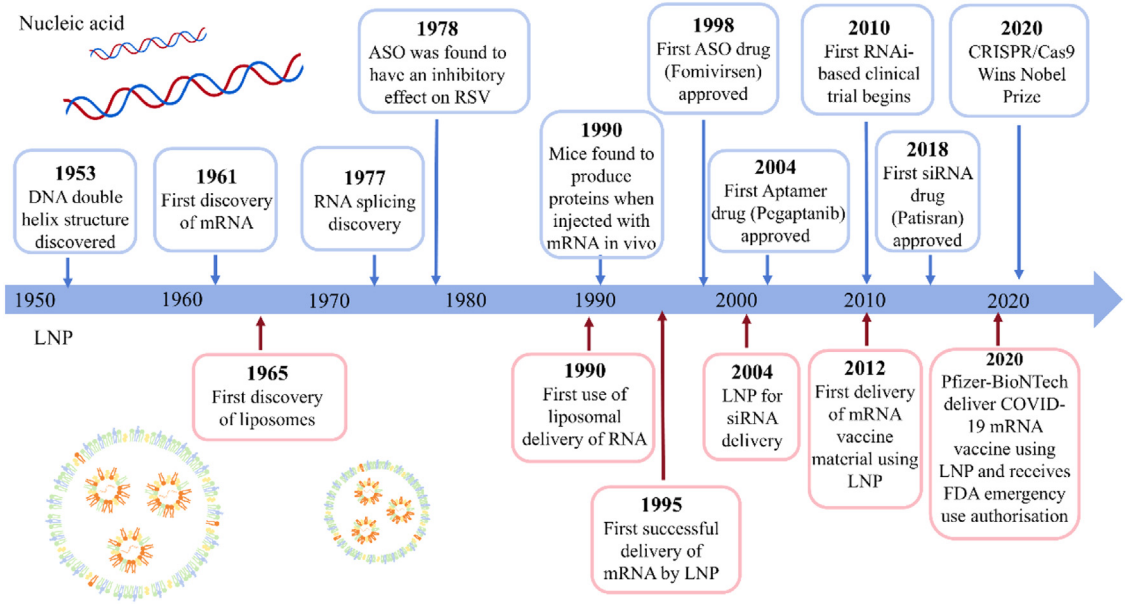


Fig. 1 – Nucleic acid drug and LNP delivery system development history.

Table 1 – Examples of marketed nucleic acid drugs.

Categorization	Trade name	Year approved	Target point	Delivery system	Indications
ASO	Vitravene®	1998	CMV UL123	Not reported	Cytomegalovirus retinitis
	Kynamro®	2013	Apo B-100		Familial hypercholesterolemia in a purest form
	Spinaraza®	2016	Exon 7 of SMN2		SMA
	Exondys 51	2016	Exon 51 of DMD		Duchenne muscular dystrophy
	Tegsedi	2018	TTR		Familial amyloid polyneuropathy (FAP)
	Waylivra	2019	ApoC III		Familial Coeliac Disease
	Vyondys 53	2019	Exon 53 of DMD		Duchenne muscular dystrophy
	Viltepso®	2020	Exon 53 of DMD		Duchenne muscular dystrophy
	Amondys 45	2021	Exon 53 of DMD		Duchenne muscular dystrophy
siRNA	Onpattro®	2018	TTR	LNP	Familial amyloid polyneuropathy
	Givlaari®	2019	ALAS1	GalNAc	Acute hepatic porphyria
	Oxlumo®	2020	HAO1	GalNAc	Primary hyperoxaluria type 1
	Leqvio®	2020	PCSK9	GalNAc	Hypercholesterolemia
	Amvuttra®	2022	TTR	GalNAc	Amyloidotic polyneuropathy
RNA adaptor	Macugen	2004	VEGF-165	Not reported	Neovascular age-related photic degeneration
dsDNA	Strimvelis	2016	ADA	γ-retroviral vector	ADA-SCID

applications of nucleic acid therapies that utilize LNPs for various diseases will be explored and insights into potential future advancements will be provided.

2. Nucleic acid therapeutics

Most research on nucleic acid therapeutics focuses on several types of nucleic acid, including ASOs, messenger RNA (mRNA), small interfering RNA (siRNA), microRNA (miRNA), and RNA aptamers [17]. ASOs bind to target mRNA to inhibit translation; siRNAs trigger RNA interference (RNAi) for gene silencing; mRNAs encode proteins for therapeutic expression; miRNAs regulate gene expression post-transcriptionally; and RNA aptamers specifically bind target molecules to modulate their activity. Nucleic acid therapeutics use these nucleic acids as active compounds for the modulation of gene expression at both the transcriptional and translational stages, which provides therapeutic benefits for targeted diseases [16]. By acting directly on gene expression, nucleic acid therapy provides a novel approach for the treatment of diseases that are difficult to treat through the use of conventional pharmaceutical therapies, which expands its potential application range [17–20]. With the growing understanding of their mechanisms and pharmacological advantages, nucleic acid therapeutics have progressed rapidly toward clinical translation. Several therapies have received regulatory approval, as summarized in Table 1. Notably, multiple agents have demonstrated compelling outcomes in late-stage clinical trials. For example, patisiran, an siRNA-based therapy for hereditary transthyretin-mediated amyloidosis, showed a 56% reduction in neuropathy progression in phase III trials and was subsequently approved by the FDA [21]. Likewise, nusinersen, an ASO for spinal muscular atrophy (SMA), significantly improved motor function in infants, with 51% achieving motor milestones compared to 0 in the control group [22]. These cases exemplify how different classes of nucleic acid therapeutics—including siRNA, ASOs and mRNA—are transitioning from laboratory research to real-world clinical success. As of 2020, at least 22 nucleic acid-based drugs had entered clinical development stages, and by 2023, >15 such therapeutics had obtained FDA or EMA approval. These approved indications span genetic disorders, cancers and metabolic diseases, underscoring the growing maturity, therapeutic value and clinical impact of nucleic acid therapeutics [16].

Despite significant progress having been made in the field of nucleic acid-based therapies, several challenges remain in this area [23,24]. The substantial molecular size and negative charge of nucleic acids serve to create significant challenges for their ability to cross cell membranes and enter cells, which limits their therapeutic potential. This highlights the need for effective delivery systems that can improve cellular uptake [25]. In recent years, significant efforts have been devoted to the development of efficient nucleic acid delivery systems [26–29]. For example, Yan et al. became aware of a marked reduction in miR-31–5p expression in diabetic mouse wounds in comparison to non-diabetic controls [30]. This observation resulted in them exploring the capacity of exogenous miR-31–5p mimics as a diabetic wound therapy. They employed milk-derived exosomes as delivery carriers and successfully encapsulated the mimics by electroporation. Their subsequent experiments demonstrated that exosome-mediated delivery improved cellular uptake, protected the miRNAs from degradation, and significantly enhanced endothelial cell function. In addition, nucleic acids face challenges relating to stability and immunogenicity *in vivo* [31]. Prior to reaching their intended cellular destinations, exogenous nucleic acids are susceptible to breakdown by extracellular nucleases. These molecules also risk being

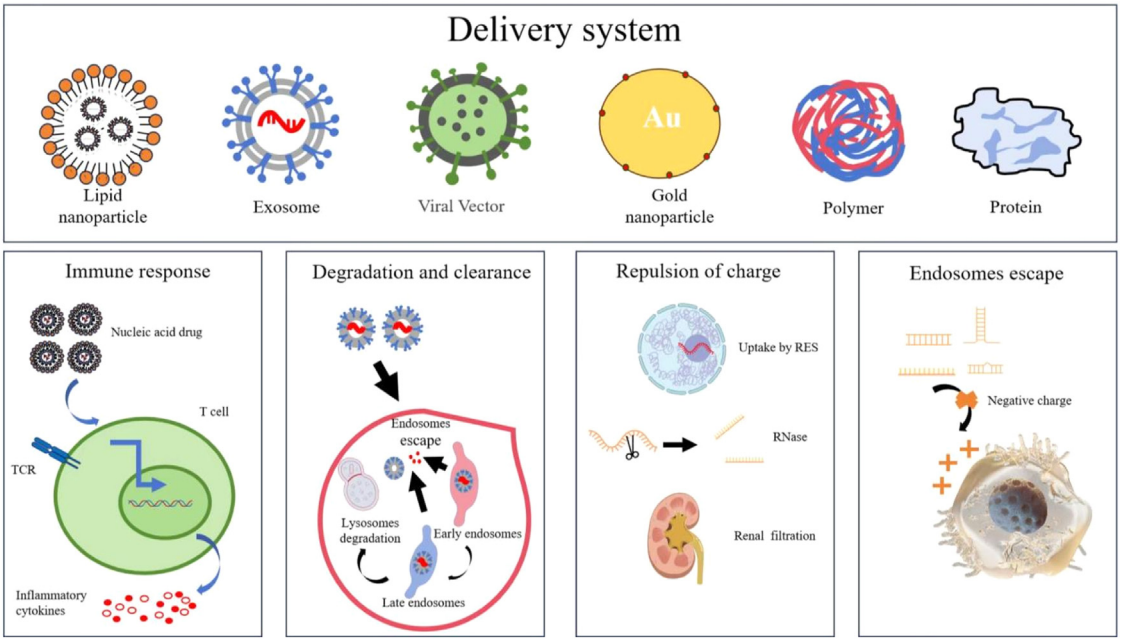


Fig. 2 – Illustration depicting several major nucleic acid delivery systems (LNPs, exosomes, viral vectors, inorganic nanoparticles, polymers and proteins) for RNA delivery. These vectors solve many challenges encountered during nucleic acid delivery, including exogenous nucleic acid-induced immune responses, nuclease degradation, rapid clearance by the liver and kidney, and ineffective endosomal escape.

recognized as foreign entities by the immune system, which could potentially lead to the activation of unintended immune responses.

Scientists have investigated a variety of chemical modification strategies and engineered a range of delivery systems that are designed to improve the stability and transport efficacy of nucleic acids in order to overcome these limitations. Chemical modifications can help improve stability and lipid solubility while also increasing targeting efficacy by attaching ligands to nucleic acid ends [32]. For example, Cas9 mRNA modification with pseudouridine and 5-methylcytosine was found to reduce the innate immune response [33]. However, the achievement of efficient cell membrane penetration and targeted delivery still requires advanced delivery carriers and technologies to be developed [34]. This review is focused on the primary nucleic acid delivery systems that are currently available, which include exosomes, viral vectors, polymers, and protein carriers, as can be seen in Fig. 2, and highlights the advantages of LNPs relative to these systems. Nevertheless, despite their promise, LNP-based platforms still face notable challenges, including limited *in vivo* stability, potential immunogenicity, and difficulties in achieving tissue-specific targeting. Addressing these issues remains critical for the successful clinical translation of nucleic acid therapies.

3. Delivery system of nucleic acid drug

Effective delivery systems are essential to address the inherent challenges of nucleic acid therapeutics, such as enzymatic degradation, low cellular uptake and off-target effects. Over the past decades, various carriers have been developed to enhance the delivery efficiency, stability and specificity of nucleic acids *in vivo*. These delivery systems differ widely in their composition, mechanisms of action and clinical potential. In the following section, we provide an overview of the major classes of delivery platforms—including exosomes, viral vectors, polymers, protein-based systems and inorganic nanoparticles—with a particular focus on LNPs as one of the most clinically advanced and versatile carriers.

3.1. *Exosomes*

Exosomes are lipid bilayer vesicles and they are generated when multivesicular bodies fuse with the cell membrane, typically ranging between 40 and 160 nm in diameter. As natural carriers, exosomes are capable of encapsulating a diverse array of therapeutic agents, and recent research has highlighted their promising function in the delivery of nucleic acid-based therapeutics [35]. It is necessary to overcome the barrier posed by the exosome membrane in order to load drugs into exosomes. Two main approaches are commonly used for this purpose: electroporation and intracellular expression encapsulation within donor cells [36–38]. However, the intracellular expression method is hindered by inefficient loading. Electroporation provides a far more straightforward and effective means of incorporating nucleic acids into exosomes, but the loading efficiency is influenced by the specific electroporation conditions that are employed [39]. For example, Cui et al. designed the bone-targeting peptide-modified exosome BT-Exo-siShn3, which

managed to successfully introduce nucleic acids into the exosome by electroporation and delivered therapeutic drugs to the bone tissues for osteoporosis treatment [40]. Derived from cells, exosomes exhibit high biocompatibility and low immunogenicity due to their natural composition [41], enabling nucleic acid drugs to evade phagocytosis and achieve significant therapeutic effects. In addition, Kooijmans et al. identified the mechanisms that underlie the accumulation of CD47-expressing exosomes and synthetic nanoparticles in pancreatic tumor cells, finding that exosomes evaded their clearance by conveying evasive signals to macrophages with the help of CD47 and released nucleic acid drugs following the preferential uptake by KRAS-transformed cells upon reaching the pancreas [42], which can be seen in Fig. 4A. Furthermore, their small size and unique membrane composition also improve their capacity to traverse biological barriers within the body [43–45]. As a result, exosomes have gained a significant amount of attention as platforms for disease diagnostics and drug delivery [46,47].

Despite these advantages, there are still significant challenges when using exosomes as nucleic acid drug carriers [48–51]. A key challenge is the large-scale production of exosomes. Exosomes that are derived from a variety of different cell sources often exhibit differences in their properties, which can cause inconsistencies in their therapeutic effectiveness. In addition, current purification techniques, which include ultracentrifugation and ultrafiltration, are expensive and time-intensive [41], This makes them less suitable for large-scale industrial production. The effectiveness of incorporating nucleic acid drugs into exosomes is also relatively low and the achievement of stable, efficient loading and retention remains a critical issue [52]. To address these limitations, recent studies have introduced several innovative strategies to improve exosome-based nucleic acid delivery. One such approach involves the engineering of exosomes through surface modification with targeting ligands or membrane fusion with synthetic materials to enhance cell specificity and uptake efficiency [53]. Additionally, exosome-mimicking nanovesicles (EMNVs), which are artificially fabricated to replicate the structural and functional characteristics of natural exosomes, offer advantages in scalability, cargo control and batch consistency [54]. These biomimetic vesicles have shown great potential in improving loading efficiency, enhancing circulation stability, and enabling targeted delivery across biological barriers. Moreover, genetic engineering of donor cells has emerged as a promising tool to produce exosomes pre-loaded with therapeutic RNA species, thereby bypassing the need for post-isolation loading [55]. Collectively, these advancements help overcome several key limitations of natural exosomes and are accelerating their translational application in nucleic acid drug delivery systems.

3.2. *Viral vectors*

With advances in genetic engineering, viral vectors offer high transduction efficiency and have become effective tools for the delivery of nucleic acid-based therapeutics [56–58]. FDA has recently granted approval to several nucleic acid therapies that are reliant on viral vectors, which include Strimvelis [59]. This treatment uses retroviral vectors as a means of introducing functional genes into bone marrow cells, which offers a therapeutic strategy for adenosine deaminase-deficient severe combined immunodeficiency [59,60]. Adenoviruses (Ad), adeno-associated viruses (AAV), and lentiviruses (LV) are currently the most commonly utilized viral vectors in this field [61–63].

