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Navigating the intricate in-vivo journey of lipid nanoparticles tailored for the targeted delivery of RNA therapeutics: a quality-by-design approach

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Abstract

RNA therapeutics, such as mRNA, siRNA, and CRISPR–Cas9, present exciting avenues for treating diverse diseases. However, their potential is commonly hindered by vulnerability to degradation and poor cellular uptake, requiring effective delivery systems. Lipid nanoparticles (LNPs) have emerged as a leading choice for in vivo RNA delivery, offering protection against degradation, enhanced cellular uptake, and facilitation of endosomal escape. However, LNPs encounter numerous challenges for targeted RNA delivery in vivo, demanding advanced particle engineering, surface functionalization with targeting ligands, and a profound comprehension of the biological milieu in which they function. This review explores the structural and physicochemical characteristics of LNPs, in-vivo fate, and customization for RNA therapeutics. We highlight the quality-by-design (QbD) approach for targeted delivery beyond the liver, focusing on biodistribution, immunogenicity, and toxicity. In addition, we explored the current challenges and strategies associated with LNPs for in-vivo RNA delivery, such as ensuring repeated-dose efficacy, safety, and tissue-specific gene delivery. Furthermore, we provide insights into the current clinical applications in various classes of diseases and finally prospects of LNPs in RNA therapeutics.

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Graphical Abstract

Introduction

RNA therapies have emerged as superior alternatives for the rapid and efficient treatment of acute and life-threatening diseases compared with other approaches. With the potential to modulate gene expression or generate therapeutic proteins, RNA therapeutics are applicable for addressing diseases characterized by specific genetic targets. They can target intracellular proteins that are otherwise challenging to reach. Moreover, RNA therapeutics can be personalized and tailored to address patient-specific genetic abnormalities, resulting in fewer side effects. They are widely considered for numerous conditions, including infectious diseases, cancers, immune disorders, heart diseases, and neurological disorders [1]. RNA therapeutics encode proteins or facilitate DNA or RNA editing and are generally categorized into two groups: non-coding oligonucleotide RNAs (e.g. siRNAs, RNA aptamers, miRNAs, sgRNAs, and ribozymes) and macromolecular coding RNAs or mRNAs synthesized through in vitro transcription (IVT) [2]. RNA therapeutics are advancing in clinical development and application worldwide (Table 1). However, the clinical translation of RNA therapies faces significant obstacles, including instability, vulnerability to RNase degradation in the bloodstream and tissues, renal clearance, uptake by the reticuloendothelial system (RES), limited cellular uptake due to size and negative charge, endosomal trapping, off-target effects, and immunogenicity [3]. Scientists have developed strategies to overcome these challenges by appropriately selecting untranslated regions (UTRs), poly-A tail addition, capping, and nucleoside modifications [4]. In addition, diverse non-viral vectors have been devised for RNA delivery, with LNPs emerging as the primary carriers for RNA therapeutics [5–7]. LNPs efficiently encapsulate RNA molecules and deliver them to target cells. They offer substantial potential for nucleic acid delivery by protecting against nucleases, avoiding the mononuclear phagocyte system, exhibiting low immunogenicity and toxicity, facilitating cellular uptake, preventing endolysosomal degradation, minimizing non-specific interactions, and enabling easy synthesis and engineering [6]. Additionally, LNP formulation can be tuned to improve stability, transfection efficacy, and extrahepatic targeting [8, 9].

In recent years, LNPs have been successfully employed for clinical applications and trials of various RNA therapeutics, such as siRNA, mRNA, and CRISPR–Cas9, to treat diseases like cancer, infections, and genetic disorders [10]. Notably, in 2020, LNP-encapsulated mRNA vaccines of BNT162b2 and mRNA-1273, produced by Pfizer-BioNTech and Moderna respectively, were developed to provoke immune responses against the SARS-CoV-2 spike protein [11]. Furthermore, Onpattro (patisiran), the first FDA-approved RNAi drug in 2018, is an LNP-based siRNA drug targeting the transthyretin gene for treating hereditary transthyretin-mediated amyloidosis. Leqvio (inclisiran) is another LNP-based siRNA drug that targets the PCSK9 gene to lower cholesterol levels and reduce the risk of cardiovascular events, receiving approval from the European Commission in 2020. NTLA-2001, an LNP-based CRISPR–Cas9 therapeutic, was introduced as the inaugural systemic in vivo

Table 1 Approved RNA therapeutics

Category	Name	Indication	Target site	Delivery system modification	Approval year
siRNA (non-coding)	Patisiran	FAPs	TTR	LNP	2018
	Givosiran	AHP	ALAS1	GalNAc	2019
	Lumisiran	PH1	HAO1	GalNAc	2020
	Inclisiran	Hypercholesterolemia	PCSK9	GalNAc-LNP	2020
	Vutrisiran	ATTR/hATTR	TTR	GalNAc	2022
	Nedosiran	PH1	LDHA	GalXc	2023
mRNA (coding)	Tozinameran	SARS-CoV-2	Spike protein	LNP	2020
	Elasomeran	SARS-CoV-2	Spike protein	LNP	2020
	Gemcovac-19	SARS-CoV-2	Spike protein	Lipid nanoemulsion	2022
	ARCoV	SARS-CoV-2	Spike protein	LNP	2022
	CORNAPCIN®	SARS-CoV-2	Spike protein	LNP	2022
	SYS6006	SARS-CoV-2	Spike protein	LNP	2023
	ARCT-154	SARS-CoV-2	Spike protein	LNP	2023
	Daichirona	SARS-CoV-2	Spike protein	LNP	2023

application for hATTR amyloidosis treatment, advancing into clinical trials in 2020. Despite the numerous advantages of LNP-RNA therapeutics, they face challenges, including immune system interactions with LNP components and the difficulty of targeting various tissues in clinical settings due to the composition of RNA-LNPs.

This updated review focuses on LNP design and application for the growing siRNA and mRNA therapeutics. Notably, it explores the in-vivo fate of RNA-LNPs, their functionalization for targeting gene expression across various diseases via different administration routes with safety and immunogenicity concerns. The review begins with the composition and fabrication methods of RNA-LNPs, considering their structural and physicochemical attributes such as morphology, size, charge, encapsulation efficiency (EE), and stability, optimized via the quality-by-design (QbD) approach. Extensive discussions cover the in-vivo fate of RNA-LNPs, including interactions with blood components, protein corona formation, biodistribution, pharmacokinetics, systemic circulation, blood clearance, intracellular trafficking, and endosomal escape mechanisms. Furthermore, it highlights current challenges and strategies in using LNPs for in-vivo RNA delivery, such as repeated-dose efficacy, safety, immunogenicity, and LNP functionalization for tissue-specific gene delivery. Lastly, the review summarizes the current clinical applications and prospects of RNA-LNPs, encompassing protein replacement therapy, genome editing, and their use in treating various diseases such as hereditary conditions, cancers, and infectious diseases.

RNA-LNP: compositions and fabrication methods

RNA molecules

RNA therapeutics, composed of adenine, cytosine, guanine, and uracil bases linked to a phosphate backbone, function as ncRNAs such as siRNAs or miRNAs, which interfere with target mRNAs through Watson–Crick base-pairing. Additionally, they can act as coding RNAs (mRNAs) [12]. siRNAs have a well-defined structure, consisting of short (usually 20–24 base pairs) RNA duplexes with two-base overhangs in the 3′ region [13]. Additionally, IVT mRNA can be used for protein replacement therapy or immunization [14], avoiding irreversible genome changes and genetic risks associated with DNA-based therapeutics [15]. CRISPR-based genome editing can modify target RNA sequences to treat specific disorders [16]. RNA aptamers can block protein activity, similar to small-molecule inhibitors and antibodies [17]. Both coding and non-coding RNAs demonstrate considerable promise in clinical contexts across diverse medical disciplines. mRNA has attracted significant interest because of its pivotal role in mRNA-based vaccines, gene therapy, and gene editing techniques such as CRISPR–Cas9. Moreover, therapeutic mRNA provides tailored treatment avenues for genetic disorders and cancer. siRNAs have seen notable market success in gene silencing and regulation across various diseases like genetic disorders, metabolic conditions, and viral infections. Among these are drugs such as patisiran (Onpattro), givosiran (Givlaari), inclisiran (Leqvio), lumasiran (Oxlumo), and glecaprevir/pibrentasvir (Mavyret). ncRNAs function as disease biomarkers for diagnosis, prognosis, and treatment response prediction. They have also emerged as therapeutic targets in oncology, cardiology, and neurology. Collectively, both coding and ncRNAs are propelling advancements in personalized and precision medicine paradigms, reshaping clinical practices.

Chemically modified RNA backbones involve structural adjustments to RNA molecules through modifications of the phosphate backbone, ribose ring, nucleotides, and 3′- and 5′-terminals (Fig. 1A). These modifications can improve the stability, efficacy, and safety of RNA therapeutics for clinical use [18]. PS modification, which replaces one oxygen atom in the phosphate group with sulfur, improves cellular uptake by increasing hydrophobicity, nuclease resistance, and serum protein binding [19]. Modifications at the ribose 2′ position, such as 2′-F, 2′-MOE, 2′-O-Me, and 2′-LNA, enhance binding affinity and stabilize siRNAs against RNases while preventing immune activation [20, 21]. The 2′-F and 2′-O-Me modifications imitate the biophysical features of 2′-OH and can stabilize siRNAs against RNases while preventing siRNAs from activating innate immune receptors (TLR, MDA-5, and RIG-I); accordingly, all therapeutic siRNAs in clinical trials have 2′-F or 2′-O-Me modifications [22]. 5′- or 3′-RNA conjugates, like GalNAc, target the liver by binding to the ASGPR [23]. More complex modifications, such as PMO [24] and PNA [25], alter linking moieties while maintaining nucleobase pairing. Chemical modifications can be applied to sgRNAs for gene editing [26]. For instance, modifications such as 2′-O-M-3′PS (MS), 2′-O-M, or 2′-OM-3′thioPACE (MSP) at the 5′ and 3′ ends enhance genome editing efficiency [27]. The 2′-OM-3′-phosphonoacetate (MP) modification reduces off-target cleavage while maintaining on-target performance [28]. The 5′-H group modification increases activity and avoids innate immune responses [29]. Additionally, incorporating bridging NAs (2′,4′-BNA^{NC}[N-Me]) and LNA at specific sites in crRNA reduces off-target DNA cleavage by Cas9 [30]. Likewise, chemically modified nucleotides or conjugates are employed to improve the stability and pharmacokinetics of RNA aptamers [31]. Most clinical trial aptamers are modified with 5′-end PEG for increasing residence time [32], 3′end-capping strategy with inverted thymidine [33], 2′-substitutions on the sugar ring, and PS modifications

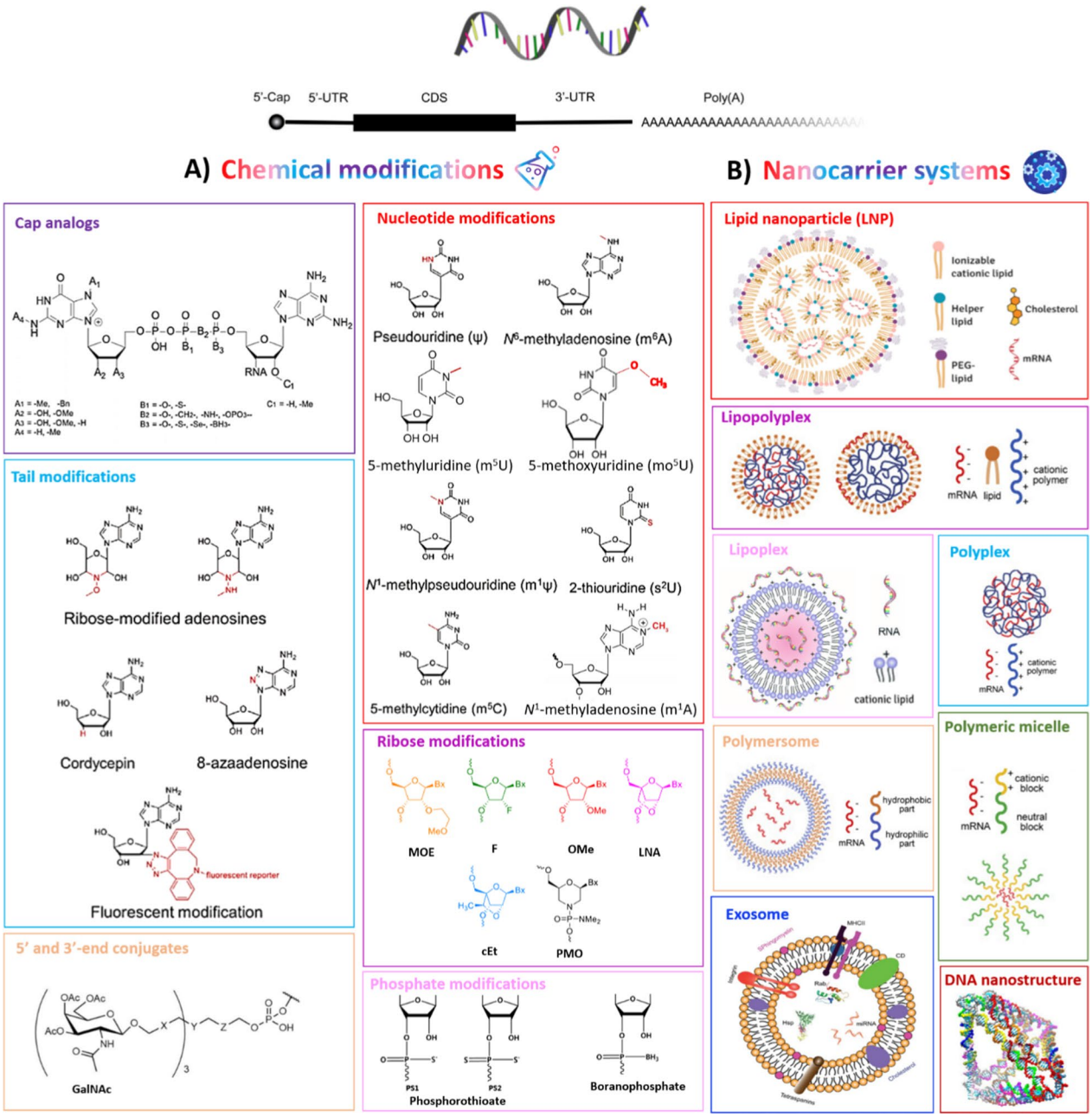

Fig. 1 Obstacles in the development of RNA therapeutics: **A** chemical modifications including alterations at the 5′ and 3′ ends (5′-capping, 3′-tail modifications, and 5′ and 3′-end conjugations), nucleotide modifications, ribose sugar substitutions at the 2′ position, and alterations in the phosphate backbone. **B** Nanocarrier delivery systems including lipid nanoparticle (LNP), lipoplex, polyplex, lipopolyplex, polymersome, polymeric micelle, exosome, and DNA nanostructures

can improve nuclease resistance and target binding affinity [34]. IVT mRNA, a ssRNA with a 5′ cap, ORF, flanking 5′ and 3′ UTRs, and a 3′ poly (A) tail, can include chemical modifications like Ψ, $m^1Ψ$, m^5C, 5hmC, m^5U, and s^2U to minimize the innate immune responses [35, 36].

Chemical modifications can enhance nuclease resistance and reduce immune activation. However, developing a safe and effective carrier to protect RNA from harmful physiological conditions and overcome gene delivery barriers is essential (Fig. 1B). Advances in nanotechnology provide potential solutions for intracellular delivery across biological barriers.

Nanovectors, such as LNPs, offer key benefits by allowing optimization of their chemical properties, including size, shape, composition, and surface chemistry. The customization of surface properties is also allowed, including the functionalization with targeting ligands, potentially improving delivery specificity and efficiency.

Lipid compositions

LNPs are regarded as leading non-viral vectors for the in vivo delivery of siRNA, mRNA, and gene editing constructs [1] due to their superior encapsulation, biocompatibility, stability, cellular uptake efficiency, and ease of large-scale production [37]. Typically, LNPs consist of ICLs, helper lipids, cholesterol, and PEG-lipids, each playing a crucial role in their functionality (Fig. 2). The molar ratios of these lipid components typically range as follows: 40–50% ICLs, 10–12% helper lipids, 38–45% cholesterol, and 1–2% PEG-lipids. The relationship between lipids and RNA is expressed by the N:P ratio, which denotes the proportion of nitrogen atoms in ICLs to phosphate atoms in RNAs. It is also expressed as a mass ratio, reflecting the ICL weight relative to RNA [38]. Below, please find additional details regarding the lipid components:

Component 1: ICLs, a critical component, consist of headgroups containing tertiary amines, linkers, and lipid tails. The chemical structure, particularly the amine head group, significantly influences mRNA encapsulation and delivery efficiency [39]. Flexibility in charge is essential during LNP preparation, maintaining a neutral surface charge at physiological pH to prevent immune responses while becoming positively charged in endosomal environments for membrane fusion and effective cytoplasmic release [40]. Optimal ICLs for in vivo activity exhibit a pKa within the range of 6.2–6.5, ensuring neutrality at pH 7.4 and positivity at acidic pH (< 6.0) [41]. Common ICLs are illustrated in Fig. 3 A. DLinKDMA, initially identified as a lead compound with a dimethyl amino headgroup, was modified to increase the spacer length to the dioxolane linker, resulting in DLinKC2DMA, which improved the efficacy of the ICL. Further optimization led to DLinMC3DMA, a gold standard ICL used in LNP formulations for NAs, including Onpattro and Leqvio [42, 43]. The linker bridging the amine headgroup to the lipid tail is pivotal, influencing transfection efficiency, stability, and biodegradability [44]. Linkers are diverse, including ethers, esters, amides, and carbamates, and their functional group, spacing, and length affect transfection efficiency [45]. Maier et al. revealed that esterification at carbons 9 and 10 in the linalyl moiety conferred a potency akin to MC3-lipid, with an accelerated clearance [44]. Shirazi et al. engineered biodegradable multivalent cationic lipids with a disulfide linkage between the head and tail groups, enhancing cell viability in vitro [46].