Ad vectors were among the first viral vectors to be used in gene delivery and they demonstrated high transduction efficiency and large packaging capacity, which made them prominent in early gene therapy applications [64–66]. However, their high immunogenicity means that they are now more commonly applied in the development of vaccines and cancer drugs [67]. For example, during the COVID-19 pandemic, the vaccines that were developed by AstraZeneca and Janssen/Johnson & Johnson, respectively used ChAdOx1 and Ad26 as vectors [68]. However, the safety profile of Ad vectors requires further optimization [65,67,69]. Studies have indicated that many clinical batches of Ad vectors contained more replication-competent adenoviruses (RCA) than anticipated, which exceeded regulatory agency standards [67]. Compared to Ad vectors, AAV vectors exhibit lower immunogenicity, enhanced safety, and the ability to infect diverse tissues with stable gene expression [70–72]. The large number of clinical trials of AAV-based gene therapy also highlights the great potential it has. Notably, Luxturna®—an AAV2-based gene therapy—was the first FDA-approved *in vivo* gene therapy for an inherited retinal disease, demonstrating sustained visual improvement in patients with RPE65 mutations [73]. Recent advances have further expanded the utility of AAV vectors through the integration of gene-editing tools such as CRISPR-Cas9, enabling precise and durable gene correction *in vivo*. For instance, engineered AAVs carrying CRISPR-Cas9 systems have demonstrated targeted editing of disease-related genes in models of Duchenne muscular dystrophy and hereditary retinal disorders, achieving high editing efficiency with reduced off-target effects [74,75]. These innovations highlight AAV's growing role as both a delivery vector and a functional gene-editing platform. Furthermore, LV vectors derived from the human immunodeficiency virus have been applied extensively in scientific research and clinical trials [76,77]. Target gene fragments are delivered into the host genome through reverse transcription by these vectors after fusing with host cells. Unlike AAV, LV vectors integrate into the host genome, which supports long-term transgene expression but may also raise safety concerns due to the risk of insertional mutagenesis [78–80]. For example, Zynteglo®—based on a LV platform—has been approved in the EU and FDA for the treatment of transfusion-dependent β-thalassemia, with trials showing over 80% transfusion independence rate among treated patients [81]. This feature enhances the safety profile of LV vectors as carriers for the delivery of nucleic acid drugs and increases the potential they have for future therapeutic applications. Research has shown that infusing autologous $CD34^+$ cells transduced with LV into infants with Artemis-deficient severe combined immunodeficiency can result in gene correction and treated infants have exhibited normal levels of T- and B-cells [82]. A novel CD3-targeted LV vector that can directly engineer human T cells without

requiring prior activation was introduced in a recent study. By incorporating CD3-specific single-chain antibody fragments on its surface, the vector selectively delivers genes to T cells and induces their proliferation, all in the absence of external stimuli. The CD3-LV vector successfully delivered chimeric antigen receptor (CAR) genes into T cells in a mouse model and caused CAR-T cell generation. These cells demonstrated robust targeting capabilities and effectively cleared specific cells [83]. The results serve to highlight the significant capacity LV have as effective vectors for nucleic acid delivery. In addition, newer viral delivery systems have emerged in recent years, including bacteriophage T4. Zhu et al. designed a series of T4 phage-based artificial viral vectors as shown in Fig. 4B, which were combined with DNA, RNA, proteins, and other substances for effective cellular delivery. These artificial viral vectors have demonstrated powerful "cargo" loading and human cell delivery capabilities. However, despite clinical trials producing encouraging results, gene therapy that uses viral vectors remains limited by immunogenicity issues and the ongoing necessity for genome integration technique improvements [84].

3.3. *Polymer*

Researches on the use of polymers in nucleic acid drug delivery have increased steadily in recent years and the main applications include cationic polymers, neutral polymers and self-assembled polymers. Researchers have devoted their efforts to enhancing the performance and targeting the precision of these delivery systems by exploring diverse polymers and their modification approaches [85–87]. Cationic polymers bind to the negatively charged phosphate backbone of nucleic acids via electrostatic interactions, facilitating their delivery [88]. Polyethyleneimine (PEI) is a typical cationic polymer and is commonly applied in gene transfection and RNAi due to its cationic charge, which increases cell penetration rate and high delivery efficiency. Kuang et al. incorporated photoactivatable platinum backbone polymers and siRNA targeting polo-like kinase 1 onto the surface of PEI-coated upconversion nanoparticles (UCNPs), creating multifunctional UCNPs functionalized with both multi-protagonist agents and siRNA, as illustrated in Fig. 3A. This study demonstrated that these UCNPs can be used for multimodal imaging and have the potential to enhance the effectiveness of synergistic cancer therapy [89]. Subsequent research has also shown that these PEI nanoparticles can efficiently deliver siRNA, which leads to the oncogene expression in breast cancer cells being successfully suppressed and a significant reduction in tumor growth *in vivo* [90]. However, cations also lead to a higher risk of cytotoxicity and cell membrane damage with this polymer, whereas neutral polymers are more biocompatible, where poly(lactic-co-glycolic acid) (PLGA) nanoparticles have also been successfully applied to deliver mRNA encoding antigenic proteins for cancer immunotherapy [91]. These nanoparticles can provide a prolonged release, which lengthens the exposure time of antigens internally as a means of enhancing the immune response [92]. Neutral polymers have commonly been used in long-acting drug delivery studies in recent years due to their slow and controlled drug release characteristics. However, self-assembled polymers are highly structurally tunable and this means that they can be designed as carriers with targeted delivery properties and smart response functions as necessary. The targeted delivery of polymer particles to specific cells or tissues can be concluded by adjusting their size and surface charge of polymer particles or modifying them with specific ligands. Certain polymer nanoparticles can also be modified with pH-responsive materials, which serve to enhance drug delivery efficiency and specificity in acidic environments, such as tumor tissues [93,94]. Studies have also shown that optimizing the formulation of polymeric carriers can serve to improve the loading and cellular delivery of large molecules. A strategy for synthesizing poly(disulfide)s through the convenient photocrosslinking polymerization of modified thioctic acid monomers, which include a zinc-coordinated diacetamide analog (ZnDPA) and guanidine (GUA), was proposed by Hei et al. The resulting polydisulfide backbone that contains disulfide bonds is sensitive to reducing environments, which facilitates the controlled release of encapsulated molecules, as is shown in Fig. 3B In addition, the polydisulfide that was produced demonstrated superior performance in comparison to commercial transfection reagents for delivering plasmid DNA and siRNA by adjusting the feeding ratios of ZnDPA and GUA (Fig. 3C). In addition to linear and self-assembled polymers, dendrimers have emerged as promising carriers for nucleic acid delivery. These highly branched, three-dimensional (3D) macromolecules provide abundant surface functional groups and allow precise control over structure, molecular weight, and nucleic acid binding affinity. Poly(amidoamine) (PAMAM) dendrimers, in particular, have been widely studied for delivering siRNA and mRNA due to their ability to form stable complexes and facilitate endosomal escape [95]. Recent studies have shown that surface-engineered dendrimers—such as PEGylated or ligand-modified PAMAMs—can reduce cytotoxicity while improving biodistribution and tumor-specific targeting. Notably, dendrimer-based RNA nanoparticles have been used to induce protective immunity against lethal infectious diseases, highlighting their versatility beyond oncology [96]. These advances reinforce the potential of dendrimers as modular and customizable platforms for safe and efficient nucleic acid delivery.

However, the application of polymers as nucleic acid carriers remains hindered. Some polymers, particularly high-molecular-weight variants, exhibit toxicity, which necessitates the chemical modification or synthesis of low-molecular-weight forms [97]. Alternatively, degradable polymeric materials such as cationic collagen and chitosan can be used so that the potential toxicity that is caused by the long-term accumulation of polymers in the body can be avoided. Despite the stability of many polymers, effective drug delivery can be hindered by degradation and morphological changes *in vivo*. Finally, although ligand modifications enhance targeting capabilities, the achievement of high targeting efficiency in complex *in vivo* environments remains challenging. This is primarily a result of physiological barriers, such as hepatic and renal clearance, which serve to diminish the proportion of the delivery system that reaches the intended target site [98,99].

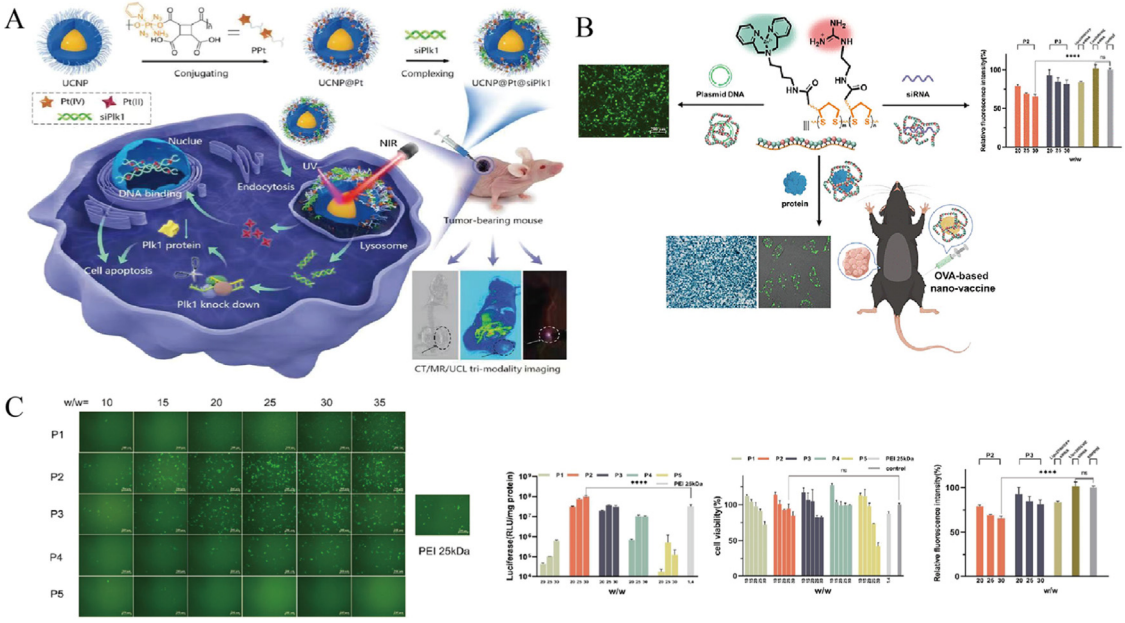


Fig. 3 – Nucleic acid drug delivery studies using polymers. (A) Modified PEI-encapsulated siRNA for cancer therapy [89]. Copyright 2021 John Wiley and Sons; (B) Modified polydisulfide encapsulated nucleic acids for tumor immunotherapy [100]. Copyright 2023 American Chemical Society; (C) *In vitro* gene transfection of polydisulfide-encapsulated nucleic acids [100]. Copyright 2023 American Chemical Society.

3.4. *Protein-based delivery systems*

Protein-based carriers have been found to have significant promise in the facilitation of the delivery of nucleic acid drugs [101]. For example, cationic proteins have been used as carriers for nucleic acids in various studies, demonstrating promising results *in vivo* applications [102,103]. A recent study utilized bovine lactoferrin (LF)-modified carriers to transport siRNA to liver tumor tissues in mice, resulting in a notable suppression of tumor growth. Cationic proteins like LF form electrostatic complexes with negatively charged nucleic acids, which not only enhance stability in biological fluids but also promote cellular uptake through endocytic pathways. Similarly, zinc finger proteins and transcription activator-like effector nucleases have been employed for the targeted delivery of specific RNA or DNA sequences, allowing precise control over gene expression. Zinc finger proteins achieve sequence-specific binding to target DNA regions via modular zinc-coordinated motifs, enabling the precise delivery and regulation of gene expression at the transcriptional level. Such systems hold substantial potential for advancements in gene therapy.

The targeted delivery mechanisms of protein-based delivery systems primarily rely on ligand-receptor interactions, electrostatic interactions, and membrane permeation [104]. Protein-based carriers interact electrostatically with nucleic acids, which forms stable complexes that shield them from degradation and facilitate cellular entry via endocytosis or direct membrane penetration [105]. It is possible to design proteins to selectively recognize and attach to specific receptors that are present in target cells through the incorporation of antibodies, ligands or fusion proteins that interact with cell surface markers. This facilitates cell- or tissue-specific delivery [106]. For example, delivery systems that use RGD-ligand proteins are able to target integrin receptors, which enables tumor cell-specific delivery [107]. In another study, an oncolytic virus-like nanoparticle, OV@FN, was developed by Li et al. [108] for tumor-targeted gene delivery. OV@FN is composed of NA-Zn@G, a nucleic acid-loaded nanocore, and FN, a heterotrimeric membrane vesicle, which expresses the lysosomal viral fusion membrane glycoprotein (mVSV-G). The FN vesicle in this system enables the transport of nucleic acid-loaded nanocores into the cytoplasm of tumor cells through membrane fusion, which is activated by the mildly acidic tumor microenvironment. The NA-Zn@G then responds to the elevated glutamate concentration in the tumor cell cytoplasm, which enables the precise release of drugs in the targeted area, as can be seen in Fig. 4C. These recent findings serve to emphasize the distinct advantages protein-based carriers have for nucleic acid drug delivery. These systems are composed of natural or recombinant proteins and exhibit high biocompatibility and low immunogenicity *in vivo*. In addition, proteins can be modified to selectively target specific cells or tissues, which improves the effectiveness of nucleic acid drugs while also ensuring that unintended side effects are minimized [109]. Protein delivery systems can also be genetically engineered or chemically modified so they can incorporate functional modules including targeting ligands, cell-penetrating peptides, or degradation protection mechanisms, which improves delivery efficiency [110].

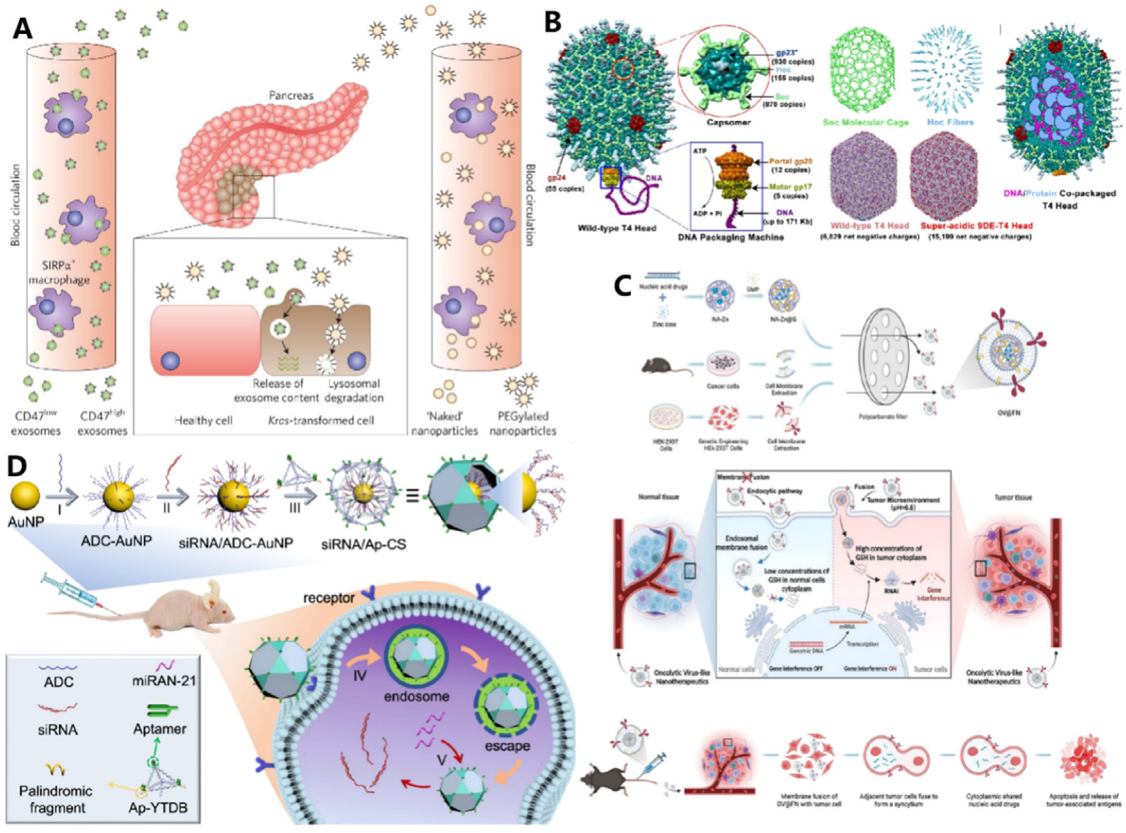


Fig. 4 – The mechanisms of action of various nucleic acid delivery systems. (A) The underlying mechanisms responsible for the buildup of CD47-expressing exosomes within pancreatic tumor cells [42]. Copyright 2017 Springer Nature; (B) Artificial vector design based on T4 phage [133]. Copyright 2023 Springer Nature; (C) Schematic illustration of preparation and performance of oncolytic virus-like nanoparticles, OV@FN [108]. Copyright 2024 John Wiley and Sons; (D) Preparation of AuNP and its application for siRNA targeted delivery and controlled release in tumor cells via an antibody-drug conjugate (ADC) strategy [124]. Copyright 2021 Springer Nature.

Despite their advantages, protein carriers face several critical challenges that limit their clinical translation and scalability [111]. First, proteins are susceptible to degradation, aggregation, and denaturation under physiological conditions, which can impair their delivery efficiency and compromise drug stability. Furthermore, the manufacturing of recombinant or modified proteins is often labor-intensive and costly, requiring sophisticated expression systems, purification protocols, and stringent quality control. These production constraints can limit scalability, especially for clinical-grade formulations. Although proteins generally exhibit low immunogenicity, certain carrier proteins or their chemical modifications may still trigger immune responses, particularly after repeated administration. These factors raise safety and consistency concerns. However, recent advancements in protein engineering and recombinant expression technologies, including cell-free protein synthesis, fusion protein design, and site-specific PEGylation, offer potential strategies to enhance protein stability, reduce immunogenicity, and streamline production workflows, which may help overcome these limitations in future clinical applications [109].

3.5. *Inorganic nanoparticles*

Inorganic nanoparticles—such as gold, magnetic iron oxide and porous silicon—have been extensively investigated for nucleic acid delivery due to their unique physicochemical properties and surface engineering potential [112]. In recent years, studies utilizing iron oxide nanoparticles to deliver siRNAs have demonstrated notable success in tumor growth inhibition [113–115]. Studies on gold nanoparticles (AuNPs) conjugated with antibodies, aptamers, or small molecules have demonstrated enhanced targeting efficiency and cellular uptake for the delivery of mRNA and siRNA. In addition to surface functionalization, AuNPs possess photothermal properties, enabling light-triggered release and the potential for combinational gene-photothermal therapies [116].

Among inorganic nanoparticles, magnetic nanoparticles (MNPs)—particularly superparamagnetic iron oxide nanoparticles (SPIONs)—have attracted attention for their ability to enable site-specific nucleic acid delivery under external magnetic fields. These particles can be magnetically guided to accumulate at diseased sites, enhancing local drug concentration while minimizing off-target effects. In recent

studies, siRNA-loaded SPIONs coated with PEI have been successfully used to knock down gene expression in tumor cells, achieving enhanced intracellular uptake and gene silencing efficiency through magnetofection [117,118]. This magnetic responsiveness not only improves delivery precision but also holds potential for integrating real-time imaging and therapy, known as theranostics. AuNPs, on the other hand, achieve targeted delivery through surface modifications with peptides or antibodies [119,120]. Additionally, their photothermal properties enable controlled drug release upon light exposure, thereby enhancing therapeutic efficacy [121–123]. For example, Xue et al. developed a siRNA delivery system with the ability to enhance serum stability and enable responsive release via endogenous miRNAs. The system uses Y-shaped backbone-rigidified triangular DNA bricks as a means of protecting siRNA from degradation and forms a multi-functional 3D DNA shell around a AuNP core. Targeted delivery is enabled by aptamers on the nanoparticle surface. The system effectively induces gene silencing and apoptosis in tumor cells *in vitro*, outperforming the Lipo3000/siRNA formulation. It completely inhibits tumor growth *in vivo*, which demonstrates the potential it has as a next-generation anticancer drug carrier for targeted therapies [124], as can be seen in Fig. 4D Porous silicon nanoparticles are widely used in nucleic acid delivery due to their high surface area and tunable pore size, which enable efficient drug loading. In addition, their biodegradability and ability to disintegrate into non-toxic silicic acid under physiological conditions make them attractive for *in vivo* applications [125,126]. These nanoparticles exhibit strong physical and chemical stability, resisting degradation and thereby preserving nucleic acid integrity during delivery [127]. In addition, many inorganic nanoparticles possess unique optical, magnetic, or electrical properties, including the photothermal effect of AuNPs and the magnetic responsiveness of iron oxide nanoparticles, which enables multimodal therapy or imaging and enhances nucleic acid drug efficacy [128]. For example, there have been studies using PEI-coated ferrite MNPs as carriers to deliver functionalized RNA nanoparticles (RNA NPs) into HEK293 cells to achieve a knock-down effect on protein expression. The findings revealed that MNPs effectively shielded RNA NPs from nuclease degradation while notably enhancing transfection efficiency through magnetic field stimulation. Specifically, the system was shown to significantly reduce GFP expression, demonstrating effective RNA-mediated gene silencing in mammalian cells [129].

However, despite their stability, some inorganic materials (*e.g.*, metal oxides) may pose toxicity risks *in vivo*, affecting biocompatibility [130–132]. Certain inorganic nanoparticles are not readily degradable and may accumulate in the body over time, potentially leading to toxicity. Additionally, achieving targeted and efficient delivery often requires complex surface modifications, which can increase manufacturing costs and complexity.

In the preceding comparison, we discussed several types of nucleic acid delivery carriers, including exosomes, viral vectors, polymers, protein carrier and inorganic particles. A selection of representative systems and their respective advantages and limitations are summarized in Table 2. However, as the need for efficient nucleic acid delivery continues to grow, enhancing the efficiency, safety and targeting of these systems has become a primary focus of ongoing research. Among the numerous delivery systems, LNPs have recently attracted considerable interest as a promising platform for drug transport. This is because of their excellent biocompatibility, highly efficient drug loading and convenience to manufacture. The following discussion will focus on the application of LNPs in nucleic acid delivery, highlighting their advantages in improving delivery efficiency and addressing the limitations of existing carriers.

4. Development of LNPs

LNPs are nanoscale delivery carriers composed of lipid-like molecules, offering controllable particle size and surface properties that ensure biocompatibility and stability in biological systems [134]. LNPs serve as the main vectors for nucleic acid drug delivery, effectively encapsulating and safeguarding nucleic acid molecules from degradation in the bloodstream [135]. Additionally, surface modifications enhance their ability to target specific tissues and cells [136]. LNPs are primarily composed of ionizable lipids, phospholipids, cholesterol, and polyethylene glycol-conjugated lipids (PEG-lipids), as shown in Fig. 5 which collectively improve carrier stability, reduce immunogenicity, and optimize *in vivo* distribution [137].

The design of LNPs facilitates nucleic acid loading through charge or pH gradients, improving encapsulation efficiency and improving the biological activity of the nucleic acids [138]. Additionally, LNP characteristics such as particle size and structure can be precisely regulated using microfluidics and other methods, including ethanol injection, thin-film hydration followed by extrusion, and high-pressure homogenization. These approaches allow fine-tuning of nanoparticle morphology and size distribution, thereby improving their *in vivo* biodistribution and cellular uptake efficiency [139]. Surface modifications like PEGylation help reduce non-specific clearance in the circulatory system, prolonging circulation time and improving targeted delivery.

Recent advancements in LNP research have significantly advanced nucleic acid drug delivery. During the COVID-19 pandemic, LNPs became central to mRNA vaccine development, improving mRNA stability, boosting immunogenicity, and ensuring effective delivery [140]. For example, the mRNA vaccines developed by Pfizer-BioNTech and Moderna employ LNPs to encapsulate mRNA, ensuring its stability *in vivo* while boosting immune responses [141]. Furthermore, LNPs are extensively used for siRNA and miRNA delivery, enabling precise regulation of gene expression. Studies have demonstrated that by modifying LNP composition and structure, siRNAs can be effectively transported to targeted organs with high efficiency to achieve gene silencing effects [142]. The incorporation of cationic lipids, such as DLin-MC3-DMA, significantly enhances the encapsulation efficiency and stability of siRNAs, facilitating their use in gene therapy [137]. LNPs function as non-viral carriers for the CRISPR-Cas9 system, enabling the effective transport of Cas9 mRNA and guide RNA (gRNA) to facilitate

Table 2 – Major delivery systems for nucleic acid medicines and their characteristics.

Delivery system	Mechanisms	Advantages	Drawbacks
Naked RNA	• Local injection • Non-specific absorption • Renal filtration	• No additional development of delivery systems required	• Easily degraded • Immunogenic • Difficult to enter cells • Short half-life • Some chemical modifications are toxic
Lipids	• Ionized lipids • Endocytosis • Endosome escape • PEG modification • Liver targeting	• High delivery efficiency • Endosomal escape • Scalable production	• Cytotoxic • Equires pretreatment with steroids, etc.
Polymer	• Charge Interaction • Controlled degradation • pH-responsive release • Targeted Modification	• Adjustable structure • High targeting capacity • Low Immunogenicity	• Endosomal escape is limited • Polymer toxicity • Limited *in vivo* degradation
Extracellular vesicle	• Load Encapsulation • Transphysiological barriers • Engineered modifications	• Highly biocompatible • High targeting ability • Cross-barrier capability • Engineered Modifications	• Complexity of production • Low loading efficiency • Limited Stability
Conjugate link	• Enhanced nucleic acid stability • Immune evasion • Optimized affinity • Targeted delivery	• Improved stability • Immune evasion • Enhanced targeting	• High modification costs • Limited delivery range • Activity may be affected

gene editing. This approach shows promise for treating genetic diseases and cancers [143].

4.1. *Composition of LNP*

4.1.1. *Ionizable lipids*

Ionizable lipids are key constituents of LNPs in nucleic acid drug delivery, aiding in the encapsulation, safeguarding and controlled release of nucleic acids [144]. A major character of ionizable lipids is their ability to change charge states under different pH conditions, making them particularly suitable for this application [145]. Ionizable lipids are typically engineered to remain neutral at physiological pH (~7.4) while gaining a positive charge in acidic conditions (*e.g.*, pH 5–6) [146].This characteristic enables LNPs to remain stable and exhibit lower toxicity at neutral pH while facilitating nucleic acid release in the acidic intracellular environment [147]. The pH responsiveness of ionizable lipids, as compared to conventional cationic lipids, minimizes positive charge exposure in circulation, thereby reducing toxicity and immune activation [148]. While cationic lipids can adsorb proteins in the bloodstream and trigger immune responses, ionizable lipids mitigate this issue by maintaining a neutral charge under physiological conditions [149]. Several lipid types are shown in the Table 3.

It has been established that the immune responses, both innate and adaptive, induced by LNPs are strongly influenced by the amine groups at the head of the molecules and the chemical structure of the ionizable lipids. Studies suggest that the provoked inflammatory signals may actually help maintain the efficacy of nucleic acid delivery. Chen et al. [150] develop a novel ionizable lipid, 4N4T, to create LNPs (4N4T-LNPs) that enhance mRNA translation efficiency compared to SM-102-LNPs. The 4N4T-LNPs deliver a DS mRNA vaccine encoding the spike protein of SARS-CoV-2 variants, triggering strong immune responses against variants like Delta and Omicron. The 4N4T-based vaccine shows superior antibody titers and induces Th1-skewed T cell responses with good safety as shown in Fig. 6A. This approach offers a more effective mRNA delivery system for SARS-CoV-2 vaccines. Sharma et al. employed similar research methods to reach the same conclusion, further supporting their findings with molecular docking simulations. They discovered that ionized lipids with amine headgroups exhibit increased binding affinities to the TLR4-MD-2 complex. This increased affinity may be associated with NF-κB activation observed in murine macrophages [151].

Ionizable lipids interact with negatively charged nucleic acids via electrostatic forces, which in turn improves the encapsulation efficiency of LNPs [152]. This interaction enables effective nucleic acid encapsulation during LNP preparation and provides protection from enzymatic degradation *in vivo*. Once taken up via endocytosis, LNPs are sequestered within endosomes. Under acidic conditions, the ionized lipids interact with the negatively charged phospholipids present in the endosomal membrane, promoting LNP fusion and rupture with the membrane. This release mechanism is mediated by the “proton sponge

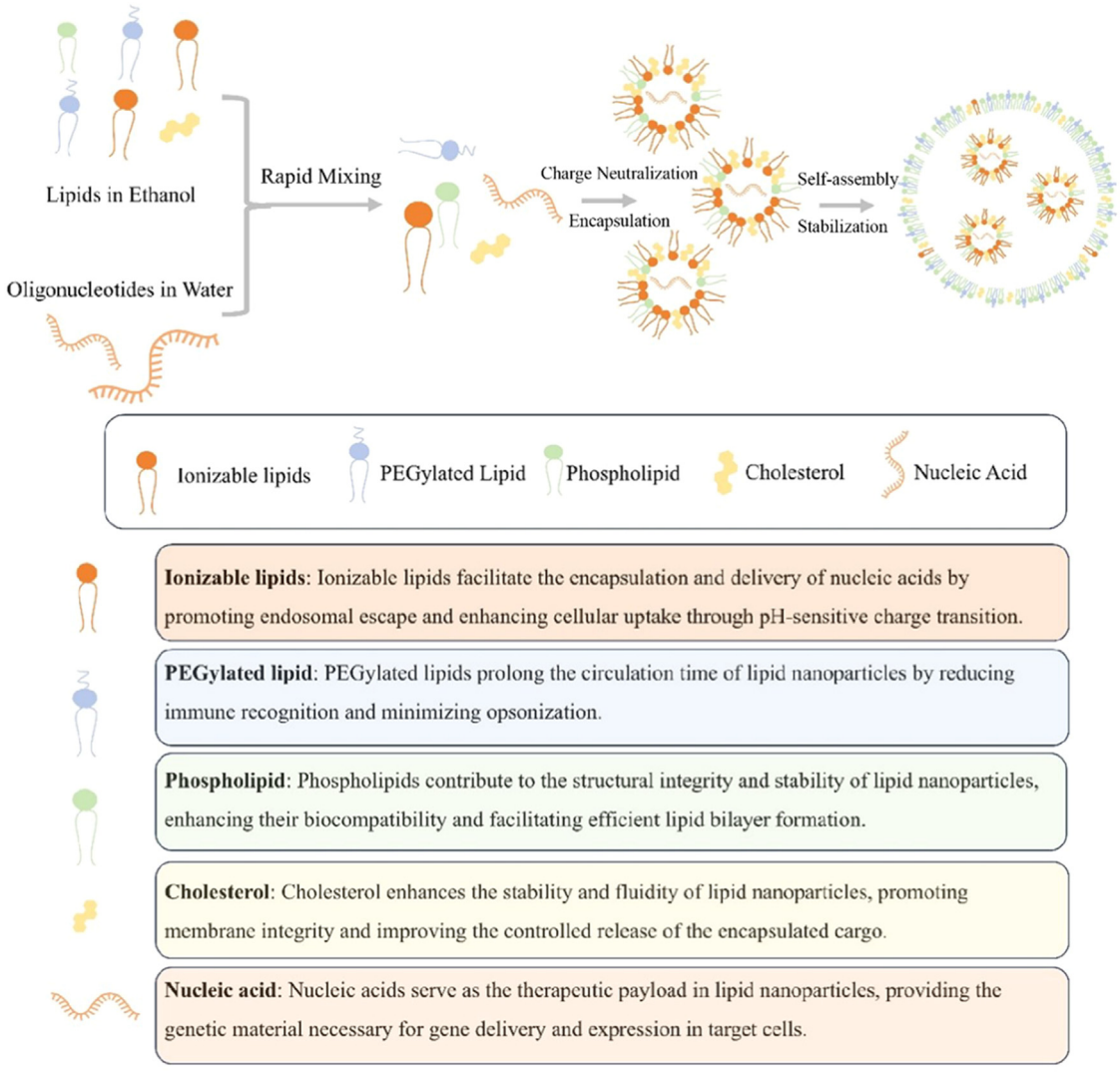


Fig. 5 – Composition and preparation process of LNPs.

Table 3 – Several lipid types.

Classification of Applications	Name	Advantages	Disadvantages
Lipids for mRNA vaccines	Dlin-MC3-DMA [166]	High transfection efficiency, good stability, suitable for mRNA delivery.	Potential cytotoxicity at high concentrations.
	SM-102 [156]	High mRNA delivery efficiency, good biocompatibility.	Expensive and less widely available.
	ALC-0315 [168]	Effective in mRNA delivery, reduced toxicity *in vivo*.	Requires optimization for large-scale production.
Lipids for siRNA delivery	C12-200 [169]	Efficient siRNA delivery, strong lipid bilayer stability.	Limited tissue specificity, potential for off-target effects.
	DLin-KC2-DMA [155]	High siRNA transfection efficiency, good *in vivo* stability.	Cytotoxicity concerns at higher doses.
Novel lipids	OF-02 [171]	Enhanced mRNA delivery efficiency, lower toxicity.	Limited clinical data.