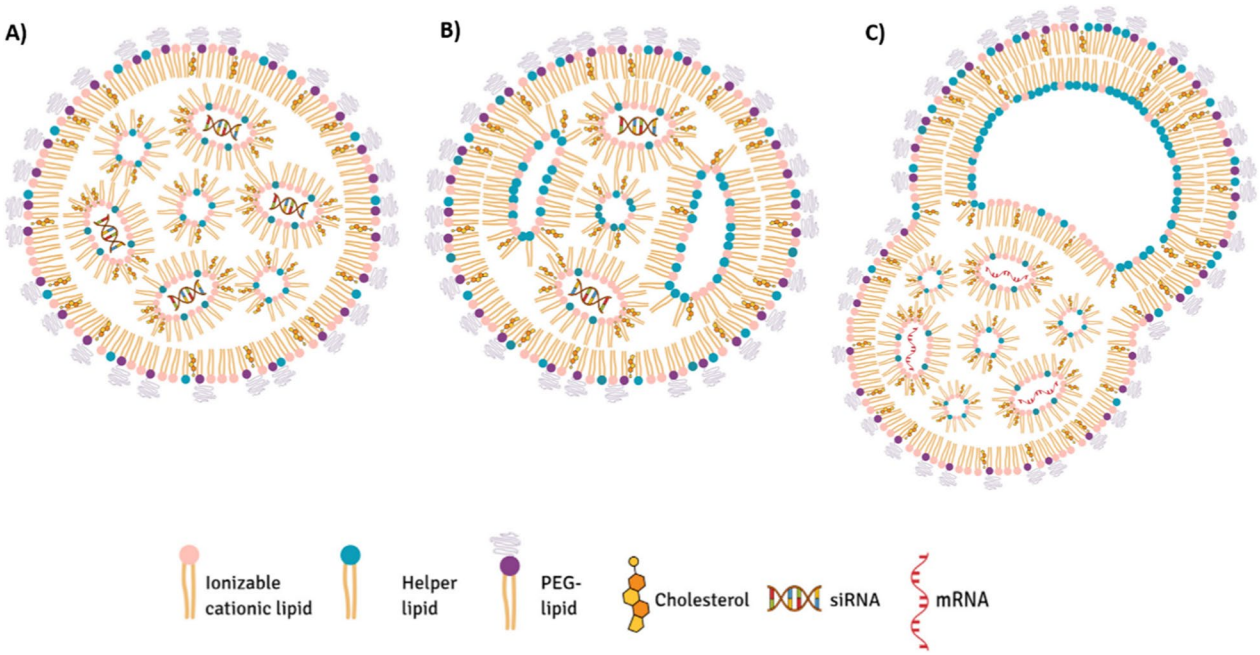

Fig. 2 Structure of lipid nanoparticle (LNP) encapsulating nucleic acids. **A** Compact nanostructured core. The LNPs exhibit a compact, electron-rich core where the siRNA is tangled with positively charged lipids forming inverted micelle structures, enclosed by cholesterol and helper lipids; **B** the multilamellar assembly features an external bilayer and a dense inner core; **C** 'Bleb' configuration. The cationic lipid predominantly interacts with the mRNA, creating a solid core, while the helper lipid is mainly partitioned into a 'bleb' bilayer (Adopted from [38])

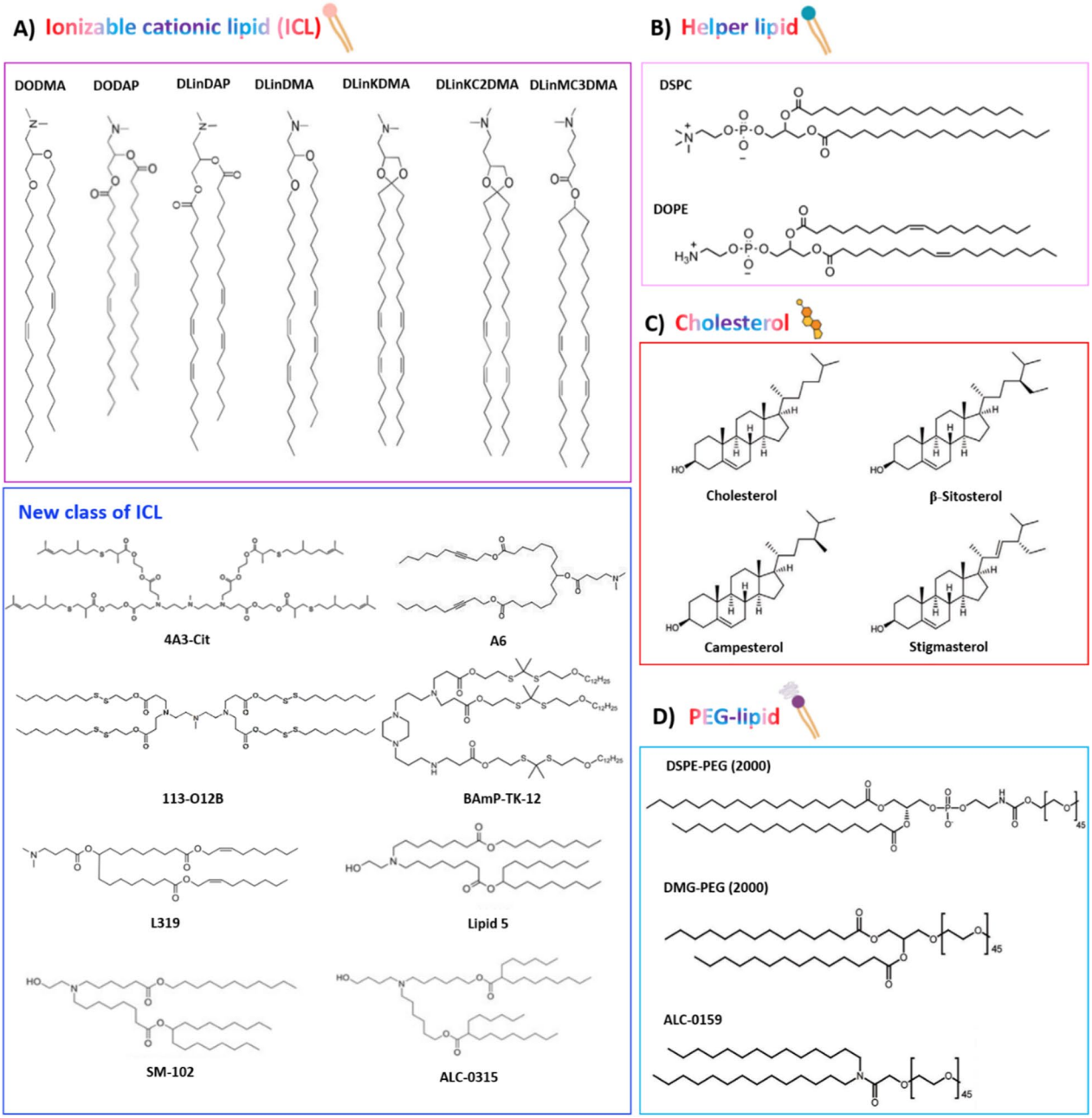

Fig. 3 Chemical compositions of lipid nanoparticles (LNPs): **A** ionizable cationic lipids (ICLs); **B** helper lipids; **C** cholesterol and its derivatives; **D** PEG-lipids

Lipid tail characteristics, such as saturation and unsaturation, length, level of substitution, and structure, influence transfection efficiency [47]. The lipid tail structure affects particle internalization, with certain lipids providing optimal cellular uptake and capability for inducing endosomal membrane-disruptive H_{II} phases [48]. When examining various degrees of lipid tail unsaturation, linoleic chains, as found in DLinDMA, offer optimal particle internalization and endosomal release [48]. Recent advancements have led to a new class of ICLs for efficient mRNA delivery, including SM-102, L319, ALC-0315, and Lipid 5 (Fig. 3A) [49, 50]. SM-102 and ALC-0315 are the ICLs used in the Moderna Spikevax and BioNTech Pfizer Comirnaty COVID-19 vaccines [49, 50]. Through combinatorial library design, novel ICLs have been identified like A6, 113-O12B, 16-I, 4A3-Cits, and

BAmP-TK-12 for targeted delivery to specific cells and improved mRNA delivery efficiency (Fig. 3A) [51, 52]. For example, Anderson et al. developed A6, an ionizable lipid with alkyne bonds, to target human erythropoietin mRNA delivery to hepatocytes [53]. LNPs composed of 113-O12B for lymph node targeting achieve mRNA delivery to 30% of APCs [54]. Moreover, ROS-sensitive LNPs containing BAmP-TK-12 exhibited targeted mRNA delivery and protein expression in cancer cells [52].

Component 2: Helper lipids, typical phospholipids like DSPC and DOPE, enhance LNP stability, cellular uptake, and endosomal release [27]. DSPC forms stable bilayer structures, which contribute to LNP stability and rigidity, while DOPE, with fusogenic characteristics, facilitates improved fusion of LNPs with endosomal membranes, thereby aiding RNA release (Fig. 3B) [55].

It was determined that ~30–40 mol% helper lipid is required to efficiently entrap siRNA within LNPs, providing additional insight into the role of these helper lipids [56]. The helper lipids serve as a mechanism for spacing out ionizable lipids to achieve a membrane surface charge of approximately +1 per nm^2 (siRNA has a surface charge of approximately −1 per nm^2). For LNP-pDNA formulations, certain unsaturated phosphatidylcholines (i.e., SOPC and DOPC) improved the LNP activity over DSPC in vitro [57]. DOPE-containing LNP-pDNA systems showed the best activity in murine serum, suggesting a potential role of helper lipids in modifying the LNP surface affinity to distinct apolipoprotein subtypes. LoPresti et al. explored helper lipids with different properties (neutral, cationic, and anionic) and demonstrated that changes in the chemistry and concentration of these lipids influence nanoparticle surface charge, affecting their distribution and efficacy in specific tissues [58].

Component 3: Cholesterol, a naturally occurring lipid, stabilizes LNPs by intercalating into bilayer structures and protecting against serum protein interactions [59]. The role of cholesterol in RNA delivery systems is not fully understood. However, particles lacking cholesterol may extract cholesterol from lipoproteins in blood circulation, potentially leading to destabilization. Cholesterol enhances intracellular delivery by promoting fusion with cellular and endosomal membranes, especially when combined with unsaturated lipids [60]. The size of LNPs is also impacted by cholesterol content, exhibiting a reduction in particle size as cholesterol levels increase within the range of 10–60 mol% [61]. Cholesterol is an exchangeable molecule that can accumulate within the LNP during circulation, significantly reducing surface-bound protein and enhancing circulation half-lives [62]. It is also crucial for nucleic acid encapsulation. Specifically, at least 40 mol% cholesterol is required to achieve nearly complete siRNA encapsulation [63].

Various cholesterol types in LNP formulations have been investigated for their effects on cell transfection efficiency (Fig. 3C). For instance, the incorporation of 20α-OH cholesterol can redirect mRNA-LNP systems from hepatocytes to hepatic endothelial cells and Kupffer cells by altering the LNP surface affinity to distinct apolipoprotein subtypes or scavenger receptors [64]. Zeng et al. discovered that using β-sitosterol instead of cholesterol in LNP formulations can improve the mRNA transfection on immune cells and enhance T-cell proliferation and IL-2 production [65]. Cholesterol derivatives with C24 alkyl tails in LNPs lead to diverse morphologies, with modifications like methyl and ethyl groups increasing multilamellar structures and double bonds causing lipid partitioning, both enhancing gene delivery efficiency [66].

Component 4: PEG-lipids serve dual functions by controlling particle size during formation and improving stability by establishing a hydrophilic spatial hindrance. PEG-lipids prevent particle aggregation, enhance in vivo circulation lifetime, and reduce rapid clearance by RES [37]. However, PEG-lipids can diminish cellular uptake, necessitating a balance for effective intracellular delivery [67]. Very short PEG (< 1 kDa) lacks efficient circulation time, while cellular uptake is diminished by very long PEG chains (> 5 kDa) or high mole fractions (> 15 mol%). Typically, PEG molecules with a molecular weight = 2 kDa at a ratio lower than 5%, preferentially 0.5 mol%, are employed for LNP formulation [68]. Lokugamage et al. found that formulations without PEG-lipids resulted in unstable, polydisperse LNPs over 200 nm in diameter [69]. Increasing the molar ratio of PEG-lipids results in significantly smaller LNPs, regardless of other lipid components. PEG-lipids influence nucleic acid encapsulation efficiency, in vivo distribution, transfection efficiency, and immune response. These characteristics are linked to the molar ratio of PEG-lipids and the structure and length of the PEG chain and lipid tail [63]. Diffusible PEG-lipids contribute to particle stability and facilitate intracellular transport [70, 71]. PEG-DMPE and PEG-DMG with 14-carbon acyl chain rapidly dissociate from LNPs during circulation, whereas PEG-DSPE and PEG-DSG with 18-carbon acyl chain remain associated, extending their half-life at high concentrations [72]. Moderna's COVID-19 vaccine contains 1.5% PEG2000-DMG, while BioNTech-Pfizer's vaccine includes 1.6% ALC-0159 (Fig. 3D).

RNA-LNP fabrication

LNP fabrication involves the orchestrated interplay of distinct lipid derivatives crucial for optimizing RNA delivery. The self-assembly of LNPs relies on the electrostatic attraction between lipids and RNA in solution.

Anionic mRNA initiates aggregation with cationic lipids, forming NPs via van der Waals forces. In an acidic buffer (e.g., pH = 4), ICLs in ethanol interact with negatively charged RNA, forming inverted micelles. Subsequent ethanol diffusion into the aqueous phase precipitates lipids, resulting in hydrophobic interactions [73]. The detailed mechanism suggests the migration of unbound ICLs into LNP cores, potentially forming multilamellar structures like onion or inverted hexagonal (H_{II}) structures [66]. A prompt and homogeneous blending of ethanol-solubilized lipids with an aqueous buffer containing dissolved RNA is essential for consistent particle generation.

Various conventional bulk mixing methods for NP synthesis, including thin-film hydration, solvent diffusion, pore extrusion, ultrasonication, melting emulsification, solvent injection, and bulk nanoprecipitation, have been employed; however, conventional approaches may yield inconsistent results (Fig. 4A). These methods for LNP synthesis have several disadvantages such as poor reproducibility due to variations in the synthesis process; requirement of a high amount of surfactant, which can affect the stability and functionality of LNPs; limited control over critical parameters like particle size, PDI, and EE; limited shelf life of LNPs due to chemical degradation of mRNA and impurities in the solution. Some traditional methods require harsh conditions that can negatively impact the integrity of the encapsulated mRNA or other therapeutic agents. These disadvantages highlight the need for improved and more controlled LNP preparation techniques to ensure consistent and effective delivery of therapeutic agents [74, 75]. Although post-treatment methods like membrane extrusion and ultrasonication have been proposed for LNP homogenization, issues remain. Payload stability and carrier deformation during these processes are still unresolved [76].

Microfluidics offers a more stable and reproducible method for producing LNP formulations. This technique provides precise tunability, short mixing time, low sample consumption, monodisperse particle size distribution, reproducibility, and the ability to scale up production. Key aspects of microfluidics include high controllability of TFR and FRR, as well as mixing conditions, scalability, real-time monitoring, and customized microchannel geometries [77]. Various mixer designs, such as T-junction, HFF, SHM, bifurcating or toroidal, baffle, and Tesla mixers, have been explored to enhance the efficiency of the mixing process and achieve rapid LNP formation [77] (Fig. 4B). The T-junction method, a basic microfluidic device, facilitates rapid mixing at high TFR by combining an aqueous medium with a lipid-organic solvent (ethanol), resulting in supersaturated lipid self-assembly into LNPs without a need for size reduction. HFF involves mixing lipid organic solutions with aqueous solutions in a microfluidic channel, allowing precise control over LNP size and EE. SHM induces chaotic mixing, offering a controlled size and shape by adjusting the TFR and FRR. Bifurcating toroidal mixers achieve large-scale production of uniform LNPs. Baffle mixers introduce successive turns for rapid mixing, allowing fine-tuning of the LNP size. The Tesla mixer efficiently achieves chaotic mixing, enabling a wide range of flow rates for smaller NP production [78, 79]. Each microfluidic design tackles distinct challenges, offering flexible choices for controlled and reproducible LNP synthesis. However, microfluidic mixing methods may pose concerns about environmental waste and expenses in case of employing single-use disposable cartridges, variability between cartridges, and the compatibility of cartridge materials with organic solvents, requiring careful design and application. Zöller et al. aimed to design charge-converting LNPs using a microfluidic mixing technique for their preparation and coating. LNPs consisting of DSPC, cholesterol, MPEG-2000-DSPE, and cationic surfactants were prepared at different flow rate ratios. Utilizing a second microfluidic chip, LNPs were coated with polyoxyethylene (9) nonylphenol monophosphate ester (PNPP) [80]. Roces et al. highlighted that microfluidics offers significant advantages for LNP manufacturing, notably in terms of scalability, reproducibility, and expedited preparation. Critical factors affecting the characteristics of LNPs were assessed, including FRR and TFR, both of which were determined to have a significant impact on particle size. Additionally, the selection of amino lipids, buffers, and nucleic acid payloads also played a crucial role in determining LNP properties. These findings serve as a valuable reference for the development and scaling of LNP systems utilizing microfluidics [61]. Ma et al. developed a scalable method for synthesizing LNPs using inertial microfluidic mixers that are scaled isometrically in three dimensions. A theoretical predictive model was employed to ensure a consistent mixing time across various chips, thereby achieving uniformity in particle size and distribution. siRNA-LNPs produced at various TFRs present a feasible approach for the scalable production of LNPs, potentially promoting the advancement of nucleic acid drug development into clinical applications [81].