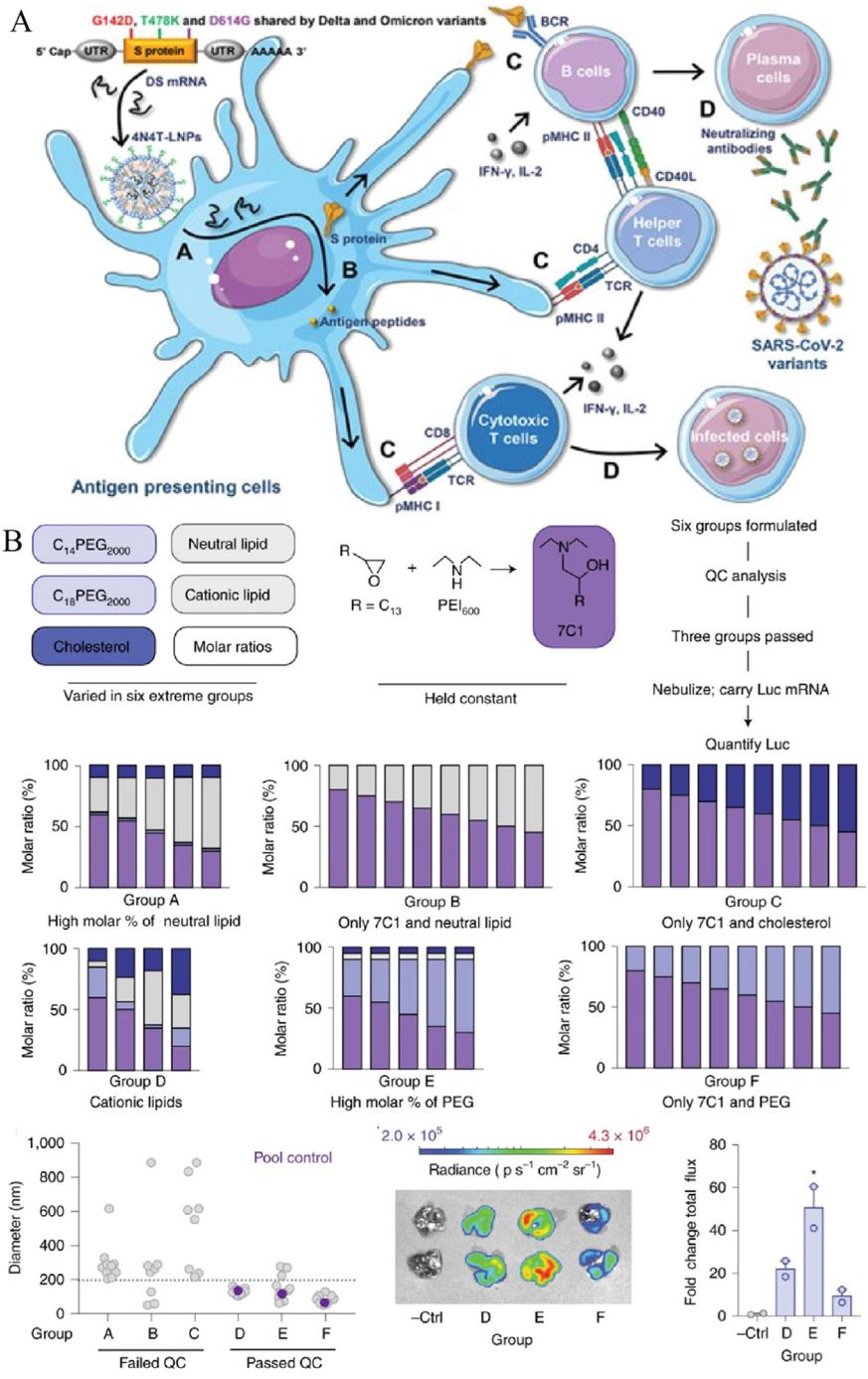


Fig. 6 – Role and mechanism of the main components of LNP. (A) Anti-SARS-CoV-2 variant mRNA vaccine based on LNP delivery from a novel ionizable lipids [150]. Copyright 2022 John Wiley and Sons. (B) Effect of varying the ratio and type of PEG to auxiliary lipids on LNP delivery [174]. Copyright 2021 Springer Nature.

effect'', whereby the positively charged lipids absorb protons in the endosome, increasing osmotic pressure and leading to endosomal membrane disruption, thus allowing nucleic acid release into the cytoplasm [153].

To fully harness the potential of nucleic acid-based therapies, it is essential to broaden the range of available LNP-based strategies. A major challenge in the development of LNP is the identification of novel ionizable lipids, which continues to be a major bottleneck, the more commonly used lipids are shown in Table 3. Li et al. [154] develop a machine learning-driven approach to accelerate the discovery of ionizable lipids for mRNA delivery. They generate a diverse library of 584 lipids and screen their mRNA transfection efficiency. The resulting data train predictive models, which identify

promising candidates from a virtual library of 40,000 lipids. Among them, lipid 119-23 demonstrates superior transfection in muscle and immune cells. This strategy streamlines ionizable lipid identification, enhancing LNP formulation for targeted mRNA therapies [157,158].

4.1.2. *Phospholipids*

Phospholipids are critical components in LNPs, where they help stabilize structure, support membrane fluidity, and assist in nucleic acid encapsulation [159]. As key elements of natural cell membranes, phospholipids exhibit excellent biocompatibility. They form bilayer membrane structures that mimic cell membrane properties, enhancing the stability of LNPs. Through self-assembly, the lipid bilayer provides effective encapsulation and protection of nucleic acid drugs [160]. The bilayer structure of phospholipids consists of hydrophilic heads and hydrophobic tails, which imparts LNPs with membrane fluidity and flexibility, enabling them to adapt to cell membrane environments and improve cellular uptake efficiency [161]. Compared to synthetic lipids, phospholipids, as natural biomolecules, generally exhibit low toxicity and superior biocompatibility, making them suitable for long-term *in vivo* applications. The bilayer structure of phospholipids forms a barrier which protects nucleic acids from enzymatic degradation within the body [162]. Additionally, phospholipids play a crucial role in stabilizing nucleic acid encapsulation in LNPs through synergistic interactions with ionizable lipids or other components. By promoting the fusion of LNPs with cell membranes, they enhance endocytosis and subsequently increase cellular uptake efficiency. The fluidity of phospholipids also enables LNPs to accumulate near endosomal membranes, assisting other components, such as ionizable lipids, in promoting endosomal escape and the release of nucleic acids into the cytoplasm [163]. Phospholipids, in conjunction with cholesterol, contribute to the overall stability of lipid membranes. Studies have shown that LNPs incorporating phospholipids demonstrate extended circulation time and greater drug-loading capacity compared to formulations lacking phospholipids. Leveraging this characteristic, they designed a multi-tailed ionizable phospholipid featuring an ionizable amine, a phosphate group, and three hydrophobic chains. By integrating the phospholipid with different auxiliary lipids, multi-component LNPs were developed to enhance targeted organ delivery. This unique composition outperformed conventional phospholipids, achieving effective mRNA transport and CRISPR-Cas9 gene editing in the spleen, liver, and lungs following intravenous administration. These results highlight its notable *in vivo* efficacy and targeted organ selectivity [164]. For example, Cheng et al. [165] developed a class of selective organ targeting (SORT) LNPs by incorporating supplemental lipids into standard LNP formulations. The inclusion of 1,2-dioleoyl-sn–glycero-3-phosphoethanolamine (DOPE) facilitated liver targeting, sphingosine promoted lung-selective delivery, and phosphatidic acid (PA) enabled spleen targeting following intravenous administration. These formulations demonstrated effective mRNA delivery and CRISPR-Cas9 gene editing in corresponding organs, highlighting the role of lipid composition in directing *in vivo* biodistribution.

4.1.3. *Cholesterol*

Cholesterol is a key component of LNPs, strengthening stability, maintaining structural integrity, and modulating membrane fluidity. Its rigid structure strengthens the LNPs, allowing them to function efficiently during circulation throughout the body. This stability is particularly vital in environments exposed to high shear forces or elevated temperatures, as it helps maintain LNP integrity and protects the nucleic acid drugs from degradation during delivery. By stabilizing the lipid bilayer, cholesterol increases the membrane's density, making it more resistant to breakdown. This helps reduce drug leakage and prolongs the circulation time of LNPs *in vivo* [166]. Moreover, cholesterol's natural lipid composition, which closely resembles that of cell membranes, ensures excellent biocompatibility and minimal toxicity, making it an ideal candidate for LNP formulations without triggering significant immune responses [167]. During endocytosis and endosome formation, cholesterol's stabilizing effect on the LNP membrane enhances its retention within the endosome [168]. A controlled amount of cholesterol helps adjust the fluidity of the LNP membrane, allowing it to circulate efficiently *in vivo* and improve cellular uptake via membrane fusion or endocytosis, thereby enhancing delivery efficiency and targeting of LNPs [169].

Endosomal entrapment of LNPs continues to be a major challenge for efficient drug delivery. Structural analysis of cholesterol analogs indicates that integrating C-24 alkyl phytosterols into LNPs (eLNPs) improves gene transfection efficiency. Crucial determinants for sustaining high transfection performance include alkyl tail length, sterol ring flexibility, and the polarity imparted by the hydroxyl (-OH) group. Both LNPs and eLNPs likely have similar initial uptake pathways, wherein ionizable lipids protonate and bind electrostatically to endosomal membranes, facilitating nucleic acid release [170]. Jung et al. develop a novel ionizable helper cholesterol analog, 3β [L-histidinamide-carbamoyl] cholesterol (Hchol), to enhance LNPs for mRNA delivery. Replacing cholesterol with Hchol in LNPs improves endosomal escape and delivery efficiency, with pKa values of $\approx$6.03 and 6.61 in MC3- and SM102-based formulations, respectively. The Hchol-LNPs also show increased red blood cell hemolysis at acidic pH, indicating enhanced endosomal membrane destabilization. In mice, Hchol-LNPs improve mRNA delivery and induce strong SARS-CoV-2 antigen-specific antibody responses. This study highlights Hchol's potential to boost LNP-mediated mRNA delivery and therapeutic efficacy [171].

4.1.4. *Surface-modified lipids*

Surface-modified lipids (SMLs) are essential in LNPs for protection, prolonged circulation time, enhanced biocompatibility, and improved targeted delivery. PEG-modified lipids, also referred to as PEG lipids, are commonly employed for surface modification of LNPs [172]. These modified lipids form a protective layer around the LNPs, shielding them from immune system detection and clearance, which prolongs their circulation time in the bloodstream and enhances the likelihood of reaching target cells. Additionally, surface modification reduces the cationic charge of LNPs, minimizing interactions with immune cells and allowing for extended circulation rather than rapid immune clearance. By

adjusting the chain length and charge density of PEG, it is possible to optimize both the biocompatibility and stability of LNPs, thereby improving targeting efficiency. Gautam et al. explore the impact of different PEG modifications on LNPs for ocular mRNA delivery. LNP variants with positive, negative, or neutral PEG-lipids showed varying cellular uptake and distribution in the retina. Notably, a zwitterionic LNP formulation named LNPx, composed of DLin-MC3-DMA, DSPC, cholesterol and PEG-lipids bearing a phosphorylcholine (PC) headgroup, demonstrated efficient gene editing in retinal pigment epithelium (RPE) cells. The PEG-PC modification endowed LNPx with a near-neutral surface charge, which contributed to its enhanced penetration and cellular uptake in ocular tissues. These findings highlight the potential of modified LNPs for targeted genome editing and gene therapy in treating blindness [173].

The hydrophilic barrier formed by PEG on the surface of LNPs prevents serum proteins from interacting with the nanoparticles, creating a "stealth effect" that reduces immune recognition and clearance, thereby prolonges extension of LNP in the bloodstream. Lokugamage et al. [174] conducted a study to optimize LNP composition by varying chemical structures and ratios of lipids. They discovered that the ratio and type of PEG to auxiliary lipids significantly influence the stability and delivery efficiency of LNPs. By changing the molar ratio of PEG and accessory lipids, the performance of LNPs in nucleic acid delivery was improved, as shown in Fig. 6B. This optimization enabled LNPs to efficiently deliver mRNA encoding broadly neutralizing antibodies against hemagglutinin, providing mice with protection against a fatal H1N1 influenza A virus challenge. Additionally, these improved LNPs exhibited superior mRNA delivery efficiency compared to earlier optimized formulations designed for systemic administration. Moreover, attaching targeting ligands to the ends of PEG chains can enhance LNP selectivity for particular cells or tissues, enabling more precise deliveryto the target sites. For example, PEG lipids conjugated to antibody fragments enable targeted delivery to cancer cell-specific antigens, thereby improving therapeutic efficacy [175]. Ocular delivery of mRNA encapsulated in LNPs facilitates efficient gene delivery and editing [173].

4.2. *Formulation methods*

LNPs have become prominent carriers for delivering nucleic acid drugs, with ongoing advancements in their design significantly enhancing both delivery efficiency and biostability. The development of LNPs has progressed from initial explorations to systematic optimization, encompassing the following stages: traditional emulsification methods, improved encapsulation techniques, and the introduction of modern microfluidic technology [176–179]. These advancements have steadily increased the nucleic acid encapsulation efficiency, stability of LNP complexes, and targeted delivery capabilities. Formulation methods are shown as Fig. 7.

In the early phases of LNP preparation, traditional emulsification methods, such as film hydration and the reverse-phase evaporation method (REV), were widely used in lipid-nucleic acid complex studies. The film hydration method entails dissolving lipids in an organic solvent to form a uniform lipid film, but it faces limitations such as nucleic acid encapsulation inefficiency and difficulty in controlling particle size [15]. On the other hand, the REV technique involves combining lipid and aqueous phases to produce LNPs. However, this approach faces challenges due to residual organic solvents and the instability of the resulting complexes. Although these early approaches highlighted LNPs' potential as nucleic acid delivery carriers, they are less suited for clinical application due to technological limitations. The subsequent introduction of the alcohol dilution method improved the homogeneity and nucleic acid encapsulation rate of LNP complexes [180]. In this method, lipids dissolved in alcohol are rapidly mixed with a nucleic acid-containing aqueous phase, allowing the lipids to encapsulate the nucleic acids. While this approach addresses some encapsulation challenges of traditional emulsification, further optimization is required to ensure alcohol removal and enhance the biocompatibility of the final complexes. The pH-gradient encapsulation method represented another advancement by leveraging a pH difference across the LNP membrane to increase nucleic acid encapsulation efficiency [181]. Negatively charged nucleic acids are effectively drawn into the acidic core of the LNPs, greatly improving encapsulation efficiency and stability. The advent of microfluidics marked a significant modernization in LNP preparation. Microfluidic devices employ nanoscale channels to regulate the mixing dynamics of lipids and nucleic acids with precision, facilitating the production of stable LNPs with uniform particle sizes [139]. This approach enhances encapsulation efficiency and particle consistency while also enabling the simultaneous loading of different nucleic acids through tailored channel configurations, thereby improving delivery precision and adaptability. Guimaraes et al. created an LNP library containing barcoded mRNA (b-mRNA) using microfluidics, which allowed for accelerated screening of mRNA delivery systems *in vivo* [182]. Furthermore, microfluidic techniques enable scalable production, making them a promising approach for clinical-grade LNP formulations. Notably, Pfizer-BioNTech and Moderna's mRNA vaccines employ microfluidic technology to ensure efficient mRNA encapsulation and maintain delivery stability.

4.3. *Influencing factor*

The physicochemical characteristics of LNPs, such as size, surface charge, and morphology, play a crucial role in determining their biodistribution and efficiency of cellular uptake. For instance, LNPs with smaller particle sizes demonstrate enhanced intracellular penetration and uniform distribution. Microfluidic and nanoscale synthesis techniques allow for precise control over particle size, with research showing that particles with a size of 60 to 100 nm are particularly effective for liver cell delivery [183]. Surface charge also affects stability and cellular uptake efficiency; adjusting the proportion of ionizable lipids to phospholipids can improve delivery efficiency across different tissues [184]. Islam et al. designed an extended-action fostemsavir (FTR) LNP formulation aimed

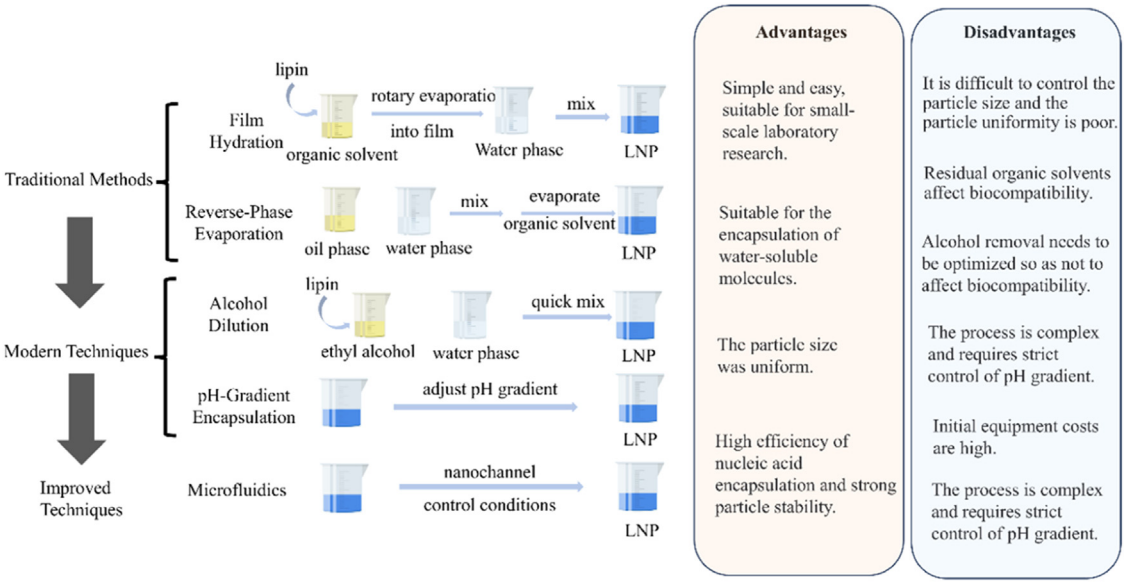


Fig. 7 – Formulation methods.

at preventing HIV-1 infection. This LNP system establishes a drug reservoir within macrophages, thereby extending the plasma half-life of FTR. Key physicochemical characteristics of the formulation, such as the incorporation of PEGylated lipids and optimized flow rate ratios, play a critical role in determining drug stability, particle size, morphology, and cellular uptake. These factors collectively contribute to enhanced antiretroviral performance. By leveraging these properties, the LNP system achieves sustained drug release, significantly improving the therapeutic efficacy of FTR against HIV-1 [185]. By co-encapsulating Cas9 mRNA and sgRNA into LNPs, the researchers successfully achieved gene editing in mouse RPE cells [139]. In recent years, scientists have investigated multiple optimized approaches to improve the efficacy of LNP complexes in nucleic acid drug delivery. This paper categorizes these strategies into four areas: enhancing nucleic acid encapsulation efficiency, promoting endosomal escape, increasing *in vivo* stability and improving targeted delivery.

4.3.1. *Nucleic acid encapsulation efficiency*

Efficient encapsulation of nucleic acids is essential to maximizing LNP delivery efficacy. To achieve high encapsulation rates, researchers have proposed several methods. A commonly employed method for improving encapsulation efficiency is pH-gradient loading. This technique generates an acidic environment within the LNP, which aids in the uptake and retention of negatively charged nucleic acids. This method is particularly advantageous for small RNA delivery, as it boosts their effectiveness by enhancing encapsulation efficiency. Another critical factor in encapsulation efficiency is the interaction between ionizable lipids and nucleic acids. Improved ionizable lipids, such as DLin-MC3-DMA, regulate protonation levels, allowing for effective nucleic acid adsorption and encapsulation. These lipids have been successfully used in mRNA vaccines and gene therapy formulations. In addition, recent advancements have been made in the development of LNP encapsulation strategies for macromolecular nucleic acid drugs, such as DNA. Renzi et al. explored a more efficient DNA delivery platform by incorporating an additional layer of DNA and plasma proteins onto the LNPs [214]. As illustrated in Fig. 8A, while unmodified LNPs achieved a 17% uptake in splenic monocytes, LNPs with the described modifications demonstrated only a 2.1% uptake, indicating enhanced cell transfection efficiency and improved immune evasion. In summary, improving encapsulation efficiency is key to enhancing LNP-based nucleic acid delivery. Techniques like pH-gradient loading and optimized ionizable lipids boost encapsulation, especially for siRNAs and mRNAs. Additionally, advancements in LNP design for DNA delivery improve transfection and immune evasion, advancing their potential in gene therapies and vaccines.

4.3.2. *Endosomal escape*

LNPs primarily enter cells through lectin- or caveolae-mediated endocytosis, facilitated by receptor-ligand interactions or non-specific adsorption [186]. Under acidic conditions, ionizable lipids within LNPs become positively charged, strengthening their interaction with cell membranes and facilitating cellular uptake. Once internalized, LNPs are trapped within endosomes, where the ionizable lipids undergo protonation, causing disruption of the endosomal membrane. This mechanism involves phenomena like the ”proton sponge effect” or lipid phase separation, facilitating the release of nucleic acids into the cytoplasm [187]. Once in the cytoplasm, the nucleic acids engage cellular machinery to induce gene silencing or activation, depending on the therapeutic application [139]. The efficiency of endosomal escape is crucial for the successful delivery of LNP-encapsulated nucleic acid drugs [187], as it ensures the release of the payload into the cytoplasm [188]. However, the

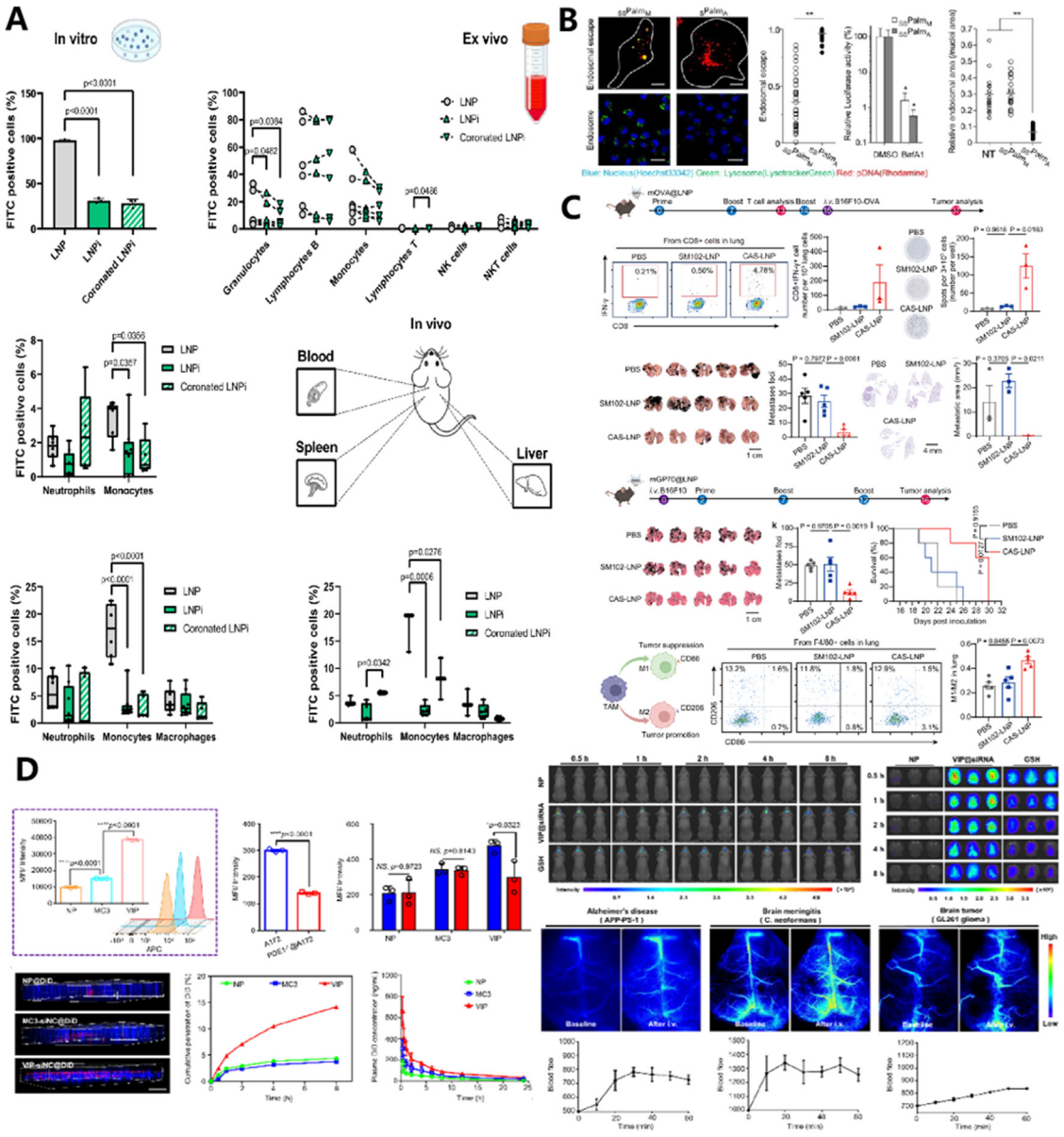


Fig. 8 – The main factors affecting the efficiency of LNP delivery of nucleic acid drugs. (A) Efficiency of immune cells to capture LNP, LNPi and coronal LNPi *in vitro* and *in vivo* [214]. (B) Influence of proton sponge effect on LNP endosomal escape efficiency [191]. Copyright Elsevier. (C) CAS-LNP as cancer vaccines [204]. Copyright 2024 Springer Nature. (D)Validation of brain targeting and related mechanism [213]. Copyright 2021 Springer Nature.

stability of endosomal membranes can act as a barrier, as LNPs may be trafficked to lysosomes, where they are vulnerable to enzymatic degradation or destruction under acidic conditions. Inefficient endosomal escape reduces therapeutic efficacy, as only a small fraction of the drug payload is released [189]. Although certain design elements, such as ionizable lipids, are incorporated to promote endosomal escape, their efficiency remains limited [190]. Furthermore, some escape-enhancing components, such as highly reactive surface lipids, may cause unintended toxicity or membrane disruption.

To improve endosomal escape, researchers often employ ionizable lipids with pH-responsive properties, such as Dlin-MC3-DMA, which protonates under the acidic conditions of the endosome [187]. This protonation destabilizes the endosomal membrane, facilitating drug release. Enhanced designs of ionizable lipids aim to improve release efficiency while minimizing toxicity. The "proton sponge effect" is another mechanism employed to enhance endosomal escape. In this strategy, weakly basic groups, such as amines, are incorporated into LNPs. These groups absorb protons from the endosome, which raises the osmotic pressure and disrupts the endosomal membrane [189]. For example, Zhang et al. developed a neutral biodegradable lipid-envelope-type nanoparticle system, incorporating the proton sponge effect to facilitate the efficient release of nucleic acid drugs. By using vitamin A-derived scaffolds and lipid components, the LNPs

were designed for optimal cellular uptake and intracellular drug release. The results demonstrated the proton sponge effect, mediated by ionizable lipids on the LNP surface, increases the endosomal pH, leading to the disruption of the endosomal membrane and subsequent release of the encapsulated nucleic acids [191], as shown in Fig. 8B. This study highlights the potential of proton sponge-based LNP systems for enhanced nucleic acid drug delivery and gene therapy applications. Additionally, the inclusion of membrane fusion enhancers promotes faster fusion between LNPs and the endosomal membrane, further facilitating the release of the drug [192]. Stimuli-responsive lipids or polymers, which alter their structure in response to changes in pH, enzymatic activity, or redox conditions, can facilitate the effective release of drugs. For example, pH-responsive polymer coatings degrade in the acidic endosomal environment, releasing their cargo. Additionally, synergistic delivery strategies, such as combining cell membrane-penetrating peptides (CPPs) or endosomal escape enhancers (*e.g.*, GALA peptides) with LNPs, have also shown promise in improving escape efficiency [193]. For example, Hołubowicz et al. [194] used the enhancement of ribonucleoprotein (RNP) delivery efficiency through CPPs in their exploration of safer and more efficient gene editing through optimized delivery of RNPs by LNPs. Further advancements include real-time monitoring of LNP intracellular pathways and endosomal escape efficiency using fluorescent labeling and live-cell imaging [195]. The high-throughput screening of extensive lipid libraries has enabled the identification of novel ionizable lipids or co-lipids that offer improved escape efficiency and lower toxicity [196]. In summary, optimizing endosomal escape is essential for enhancing the effectiveness of LNP-mediated nucleic acid delivery. Strategies such as pH-responsive ionizable lipids, the proton sponge effect, and membrane fusion enhancers help disrupt endosomal membranes and facilitate drug release. Additionally, incorporating stimuli-responsive materials and synergistic delivery methods, like CPPs, further enhance escape efficiency. Ongoing advancements in lipid design and real-time monitoring are helping to improve both the safety and effectiveness of these systems.

4.3.3. In *vivo stability*

In vivo stability and immunogenicity are critical factors influencing the therapeutic efficacy of nucleic acid drugs, optimizing these aspects enhances both safety and effectiveness. Cellular pattern recognition receptors (PRRs) can detect nucleic acid drugs, potentially activating the innate immune response and inducing the secretion of pro-inflammatory factors [197]. Furthermore, the lipid components of LNPs can activate immune cells, including macrophages and dendritic cells (DCs), potentially triggering an inflammatory response or even a cytokine storm [198]. The lipid component in PEG-modified LNPs can also provoke the release of pro-inflammatory factors. For example, highly active ionized lipids may damage hepatocyte membranes, cause mitochondrial dysfunction or apoptosis, and contribute to liver inflammation or toxicity. Some PEG-lipids may be difficult to fully degrade, leading to prolonged accumulation and potential toxic reactions or interference with hepatic metabolic functions. High doses of LNPs may also provoke inflammatory responses or impair the function of the spleen, lungs, or kidneys due to systemic distribution [199].

Recently, several strategies have been suggested by researchers to enhance the stability and biocompatibility of LNP delivery systems [200]. For example, chemical modification of nucleic acid drugs—such as the use of pseudouridine and 5-methylcytidine in place of natural bases in mRNA—can reduce their immunogenicity [201]. The use of closed-loop RNA (circRNA) or DNA-RNA hybrids can further minimize immune recognition. Employing lipid materials with low immunogenicity or natural origins, such as cholesterol derivatives, can reduce immune activation. The use of biodegradable ionized lipids, such as ester-bonded ionized lipids, can help minimize long-term cumulative toxicity. PEG surface modification significantly improves LNP stability in circulation by reducing protein adsorption and nonspecific clearance. This approach has been widely implemented in the preparation of LNP delivered drugs, such as in the COVID-19 mRNA vaccines [202]. The use of biocompatible materials and controlled degradation rates further supports *in vivo* stability while minimizing immune system activation [174]. Recently, novel lipid materials have been developed to reduce LNP immunogenicity, ensuring safety for long-term therapies [136]. Accordingly, the newly developed lipid materials can be designed with low immunogenicity to avoid the recognition and clearance of LNP by immune cells or the immune recognition of LNP can be significantly reduced by optimizing its molecular structure and hydrophilic/hydrophobic balance. These optimization strategies collectively enhance LNP performance as nucleic acid delivery carriers, advancing their potential in clinical applications [203]. For example, poor delivery resulting from the decomposition and aggregation of LNPs during nebulization poses a significant challenge. To solve this problem, Liu et al. introduced a charge-assisted stabilization (CAS) approach to promote electrostatic repulsion among LNPs. To investigate whether the enhanced stability of CAS-LNPs during atomization resulted from improved individual nanoparticle stability or the induced electrostatic repulsion, the researchers employed liquid-phase atomic force microscopy. This technique was utilized to assess and compare the mechanical properties of CAS-LNPs with those of SM102-LNPs. The results showed that the Young's modulus and the maximum force required to break individual CAS-LNPs and SM102-LNPs were nearly identical, indicating that the negatively charged peptide-lipid conjugate (DSSC-DOPE) did not significantly alter the mechanical stability of individual LNPs. Therefore, the inclusion of DSSC-DOPE did not enhance the mechanical stability of the LNPs themselves but rather increased electrostatic repulsion between the particles, thereby preventing aggregation during nebulization. To evaluate the therapeutic potential of CAS-LNPs, the researchers engineered an mRNA sequence encoding the B16F10 tumor antigen glycoprotein 70 (GP70). This mRNA (mGP70) was encapsulated within either CAS-LNPs or SM102-LNPs and delivered intravenously to mice bearing B16F10 tumors. Additionally, the LNPs were administered via inhalation on Day 2, 7 and 12 following tumor inoculation to further assess their efficacy. As shown in Fig. 8C, the results demonstrated that inhaled CAS-LNPs acted as an

effective therapeutic vaccine, promoting pro-inflammatory macrophage activation and significantly inhibiting tumor metastasis [204].

4.3.4. *Targeted delivery*

Non-specific endocytosis often occurs during LNP-mediated drug delivery, with preferential uptake by non-target liver cells [205]. This reduces access to targeting cells and can lead to premature degradation of nucleic acids. Inefficient endocytosis can result from weak interactions between LNPs and cell membranes (*e.g.*, due to hydrophobic or electrostatic repulsion), insufficient receptor availability on target cells, or poorly designed ligands [206]. Additionally, to prevent enzymatic degradation and premature aggregation, stabilizing components like PEG lipids are integrated into LNP formulations [207]. Adsorption of serum proteins, such as apolipoproteins, onto the LNP surface forms a "protein corona,'' influencing biodistribution and receptor recognition (*e.g.*, LDL receptors binding ApoE) [208]. LNPs naturally accumulate in tissues exhibiting enhanced permeability and retention (EPR) effects, such as liver sinusoids and tumors [209]. By optimizing particle size (50–100 nm) and surface charge, researchers have achieved prolonged circulation time and enhanced extravasation into target tissues. Modifying the surface charge of LNPs to a weakly negative or neutral state reduces nonspecific adsorption and enhances target cell binding. Additionally, optimizing the hydrophobic–hydrophilic balance of LNPs can improve their stability and membrane interactions. Controlling particle size and shape is another critical strategy; for example, LNPs in the range of 50–100 nm in size are ideal for liver sinusoidal pores and are efficiently internalized by hepatocytes. Furthermore, altering the LNP shape (*e.g.*, from spherical to oval) can optimize endocytosis pathways [210].