Physicochemical attributes of RNA-LNPs

The physicochemical attributes of LNPs are crucial in determining their stability and functionality, directly influencing in vivo fate and gene delivery efficiency [55]. LNP structure and morphology are influenced by various factors, including lipid components and their ratios, preparation methods, and the type of NA.

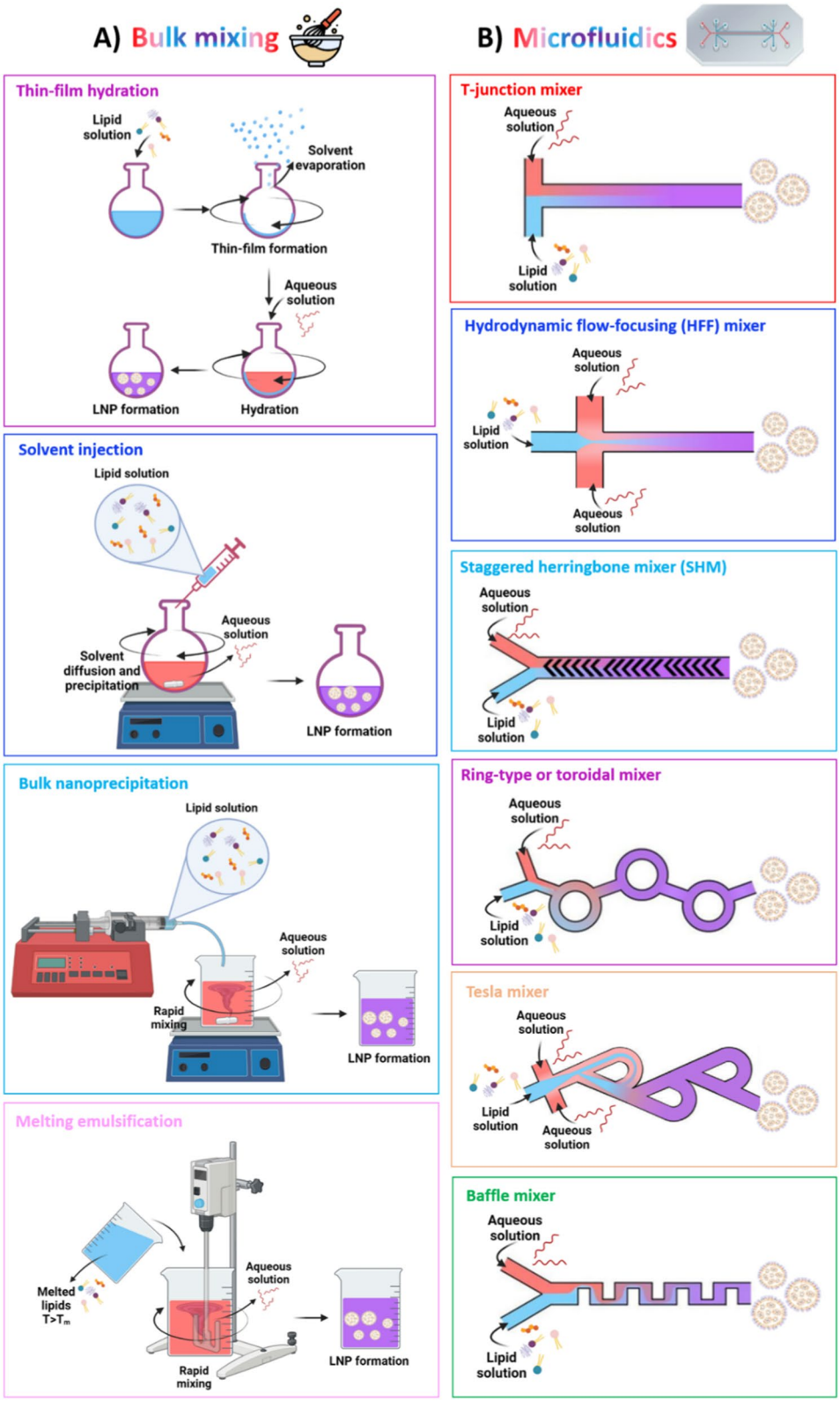

Fig. 4 Schematic representation of various lipid nanoparticle (LNP) fabrication techniques: **A** bulk mixing methods including thin-film hydration, solvent injection, bulk nanoprecipitation, and melting emulsification; **B** microfluidic methods utilizing distinct mixer designs such as T-junction, HFF, staggered herringbone, ring-type, Tesla, and baffle mixer

Morphology

The morphology of LNPs affects cellular uptake, biodistribution, and toxicity. Common methods for assessing LNP morphology include TEM, SEM, and AFM. Cryo-TEM and scattering techniques, such as XRD, SAXS, and SANS, offer valuable insights into the inner structure, shape, and dimensions of LNP forms that may be challenging to identify through other means [82]. LNPs containing a high amount of ICL (more than 40 mol%), DSPC, and cholesterol can form inverted micelles around RNA therapeutics. Conversely, those with a lower amount of ICL (20 mol%) and a higher DSPC content (30 mol%) result in solid cores primarily composed of inverted micelles, with ICL, cholesterol, and DSPC accumulating in the bilayer area [83] (Fig. 2B). The use of DSPC and cholesterol in LNP formulations is strategically based on the interaction of DSPC with ICL through its phosphate group and its complexation with RNA through its choline group. Cholesterol occupies the space within the curvature created by the interaction of ICL with RNA-based therapeutics [45, 84]. PEG-lipids cover the outer layer of LNPs because of their hydrophilic nature and steric size [68]. Unlike siRNAs, mRNA leads to bleb formation, influencing morphology and EE [85] (Fig. 2C). Moreover, substituting DOPE for DSPC resulted in the absence of the bleb structure, developing a condensed core comprising inverted micellar structures [45].

Size and polydispersity

The size of LNPs significantly affects their stability, EE, and biological performance. Techniques such as NTA, DLS, and TEM are commonly used to measure LNP size. Smaller LNPs typically exhibit better stability, lower aggregation, higher EE, and longer circulation time than larger LNPs. However, very small LNPs may reduce gene expression efficiency and increase clearance. An ideal size for cellular internalization is approximately 100 nm, falling within the acceptable size range of 20–200 nm [40]. Factors like lipid type and ratio, NA concentration, preparation method, and storage conditions affect LNP size. Increasing the ICL content from 50 to 80 mol% while decreasing the cholesterol content from 37.5 to 7.5 mol% results in LNPs with sizes increasing from 50 to 100 nm [83]. In addition to mean size, the PDI) indicates size distribution homogeneity and is evaluated using techniques like DLS and NTA. High PDI can lead to inconsistent transfection efficiency, reduced stability, increased aggregation, and heightened immune recognition.

Zeta potential

Zeta potential, which influences LNP circulation and tissue uptake, can be modified by lipid type and ratio, particularly those with ionizable amine groups or PEG chains. Techniques like zeta potential analysis and electrophoretic light scattering measure the LNP charge. While positively charged LNPs have a higher affinity for negatively charged NAs, facilitating their encapsulation and protection, they may also pose higher toxicity risks due to increased internalization, interaction with the cell membrane, lower stability, higher aggregation, and stronger immune response compared with neutral or negatively charged LNPs. An elevated surface charge, whether +30 mV or −30 mV, usually leads to rapid elimination from the bloodstream and increased sequestration by the RES. In contrast, LNPs with a neutral charge, between −10 and +10 mV, tend to circulate in the blood with a reduced uptake by the RES.

Encapsulation efficiency (EE)

EE, a critical determinant of gene delivery effectiveness and cost-effectiveness for LNPs, reflects the degree to which NAs are safeguarded from degradation and successfully delivered to target cells. Various established techniques, including ultracentrifugation, agarose gel electrophoresis retardation assay, UV–vis spectrophotometry, fluorescence spectrophotometry, and ethidium bromide intercalation assay, are commonly used to quantify EE. Factors such as the type and ratio of lipids, concentration and molecular weight of NAs, preparation method, and storage conditions influence EE%. For example, changes in ICL content notably impact EE, as evidenced by a 30% increase in the molar ratio of ICL, leading to a proportional reduction in cholesterol amount and subsequent decline in siRNA EE [45]. Inadequate molar ratios, particularly cholesterol, diminish the interaction of ICLs with NAs, resulting in decreased EE. In addition, high proportions of PEG-lipid (2.5 or 5 mol%) reduce EE [83].

Stability

Stability studies are vital for translating LNP-RNA formulations from research to practical application, ensuring the determination of shelf life and optimal storage conditions. Unstable LNPs may undergo issues like aggregation, degradation, leakage, or phase transition, jeopardizing their gene delivery efficacy and safety. Stability testing should be conducted early in preclinical development. Common techniques for stability assessment include DLS, zeta potential analysis, TEM, UV–vis spectrophotometry, fluorescence spectrophotometry, and in vitro/in vivo gene expression assays, aligned with the ICH guidelines [50, 86]. The stability of LNP-RNA formulations depends on factors such as pH, temperature, light, humidity, ionic strength, lipid type and ratio, and NA properties [39, 50]. To augment the stability of

LNP-RNA constructs, techniques such as the incorporation of buffers and surfactants, application of stringent processing controls, and using cryoprotectants during lyophilization are employed [86]. For example, both Moderna and BioNTech/Pfizer use sucrose as a cryoprotectant in their mRNA COVID-19 vaccines, incorporating specific buffering systems. Moderna employs Tris HCl buffer to maintain the final pH between 7 and 8, whereas BioNTech/Pfizer uses phosphate buffer. Choosing the appropriate buffering agent and osmolyte is of paramount importance because of the potential pH changes during freezing and storage at ultra-low temperatures [50, 87].

Overall, controlling the quality attributes in LNPs, such as average size, size distribution, zeta potential, structural integrity, loading capacity, and stability, is crucial for the successful delivery and therapeutic application of RNA. An in-depth examination of the physicochemical characteristics of LNPs supports the QbD approach by identifying critical attributes, facilitating risk evaluation, directing experimental frameworks, and ensuring effective control measures. These elements collectively contribute to the development of high-quality pharmaceutical products [88], which will be elaborated in the subsequent section.

RNA-LNP QbD

QbD is a systematic approach used in pharmaceutical development to ensure consistent adherence to predefined quality standards, while also aiming to improve efficacy and safety [89]. The QbD process begins with formulating the QTPP, which outlines the desired attributes of the final product, encompassing efficacy, safety, and other pertinent characteristics. Subsequently, CQAs are identified, representing the specific traits essential for guaranteeing product quality and performance. To attain these CQAs, a comprehensive understanding of CPPs and CMAs throughout drug development and manufacturing is imperative. CMAs pertain to the inherent characteristics of raw materials used in the manufacturing process, such as the properties of the drug and excipients. Conversely, CPPs encompass the pivotal variables within the manufacturing process that must be regulated to ensure the desired product quality. Risk assessment methodologies are frequently employed to prioritize and evaluate the impact of diverse CMAs and CPPs on CQAs, facilitating the identification of the most critical factors necessitating control or optimization to achieve the desired product quality. Once these critical factors are identified, a DoE approach is used to systematically explore how each factor influences product quality. The DoE conducts a series of experiments testing different combinations of CMAs and CPPs to establish a mathematical model of the manufacturing process. This model aids in understanding how alterations in input parameters affect the output (product quality), facilitating the development of robust manufacturing processes [90]. Statistical experimental designs, such as full or fractional factorial designs, RSM, and Box–Behnken designs are employed for DoE [91]. Factorial Designs involve studying the effects of two or more factors simultaneously. Full factorial designs consider all possible combinations of factors at different levels and provide comprehensive information about the interaction between factors. A full factorial design can be used to investigate the effects of multiple formulation variables, such as lipid type, lipid concentration, and solvent type on the particle size and encapsulation efficiency. This approach helps in identifying the most significant factors and their interactions [92]. Fractional factorial designs consider only a subset of the full factorial design that includes only a fraction of the total possible combinations. This design is useful when the number of factors is large, and a full factorial design would be impractical. For example, it can be used to quickly identify which formulation parameters (e.g., lipid type, surfactant type, solvent type) have the most significant impact on particle size and stability [93, 94]. RSM is used to explore the relationships between several explanatory variables and one or more response variables. It helps in identifying the optimal conditions for the desired response. For example, researchers might use RSM to determine the optimal lipid-to-drug ratio and surfactant concentration to maximize encapsulation efficiency and minimize particle size [95]. Box-Behnken is a type of RSM that does not include combinations where all factors are at their extreme values simultaneously. This design is efficient and requires fewer runs than a full factorial design [96, 97]. For instance, it can help in understanding how the interactions among lipid type, stirring speed, and temperature affect the stability and size distribution of the NPs [98, 99]. Factors affecting responses were validated statistically, using ANOVA, and different models (linear, two-factor interaction (2FI), cubic, quadratic) were applied. Three-dimensional surface and contour plots were used to illustrate significant effects [100].

In the QbD approach for LNP formulation, several key concepts are used to ensure the QTPP (efficacy, quality, and safety) of the final product. CQAs, representing specific traits of LNPs crucial for ensuring their QTPP, encompass factors like particle size, polydispersity, zeta potential, EE, and stability. On the other hand, CMAs, denoting inherent characteristics of the raw materials used in LNP manufacturing, may comprise lipid type and concentration, RNA integrity and concentration, stabilizer, and the presence of impurities or contaminants. CPPs are pivotal variables within the manufacturing

process that directly shape the formation, characteristics, and performance of LNPs. Examples of CPPs in LNP formulations may involve parameters such as mixing speed and duration, temperature, pH, lipid-to-NA (N/P) ratio, solvent type and concentration, and the methodology employed for NP characterization (Fig. 5). By effectively identifying and managing these CQAs, CMAs, and CPPs throughout the development and manufacturing stages of LNP formulations, researchers can optimize the quality, efficacy, and safety of the final product. Kauffman et al. optimized erythropoietin (EPO)-mRNA-loaded LNPs. Six input variables were considered: C12-200:mRNA weight ratio, phospholipid type, C12-200, cholesterol, and PEG molar contents. Output variables included %EE, particle size, PDI, and EPO serum concentration. Initial screening determined the optimal phospholipid type. Further screening refined the C12-200:mRNA ratio, C12-200%, phospholipid %, PEG %, and cholesterol %. Final optimization identified the best composition: 10:1 C12-200:mRNA weight ratio, 35% C12-200, 16% DOPE, 46.5% cholesterol, and 2.5% C14-PEG2000. The optimal LNPs had a particle size of 102 nm, PDI of 0.158, 43% encapsulation efficiency, pKa of 6.9, and zeta potential of −5.0 mV. The in vivo efficacy achieved with the injection of 15 µg EPO mRNA was measured at 7065 ± 513 ng/µL [93]. In another study, Blakney et al. optimized SAM LNPs using a full factorial design. They examined five variables: lipid type (C12-200, DDA, DOTAP, cephalin), lipid-to-RNA ratio (1:1, 4:1, 18:1, 90:1), lipid concentration (high, medium, low), particle concentration (high,

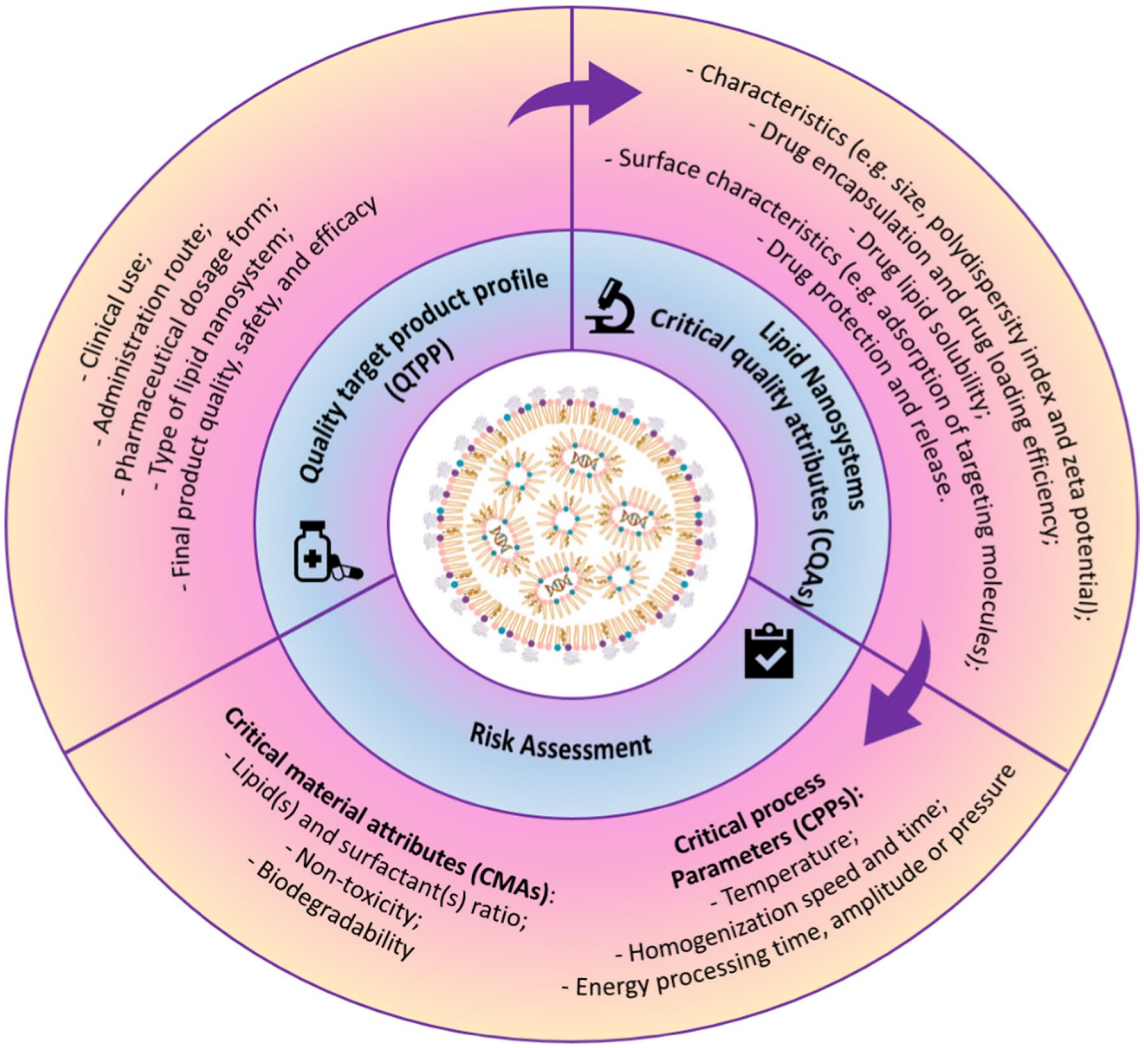

Fig. 5 Fundamental procedures for applying the quality-by-design (QbD) approach in developing lipid nanoparticles (LNPs) which includes quality target product profile (QTPP), critical quality attributes (CQAs), critical process parameters (CPPs) and critical material attributes (CMAs). In the QbD approach by using statistical, analytical, and risk-management methodologies optimized products can be designed and developed

medium, low), and cationic to zwitterionic lipid ratio. The key outcome was luciferase expression after 10 days. Cephalin lipids at an 18:1 lipid-to-RNA ratio, with low lipid and medium particle concentrations, resulted in a sevenfold increase in luciferase expression compared to the original formulation [92]. Ly et al. optimized LNPs for SAM expression and cellular activation using a Box–Behnken design. The study tested formulations varying in phospholipid content, ionizable lipid type, ionizable lipid content, and pH. The N/P ratio, DOPE and DMG-PEG-2000 contents, flow rate, and temperature were fixed. Output variables included particle size, PDI, EE%, charge, and protein expression. RSM modeling utilized second-order OLS regression, with a Box–Cox transformation to improve accuracy. Optimal conditions were determined using the desirability function and BFGS optimization [98]. Bastogne et al. optimized cationic nano-lipid formulations for siRNA transfection using DoE based on a D-optimal mixture design, testing 36 formulations. The study varied the proportion of DOTAP, PEG surfactant concentration, lecithin proportion, and LNP size. Key outputs included LNP stability, siRNA transfection rate (minimum 30%), and PDI. Polynomial models were employed to link input and output variables, and evaluated using Bayesian estimation, posterior predictive checks, and leave-one-out cross-validation [101]. Young et al. optimized LNP compositions for mRNA delivery to the placenta using a factorial design study. They created 18 unique LNPs (A1–A18) via definitive screening design (DSD). Factors included ionizable lipid type (C12-200, DLin-MC3-DMA), phospholipid type (DSPC, DOPE), and various molar percentages of lipids and cholesterol. Key metrics measured were hydrodynamic diameter (72.2–171.5 nm), polydispersity index (0.120–0.317), mRNA encapsulation efficiency (35.6–83.2%), transfection efficiency, and apparent pKa (5.3–7.1). The optimal formulation contained 35% C12-200, 10% DOPE, 1.5% PEG, and 53.5% cholesterol, with a hydrodynamic diameter of 130.2 nm, PDI of 0.064, EE of 56.5%, and pKa of 6.6 [102].

Overall, by identifying the desired QTPP and managing CQAs, CMAs, and CPPs throughout the development process, along with implementing a risk control strategy for manufacturing and continuous process improvement, researchers can enhance the quality, efficacy, and safety of the final product [103].

In-vivo fate of RNA-LNPs

The in-vivo fate of LNPs and RNA cargo depends on several factors, including LNP composition, size, surface charge, PEGylation, injection route, target tissue, and immune response. Concerning LNP composition, the choice of ICLs, helper lipids, cholesterol, and PEG-lipids affects stability, fusogenicity, endosomal escape, cellular uptake, and transfection efficiency. Particle size affects circulation time, biodistribution, aggregation, and clearance. Larger particles enhance gene delivery efficiency, whereas smaller particles provide better control over body disposition. Surface charge, determined by ionizable lipids and their pKa values, influences interactions with serum proteins, cellular membranes, endosomes, and immune cells. Optimal gene delivery requires a balance between positive and negative charges. PEGylation, achieved through PEG-lipids, forms a hydrophilic steric barrier, preventing aggregation and improving circulation lifetimes, but potentially inhibiting cellular uptake. The chemistry and density of PEGylation on the surface of LNPs can also affect membrane permeability, biodistribution, and immune response. The injection route influences the initial exposure of LNP to the blood circulation and organs, yielding varied biodistribution patterns and gene expression levels. Target tissue, the intended organ or cell type for mRNA delivery, impacts LNP biodistribution by influencing interactions with tissue-specific receptors, transporters, enzymes, and immune cells. The immune response, involving innate and adaptive systems, influences LNP biodistribution through opsonization, phagocytosis, cytokine production, and Ab formation. LNPs with high surface charge or PEGylation can activate complement and undergo macrophage uptake, whereas those with low surface charge or PEGylation may evade immune recognition, extending circulation time [104]. Several rational design strategies can enhance the fate of RNA therapeutics loaded into LNPs. For example, adding electrolytes, such as sodium chloride, can mitigate the repulsion between cationic liposomes and lipoplex intermediates during complex formation. This contributes to stabilizing mRNA encapsulation, reducing particle size, preventing aggregation in saline mixtures, enhancing mRNA stability in serum, and improving gene expression administration [105]. Therefore, advancements in the rational design and manufacturing of LNPs offer promising avenues for enhancing in vivo performance in delivering RNA therapeutics.

Preclinical evaluation tools

Achieving successful translation of LNPs requires careful control of their properties, which influence colloidal stability, decomposition, biodistribution, and cellular interactions. These properties can change when LNPs are exposed to biological fluids. Therefore, conducting in-vitro assessments of LNPs in media that simulate biological conditions is essential for accurately predicting their physiological behavior [106]. In-vivo characterization studies of LNPs encompass a variety of methods to evaluate their performance, distribution, effectiveness, and

safety. For biodistribution and pharmacokinetic analysis, which entails examining how LNPs distribute throughout the body after administration, various techniques are employed, such as radiolabeling, luminescence or fluorescence bioimaging, microscopy, and organ perfusion techniques. Radiolabeling is the least cumbersome approach for quantifying LNP biodistribution [107]. Measuring fluorescence encounters challenges from biological autofluorescence but using probes like Cy7 allows for tracking LNPs in live mice organs via confocal microscopy [108]. Liver perfusion is particularly effective for evaluating LNPs' local distribution, offering simplicity compared to other tissue perfusion methods for organs like the brain, lungs, kidneys, muscles, and tumors [109]. Preclinical biodistribution data for approved RNA therapeutics can be accessed in the FDA's Application Review Files or the European Public Assessment Reports. Reporter genes, such as Fluc, offer a high-sensitivity method for analyzing in-vivo gene expression. QWBA and MAR map the dispersion of radiolabeled compounds in tissue sections, providing cellular distribution data. They rely on specific radiolabeling procedures. For example, preclinical studies for Onpattro, Oxlumo, Leqvio, Givlaari, and COVID-19 vaccines utilized QWBA in rats. LC–MS/MS, a gold standard for bioanalytical chemistry, measures siRNA levels (e.g., Onpattro and Givlaari [110, 111]) and evaluates the pharmacokinetics and biodistribution of synthetic lipid components (e.g., Onpattro and the Pfizer/BioNTech COVID-19 vaccine [112, 113]). However, MS-based quantifications face challenges with high molecular weight and anionic RNA therapeutics. It enables the visualization of single-molecule RNA and multiple target mRNAs simultaneously, while RT-qPCR and ddPCR offer improved sensitivity, precision, and reproducibility for RNA biodistribution studies. In vivo BLI provides insights into mRNA-LNP biodistribution and translation kinetics using luminescent and fluorescent reporter proteins or labeled LNPs (Fig. 6). Therefore, the choice of evaluation tools depends on the specific requirements and challenges associated with RNA therapeutics, each offering unique advantages and limitations [114].

Blood components forming protein corona

Upon entry into the body, naked genes engage with various biological components, depending on the specific biological fluids at the administration site, such as lung surfactants during inhalation, interstitial fluid with local injection, and blood plasma post-intravenous administration [115]. The efficacy of in vivo gene delivery significantly hinges on interactions with blood components, encompassing blood cells, ions, and plasma proteins. Cationic non-viral vectors, like lipoplexes, may aggregate in ionic physiological media that proteins like serum albumin can prevent. Similarly, erythrocytes in phosphate-buffered saline exhibit comparable interactions, which can be mitigated by adding proteins [116]. Plasma proteins binding to LNPs form a "biomolecular corona," comprised of diverse biomolecules such as apolipoproteins, complement elements, immunoglobulins, and coagulation factors [117]. The composition of this corona, determined by LNP characteristics and the presence of NAs, critically alters their physiological attributes, impacting distribution, longevity in the bloodstream, cellular internalization, immune recognition, stability, and pharmacokinetics [118, 119]. LNP surface is predominantly coated with biomolecules like ApoE, vitronectin, albumin, and β2-glycoprotein I, which form stable associations influencing further interactions. The protein corona comprises a firmly attached "hard" corona and a more transient "soft" corona, dynamically interacting with surrounding proteins. Equipped with inherent targeting abilities, the protein corona enhances receptor-specific interactions and uptake via endocytosis [120]. Factors such as biofluid type, concentration, temperature, and origin crucially shape the corona, dictating LNP clearance, tissue targeting, and therapeutic effectiveness. ICLs, altering their charge based on pH, utilize ApoE to enhance liver-targeted gene delivery, where the level of ApoE influences delivery efficiency, while lipoproteins generally suppress gene expression. In liposomes containing DOTAP and cholesterol, fibronectin plays a pivotal role in gene expression in the lung, as does albumin corona in inflammatory liver and tumor conditions [121]. Hence, designing LNPs necessitates careful modulation of protein interactions for natural targeting or minimizing blood cell interactions for passive or active targeting.

Liver accumulation and extrahepatic distribution

The distribution of non-viral vectors in target tissues is heterogeneous and not completely understood, influenced by variations in blood flow and the diversity of receptor expression. The vascular barrier poses a significant challenge, hindering gene delivery to cells beyond the blood vessels. However, some tissues, such as the liver, spleen, and tumors, feature endothelial fenestrations that aid in delivering genes to cells outside the bloodstream. Notably, the size of liver sinusoidal endothelial fenestrae ranges from 100 to 160 nm, with variations across different species [122]. For effective hepatocyte delivery, carriers must be smaller than the endothelial fenestrations. NPs pass through murine tumor vessel walls more easily than human tumors, which show variable permeability depending on the cancer type [123]. Researchers should adjust NP size for optimal vascular permeability during pre- and post-administration phases [124]. When dealing with tight vascular structures like the blood–brain

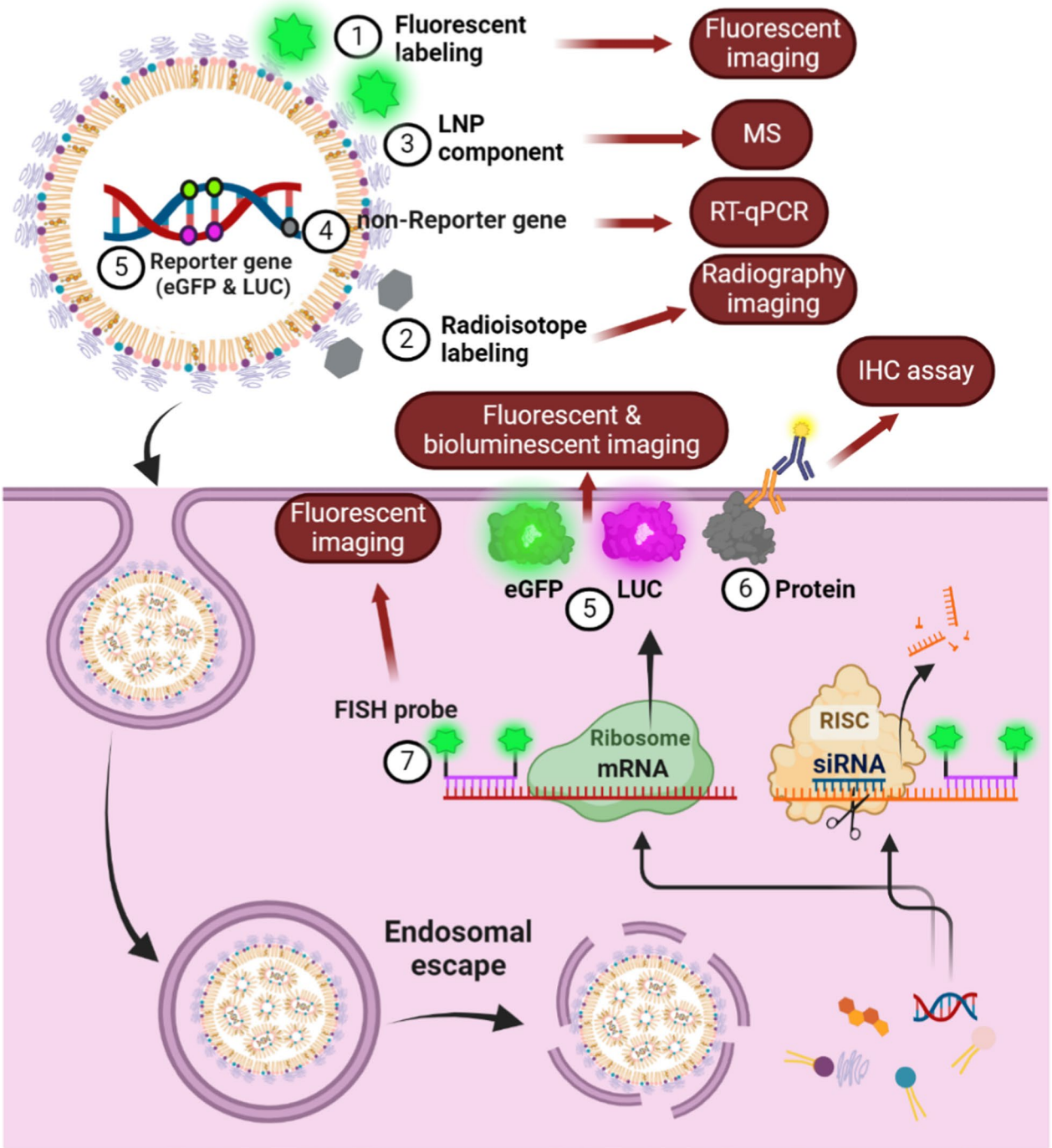

Fig. 6 Summary of common labeling and identification techniques for LNPs, RNAs, and protein products. Lipid nanoparticles (LNPs) are labeled with fluorescent and radioisotope tags for imaging purposes (1 and 2). LNP components are analyzed using mass spectroscopy (MS) (3). Specific nucleic acid sequences are detected using reverse transcription-quantitative PCR (RT-qPCR) (4). Reporter gene expressions, such as enhanced green fluorescent protein (eGFP) and luciferase (LUC), are visualized through fluorescent and bioluminescent imaging (5). Immunohistochemistry (IHC) techniques identify proteins produced by mRNA-LNP (6) or suppressed by siRNA-LNP. Fluorescence in situ hybridization (FISH) probes bind to specific nucleic acid sequences within cells, enabling their visualization (7)

barrier, a simple particle size control is insufficient. An effective strategy may involve leveraging transcytosis by endothelial cells and targeting specific receptors such as the transferrin receptor [125]. Alternatively, physical stimuli such as ultrasound can disrupt the vessel wall, facilitating gene transfer into the tissue [126].

LNPs naturally exhibit a strong affinity for the liver, making them ideal carriers for hepatic delivery [127]. The presence of ApoE in the protein corona of LNPs facilitates their interaction with cell receptors such as LDLR, VLDLR, and LRP, predominantly in the liver. The high affinity of ApoE for LDLR on hepatocytes enhances LNP uptake via receptor-mediated endocytosis. Cheng et al. observed that the PEG-lipid component of LNPs can detach, allowing ApoE to adsorb onto the LNP surface [128]. Beyond liver targeting, different components of the protein corona can direct LNPs to extrahepatic tissues or organs [129, 130]. HDL on the surface of LNP interacts with the SR-BI receptors on lymphatic endothelial cells [131]. Vitronectin facilitates targeted delivery of LNPs to tumor cells through interactions with $\alpha_v\beta_3$ integrins [132]. LNPs with a vitronectin-rich corona show reduced delivery to HepG2 cells with LDL receptors compared to those with an apolipoprotein-rich corona [133]. Coronas rich in albumin extend circulation time and facilitate lymphatic drainage [134]. Gp60 and SPARC receptors are the key targets for delivering LNPs with an albumin-rich corona [135]. A vitronectin-rich corona may inherently target the lungs and tumors, while the affinity of b2-GPI for phosphatidylserine allows for spleen targeting by LNPs [132]. Figure 7 illustrates LNP natural targeting via protein corona formation, highlighting how the corona composition serves as a strategy for natural LNP targeting.