Another approach involves modifying the LNP surface with some specific ligands, such as N-acetylgalactosaminogalactan (GalNAc). These ligands selectively bind to receptors on the surface of hepatocytes, such as asialoglycoprotein receptors (ASGPR), thereby enhancing the efficiency of liver cell uptake. The specificity of LNP delivery can also be enhanced by incorporating novel ligands, including short peptides and antibody fragments. For instance, one researcher developed a novel LNP system designed to target the shikimoylated mannose receptor, aiming to improve antigen presentation to DCs. This system efficiently delivers mRNA to DCs, enhancing their ability to present antigens [211]. Ligand density also affects binding specificity and delivery efficacy; an increase in surface ligand density can strengthen receptor binding and improve delivery efficiency [212]. Bian et al. develop a vinpocetine-derived ionizable-lipidoid nanoparticle system, inspired by vinpocetine's ability to regulate cerebral blood flow, the system efficiently crosses the blood-brain barrier (BBB), exhibits high gene-loading capacity, and promotes endosomal escape, as shown in Fig. 8D The nanoparticles demonstrate minimal immunogenicity and potent brain-protective effects, with promising potential for synergistic treatment of brain disorders, as shown in APP/PS1 mice. This self-enhanced delivery system addresses key challenges in drug targeting and brain biodistribution [213].

5. Application of LNPs for nucleic acid delivery in disease treatment

5.1. *Hepatic targeting*

LNPs represent a highly efficient delivery platform for nucleic acid-based therapeutics targeting the liver, owing to the unique physiology of hepatic tissue and the biochemical adaptability of LNPs [215]. The liver's fenestrated sinusoidal endothelium, with pores approximately 100–150 nm in diameter, allows LNPs to extravasate efficiently into the space of Disse [216]. Kupffer cells, liver-resident macrophages, rapidly engulf LNPs via phagocytosis, while hepatocytes internalize LNPs predominantly through clathrin-mediated endocytosis, often enhanced by opsonins or GalNAc-mediated interactions with the ASGPR [217]. Ionizable lipids within LNPs enable endosomal escape, ensuring cytoplasmic release and effective therapeutic action. Recent progress in hepatic targeting includes the clinical approval of Patisiran, an LNP-delivered siRNA drug for hereditary transthyretin amyloidosis, which inhibits liver-derived transthyretin protein expression [218]. Additionally, LNPs have been employed for the delivery of mRNA to produce therapeutic proteins, with applications extending to liver disorders such as alpha-1 antitrypsin deficiency [219]. Hepatic LNP delivery systems have also facilitated siRNA-based therapies for metabolic and viral diseases, including hepatitis B and non-alcoholic steatohepatitis (NASH) [220]. Zhang et al. [221] developed an mRNA-based liver-targeted antibody therapy to address metabolic dysfunction-associated steatohepatitis (MASH) and liver fibrosis, utilizing a specialized LNP delivery system (Fig. 9B). By encapsulating mRNA encoding for IL-11 single-chain variable fragment (scFv), the targeted AA3G LNP (referred to as mIL11-scFv@AA3G) was able to specifically accumulate in the liver, where it secreted IL-11 scFv to neutralize the excess IL-11 in the hepatic environment (Fig. 9D). This effectively inhibited the IL-11 signaling pathway in both hepatocytes and hepatic stellate cells. In a fibrotic MASH model, mIL11-scFv@AA3G significantly alleviated steatosis and resolved liver fibrosis and inflammation. These results highlight the potential of this multifunctional LNP platform to target specific hepatocyte subtypes for the sustained expression of therapeutic antibodies, offering a promising approach for treating MASH and liver fibrosis. The mRNA-based liver-targeted antibody therapeutics developed for MASH hold great potential and could be expanded for the treatment of various other diseases. Chen et al. [222] created a liver-targeted LNP that delivered siRNA targeting the type I TGF-β receptor (TGFβRI) mRNA, demonstrating therapeutic efficacy against liver fibrosis in mice. Treatment with LNP-siTGFβRI resulted in a significant, dose-dependent reduction in the density of fibrotic areas, without inducing systemic toxicity in major organs (Fig. 9A). The strategy also demonstrated efficacy in reducing granulomatous lesions associated with schistosomiasis-induced liver fibrosis, without any observable side effects. Berti et al. [223] develop LNP formulations for co-delivery of mRNA and dexamethasone (DX) to enhance protein replacement therapy while mitigating inflammation.

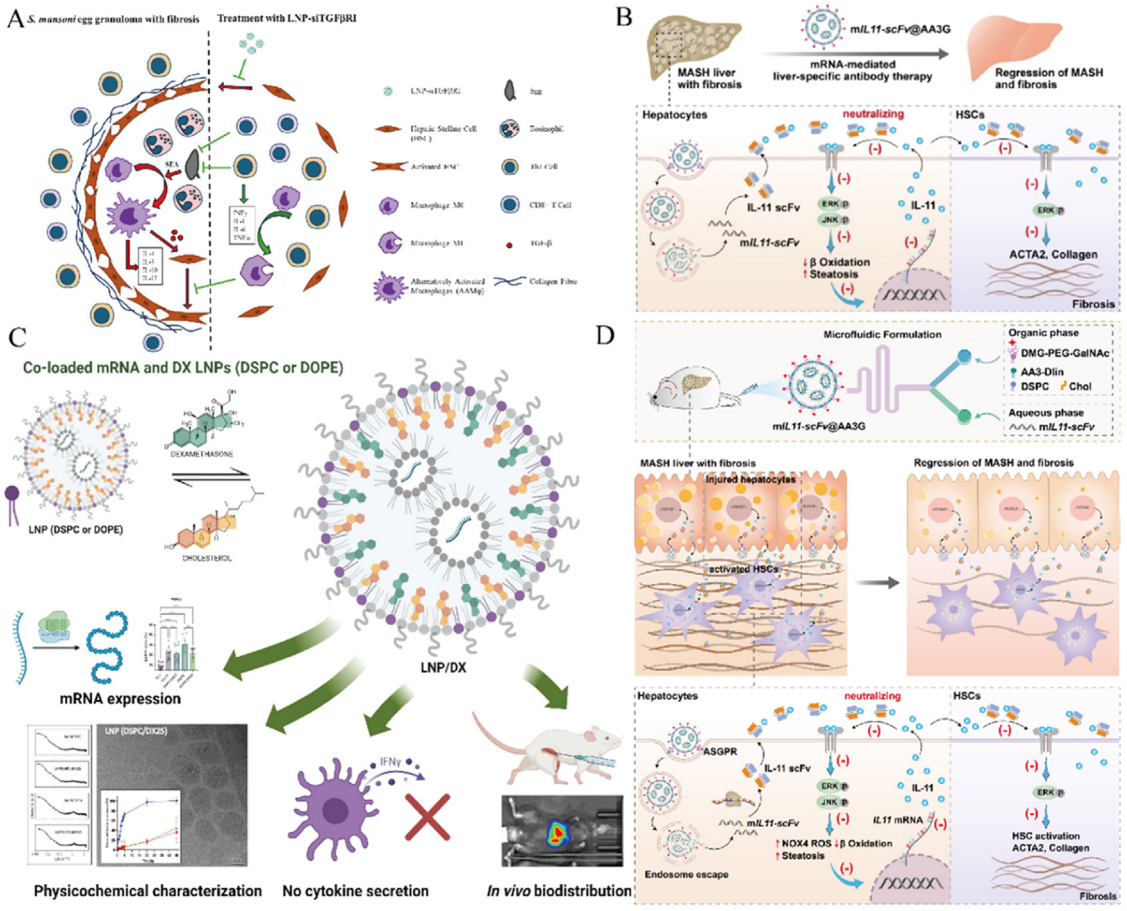


Fig. 9 – Application of LNP to deliver nucleic acid drugs for the treatment of liver diseases. (A) Use of LNP-encapsulated TGFβRI-siRNA drug for the treatment of schistosomiasis-induced liver fibrosis [222]. Copyright 2024 Public Library of Science; (B) LNP-encapsulated mRNA drugs mediate the progression of steatohepatitis and liver fibrosis [221]. Copyright 2024 American Chemical Society; (C) Encapsulation of dexamethasone into mRNA LNPs for the development of liver-targeted anti-inflammatory therapies [223]. Copyright 2024 Multidisciplinary Digital Publishing Institute; (D) Schematic representation of liver-targeted antibody therapy mediated by mIL11-scFv, utilizing AA3G LNPs, for the treatment of MASH and hepatic fibrosis [221]. Copyright 2024 American Chemical Society.

By replacing 25% of cholesterol with DX, two LNP variants incorporating either DSPC or DOPE as helper lipids were created. These LNPs demonstrated high stability, efficient mRNA encapsulation, and effective transfection across multiple cell types. DX incorporation reduced cytokine release while preserving mRNA expression (Fig. 9C). *In vivo* studies confirmed liver-targeted biodistribution after intramuscular injection. This strategy offers an approach for treating liver-related metabolic diseases with reduced inflammatory side effects.

LNPs enable efficient liver-targeted delivery due to the liver's unique physiology and LNPs' adaptability. Recent advances encompass both approved therapies—such as Patisiran for the treatment of hereditary transthyretin amyloidosis—and experimental delivery systems targeting metabolic and fibrotic diseases [21,224]. Recent studies demonstrated LNPs delivering mRNA or siRNA to treat MASH, fibrosis and liver disorders, achieving sustained therapeutic effects and reduced inflammation, highlighting their potential as versatile liver-targeted platforms [225–227]. Clinical trials investigating the use of RNA-based therapeutics for liver diseases are summarized in Table 4.

5.2. *Pulmonary delivery*

The lungs, as critical respiratory organs responsible for gas exchange, are directly exposed to external air. Their complex structure and extensive vascular network make them particularly susceptible to pathogens, pollutants, and allergens [228]. The lung's extensive capillary network and specialized epithelial cell structure make it a target for both systemic and localized drug delivery, as drugs can readily diffuse upon reaching lung tissues [229]. However, effective treatment for lung diseases is often constrained by challenges such as uneven drug distribution, rapid clearance, and limited targeting efficiency. These challenges have driven the development of nanocarriers, positioning nanotechnology particularly LNPs, as a valuable approach for lung drug delivery [174]. LNPs protect nucleic acid drugs from rapid degradation by encapsulating them.

Table 4 – Clinical trials for treatment of liver diseases using RNA-therapeutics.

Brand name	Delivery system	Disease	Status
APOC-III-L-Rx	Conjugate (GalNAc)	Elevated triglycerides	pH 1 completed
IONIS ANGPTL3-LRx	/	Elevated triglycerides/Familial hypercholesteremia	pH 1 completed
IONIS-TTR-Rx (Inotersen)	Naked (modified)	FAP	pH 2 completed
ALN-TTR01	LNP	Transthyretin mediated Amyloidosis (ATTR)	pH 1 completed
ND-L02-s0201	LNP	Hepatic fibrosis	pH 2 completed
MRT5201	LNP	Ornithine Transcarbamylase (OTC) deficiency	pH 1/2 withdrawn
mRNA-3704	LNP	Isolated methylmalonic acidemia (MMA)	pH 1/2 withdrawn
mRNA-3927	LNP	Propionic Acidemia	pH 1/2 Active

Additionally, the properties of LNPs can be tailored to suit the unique physiological environment of the lungs, thereby improving delivery efficiency and minimizing nonspecific uptake [162]. Mechanistically, two primary strategies are employed to deliver nucleic acid drugs to the lungs via LNPs: intravenous injection, which leverages the lung's capillary network for distribution, and aerosolized inhalation, which facilitates direct deposition in the lung mucosa and epithelial cells [230]. The former enables broad systemic distribution, while the latter achieves localized delivery and increased drug concentration within the lungs. This LNP-based delivery strategy effectively reduces nucleic acid drug degradation, enhances lung-specific targeting, and improves cellular uptake efficiency [231]. Qiu et al. [231] engineered N-series LNPs featuring amide-bonded tails to achieve selective mRNA delivery to the lungs, addressing the liver-centric targeting limitations of conventional LNPs. Through protein corona analysis, the researchers identified unique plasma proteins that may play a role in organ-specific targeting, such as apolipoprotein E (ApoE) for liver targeting, clusterin for kidney accumulation, and vitronectin for lung tropism. By altering the headgroup structure of the LNPs—specifically by incorporating phosphocholine or dimethylamino headgroups—they demonstrated the ability to target distinct pulmonary cell populations. In a preclinical model of lymphangioleiomyomatosis (LAM), N-series LNPs effectively delivered Tsc2 mRNA, restoring TSC2 protein expression and markedly reducing tumor growth. This work underscores the potential of lung-targeting LNPs as a promising mRNA-based therapeutic strategy for LAM and other respiratory disorders. Zeng et al. [232] introduced a cationic lipid pair (CLP) strategy that combines liver-targeted ionizable lipids with their derived quaternary lipids to enhance the hepatic-pulmonary affinity of four-component LNPs for efficient *in vivo* mRNA delivery. Through structure-activity relationship studies, they found that the optimal CLP design for improving lung-targeted mRNA delivery involved using ionizable lipids such as 5A2-SC8 and its derivatives. These lipids, originally developed for hepatic targeting, unexpectedly facilitated efficient pulmonary delivery and demonstrated superior mRNA transfection efficiency in lung tissues. The CLP approach was also shown to be adaptable to clinically used ionizable lipids, such as SM-102 and ALC-0315, enabling the development of lung-targeted LNP delivery systems. Additionally, the study confirmed that CLP-based LNPs are safe and efficient for mRNA transfection in both lung endothelial and epithelial cells as shown in Fig. 10A. This work presents a robust CLP strategy that shifts the delivery preference of LNPs from the liver to the lungs, offering significant potential to expand the therapeutic applications of mRNA-based treatments. Recently, many advancements have been made in utilizing LNPs for delivering nucleic acids for lung diseases [233–234]. For instance, non-small cell lung cancer (NSCLC) is commonly associated with KRAS mutations in 25%–30% of patients [235]. Cina et al. developed a novel siRNA-based LNP formulation, NBF-006, targeting glutathione S-transferase pi (GSTP), a critical regulator of oncogenic RAS signaling in KRAS-mutant NSCLC. The LNP composition includes ionizable lipids, phospholipids, cholesterol, and PEG-lipids—components optimized for efficient siRNA encapsulation and systemic delivery. Designed for intravenous administration, the LNPs exhibit physicochemical properties such as a particle size of ~80–100 nm and near-neutral surface charge, facilitating passive accumulation in lung tissues and tumor microenvironments via the EPR effect. *In vivo* studies demonstrated that NBF-006 selectively accumulated in the lungs and tumor sites, while exhibiting minimal hepatic distribution. In both subcutaneous and *in situ* lung tumor models, NBF-006 achieved dose-dependent tumor growth inhibition and significantly prolonged survival, underscoring its therapeutic potential and organ-targeting capability through physicochemical optimization strategies [236]. In another study, Zimmermann et al. performed dry powder lung administration with the help of DLin-MC3-DMA-containing LNP encapsulated siRNA and the result showed 50% silencing of the house keeping gene GAPDH was exhibited in lung sections [237]. This also demonstrates the great potential of LNP to deliver nucleic acid drugs in lung diseases. In summary, LNPs represent a promising strategy for delivering nucleic acid-based therapies for lung diseases. Recent advancements, such as the development of lung-targeted LNP formulations and surface functionalization strategies, have significantly improved the specificity and efficacy of drug delivery. Preclinical studies in diseases like NSCLC demonstrate the potential of LNPs to overcome the challenges of lung drug delivery, providing a strong foundation for future clinical applications.