The protein corona composition on LNPs, enriched with specific proteins, is primarily determined by the controlled attributes of the LNPs. Notably, differences in the protein corona between liver and lung-targeted LNPs

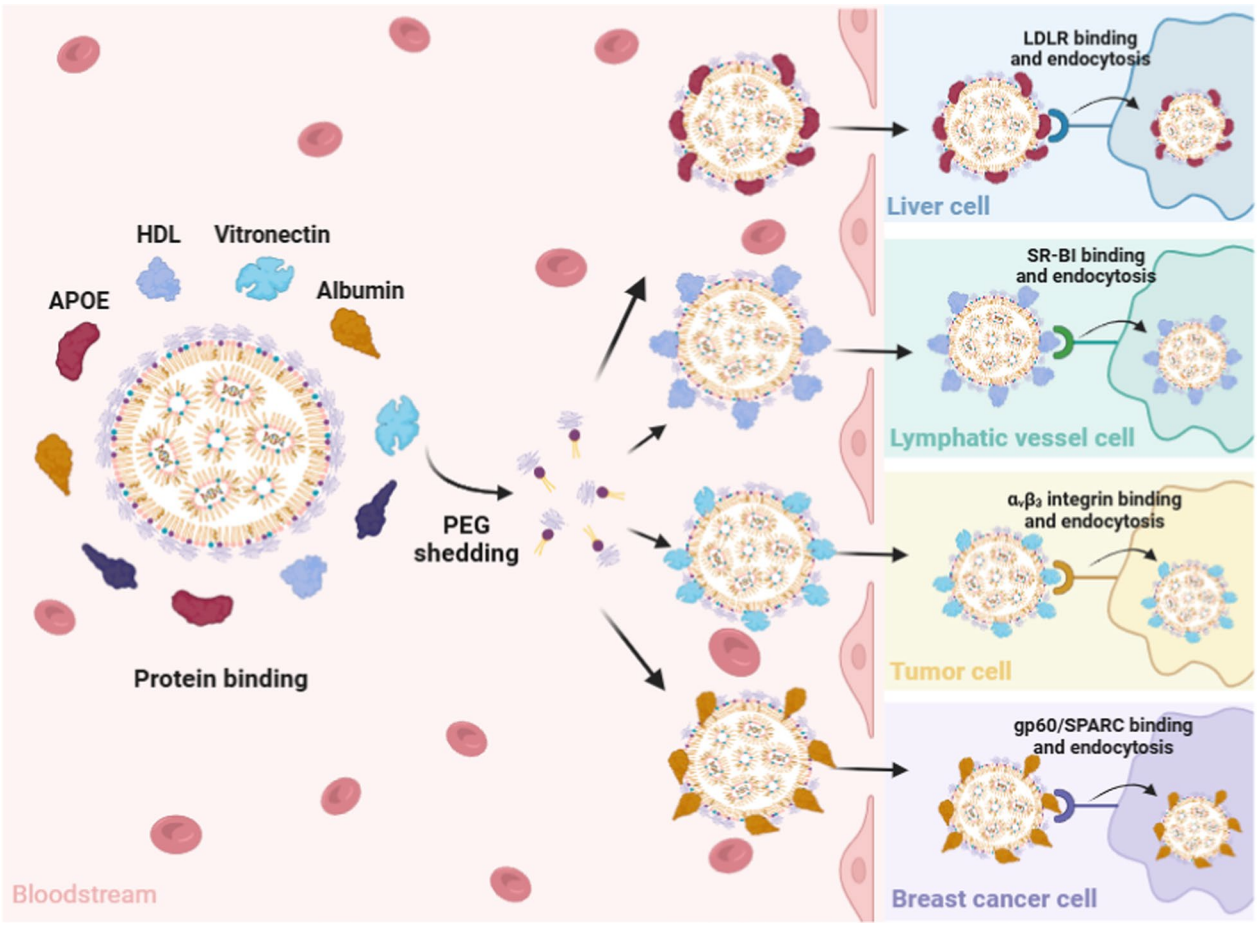

Fig. 7 Natural targeting of various cells by LNP-RNA therapeutics influenced by PEG-lipid shedding and protein corona composition. ApoE, high-density lipoprotein (HDL), vitronectin, and albumin can naturally target LNPs to the liver parenchyma, lymphatic vessel, and tumor and breast cancer cells, respectively

are unrelated to surface charge, suggesting that the introduction of amide bonds influences the corona composition, thereby facilitating lung-specific mRNA delivery [136]. Dahlman et al. have demonstrated that altering the length of the hydrophobic tail in lipids derived from 2,5-piperazinedione enables RNA delivery independent of ApoE or LDLR, highlighting the significance of lipid chemical structure in directing LNP navigation [137]. Another study found that modifying LNP helper lipids can change tissue tropism: DSPC-containing LNPs accumulate in the spleen, while DOPE-containing LNPs target the liver due to different affinities for ApoE [118]. The presence of proteins in the LNP corona does not guarantee targeted receptor interaction. Factors such as protein availability, conformation, and orientation are crucial, as not all proteins function as targeting agents; so, understanding how adsorbed proteins on LNPs affect cellular recognition is vital for targeted delivery [138]. Chan et al. used the corona "fingerprint" of gold NPs, suggesting that cellular uptake prediction is more accurate when based on the corona rather than particle size, charge, or clustering. However, in LNPs, only a minority of corona proteins enhance cellular association, highlighting the complexity of developing targeted delivery systems for gene therapies beyond liver applications [139]. Approaches like grafting targeting moieties onto LNP surfaces or adjusting surface chemistry enhance tissue- or cell-specific delivery [139].

Systemic circulation and blood clearance

The fate of LNPs within the body is determined by their stability, circulation, interaction with the immune system, and clearance from the bloodstream, all of which are crucial for the success of gene therapy [140]. Some LNP formulations are ineffective in transporting genetic material due to their instability in biological fluids, highlighting the need to evaluate their stability and integrity under these conditions. For example, cholesterol-free LNPs containing pH-sensitive cationic lipids, egg sphingomyelin, and PEG-lipid demonstrate instability in blood, resulting in premature siRNA release [141]. The adsorption of proteins onto LNPs triggers coagulation processes and opsonin attachment, leading to their recognition and elimination by the MPS [142]. These events reduce NP delivery to target organs and decrease therapeutic effectiveness. Electrostatic attraction between positively charged LNPs and negatively charged proteins causes particle agglomeration, which shortens their circulation time and reduces cellular internalization. Additionally, the protein corona can compromise particle integrity, leading to premature cargo release and affecting biodistribution and circulation time [143]. Serum proteins can destabilize LNPs, necessitating thorough testing for agglomeration, premature RNA release, and dye leakage in biological fluids.

Furthermore, LNPs possess similar characteristics to viruses, including a lipid envelope and a comparable size and curvature. Viruses also exhibit a biomolecular corona that affects infectivity and DC activation [144], indicating an evolutionary role in evading the immune system. Consequently, a significant challenge in LNP delivery is evading the immune system [145]. NPs often face challenges in (pre)clinical trials due to strong immune responses after injection, with subsequent administrations potentially triggering an adaptive immune reaction.

Various strategies, including modifying LNP composition by adding cholesterol with high-phase transition temperature phospholipids, can reduce LNP clearance by altering protein adsorption on the NP surface [146]. PEGylation technology induces a "stealth effect" on NPs by hindering protein adsorption and opsonization. This enhances LNP circulation stability, reduces macrophage phagocytosis, and prolongs blood residency. Proteins like clusterin in the PEGylated NP corona further enhance the stealth effect. The efficacy of PEG-lipid depends on its quantity, molecular size, and surface density. Specifically, increasing the PEG-lipid content with longer chains (C18) on LNPs boosts circulation duration, while shorter chains (C14) do not have the same effect. At least 1.5 mol% PEG-lipid is necessary to alter LNP pharmacokinetics. Additionally, high PEG content reduces protein interactions, while the chain length influences the protein corona composition [147, 148]. Utilizing PEG and related modifications requires meticulous assessment due to potential limitations. The "PEG dilemma" refers to the reduced cellular internalization and endosomal release associated with PEGylation. Solutions to this issue involve using pH-sensitive, enzyme-cleavable, and detachable PEG-lipids. Research on de-PEGylation kinetics for siRNA delivery indicates that PEGs with short, saturated tails are essential for effective de-PEGylation and gene silencing, exemplified by Onpattro. Such PEGs can swap with proteins like ApoE post-administration, improving hepatocyte targeting and demonstrating the strategic use of corona formation for circulation control and tissue targeting [149].

Cellular uptake and intracellular trafficking

Non-viral vectors are typically internalized through endocytosis, utilizing various pathways such as clathrin-mediated, caveolae-mediated, and macropinocytosis [150]. Endocytosis efficiency is influenced by receptor types and densities, with receptors like LDL and ASGPR known for their high uptake capacities [151]. Once internalized, particles are transported to endosomes, undergo degradation, and are eventually directed to lysosomes

upon acidification. Achieving endosomal escape is crucial for effective gene delivery, as some pathways can circumvent lysosomal degradation. Intracellular transfer mechanisms, independent of endocytosis, include membrane fusion, as observed in viruses like the Sendai virus and HVJ envelope vectors [152]. Fluorescence imaging is a suitable method for tracking the subcellular distribution of non-viral vectors, particularly in monolayer-cultured cells, enabling quantitative analysis and visualization [153]. However, challenges arise in three-dimensional cultures like spheroids due to low fluorescence detection, and assessing subcellular distribution in living organisms using tissue sections remains challenging [154].

Endosomal escape efficiency is pivotal for successful gene delivery. LNPs are typically trapped in endosomal compartments, with only a small fraction managing to escape [155]. Although the precise mechanism is not fully understood, positively charged lipids may enhance electrostatic interactions and fusion phenomena with negatively charged endosomal membranes, releasing mRNA molecules into the cytoplasm [156]. Jiang et al. [157] introduced a protein probe, ddRLuc, composed of a signal sequence fused to a human IgG1 Fc fragment. This probe binds cells via the Fc receptor. Once expressed in cells, the fusion protein (ddRLuc-Fc) undergoes glycosylation in the endoplasmic reticulum and is subsequently secreted. Activation of ddRLuc-Fc depends on deglycosylation mediated by the cytosolic enzyme, NGLY1, following cellular entry and endosomal release [158, 159]. Common strategies to enhance endosomal escape use pH-sensitive polymers, fusogenic lipids or peptides, and the design of more potent and safer ICLs. Endosomal escape can be optimized by adjusting the pKa of ionizable lipids [160]. Additionally, the properties of lipidic tails can influence the endosomal escape of LNPs [85]. For instance, lipids with branched tails demonstrate improved endosomal escape compared to those with linear tails due to stronger protonation at endosomal pH [161]. Moreover, the type of helper lipids and the ratio of lipids affect endosomal escape [162]. Among helper lipids, DOPE demonstrates high gene delivery efficiency in vitro by inducing fusion with the endosomal membrane. However, fusion with erythrocytes occurs in vivo, reducing efficiency [163]. PEI facilitates endosomal escape through the proton sponge effect, inducing disruption of endosomes due to chloride ion influx [164]. Additionally, fusogenic peptides like GALA aid in pH-sensitive endosomal release. Furthermore, inorganic salts such as calcium phosphate promote endosomal escape through acidification and dissolution [165]. Additionally, certain techniques can achieve efficient gene delivery without requiring endosomal escape by creating temporary pores in the cellular membrane using external forces. These techniques include electroporation, microbubbles, ultrasound application, and the hydrodynamic strategy involving rapid injection of a substantial volume of NA solution [166–168]. Following entry into the cytosol, LNP diffusion is hindered by cytoskeletal structures [169, 170]. In dividing cells, exogenous NA localizes in the nucleus during cell division when the nuclear membrane disintegrates. Conversely, non-dividing cells experience limited mass transport due to the intact nuclear membrane. Nuclear pores allow the diffusion of molecules up to 60–100 kDa, requiring NLS for larger materials [171]. The nuclear transport of RNA therapeutics is crucial for influencing intranuclear gene activity. Tammam et al. demonstrated that chitosan conjugated with an NLS peptide effectively transported 150 nm into the nucleus [172].

An ideal nanovector must tightly load mRNA before entering the cytoplasm for optimal protection and delivery, and then rapidly release the mRNA once in the cytoplasm. Various methods, such as FRET, gel electrophoresis, flow cytometry, and qPCR, can investigate the mechanisms and kinetics of mRNA dissociation from LNPs. These methods provide insights into the location, extent, and rate of mRNA release from LNPs and correlate with gene expression levels.

To achieve controlled unpacking of RNA-LNPs and to regulate in vivo behavior, stimuli-responsive nanovectors have been developed to respond to intracellular triggers such as pH, redox, ATP, and enzymes and external triggers like magnetic fields, light, and ultrasound [173]. These nanovectors can stably encapsulate RNA in physiological environments and non-target sites but release RNA cargo upon structural changes triggered by specific stimuli in target cells [174, 175] (Fig. 8). pH-responsive LNPs remain stable at physiological pH but become positively charged in acidic environments, enhancing gene release and endosomal escape. This adaptability aids in targeted delivery and reduces cytotoxicity [176]. For example, using a pH-catalyzed hydrolysis strategy, Tanaka et al. designed a self-degradable ionizable lipid (ssPalmO-Phe) containing disulfide bonds and phenyl esters. This strategy promotes LNP degradation and mRNA release through intra-particle enrichment of reactants, enhancing transfection efficiency [158]. In addition, DOP-DEDA, a novel charge-reversible lipid derivative, shows different charges at varying pH levels, ensuring stability at physiologic pH without PEG-lipids. DOP-DEDA LNPs effectively suppress PLK1 mRNA and protein expression and enhance siRNA delivery with apoE3 [177]. Additionally, Siegwart et al. studied PEGylated BODIPY dyes (PBDs) as surface-stabilizing agents for LNP formulations with pH-responsive mRNA release. PBD LNPs greatly enhanced cytoplasmic protein

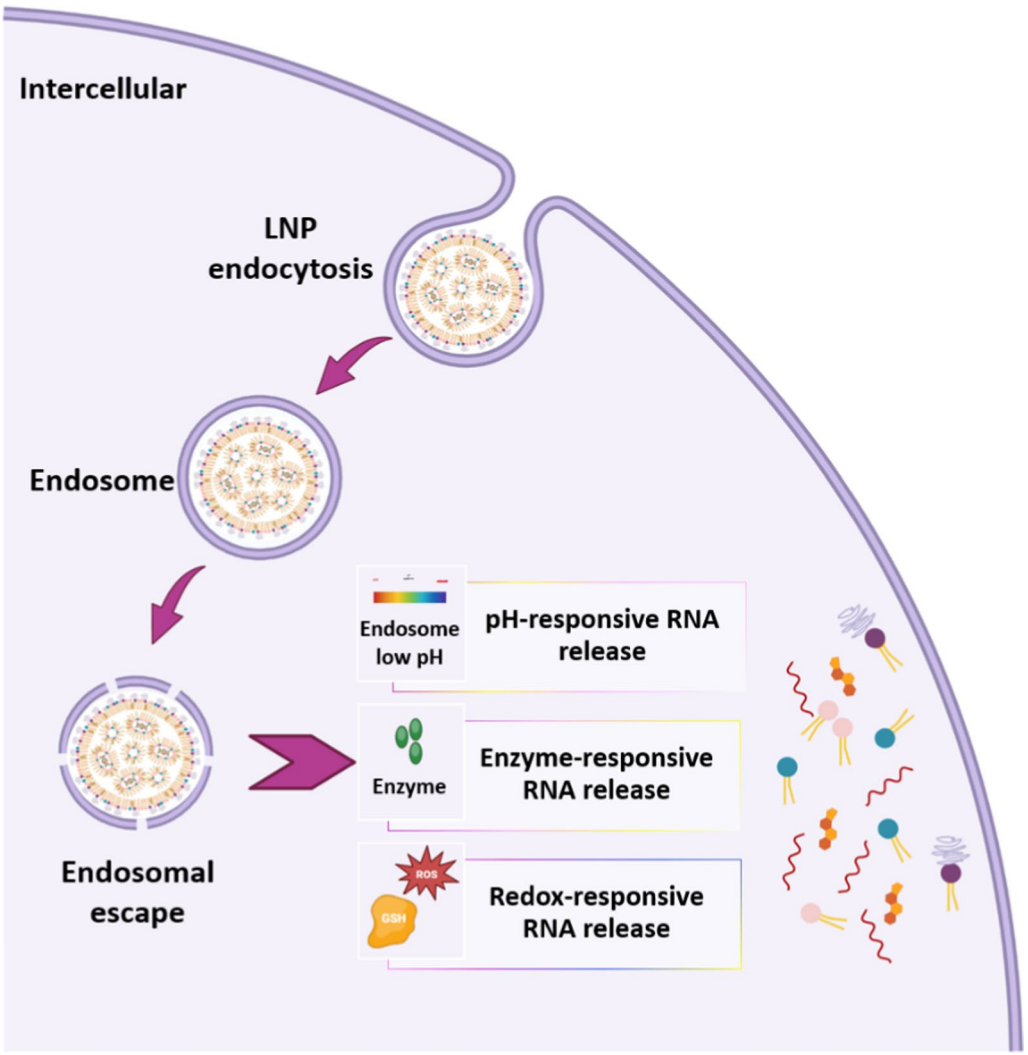

Fig. 8 Illustration of endosomal escape and stimuli-responsive RNA release mechanisms; pH-, enzyme-, and redox-responsive RNA release can be achieved following LNP endocytosis

production and better mRNA delivery in the liver compared to PEG-DMG LNPs [178]. Furthermore, a novel pH-sensitive LNP called BAMPA-O16B has been developed to enhance siRNA delivery and endosomal escape in GBM cells. This LNP effectively delivered siRNA against CD47 and PD-L1, improving T cell-dependent antitumor immunity in mice [176].

Higher redox potential in the cytoplasm compared to the extracellular environment can trigger RNA release. The higher GSH concentration in the cytoplasm facilitates the disruption of disulfide bonds within LNP structures incorporating the CR8C peptide, enabling improved gene therapy [165]. An ionizable lipid with a degradable linker (4A3-SCC-PH) and branched tails significantly enhanced mRNA transfection, showing a 15.5-fold improvement in FLuc mRNA delivery compared to MC3 LNP. This was due to the asymmetric alkyl chains attached to the thioether and the GSH-responsive disulfide bond, creating a conical shape that facilitated efficient mRNA delivery in malignant cells [179]. Kamath et al. reported a redox-responsive LNP loaded with TP53-coding mRNA for inducing enhanced apoptosis in TP53-deficient Hep3B HCC and H1299 NSCLC cells in vitro and in vivo [180]. Also, ROS is significantly higher in cancer cells than in normal cells, making ROS a specific stimulus for triggering mRNA release [181]. Similarly, boric acid ester [182–184] and thioketal linkages [185] can create ROS-sensitive LNPs for controlled gene release. Cai et al. synthesized an ionizable lipid, BAmP-TK-12, containing ROS-degradable thioketal moieties for

mRNA delivery. High ROS concentrations in tumor cells induce the oxidation and degradation of TK-12 moieties in BAmP-TK-12-based LNPs, facilitating mRNA release and subsequent translation. This approach successfully delivered mRNA encoding RAS protease DUF5 for RAS mutant depletion, suppressing tumor growth in A549 tumor-bearing mice and demonstrating the potential of ROS-responsive LNPs for tumor-specific mRNA delivery [52, 186].

Enzyme-responsive nanocarriers can trigger gene release, carrier dissociation, and improved cellular uptake in tumor environments. For example, esterase-responsive cationic polymers can reverse their charge to release therapeutic RNA in the presence of intracellular esterase [187]. Similarly, matrix metalloproteinase-sensitive nanocarriers can release their payload specifically in tumor tissues, enhancing therapeutic efficacy [188].

ATP-responsive LNPs for RNA delivery leverage the high ATP levels in tumor cells to achieve targeted and efficient gene release. The phenylboronic acid group in these nanocarriers can form and break ester bonds with ATP, leading to nanoparticle dissociation and RNA release [189]. Other examples include phenylboronic acid-modified polymers and PEI crosslinked with alginate, which enhances transfection efficiency and gene silencing in tumor cells [190, 191].

Multi-stimulus responsive LNPs for RNA delivery are designed to respond to multiple stimuli, enhancing accuracy and specificity. For example, Gao et al. developed a pH/redox dual-responsive polyplex for delivering MDR1 siRNA and doxorubicin, which releases its payload in acidic and high GSH environments, demonstrating potent antitumor efficacy [192]. Similarly, Zhang et al. designed a dual-locking nanoparticle responsive to pH and H_2O_2 levels in tumor microenvironments to control CRISPR/Cas13a gene editing selectively [193].

External-stimuli responsive systems are also being explored for RNA delivery, although less attention has been given to LNP formulations. Magnetic field-responsive LNPs can accumulate at tumor sites when guided by an external magnetic field. Studies with magnetic mesoporous silica Nps and magnetic Fe_3O_4 NPs coated with polydopamine have shown increased tumor targeting and therapeutic efficacy [194, 195]. UV light-responsive systems like coumarin-anchored PAMAM dendrimers, release their payload upon UV exposure [196]. Mo et al. developed a LASER strategy using porphyrin-LNPs, which generate ROS when exposed to near-infrared light, enhancing membrane disruption and endosomal release of siRNA. This method improved siRNA escape and target knockdown in prostate cancer cells. In vivo, porphyrin-LNPs with gold NPs confirmed the LASER effect, enhancing RNA therapeutic delivery [197]. Far-red light-mediated nanocarriers, such as those developed by Wang et al., utilize photosensitizers to produce ROS, promoting endosomal escape and gene release [198]. Another example is photolabile spherical nucleic acids that disassemble and release siRNA upon NIR light-triggered ROS production [199]. Ultrasound-targeted gene delivery employs ultrasound to direct genes to target sites, enhancing cellular uptake by increasing membrane permeability. Genes are loaded onto ultrasonic NPs using cationic lipids like DPPC, DSPC, and DOTAP. For example, mannose-modified bubble lipoplexes showed efficient DNA vaccination under ultrasound, achieving potent antitumor effects [200]. Biosynthetic nanobubbles from Halobacterium NRC-1 also improved gene transfection efficiency [201]. Ultrasound-enhanced ROS nanocarriers decorated with sonosensitizers like IR780 facilitate gene release through ROS generation [202].

Biodegradability plays a vital role in reducing the toxicity of nanovectors, and LNPs composed of ionizable lipids that break down into non-toxic metabolites are favored. Introducing ester bonds increases lipid biodegradability, as shown for lipids like L319 [44], Lipid 5 [160], SM-102 [41], and ALC-0315 [203]. Notably, SM-102 and ALC-0315 are used in COVID-19 vaccines [204]. Combining ester and disulfide motifs further accelerates degradation through disulfide bond cleavage, driving intraparticle nucleophilic attack on ester linkers [158, 205, 206]. Whitehead et al. synthesized a large library of degradable lipidoids created via the conjugate addition of alkyl-amines to alkyl-acrylate tails. Lipidoids have been formulated with cholesterol, DSPC, PEG2000-DMG, and siRNA for LNP preparation. The researchers discovered that particular structural and pKa parameters can predict over 95% of protein silencing in vivo, indicating that these criteria may serve as alternatives to expensive cell culture assays and animal testing [207]. Other advances include pH-sensitive PEG detachment [208] and employing materials like black phosphorus [209] that degrade under specific conditions, releasing their payload effectively. The use of biodegradable components like hyaluronic acid [210], polydisulfides [211], thioketal-crosslinked PEI [198], PEI-modified nanobubbles [201], and responsive materials like boric acid esters [182] enhances the efficacy and safety of RNA delivery. Therefore, properly designed LNPs can ensure precise control of targeted gene delivery while maintaining biocompatibility and safety [212].

Selection of the administration route

The route of administration significantly impacts the distribution, kinetics, and efficacy of RNA-LNP [6]. Intravenous gene delivery without a carrier leads to rapid degradation in the blood and minimal gene expression in liver non-parenchymal cells [213]. In contrast, local

injections, particularly into muscle tissue, result in traceable gene expression [214]. After intravenous injections, LNPs predominantly localize within various liver cell types, including hepatocytes, LSECs, and Kupffer cells [215]. LNPs primarily target the liver because of their physicochemical properties and anatomical and physiological features, such as high blood flow and discontinuous hepatic capillaries [216]. To broaden the therapeutic potential of LNPs, achieving biodistribution to organs beyond the liver is essential. Intra-arterial and intraportal injections can alter the first-pass effect, whereas intravenous injection primarily targets the lungs as the first-pass organ. Intraperitoneal delivery, commonly used in animal research rather than human studies, is associated with lower systemic toxicity than intravenous methods. Melamed et al. observed that this administration route, when applied to LNPs with cationic phospholipids, resulted in significant protein expression in the pancreas. This finding suggests potential new treatments for pancreatic diseases, including cancer and diabetes [217].

Local administration of LNPs, including intradermal, subcutaneous, and intramuscular injections, can elicit systemic immune responses by delivering mRNA to lymph nodes, where APCs and T cells are present [6]. Compared with intravenous injection, these routes often lead to prolonged protein expression at the injection site [41], potentially bypassing systemic circulation and offering sustained therapeutic effects. However, mRNA and/or lipid components may also disseminate to other tissues such as the lungs, liver, non-draining lymph nodes, and spleen [41]. LNPs employed in mRNA vaccines, including COVID-19 vaccines, predominantly generate antigen expression in the deltoid muscle and draining lymph nodes [218]. The intramuscular route allows for larger injection volumes compared with the intradermal and subcutaneous routes [38], whereas the intranodal route holds promise for naked mRNA delivery [219].

Intraocular delivery is an effective strategy for addressing ocular diseases, as conventional methods fail to reach the posterior region of the eye [198]. Techniques such as intravitreal and subretinal injections have proven successful in targeting the retina and retinal pigment epithelium with LNPs [220]. Research by Patel et al. demonstrated that ICLs with specific chemical features, such as low pKa and unsaturated tails, enhance gene expression in the retinal pigment epithelium following subretinal injection [221]. Similarly, LNPs designed with minimal PEGylation have shown increased expression in retinal cells after subretinal and intravitreal administration. Targeting the neural retina and photoreceptors, which are typically difficult to access, has been achieved through receptor-mediated uptake using LNPs modified with retina-specific peptides, as demonstrated by Herrera et al. This method holds significant potential for advancing treatments for hereditary retinal diseases [222].

Pulmonary routes such as intratracheal, intranasal, and aerosol inhalation offer direct lung-targeted delivery of therapeutics, reducing systemic adverse effects. This method circumvents the sequestration of mRNA-LNPs by Kupffer cells in the bloodstream [223]. Intratracheal administration of LNPs, whether unmodified, cationic, or ligand-tethered, is effective for siRNA delivery, with mannose-modified LNPs being particularly efficacious against pulmonary fibrosis [224]. Lokugamage et al. refined LNP formulations for pulmonary delivery through a nebulizer, termed NLD1, enriched with PEG-lipid and helper cationic lipids, achieving superior mRNA delivery to the lungs compared to LNPs designed for systemic circulation [69].

Oral and transdermal routes present significant challenges for LNP delivery due to physiological barriers. Microneedles offer a promising solution for delivering NAs across the skin [225]; however, oral delivery of LNP-NA formulations faces various hurdles in the gastrointestinal tract, including acidity, bile salts, digestive enzymes, mucus, and membrane permeability [226]. Various formulation strategies have been explored to overcome these challenges, with modifications aimed at enhancing stability, retention, and efficacy [227]. For instance, pH-responsive polymer coatings can prevent pepsin digestion and bile salt interaction, whereas lyophilization enables regular oral administration via solid dosage forms [227]. Orally available siRNAs have shown promise in preclinical and clinical studies, attributed to conjugation technologies like GalNAc conjugation and formulations containing intestinal permeation enhancers [228].

Overall, the physiological barriers to LNP delivery should be carefully considered to develop non-invasive administration methods, refine LNP-NA interactions, and enhance therapeutic effects.

LNP for tissue-specific gene delivery

LNPs exhibit promise as carriers for RNA therapies in living organisms, notably highlighted by the emergency authorization of mRNA COVID-19 vaccines. However, challenges persist, including achieving extrahepatic delivery due to liver accumulation via ApoE-LDL interaction and LNP clearance by the hepatic or renal systems or MPS [229]. Effective strategies for targeting specific organs and tissues are vital for expanding RNA-LNP applications. Recent research has concentrated on routes of administration and engineering functionalized LNPs to tackle these challenges [230]. Targeted drug delivery systems aim to precisely transport therapeutic agents to specific cells or tissues, improving treatment efficacy and minimizing mRNA-related side effects, particularly

in genome editing. Controlling the distribution of LNPs is crucial for addressing conditions localized to specific tissues or organs. Active and passive targeting are two strategies used to improve the efficacy and specificity of therapeutic agents. Pathophysiological conditions can create unique environments for tissue-specific drug delivery. Passive targeting relies on the natural physiological and pathological differences between healthy and diseased tissues. Tumors often have leaky vasculature and poor lymphatic drainage, so the EPR effect allows NPs to accumulate more in tumor tissue than in normal tissue. In ischemic conditions, areas with reduced blood flow can be targeted as NPs can accumulate in these regions due to the disrupted blood flow. Active targeting involves the use of specific ligands that bind to receptors on diseased cells. Tumors often have abnormal vasculature, overexpressed receptors, and an acidic environment. These features can be targeted by designing functionalized and stimulus-responsive LNPs. Inflammatory conditions like rheumatoid arthritis or inflammatory bowel disease have overexpressed inflammatory markers that can be targeted with specific ligands conjugated with LNPs [231]. Other pathophysiological changes, including glutamate excitotoxicity, oxidative stress, mitochondrial dysfunction, cell death, driver gene mutation, angiogenesis, and aberrations in the cell cycle and signaling pathways elucidate potential targets for nanoparticle-based drug delivery systems [232, 233].

The following section elaborates on the latest advancements in LNP passive and active targeting approaches.

Passive targeting

Passive targeting in LNPs is mainly influenced by the adsorption of the protein corona, which dictates their biodistribution. Biodistribution is also determined by the LNPs' physicochemical characteristics, including size, charge, morphology, composition, and ratio of components. LNPs interact with ApoE, after PEG detachment, facilitating passive delivery. Modulating particle size is one approach to achieving targeted organ delivery [234, 235]. LNPs under 10 nm in size are swiftly eliminated by renal clearance, predominantly gathering in the liver, and traversing into the lymphatic system. In contrast, larger LNPs tend to accumulate in the spleen and lungs or may persist at the administration site [234]. LNPs of intermediate sizes, around 78 nm, exhibit optimal delivery efficiency targeting the liver. Adjusting particle size via formulation parameters is essential for targeted delivery. LNPs incorporating pSar instead of PEG lipid showed elevated mRNA expression levels in the spleen, with a tendency for increased spleen accumulation as their size diminished [236]. The leaky nature of tumor vasculature allows for the passive targeting of NPs to tumors via the EPR effect [237]. However, the efficacy of the EPR effect is generally limited in human tumors [123]. Consequently, smaller NPs (specifically those under 50 nm) are considered more effective for human cancer treatment. Additionally, the LNP surface charge significantly impacts biodistribution and mRNA delivery efficiency. Positively charged LNPs target the lung, neutrally charged LNPs preferentially enter the liver, and negatively charged LNPs mostly accumulate in the spleen [238, 239].

LNP components, especially ICLs, which interact with specific serum proteins, are crucial for designing new delivery materials targeting organs. For instance, LNP multi-tailed iPhos have been developed to enhance endosomal escape and mRNA/sgRNA delivery for in vivo genome editing. These lipids can function together with variously charged helper lipids to allow organ-specific delivery [240]. Modifying the ICL linker structure impacts protein adsorption, consequently influencing mRNA delivery from the liver to the lungs [136]. LNPs having alkyne lipid tails can significantly increase membrane fusion to enhance mRNA release in the liver [53]. Lee et al. chemically synthesized 4A3-Cit using unsaturated thiols, resulting in an 18-fold improvement in transfection efficiency in vitro and significant liver targeting [85]. Lokugamage et al. synthesized a constrained adamantane cationic lipid, forming an armchair structure, capable of delivering siRNA to T cells in vivo [241]. The ionizable lipid OF-Deg-Lin with ester linkages generates a non-toxic fatty acid facilitated targeting of the spleen and transfection of B lymphocytes [239]. Qiu et al. utilized N-series LNPs containing an amide bond in the tail, selectively delivering mRNA to the mouse lung [136]. Ding et al. showed enhanced in vivo mRNA delivery by editing lipid amine-head groups, with spermine-derived head groups exhibiting the strongest cellular uptake, endosomal escape, and transfection potency of IL-12 siRNA in acute liver failure [242]. A study by Mitchell et al. demonstrated that suitable adjustments to the chemical structure of ionizable lipids by incorporating siloxane composites into the ionizable lipids can direct LNPs to specific tissues such as the liver, lungs, and spleen [243]. Zhang et al. demonstrated the effectiveness of functionalized *N*1,*N*3,*N*5-tris(2-aminoethyl) benzene-1,3,5-tricarboxamide derivatives (FTT) in promoting in vivo mRNA delivery. They found that FTT-lipids with branched ester chains, such as FTT2 to FTT6, facilitated efficient mRNA delivery to the liver and spleen compared with linear ester chain analogs like FTT7 to FTT10 [244]. In another study, Kowalski et al. demonstrated that amino polyesters synthesized from tertiary amino alcohol and lactone could more effectively express mRNA in the lung endothelium and liver hepatocytes,

resulting in nearly tenfold higher systemic mRNA delivery efficacy [245].

Cholesterol modification is another option for passive targeting. Paunovska et al. formulated LNPs with esterified cholesterol, demonstrating enhanced efficiency in gene delivery to liver microenvironment cells compared to regular or oxidized cholesterol [64]. LNPs containing cholesteryl oleate efficiently delivered CRISPR sgRNAs to liver endothelial cells in vivo [246]. Jung et al. revealed LNPs with histidinamide-modified cholesterol, resulting in improved intracellular delivery of SARS-CoV-2 spike mRNA [247]. CHEMS, an anionic phospholipid, was combined with other lipids to target lymph nodes [234].

Passive targeting of LNPs can also be achieved through phospholipid and PEG-lipid modification. Gan et al. developed LNPs containing constrained phospholipids with adamantyl groups, enhancing targeting to liver endothelial cells and Kupffer cells compared to unconstrained phospholipids [248]. Replacement of phospholipids with 18:1 BMP (S, R), imparting a negative charge, resulted in spleen targeting [249]. Sago et al. altered the lipid tail of PEG-lipid, thereby modifying the tropism of LNPs to target BMECs [250]. Furthermore, lipid-polymer hybrid NPs have shown effectiveness in delivering RNA through the mucus-covered epithelium in the human airway, indicating their potential in inhaled siRNA therapy for CF [251].

The SORT approach entails incorporating an additional SORT molecule into standard LNP components, guiding them to precise anatomical sites based on their biophysical characteristics. The SORT molecule can manipulate the internal and/or external charge of formulated LNPs, enabling targeted gene delivery to the lung, spleen, or liver. For instance, adding higher percentages of permanently positively charged DOTAP alters tissue tropism from the liver to the lungs. Conversely, incorporating a negatively charged 18PA SORT molecule at 10–40% in an LNP formulation leads to spleen-specific Luc expression [128]. Figure 9 shows the factors affecting passive targeting.

Active targeting

Active targeting utilizes specific ligands or molecules that bind to receptors or surface structures on target cells, enhancing the uptake of the delivery system [252]. Common ligands, including proteins, antibodies, peptides, carbohydrates, aptamers, and vitamins, can be incorporated into LNPs through non-covalent interactions or covalent bonds, primarily to PEG-lipids [253]. Generally, several conjugation strategies can be employed to attach targeting ligands to the surface of LNPs or lipid compounds. These strategies include direct covalent conjugation via carbodiimide, maleimide, or click chemistry, and non-covalent conjugation through electrostatic, hydrophobic, or hydrogen bonding interactions. Additionally, lipid anchor incorporation of lipopeptides or lipids modified with targeting moieties can be incorporated into the lipid bilayer during fabrication or post-insertion into preformed LNPs. Hybrid strategies combine multiple conjugation approaches to design ligand-targeted mRNA-LNPs with improved therapeutic properties. These ligands facilitate interactions with target cell surface molecules, such as ligand-receptor or Ab-antigen pairs, promoting retention at the target site and NP uptake by target cells [254].