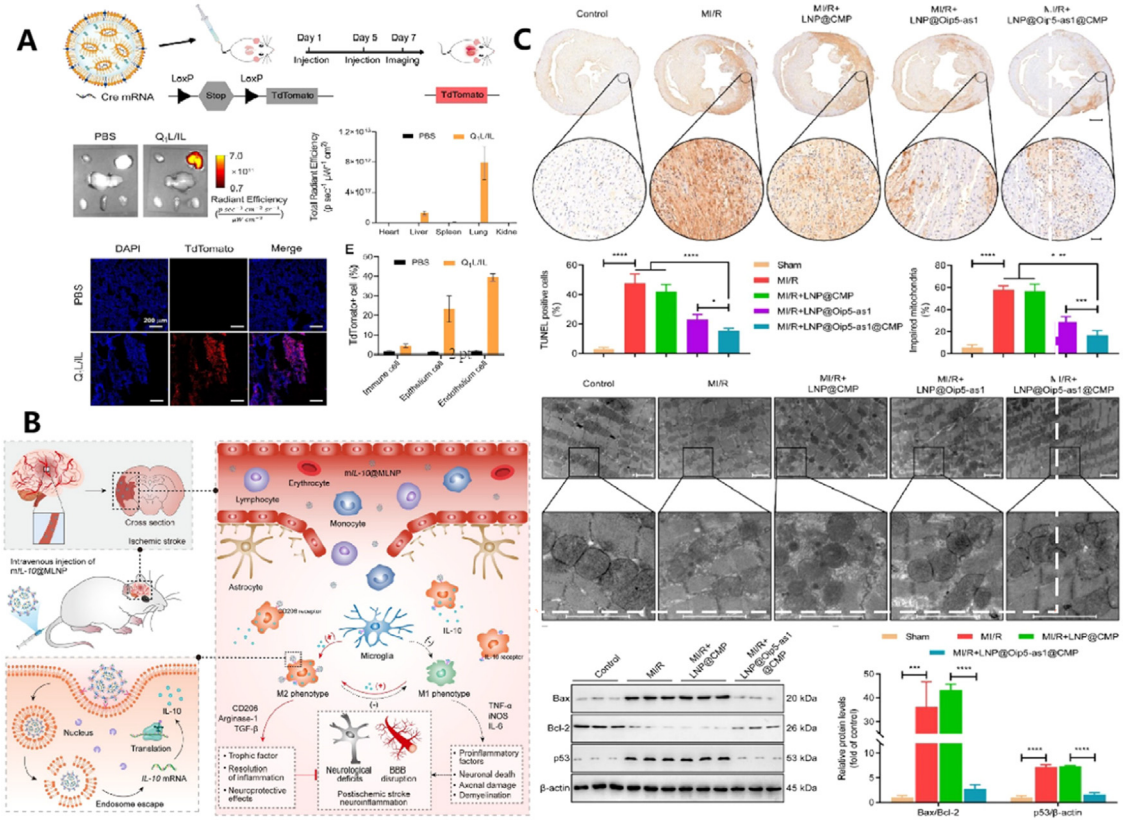


Fig. 10 – Main application organ for LNP delivery of nucleic acid drugs. (A) CLP strategy to facilitate efficient lung-targeted mRNA-LNP delivery [232]. Copyright 2024 American Chemical Society; (B) mIL-10@MLNPs cross the BBB and selectively target M2-polarised microglia located in ischaemic brain regions [242]. Copyright 2024 American Chemical Society; (C) LNP@Oip5-as1@CMP protects the heart by inhibiting the p53 signaling pathway [258]. Copyright 2024 American Chemical Society.

5.3. *CNS delivery*

Treating central nervous system (CNS) disorders presents numerous challenges, primarily because the BBB restricts the entry of most macromolecular drugs, including nucleic acids, into the brain [238,239]. Additionally, unique CNS clearance and metabolic mechanisms further limit drug retention and efficacy. Consequently, developing an effective carrier system to overcome these barriers is a central focus in therapeutic development [240]. Encapsulating nucleic acid drugs in LNPs helps prevent their rapid degradation *in vivo*, while modifications to LNP surface properties enable targeted delivery to the CNS [241]. Gao et al. develop an M2 microglia-targeting lipid nanoparticle (MLNP) system for selective mRNA delivery to ischemic brain regions, as shown in Fig. 10B This approach delivers IL-10-encoding mRNA (mIL-10@MLNPs) to promote microglial M2 polarization, creating a positive feedback loop that enhances neuroprotection. In ischemic stroke models, intravenous administration of mIL-10@MLNPs reduces neuroinflammation, restores BBB integrity, and prevents neuronal apoptosis, leading to improved sensorimotor and cognitive outcomes. The therapy remains effective up to 72 h post-stroke, highlighting its potential as a targeted mRNA-based intervention for stroke and related neurological disorders [242]. Notably, LNP surfaces can be functionalized with specific ligands, such as transferrin or LF, which can bind to receptors on the BBB and facilitate translocation. For certain CNS diseases, LNPs can be administered intravenously or intranasally, allowing non-invasive delivery across the BBB [243,244]. Khare et al. modified LNPs by incorporating ionic liquids (ILs) to minimize plasma protein adsorption and potentially enhance the accumulation of LNPs in challenging CNS targets, including brain endothelial cells (BEC) and neurons. The team employed two strategies to re-engineer the LNPs with trans-2-hexenoate choline IL: the first involved optimizing the IL encapsulation process within a standard LNP formulation, while the second substituted the PEG-lipid component of the standard formulation with IL. Both IL-encapsulated and IL-adopted LNPs exhibited colloidal stability and retained a morphology similar to that of conventional LNPs. Compared to standard LNPs, the IL-encapsulated LNPs showed enhanced uptake by mouse BECs and neurons, alongside reduced plasma protein adsorption *in vivo*. These results underscore the potential of using highly customizable biomaterials like IL to re-engineer clinically approved LNP system. The study also highlights the crucial role of LNP surface properties in influencing their affinity and uptake by hard-to-target cell types [241].

Overall, LNPs provide considerable advantages in protecting nucleic acid drugs from degradation and achieving targeted delivery to the brain through tailored modifications [245–246]. In one study, researchers used LNPs as carriers to

deliver miRNAs that inhibit genes implicated in Alzheimer's disease [247]. By modifying LNP surfaces with specific ligands such as transferrin and RVG peptide, they enhanced BBB penetration and achieved targeted delivery via intravenous injection. The results showed that LNP-loaded miRNAs successfully crossed the BBB, achieving efficient brain distribution in a mouse model. The delivered miRNAs specifically regulated genes involved in β-amyloid production, significantly reducing β-amyloid plaque accumulation in the brain. These findings indicate that LNP-delivered miRNAs can remain stable *in vivo*, penetrate the CNS, and effectively inhibit Alzheimer's disease pathology, highlighting a strategy for nucleic acid drug-based Alzheimer's therapy. In another study, scientists developed an LNP-based strategy to deliver siRNA for Parkinson's disease gene therapy [248]. This approach involved conjugating LNPs with ligands targeting dopamine neurons, enabling siRNA delivery to the lesion site via an intranasal route [249]. The study demonstrated that intranasally administered LNPs successfully crossed the BBB and localized to specific brain regions. In a mouse model, the siRNA successfully inhibited the expression of α-synuclein, whose overexpression is a key characteristic of Parkinson's disease pathology. The LNPs were composed of IL (DLin-MC3-DMA), DSPC, cholesterol and PEG-lipid, and were surface-functionalized with RVG peptide, enabling targeted delivery to neurons. Additionally, the treated mice demonstrated notable improvements in motor function, indicating that LNP-mediated siRNA delivery holds promise as a therapeutic approach and offers new possibilities for gene therapy in Parkinson's disease [250]. In summary, LNPs represent a promising strategy for overcoming the BBB and delivering nucleic acid-based therapeutics to the CNS. By modifying the surface properties of LNPs with specific ligands, their ability to cross the BBB and target specific CNS regions can be enhanced. Studies have demonstrated the potential of LNPs in treating CNS disorders by effectively delivering mRNA, miRNAs and siRNAs to relevant brain regions, leading to improved therapeutic outcomes. These findings underscore the versatility and effectiveness of LNP-based drug delivery platforms in addressing CNS-related diseases.

5.4. *Cardiovascular delivery*

Cardiovascular diseases involve damage to blood vessels in the heart and brain, leading to impaired blood flow, hypoxia and tissue injury [251]. These diseases progressively worsen due to endothelial injury and the accumulation of lipids and inflammatory cells within blood vessels, potentially resulting in irreversible damage to heart or brain tissue [252]. In the context of cardiovascular and cerebrovascular diseases, LNPs offer promising potential for targeted drug delivery. By modifying LNP surface structure (*e.g.*, incorporating cardiovascular-specific ligands) and optimizing physicochemical properties, LNPs can be directed to target tissues [253]. Administered intravenously, LNPs circulate in the bloodstream and can bind to receptors on vascular endothelial cells to achieve targeted delivery [254,255]. For example, in one study, researchers developed an LNP-based vector for miRNA delivery to modulate inflammation following myocardial infarction (MI) [252]. The methodology involved surface modification of LNPs with cardiac-targeting ligands, followed by intravenous injection into a mouse model of MI. Results showed that the LNPs successfully targeted damaged cardiomyocytes, where miRNAs effectively regulated inflammatory gene expression associated with MI, thereby reducing cardiac inflammation. This research demonstrated that LNP-delivered miRNAs are capable of penetrating cardiac tissue and modulating gene expression associated with pathological changes, presenting a novel approach for gene therapy in MI [252]. Additionally, another study explored the use of LNPs for siRNA delivery aimed at inhibiting the progression of atherosclerosis [256]. This method included the conjugation of vascular endothelial cell-targeting ligands to the LNP surface, followed by tail vein injection in a mouse model of atherosclerosis. Results indicated that the siRNA-loaded LNPs entered the arterial wall, specifically targeted and inhibited inflammatory molecules linked to atherosclerosis, reduced plaque formation, and slowed disease progression. Furthermore, the LNP delivery system minimized systemic toxicity and enhanced the safety profile of nucleic acid-based treatment [257].

In recent years, many researchers have evaluated the ability of LNP to deliver nucleic acid drugs for cardiovascular and cerebrovascular diseases. Niu et al. [258] investigated the therapeutic effects and safety of an LNP system co-loaded with Oip5-as1 and a cardiomyocyte-specific binding peptide (LNP@Oip5-as1@CMP) in a mouse model of myocardial ischemia/reperfusion (MI/R) injury. Their findings demonstrated the successful synthesis of an LNP formulation designed to target cardiomyocytes and facilitate efficient delivery of Oip5-as1. Treatment with LNP@Oip5-as1@CMP notably decreased mitochondrial apoptosis in HL-1 cells subjected to hypoxia/reoxygenation (H/R) injury. When administered intravenously, LNP@Oip5-as1@CMP significantly reduced the size of MI and enhanced cardiac function in the MI/R mouse model, as shown in Fig. 10C. Importantly, no significant organ damage was observed following LNP@Oip5-as1@CMP administration. This study underscores the potential of LNP-mediated Oip5-as1 delivery as a therapeutic strategy for mitigating MI/R injury, paving the way for new treatments for cardiovascular diseases, especially those based on lncRNA. In summary, LNPs demonstrate significant potential for targeted drug delivery in cardiovascular and cerebrovascular diseases. By modifying LNP surface properties, they can selectively target affected areas such as the heart or blood vessels, enabling efficient delivery of therapeutic nucleic acids like miRNAs, siRNAs, and modRNA. Studies have shown that LNPs can modulate inflammation, reduce plaque formation, and enhance tissue repair in models of MI and atherosclerosis, while also minimizing systemic toxicity. These findings highlight LNPs as a promising platform for targeted treatments in cardiovascular and cerebrovascular disorders.

5.5. *Immune system delivery*

Immune system disorders are characterized by an attack by the immune system on its own tissues, leading to persistent autologous tissue damage and impaired function.

These diseases are chronic, progressive, long-lasting, and difficult to treat, and treatments are often associated with immunosuppressive side effects. As a result, identifying safe and effective targeted therapeutic approaches has become a key area of focus in the research of immune system diseases [259].

LNPs as delivery carriers for nucleic acid drugs, have low toxicity, high stability, excellent intracellular delivery ability and drug protection properties, which make them show great potential for drug delivery in immune system diseases. By modifying the LNPs, such as by attaching ligands specific to immune cells, targeted delivery of nucleic acids to particular immune cells—such as macrophages or T-cells—can be achieved. This strategy enables disease-specific modulation of immune responses [136]. The delivery mechanism of LNPs in immune system diseases primarily depends on the endocytosis of target cells. The surface-modified targeting molecules on LNPs are designed to interact specifically with immune cell surfaces, providing protection against rapid degradation. When these targeting molecules bind to receptors on immune cells, the LNPs are internalized via endocytosis. Once inside, they release nucleic acid drugs in the acidic intracellular environment, effectively inhibiting or modulating the expression of genes involved in immune responses. This mode of delivery not only reduces the systemic toxicity of nucleic acid drugs, but also enhances the therapeutic effect of targeting immune cells [260]. In summary, by modifying LNP surfaces with ligands specific to immune cells, they can selectively deliver nucleic acids to immune cells, thereby modulating immune responses. Studies have highlighted the therapeutic potential of LNPs in treating diseases like multiple sclerosis and systemic lupus erythematosus [261]. These nanoparticles efficiently deliver siRNAs and miRNAs to specific inflammatory pathways, leading to a reduction in disease severity and the alleviation of associated symptoms. The findings underscore the potential of LNPs for immune system diseases, providing a safer, more effective alternative to conventional immunosuppressive treatments.

5.6. *Clinical translation*

Recent progress in the clinical application of LNP-encapsulated nucleic acid drugs has highlighted their potential in cancer immunotherapy, infectious disease prevention, and targeted treatments for rare diseases [262]. The *in vivo* mechanism of LNP-mediated nucleic acid delivery for cancer treatment is illustrated in Fig. 11. In cancer gene therapy, LNPs are employed to deliver nucleic acid drugs, targeting specific oncogenes or modulating pathways associated with cancer progression [263]. By optimizing the LNP surface structure and ligands, the study achieved efficient siRNA delivery and substantial KRAS silencing. Furthermore, the safety and efficacy of siRNA-loaded LNPs for targeting refractory cancers have been evaluated in several clinical trials. Alnylam Pharmaceuticals, for instance, has advanced an LNP-siRNA product into clinical trials to address genetic mutations in liver cancers [264].

The localized delivery of mRNA-based immunotherapies presents a promising strategy due to its capacity to generate targeted immunomodulatory proteins. These proteins help activate the immune system, enabling it to identify and eliminate cancer cells, while reducing the risk of toxicity. Hamouda et al. [265] develop a lipid-based nanoparticle system for local delivery of an mRNA mixture encoding interleukin (IL)-21, IL-7 and 4–1BB ligand (Triplet LNP) to stimulate the immune response in cancer therapy. This method markedly boosts the secretion of granzyme B and IFN-γ by $CD8^+$ T cells infiltrating tumors, resulting in the elimination of tumors and the establishment of enduring immunological memory. The effectiveness of the Triplet LNP is contingent upon the role of tumor-draining lymph nodes in facilitating the migration of $CD8^+$ T cells. In multiple tumor models in female mice, Triplet LNP outperforms immune checkpoint blockade therapies, offering improved overall survival, highlighting its promising potential for cancer treatment. Fei et al. developed a platform for selective delivery of mRNA to desired cells in tissues by combining LNP-based targeted delivery with mRNA sequence-controlled expression. Through system optimization, a three-component LNP platform was developed to enable targeted mRNA delivery to the lung, liver and spleen. By incorporating unique miRNA target sequences into the mRNA scaffold, this approach further enhances the regulation of protein translation in specific cells within the target tissues. This strategy, known as SELECT (Simplified LNP with Engineered mRNA for Cell-type Targeting), has proven effective in distinguishing mRNA expression levels between tumor and normal cells, based on the abundance of intracellular miRNAs. In a mouse model of melanoma lung metastasis, SELECT encapsulated mRNA encoding the tumor-specific cytotoxic protein human ELANE, enabling selective mRNA delivery to tumor sites and significantly inhibiting tumor growth. These findings indicate that SELECT can be a precision therapy for tumors and could also be applied to other mRNA-based treatments targeting specific cell types [266].