Sugar modification has emerged as a valuable tool for targeting specific cell types. Galactose and GalNAc target liver parenchyma cells, whereas mannose modification targets non-parenchyma cells and APCs [255]. Ligands like GalNAc and mannose have been coupled to PEG lipids for efficient liver and hepatic sinusoidal endothelial cell targeting [256]. Combinations like mannosylation of bubble lipoplexes with ultrasound irradiation have shown effective delivery of NF-κB decoy to TAMs, enhancing survival in animal models [257]. LNPs functionalized with HA offer promise for active targeting of CD44-positive cells in various cancers and certain types of inflammatory and immune cells [258].

Conjugation of antibodies, aptamers, or peptides to LNPs enhances targeted delivery [259, 260] (Table 2). For example, Rurik et al. showed that modifying LNP surfaces with T cell-targeting antibodies enables mRNA delivery for specific targeting of cardiac fibroblasts, a novel approach for heart failure treatment [261]. LNPs functionalized with CD38 mAb specifically target lymphoma cells [262], whereas LNPs modified with CD5 antibodies deliver mRNA to CAR-T cells for heart disease treatment [261]. Coupling LNPs with CD31 antibodies achieves targeted therapy of pulmonary endothelial cells [263]. Lipoprotein incorporation into siRNA-loaded LNPs creates ASSET, facilitating interaction with targeting antibodies. Arginine-rich cell-penetrating peptides, combined with targeting ligands, enhance gene delivery [264]. Kwong et al. engineered APNs that target $CD8^+$ T cells, facilitating the in vivo delivery of mRNA to antigen-specific T cells [265]. Ligands like RGD peptide and HER-2-targeting peptide show promise for active tumor targeting [266, 267]. LNPs conjugated with ligands specific to T cell markers (CD3, CD4, CD5, and CD8) enhance the targeting efficiency. In addition, functionalized polymers or polymerized peptides with GALA repeats, which provide fusogenicity to APCs, have been explored as non-liver RNA-delivery therapeutics [268]. Furthermore, the CH6 aptamer enables targeted gene delivery to osteoblasts through LNPs [269].

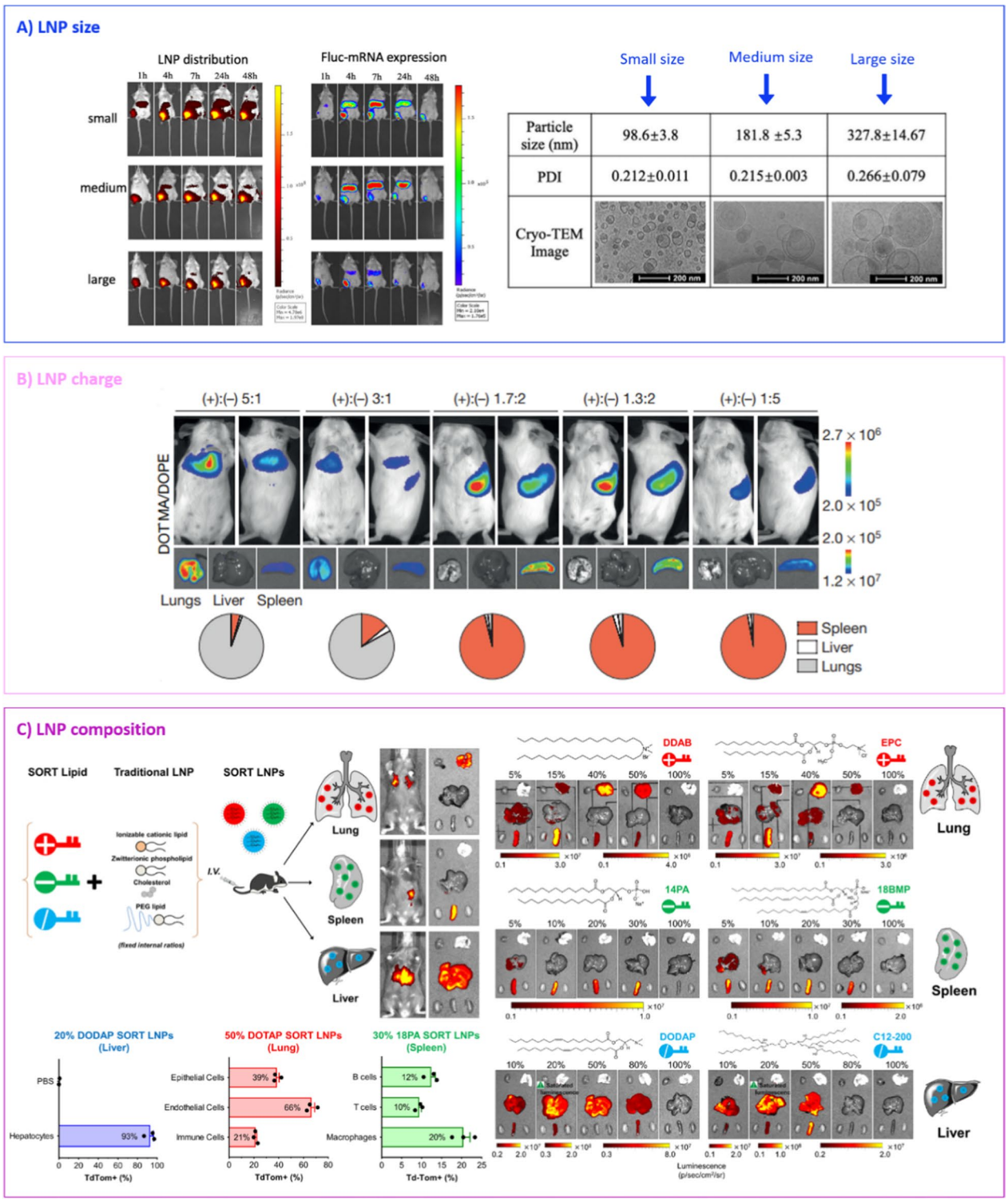

Fig. 9 Factors affecting lipid nanoparticle (LNP) passive targeting: **A** Fluorescence and bioluminescence biodistribution patterns of 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindotricarbocyanine iodide (DiR)-tagged Fluc-mRNA LNPs varying in size and the corresponding luciferase expression after intramuscular administration [235]; **B** biodistribution of Luc-LNP at various charge ratios in BALB/c mice 24 h after intravenous administration [238]; **C** incorporating an additional element, known as a selective organ targeting (SORT) molecule, into conventional LNPs modifies their distribution within the body and facilitates targeted delivery to specific tissues [128]

Table 2 Active targeting strategies for LNP-RNA therapeutics

Ligand	Receptor	Condition/target cell or organ	Payload	Ref.
Glu-urea-Lys PSMA	Androgen receptor	Prostate tumor	AR21 and AR25 siRNAs	[270]
MAdCAM-1-Fc protein	α4β7 integrin	Inflammatory gut-homing leukocytes	siRNA	[271]
aCD3 mAb (in vitro) aCD3 F(ab')2 (in vivo) Plasmalemma	Cd3	T cells in the tumor environment	mRNA	[272]
PV1	Caveolae	Lung	mRNA	[273]
Anti PECAM-1 (CD31) mAb	Lung endothelial cells	Lung	mRNA	[263]
DEC205 scFv	DEC205 + murine DCs	Spleen	siRNA	[274]
aCD4 mAb	CD4	T cell	mRNA	[275]
aCD5	Splenic T cells	Cardiac fibrosis	mRNA	[261]
pMHC1	Antigen-specific $CD8^{+}$T cells	Pr8 influenza virus	mRNA	[265]
CH6 Aptamer	Osteoblast	Metabolic skeletal disorders associated with impaired bone formation (e.g. osteoporosis)	siRNA	[269]
Anti-VCAM-Ab (CD106)	VCAM1	Brain neutrophils/glioblastoma	mRNA	[276]
aCD177	C5a	Neutrophils	siRNA and ASOs	[277]
Anti-Ly6C antibodies by ASSET	IRF8 mRNA	Ly6C + inflammatory leukocytes/DSS colitis	siRNA	[278]
aEGFR	EGFR	Tumor	Cas9-mRNA/ sgRNA	[279]
BP	Monocytes in bone marrow	Bone	mRNA	[280]
Mannose	Langerhans cells, dermal DCs, and resident DCs in the skin-draining lymph node	Influenza virus	SAM	[281]
Mannose	Hepatocytes and LSEC	Liver	mRNA	[256]
α-Galactosyl ceramide	Invariant natural killer T cells	Tumor	mRNA	[282]
Mannose	DCs in lymph node	Tumor	mRNA and siRNA	[283]
Tri-GalNAc	ASGPR	Hepatocytes in liver	siRNA	[284]
Cyclic peptide pPB	PDGFR β	Hepatic stellate cells in liver	siRNA	[285]
Mannan	Mannose receptor	DCs	SAM	[286]
Anti-β7-mAb	Integrin β7	T-lymphocytes	siRNA	[287]
Angiopep-2	LRP1	Brain	siRNA	[288]
RVG-9r	nAChR	Brain	siRNA	[289]
Azide-modified antibodies (CLIP approach)	LYVE1	LECs	siRNA	[290]
Doxorubicin	Bcl-2	Tumor	siRNA	[291]
LDV	α4β1 integrin	Leukemic cells	siRNA	[292]
Peptide ligands	PRs	Neural retina	mRNA	[222]
Transferrin	Transferrin receptor	Brain	miRNA	[293]
PD-L1	PD-L1	Breast cancer	mRNA	[294]
Anisamide	Sigma receptor of HSCs	Liver fibrosis	siRNA	[295]

Exploring small-molecule ligands has diversified targeted delivery with LNPs. Folate-functionalized LNP shows promise for brain and breast cancer therapy [296]. LNPs modified with phenylboronic acid target cancer cells by interacting with surface sialic acid residues, enabling selective binding and uptake [297]. LNPs modified with imidazole target splenic T lymphocytes for mRNA delivery, facilitating T cell immunotherapy [298]. LNPs equipped with piperazine-derived lipid target immune cells in the liver and spleen [299]. LNPs modified with heterocyclic rings in their lipid head groups enhance mRNA delivery and IFN-γ secretion, subsequently activating the cGAS-STING type I IFN signaling pathway [300]. LNPs functionalized with alendronate offer targeted delivery to bone cells via calcium chelation [297]. Vitamin-conjugated LNPs deliver antimicrobial peptides and mRNA-encoding cathepsin B to macrophages [301]. BP lipid-like compounds demonstrated exceptional

targeting of the bone microenvironment and efficiency in mRNA delivery [280]. An LNP targeting secondary lymphoid tissues has been developed with novel phosphatidylserine derivatives, enhancing mRNA delivery to the spleen. The phosphatidylserine-LNP showed increased uptake by macrophages in the spleen's red pulp and marginal zone [302]. In another study, anisamide-tethered lipidoid (AA-T3A-C12) was employed for the fabrication of siRNA-LNP to activate HSCs for silencing HSP47 in liver fibrosis [295]. Figure 10 depicts LNP modifications with targeting moieties for active targeting.

While active targeting offers advantages, challenges such as variability in ligand-receptor specificity among patients, biomolecular corona interference, and complex formulation should be addressed for efficient delivery. Continued research into effective ligand-receptor combinations is essential for optimizing this strategy. In addition, combining multiple targeting ligands on LNP surfaces can enhance specificity and binding affinity.

Current clinical applications of RNA-LNPs

Initially considered impractical because of instability and immunogenicity, mRNA has become a focal point in therapeutics because of advancements in stabilizing modifications and delivery systems. This has led to its potential to replace plasmid DNA or recombinant proteins [303]. Moreover, the widespread success of mRNA-LNP vaccines for COVID-19 has catalyzed further exploration of mRNA therapeutics, including protein replacement therapy, regenerative medicine, vaccines for infectious diseases, anticancer therapies, and organ-specific disease targeting (e.g., CF, chronic obstructive pulmonary disease, or asthma), as well as anti-inflammatory and gene editing applications (Table 3). Beyond therapeutic use, RNA-LNPs have found utility in diverse bioengineering realms such as medical imaging, bioprinting, and basic scientific research.

Protein replacement therapy

Protein replacement therapy, aimed at generating missing or defective proteins, has traditionally relied on recombinant proteins. However, mRNA encoding specific proteins or antibodies has emerged as a promising alternative, offering advantages such as rapid production, flexible design, and lower risk of contamination [324]. This approach has garnered interest across various fields, including hematology, metabolism, cardiology, pulmonology, neurology, and oncology. For instance, mRNA-loaded LNPs have been explored for treating hemophilia, a genetic bleeding disorder, by encoding missing clotting factors [325]. LNPs carrying mRNA for factor VIII or factor IX variants have shown promising results in mouse models, rapidly inducing and sustaining therapeutic protein levels [326]. Similarly, metabolic disorders like hepatorenal tyrosinemia and alpha 1-antitrypsin deficiency have been targeted using LNPs encapsulating mRNA for deficient enzymes, leading to improved organ function and increased protein levels [327]. Moreover, mRNA-LNPs have been investigated for lysosomal storage diseases such as Fabry disease, where high levels of therapeutic proteins were achieved systemically, demonstrating the potential for disease management [328]. Additionally, LNPs for delivering mRNA-encoding enzymes have shown efficacy in acute intermittent porphyria, methylmalonic acidemia, and glycogen storage disease, significantly reducing disease symptoms and improving survival rates [311].

Beyond liver-related diseases, mRNA-LNPs have been explored as therapeutic proteins. For instance, LNPs carrying mRNA encoding vascular endothelial growth factor have been studied for cardiac repair after myocardial infarction and for improving placental function in pre-eclampsia and fetal growth restriction [329]. Furthermore, LNPs for delivering mRNA encoding human frataxin have shown potential for treating Friedreich's ataxia, a neurological disorder [330]. Moreover, mRNA-LNPs have been investigated for lung diseases through nasal administration, showing promise in restoring lung function in conditions like CF [331]. Davies et al. demonstrated that subcutaneous administration of mRNA within LNPs can effectively deliver therapeutic proteins, but it causes inflammatory responses. The integration of

(See figure on next page.)

Fig. 10 **A** Schematic representation of common targeting ligands used for specific LNP-RNA delivery; **B** examples of recent LNP surface modification for active targeting: **1** bisphosphonate (BP) lipid-like component for mRNA delivery to the bone microenvironment: **a** illustration depicting the construction of BP-LNPs for mRNA delivery to the bone environment, leveraging the interaction of BP-LNPs with Ca^{2+}; **b** Cryo-TEM image showing BP-LNP morphology (100 nm scale bar) and ex-vivo imaging of bones post-LNP delivery. LNPs encapsulate luciferase (Luc) mRNA and are labeled with 1′-dioctadecyl-3,3,3′,3′-tetramethylindotricarbocyanine iodide (DiR) dye [280]; **2** phosphatidylserine-loaded LNP for delivering mRNA to secondary lymphoid tissues: **a** illustration outlining the preparation of phosphatidylserine LNPs and strategies for mRNA delivery to macrophages in secondary lymphoid organs; **b** images of Luc activity in tissues from mice treated with subcutaneous and intravenous injections of phosphatidylserine-LNPs encapsulating Luc mRNA [302]; **3** ligand-tethered lipid nanoparticles for RNA delivery to treat liver fibrosis: **a** fabrication of AA-T3A-C12/siHSP47 LNP by microfluidic mixing and specific delivery to activated hepatic stellate cells (HSCs) for silencing heat shock protein 47 (HSP47) in liver fibrosis; **b** immunofluorescence staining of HSP47 in LNP-treated activated 3T3 fibroblasts [295]

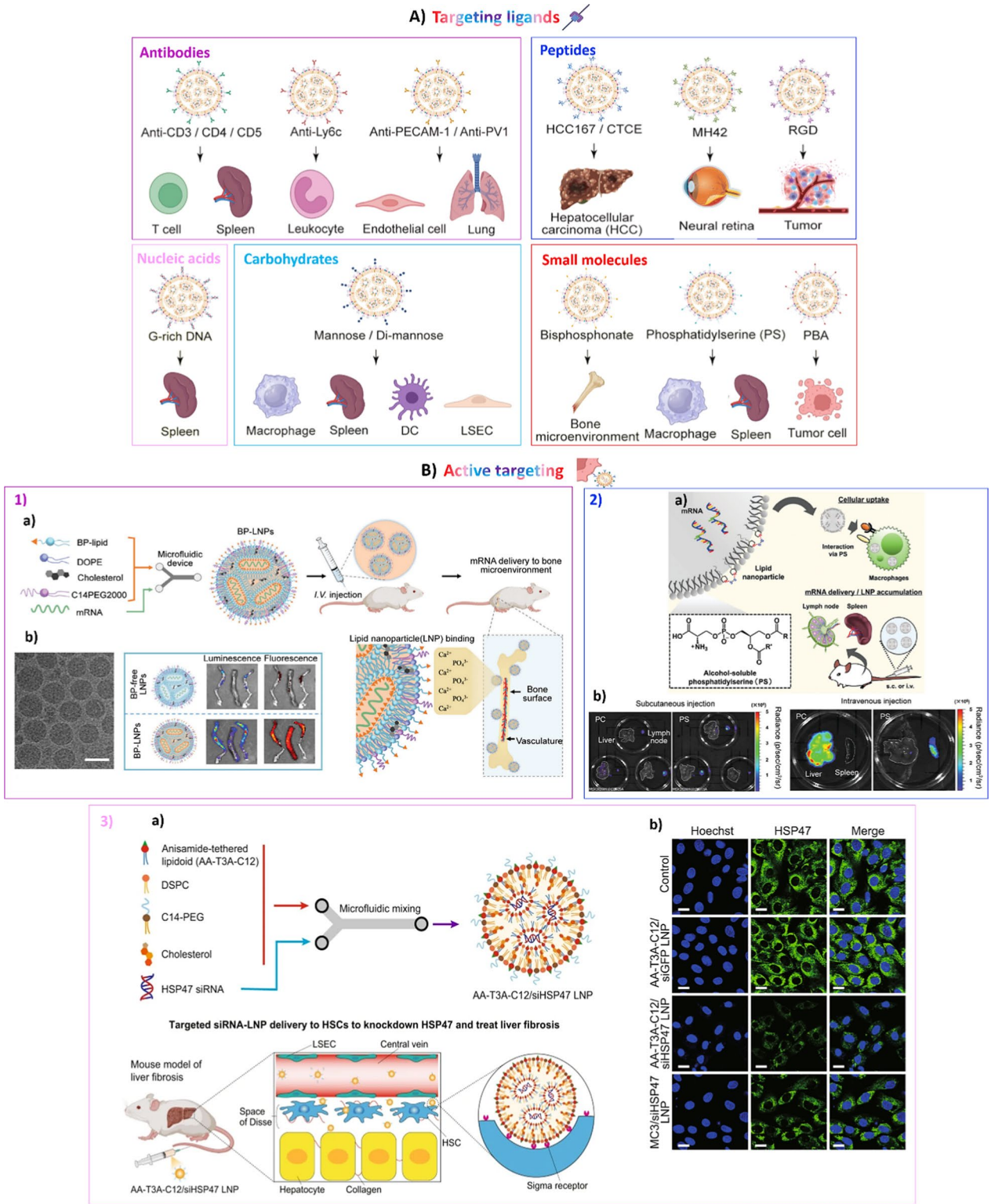

Fig. 10 (See legend on previous page.)