In the field of LNP-based vaccine research for cancer and infectious diseases, LNP-loaded mRNA, DNA, siRNA and other nucleic acid drugs are being investigated as potential vaccine systems aimed at stimulating the human immune system by delivering targeted antigens [202]. This approach holds promise for effectively preventing or treating cancer and infectious diseases. For cancer vaccines, research focuses on using LNPs to deliver mRNAs encoding tumor-associated antigens, such as neoantigens, to enhance anticancer immune responses [267–269]. Notably, companies like BioNTech and Moderna have advanced LNP-mRNA-based personalized cancer vaccines to clinical trials, showing significant immune responses, particularly in melanoma and NSCLC. Several phase I/II trials are ongoing to further assess safety and efficacy [270]. Shi et al. [271] introduce a personalized tumor vaccine using LNPs (C5 LNP) loaded with membrane protein antigens (mAg) from tumor tissue. In murine models, the vaccine triggered strong immune responses, suppressed tumor growth and extended survival. When administered in conjunction with anti-PD-1 therapy, this approach significantly elevated the rates of complete remission. Mice showed 100% resistance to tumor rechallenge, demonstrating long-term immune memory. This personalized mAg-LNP vaccine offers a cost-effective and promising

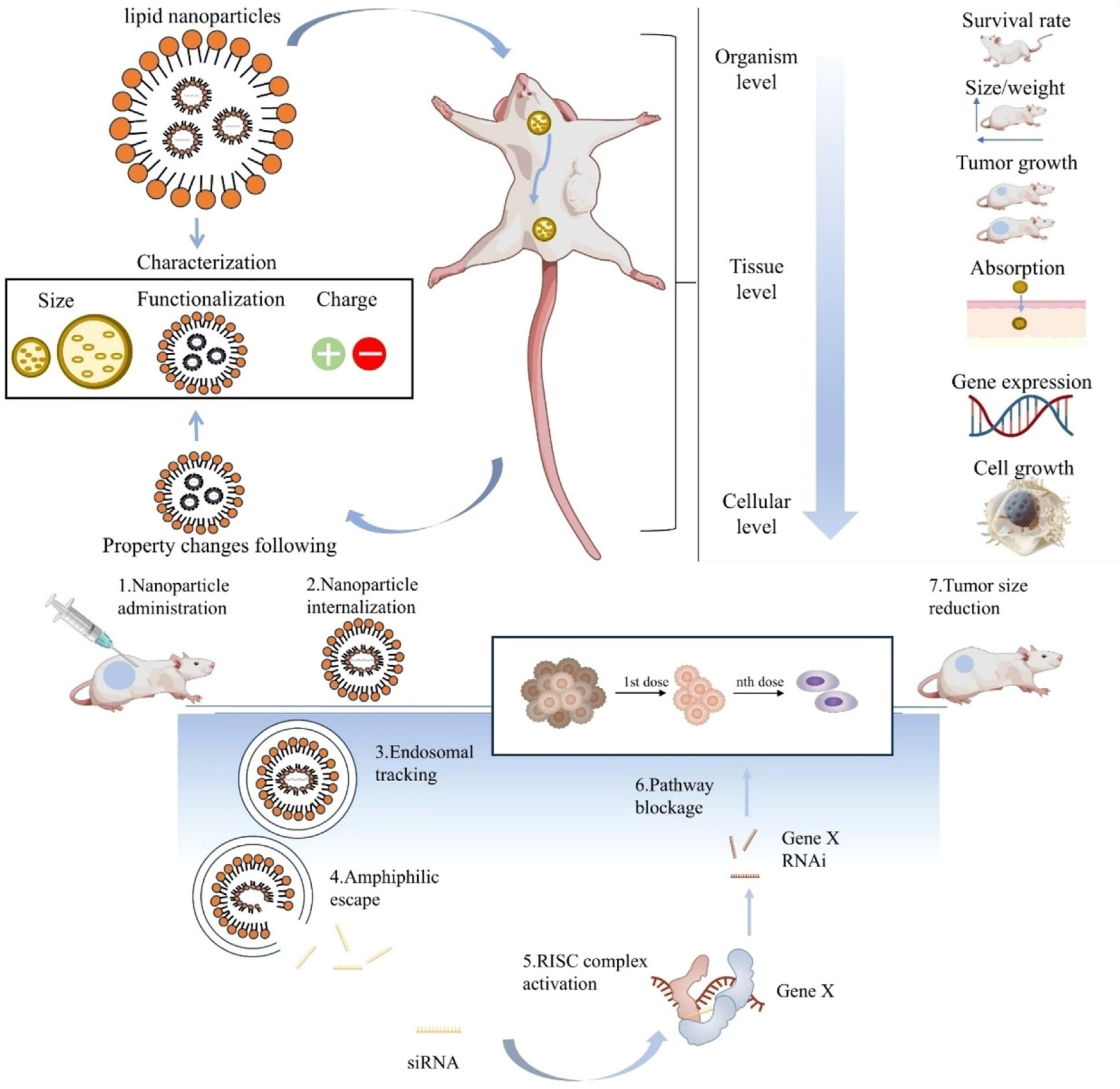


Fig. 11 – Structure of LNPs and their therapeutic process in delivering nucleic acid drugs.

approach for cancer treatment and prevention. In parallel, LNP-mRNA vaccines have achieved considerable progress in infectious disease vaccine development, especially in response to the SARS-CoV-2 pandemic. Pardi et al. demonstrated the potential of LNP-delivered mRNA in combating infectious diseases by developing an LNP-based mRNA vaccine encoding the hemagglutinin antigen of the influenza A virus [4]. Their study revealed that this vaccine effectively stimulated a robust neutralizing antibody response in mice. This pivotal work laid the groundwork for the swift creation of COVID-19 vaccines by Moderna and BioNTech, resulting in the development of mRNA-1273 (Moderna) and BNT162b2 (BioNTech/Pfizer) [2022]. These vaccines induced strong immune responses and were granted emergency use authorization worldwide, becoming the first LNP-mRNA vaccines approved for mass distribution. This marked a major achievement in the use of LNP-based nucleic acid vaccines for the control of infectious diseases [272].

In rare disease treatment, advances in LNP delivery of nucleic acid drugs have leveraged gene editing and RNAi technologies to correct the expression or functional defects of disease-causing genes [137]. Many rare diseases result from mutations in specific genes, so LNP-delivered nucleic acid drugs hold promise for targeted therapies [273–275]. Recent research has made significant progress in using LNPs to deliver gene-editing tools for rare diseases [276,277]. For example, Musunuru et al. investigated an LNP-CRISPR-Cas9 system targeting familial hypercholesterolemia (FH), a rare cardiovascular disorder. This study developed an LNP-based delivery system to deliver Cas9 and single-guide RNA (sgRNA) into liver cells, enabling the editing of the PCSK9 gene and resulting in a reduction of cholesterol levels. In a mouse model, this approach significantly lowered blood low-density lipoprotein (LDL) cholesterol, demonstrating LNP-Cas9's potential in treating FH [278]. Building on this work, Verve Therapeutics has advanced LNP-CRISPR therapies for gene editing in specific rare cardiovascular diseases, such as FH, aiming for a one-time treatment to permanently lower patients' cholesterol levels, with a phase I clinical trial currently in preparation. RNAi therapies have also shown promise for rare diseases [279]. Adams et al. explored LNP delivery of siRNA for treating familial amyloid polyneuropathy

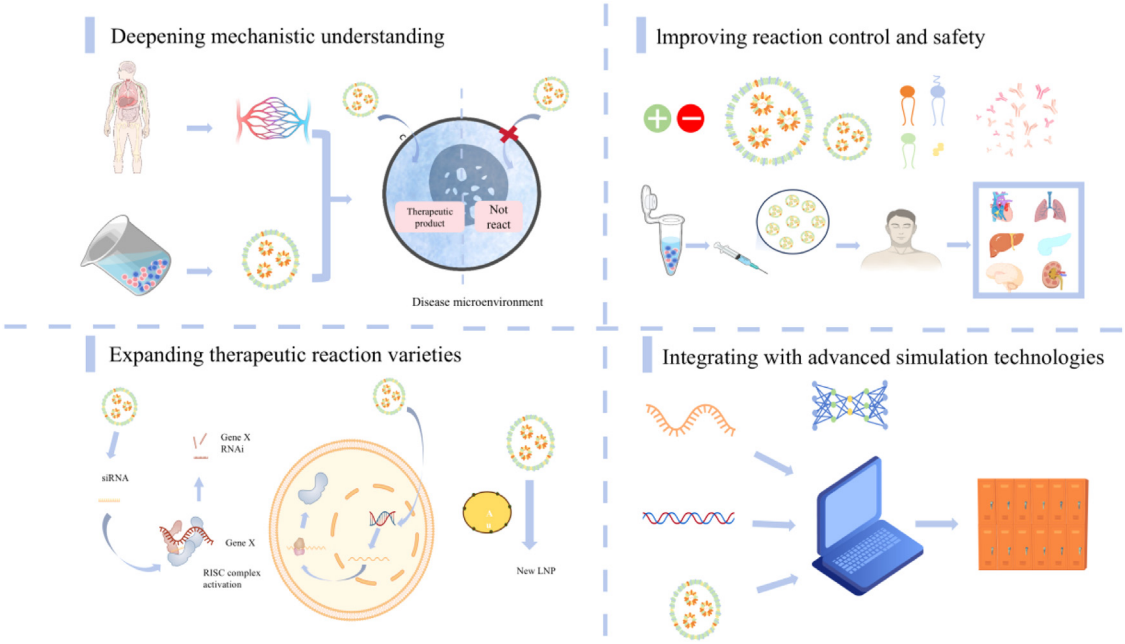


Fig. 12 – Key challenges and perspectives.

Table 5 – Pharmaceutical companies utilizing LNP technology: composition, payload, targeting, and manufacturing approaches.

Company	LNP composition	Payload type	Targeting strategy	Manufacturing technology
Moderna	SM-102 (IL), DSPC, cholesterol, DMG-PEG2000	mRNA (*e.g.*, COVID-19 vaccine)	Passive targeting to muscle tissue via intramuscular injection	Microfluidic mixing (NanoAssemblr platform)
Pfizer/BioNTech	ALC-0315 (IL), DSPC, cholesterol, ALC-0159 (PEG-lipid)	mRNA (*e.g.*, COVID-19 vaccine)	Passive targeting through intramuscular administration, local accumulation at the injection site	Microfluidic mixing with ethanol injection
Alnylam Pharmaceuticals	DLin-MC3-DMA (IL), DSPC, cholesterol, PEG-lipid	siRNA (*e.g.*, Onpattro®)	Hepatocyte targeting via ApoE-mediated endocytosis	T-junction microfluidics
Beam Therapeutics	Proprietary IL, DSPC, cholesterol, PEG-lipid	mRNA-encoded base editors and sgRNA	Active liver targeting via GalNAc-conjugated lipids	Ethanol-based microfluidics
Acuitas Therapeutics	Proprietary IL, DSPC, cholesterol, PEG-lipid	mRNA, siRNA, other nucleic acid cargos	Tissue-specific delivery design (*e.g.*, liver, spleen, lung)	Microfluidic mixing

(hATTR), a rare disease caused by TTR gene mutations. The LNPs used in this study were composed of DLin-MC3-DMA (ionizable lipid), DSPC, cholesterol, and PEG-lipid, forming a stable siRNA-encapsulated system. The LNPs achieve hepatocyte targeting primarily through ApoE-mediated endocytosis following systemic administration, which facilitates their preferential accumulation in the liver. LNP-mediated delivery of siRNA to the liver effectively suppresses the production of TTR protein, resulting in a significant decrease in the accumulation of abnormal amyloid deposits [280]. This study showed that LNP-based siRNA delivery significantly lowered TTR protein levels and alleviated neuropathy symptoms in patients. Building on these findings, Alnylam's Patisiran became the first approved RNAi therapeutic for hATTR, marking a milestone in RNAi therapy for rare diseases and paving the way for further LNP-RNAi treatments [216]. An alternative vaccine platform is known as self-amplifying RNA (saRNA). Vijayan et al. assessed the immunogenic potential of a saRNA vaccine formulated with LNP, named SMARRT. Their study found significant correlations between the levels of key analytes, including IL-15, CCL4 and CXCL10, following the administration of SMARRT. These results suggest that saRNA-based vaccines could serve as effective preventive options for respiratory syncytial virus (RSV), as they elicit strong innate, cellular, and humoral immune responses [281]. The SMARRT LNPs were

composed of ionizable lipid (SM-102), DSPC, cholesterol and PEG-lipid. These particles were administered intramuscularly and passively targeted antigen-presenting cells (APCs) at the injection site. The composition facilitated efficient saRNA encapsulation, endosomal escape, and translation, ultimately promoting robust immune activation. In summary, recent advancements in LNP-based nucleic acid delivery systems have demonstrated their broad therapeutic potential across multiple fields, including cancer immunotherapy, infectious disease prevention, and treatment of rare diseases. Clinical applications have shown promising results, with LNPs effectively inhibiting tumor growth, enhancing immune responses, and advancing personalized cancer vaccines. Furthermore, LNP-based vaccines highlight their capability in infectious disease control. In rare diseases, LNPs have facilitated gene editing and RNAi therapies, offering potential cures for genetic disorders. These findings emphasize the rising value of LNPs as a flexible and powerful platform in modern therapeutics, offering novel approaches for addressing a broad spectrum of diseases.

6. Conclusion and future perspectives

Recent developments in nucleic acid drugs have ushered in a transformative period for nucleic acid therapy, leading to increased research focus on both nucleic acid-based therapeutics and the creation of effective delivery systems [282]. To ensure delivery that is safe, efficient, and adaptable, researchers have explored a variety of different approaches, which has driven innovations in delivery materials and chemical modifications [283]. Various nucleic acid delivery carriers offer distinct advantages and limitations. For example, exosomes have excellent biocompatibility and low immunogenicity, which allows them to evade immune responses. However, their production is quite complex, they have low yields, and it is challenging to scale them up. Viral vectors are known for their high efficiency and their ability to target specific cells, which makes them ideal for sustaining long-term gene expression. However, their use is associated with risks, which include immunogenicity and the potential for insertional mutagenesis. Polymeric nanoparticles provide versatility regarding structure and function, which enables tailored release profiles and specific targeting. However, their high cytotoxicity and limited biodegradability present significant safety concerns despite these advantages. Protein-based systems enhance cellular uptake and targeting through protein interactions, but they have limited stability *in vivo*, rapid clearance, and restricted delivery efficiency. Inorganic nanoparticles are known for their structural stability, high nucleic acid loading capacity, and photothermal responsiveness and they have great multifunctional therapy and imaging application potential. However, due to their limited biodegradability and the associated toxicity risks from prolonged accumulation in the body, challenges persist.

LNPs have emerged as a promising solution for nucleic acid drug delivery. They demonstrate efficient nucleic acid encapsulation, high loading capacity, and protection against degradation. LNPs are composed of natural or synthetic lipid materials, are biocompatible, have low toxicity, and interact effectively with cellular membranes in order to deliver nucleic acids into the cytoplasm by endocytosis. In addition, LNPs can modulate immune responses, which reduces adverse immune reactions. Surface modifications enable LNPs to achieve targeted delivery, which enhances efficiency for specific tissues or cell types.

However, clinical translation of LNPs remains hindered by challenges such as *in vivo* instability, dose-dependent toxicity, and complex manufacturing processes [284,285]. Strategies such as the optimization of lipid formulations, the controlling of particle size, and the incorporation of specific targeting ligands have been suggested as means of overcoming these challenges and improving LNP efficacy [286–288]. Future LNP research in nucleic acid drug delivery should focus on the following four key areas (Fig. 12). To further illustrate the practical application and industrial adoption of LNPs, Table 5 summarizes the LNP compositions, payload types, targeting strategies, and manufacturing technologies employed by representative pharmaceutical companies.

6.1. *Deepening mechanistic understanding*

Elucidating the molecular mechanisms that underlie LNP interactions with biological systems is essential for improving delivery efficiency. Detailed studies on LNP interactions with cellular membranes, endosomal escape pathways, and immune responses would provide insights for rational design. In addition, the exploration of individual variability (*e.g.*, age, genetic background) in LNP performance could enable personalized delivery strategy development.

6.2. *Improving reaction control and safety*

Safety is still a major challenge for LNP clinical applications. The development of more precise formulation control methods for mitigating dose-dependent toxicities, particularly hepatotoxicity, is essential. Fine-tuning key parameters such as particle size, surface charge, and lipid composition, together with incorporating safer lipid components, could enhance biocompatibility. Furthermore, strategies for improving LNP stability during storage and transportation would facilitate broader application.

6.3. *Expanding therapeutic applications*

LNPs have primarily been used in mRNA vaccines and RNAi, but their potential extends far beyond these areas. Future research should explore the use of LNPs for the delivery of DNA vaccines, gene-editing tools, and nucleic acid-encoded antibodies. By combining LNPs with multifunctional materials, such as stimuli-responsive polymers or inorganic nanoparticles, their scope could be expanded, which would enable integrated therapeutic and diagnostic applications.

6.4. *Integrating with advanced technologies*

Advanced computational techniques, which include molecular dynamics simulations and AI-based algorithms, offer substantial improvements in LNP design and optimization. Virtual screening and high-throughput modeling can predict lipid formulations' performance,

reduce trial-and-error in formulation, and accelerate clinical translation. In addition, the integration of simulation technologies with multi-omics data could support the precise design of LNPs for specific applications.

In summary, although significant advancements have been made in LNP-based nucleic acid carrier development, further improvements are required to ensure their successful translation into clinical gene therapy applications. Key strategies that can be used to enhance the safety and efficiency of LNPs include the optimization of lipid formulations, the controlling of particle size, the incorporation of excipients, and the introduction of specific targeting ligands. Through iterative design, mechanistic insight, and technological integration, LNP-based delivery systems are poised to play a central role in the next generation of nucleic acid medicines.

Conflict of Interest

All authors declare they have no conflicts of interests.

CRediT authorship contribution statement

Xuan Lin: Writing – original draft. **Hongguang Xiang:** Investigation. **Jiong Wu:** Writing – original draft. **Ruixi Liao:** Supervision. **Yuhao Li:** Methodology. **Bohui Xu:** Conceptualization. **Ying Xu:** Data curation. **Yan Shen:** Writing – review & editing. **Qian Li:** Project administration. **Yu Tian:** Writing – review & editing.

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