Table 3 Application of LNP-RNA therapeutics based on the target disease

LNP formulation	Payload	Specific organs/cells	Disease	Ref.
5A2-SC8, cholesterol, DMG-PEG, DOPE	CRISPR/Cas9	Bone marrow (hematopoietic stem cells, leukemic cells, and mature blood cells)	Sickle cell disease and acute myeloid leukemia	[304]
DODAP, cholesterol, DMG-PEG, DSPC	siRNA	Liver	Target the FVII gene in the liver	[305]
BP-lipid, cholesterol, C14PEG2000, DOPE	mRNA	Bone microenvironment and bone marrow (bone cells, cells of the hematopoietic and immune systems, fibroblasts, stromal cells, endothelial cells, monocytic lineage, B cell lineage, T cells, monocytes, granulocytes, B cells, and hematopoietic stem cells)	Skeletal diseases and age-related bone abnormalities (osteoporosis, osteoarthritis, osteomyelitis, and bone cancer)	[280]
4A3-SC8, DOPE, Cholesterol, DMG-PEG, DOTAP	NG-ABE8e mRNA–sgR553X	Lungs	Genetic lung diseases, such as cystic fibrosis	[306]
5A2-SC8, DOPE, cholesterol, DMG-PEG, 10% 18:1 PA	mRNA	$CD4^+$ and $CD8^+$ T cells in spleen	B cell lymphoma	[307]
Sazo/TAzo lipidoid, DOPE, cholesterol, DMG-PEG	mRNA	Dendritic cells	Melanoma	[308]
PEG-lipids, ionizable lipids, structural lipids, cholesterol	HNF4A mRNA	Liver	Liver fibrosis and cirrhosis	[309]
246C10, DOPE, cholesterol, PEG-ceramide lipids	Cas9 mRNA and mouse AT-targeted sgRNA	Liver	Hemophilia	[310]
2-(Dinonylamino)-1-(4-(*N*-(2-(dinonylamino)ethyl)-*N*-nonylglycyl)piperazin 1-yl)ethan-1-one, DSPC, cholesterol, PEG-DMG	hPBGD mRNA	Liver	Acute intermittent porphyria	[311]
Dlin-MC3-DMA-based lipids, helper lipids, cholesterol, PEG-lipids	BisCCL2/5i mRNA	Liver	Liver cancer	[312]
MC3 lipids, cholesterol or β-sitosterol, DMG PEG2000, DSPC	hsACE2mRNA	Liver	SARS-CoV-2	[313]
5A2-SC8, DOPE, cholesterol, PEG-lipids	Cas9 mRNA, PD-L1 sgRNA, and FAK siRNA	Liver	Liver cancer	[314]
G0-C14, PDSA, DSPE-PEG, DMPE-PEG	p53 mRNA	Liver	Liver cancer	[315]
DLin-MC3-DMA, DSPC, cholesterol, PEG2000-DMG	sPD-L1 mRNA	Lung	Acute respiratory distress syndrome	[316]
306-N16B + 113-N16B, cholesterol, DOPE + DSPC, DMG-PEG2000	Cas9 mRNA, sgRNA, and Tsc2 mRNA	Lung	LAM	[136]
DLin-MC3-DMA, DPPC, cholesterol, DSPE-PEG	eGFP mRNA	Lung	Idiopathic pulmonary fibrosis	[317]
5A2-SC8, DOPE, cholesterol, DMG-PEG	Cas9 mRNA, sgRNA, and donor ssDNA templates	Lung	Cystic fibrosis	[318]
Modified PEIcompound7C1, cholesterol, DMG-PEG2000, cationic lipid DOTAP	mRNA encoded with broadly neutralizing antibody targeting hemagglutinin	Lung	H1N1 infection	[69]
Lipids with bisphosphonate head groups	BMP-2 mRNA	Bone	Skeletal diseases	[280]
DALs, DOPE, cholesterol, DMG-PEG	IL-12 mRNA	Tumor	Melanoma	[319]
Ionizable cationic lipids, phosphatidylcholine, cholesterol, PEG-lipids	Rituximab mRNA	Tumor	Lymphoma	[320]
cKK-E12, DSPC, cholesterol, PEG2000-DMG	HER2 antibody mRNA	Tumor	Breast cancer	[321]

Table 3 (continued)

LNP formulation	Payload	Specific organs/cells	Disease	Ref.
Anti-CD5 antibody, ionizable lipids, phosphatidylcholine, cholesterol, PEG-lipids	FAP-CAR mRNA	T-cell	Cardiac fibrosis	[261]
Anti-Ly6cmAbs, MC3, DSPC, cholesterol, DMG-PEG, DSPE-PEG	IL-10mRNA	Leukocyte	Inflammatory bowel disease	[322]
MC3, DSPC, cholesterol, DSPE-PEG2000-mannose	Ara h2 mRNA	LSECs	Peanut allergy	[323]

hsACE2 human angiotensin-converting enzyme 2, *HNF4A* human hepatocyte nuclear factor alpha, *hPBGD* human porphobilinogen deaminase, *LAM* lymphangioleiomyomatosis, *LSECs* liver sinusoidal endothelial cells

anti-inflammatory steroid prodrugs within LNPs has led to a reduction in inflammation and an extension of protein expression duration, thereby rendering this approach appropriate for chronic treatment regimens and self-administration [332]. DeRosa et al. demonstrated that delivering mRNA encoding human α-galactosidase protein via LNPs significantly increased serum GLA protein levels in mice and non-human primates. This method shows promise as a new treatment approach for Fabry disease, potentially surpassing current therapies [328]. Overall, the versatility and efficacy of mRNA-LNPs for protein replacement therapy offer promising avenues for treating several genetic and acquired diseases, representing a significant advancement in medical therapeutics.

Genome editing

Gene therapy, which encompasses genome editing, seeks to deliver mRNA to specific targets for gene modulation, including silencing or expression. CRISPR/Cas9 has gained prominence for gene editing due to its precision and versatility [333]. However, challenges in delivering pDNA or the Cas9 protein/sgRNA complex have prompted the exploration of mRNA-LNP platforms for efficient and safe genome editing [334]. CRISPR/Cas9-mediated gene editing using LNPs has shown feasibility and efficacy, particularly in liver-related disorders [335]. For instance, hypercholesterolemia, characterized by elevated LDL levels, has been targeted by reducing PCSK9 expression. Studies have demonstrated significant reductions in serum PCSK9 and cholesterol levels following systemic delivery of Cas9 mRNA and sgRNA using LNPs [333]. LNPs have also been employed for base editing, achieving efficient PCSK9 knockdown and LDL reduction in animal models [336]. Furthermore, LNPs have been used to target other genes implicated in various diseases. For example, Angptl3 and transthyretin have been targeted to reduce serum protein levels associated with cardiovascular and hTTR amyloidosis diseases, respectively [337]. CRISPR/Cas9 LNPs have also shown promise in addressing hemophilia and metabolic disorders like PKU and tyrosinemia type 1 [338]. Beyond liver-related conditions, LNP-mediated CRISPR/Cas9 delivery has been explored in extrahepatic organs. Intramuscular administration of LNPs has been investigated for Duchenne muscular dystrophy treatment, whereas LNPs targeting PLK1 and EGFR have shown efficacy against glioblastoma and ovarian cancer cells, respectively [339]. Im et al. developed ionizable LNPs to effectively deliver CRISPR/Cas9 ribonucleoproteins (RNPs), achieving high-efficiency gene editing in vitro and in vivo, potentially addressing diseases like cancer and genetic disorders [340]. Herrera-Barrera formulated an array of enhanced LNP (eLNPs) to deliver prime editors (PEs) RNA efficiently for gene editing, achieving a 54% prime editing rate. eLNPs led to improved endosomal escape, eventually causing onset of editing within 9 h and reaching maximum efficiency after 24 h. This technology showcased potential for new therapies targeting various disease-causing mutations [341]. Overall, LNP-mediated CRISPR/Cas9 gene editing holds great potential for treating various diseases, offering a promising avenue for precise and efficient therapeutic interventions.

Cancer

Recent investigations into cancer have highlighted the therapeutic potential of mRNA-based LNPs for various cancer types, including melanoma, lymphoma, hepatocellular carcinoma, colon cancer, breast cancer, and prostate cancer [342]. In non-Hodgkin's lymphoma, targeting lymphocytes with mRNA delivery presents a rational approach, with the spleen and lymphoid glands serving as potential delivery sites [343]. mRNA-based LNPs have shown promise in melanoma, leveraging specific tumor antigens like human MART1 and LAMP1 to induce robust antitumor immune responses and enhance survival rates in murine models [344]. In addition, cationic liposomes carrying Bax-mRNA demonstrated superior antitumor effects compared with Bax-plasmid cationic liposomes, both in vitro and in vivo [345]. Modified OVA mRNA-lipid formulations stimulate $CD8^+$

T-cell responses against melanoma models, with specific lipid characteristics (e.g., addition of sodium lauryl sulfate) influencing clonal expansion and function of $CD8^+$ T-cells [346]. In Ai14D reporter mice, delivery of Cre-recombinase mRNA via optimized LNPs resulted in distinct protein expression rates in APCs. In addition, comparing unmodified and modified OVA mRNA revealed enhanced $CD8^+$ T cell production, particularly through IFN induction. Moreover, OVA mRNA-modified liposomes containing galactosyl ceramide as an immune adjuvant significantly reduced tumor growth in the B16-OVA melanoma model, albeit with a moderate survival rate [282]. For hepatocellular carcinoma, LNP-mediated mRNA delivery has shown efficacy in reducing tumor size and increasing survival in a transgenic mouse model with an overexpressed MYC oncogene, with low toxicity in normal cells [347]. Modifications of LNPs have been explored in colon cancer, where protamine complexation enhanced mRNA condensation, lowered enzymatic degradation, and improved delivery efficiency [348]. In breast cancer, mRNA delivery systems have shown superior pharmacokinetic profiles compared with synthetic antibodies that results in increased serum levels of therapeutic agents and improved tumor suppression with minimal adverse effects [349]. For instance, compared with synthetic trastuzumab (Herceptin), in vivo PK evaluation of the mRNA-based system in C57BL/6 mice showed significantly higher serum trastuzumab levels, despite lower dosages. In addition, $HER2^+$ mice exhibited higher morbidity-free survival, reduced average tumor volume, and no significant toxicity with trastuzumab mRNA compared to Herceptin [321]. In prostate cancer, delivery of modified PTEN mRNA via PEG-coated polymer-lipid hybrid NPs has demonstrated promising results in increasing tumor suppression potential, inducing apoptosis, inhibiting cell viability, and suppressing the PI3K pathway [350]. Silva et al. compared three different mRNA vaccine modalities to target HPV-16-related tumors in mice. These vaccines, encapsulated in LNPs, showed strong activation of E7-specific $CD8^+$ T cells, prevented tumor relapses, and eradicated subcutaneous tumors. All three mRNA vaccines were superior to DNA and protein vaccines, indicating their promising potential for further clinical trials [351]. Chen developed an endogenous lymph node-targeting LNP named 113-O12B for mRNA cancer vaccines. The LNP increased $CD8^+$ T cell response and showed excellent tumor inhibition, with long-term immune memory in treated mice [54]. These findings underscore the potential of mRNA-based therapies across a spectrum of cancer types, offering new avenues for effective and targeted cancer treatment.

Infectious diseases

mRNA vaccines encapsulated in LNPs have emerged as a groundbreaking technology for combating infectious diseases. Their appeal lies in several advantages, including ease of manufacturing, acceptable immunogenicity, and excellent safety profile [352]. This innovation represents a significant leap forward in vaccine development, particularly for diseases characterized by high genetic instability and infectivity. The development of mRNA-based vaccines traces back to 1989 when they were first explored as a therapeutic approach [353]. mRNA encapsulation within LNPs controls biodistribution and targeting specific cells. Notably, in response to the COVID-19 pandemic, mRNA vaccines developed with LNPs entered clinical trials, with mRNA-1273 (Moderna vaccine) and BNT162b2 (Pfizer/BioNTech vaccine) receiving emergency use authorization from the FDA in 2020 [354]. Beyond COVID-19, mRNA-LNP vaccines hold promise for various infectious diseases. Candidates targeting viruses such as HIV, seasonal influenza, Zika virus, RSV, and EBV are undergoing preclinical and clinical evaluation [355]. Studies have demonstrated the effectiveness of mRNA-based influenza vaccines in eliciting protective immune responses against diverse influenza virus strains [356].

In addition to vaccination, mRNA therapeutics can address infectious diseases. For example, mRNA encoding anti-HIV antibodies delivered via LNPs has shown promise for passive immunotherapy against HIV-1 [357]. The efficacy of mRNA delivery systems is influenced by factors such as the type of delivery system and administration route, which affect biodistribution. Studies have shown that mRNA-LNP favors spleen accumulation after intravenous administration [358]. In addition, biodistribution studies in mice have revealed distinct expression patterns following various administration routes, highlighting the importance of route selection in optimizing efficacy [41]. Li et al. developed a multiply adjuvanted mRNA vaccine using LNPs for optimized mRNA delivery and enhanced immune responses. In mice, this vaccine increased antibody titers against SARS-CoV-2 tenfold compared to unadjuvanted vaccines, indicating a potential for improved efficacy and safety in mRNA-based immunization [359]. SAM vaccine delivered by LNPs demonstrated a robust immune response and favorable safety profile in preclinical models, neutralizing multiple SARS-CoV-2 variants and reducing viral load. It has progressed to phase 1 clinical trials [360]. Clinical studies investigating mRNA-LNP-based vaccines for both cancer and infectious diseases are underway, further underscoring the potential of this innovative approach in advancing preventive and therapeutic interventions.

Inflammation

LNPs can trigger an immune response by interacting with receptors of the innate immune system, such as PRRs or TLRs, potentially diminishing therapeutic efficacy [361]. Incorporating anti-inflammatory agents like dexamethasone into LNPs can mitigate inflammation while maintaining therapeutic effectiveness. Zhang et al. demonstrated that the co-delivery of dexamethasone with mRNA-LNPs suppressed local inflammation while enhancing hepatic mRNA expression [361]. LNPs containing dexamethasone at a molar ratio of 9:1 with cholesterol (referred to as C9D1 LNP) showed reduced serum TNF levels compared with dexamethasone-free LNPs, without compromising transfection efficiency or inducing cytotoxicity. Davies et al. incorporated hydrophobic aliphatic ester prodrugs, including anti-inflammatory compounds like rofleponide and budesonide, within LNPs to mitigate innate immune responses [332]. Rofleponide-C14 prodrug, predominantly distributed in the outer shell of LNPs, significantly reduced local and systemic inflammation when included in mRNA-LNPs encoding a model-secreted protein (hFGF21). Similarly, budesonide conjugated with longer chain length esters (C16) improved local tolerability compared with shorter chain lengths. These findings indicate the potential of LNPs to suppress inflammatory responses. In addition to the direct incorporation of corticosteroids and chemically modified substances, modifications to ionizable lipids or optimized mRNA sequences have been explored to reduce anti-inflammatory reactions [362]. Kawase et al. showed that LNPs loaded with Irf5 siRNA effectively downregulated inflammatory factors (TNF and IL-6) in macrophages using biodegradable ionized lipid L120 [363]. Verma et al. engineered gemini LNP (GLNPs) to deliver siRNA effectively to colon cells and mitigate gut inflammation by reducing TNF-α expression. These GLNPs demonstrated stability, reduced toxicity, and inhibited key immune cell infiltration, highlighting their potential for next-generation nucleic acid delivery in treating inflammatory bowel disease [364].

Regenerative medicine

LNPs have versatile applications beyond therapeutics, spanning bioengineering fields such as medical imaging [73], bioreactors [365], and bioprinting with 3D reconstructed scaffolds [366]. LNPs play a pivotal role in life science studies [367] and intracellular trafficking [368]. For instance, bio-printed nanofibers facilitate the design and validation of optimal RNA-LNP vaccines, thereby enhancing gene editing efficiency [369]. LNPs are also employed in phototherapy and chemiluminescence imaging, particularly in conjunction with quantum dots [370]. In tissue engineering and regenerative medicine, LNPs enable spatially controlled payload release in both 2D and 3D tissue models, addressing challenges in programable cell-to-cell signal transport [366]. Yang et al. integrated LNP-mediated gene editing complexes into a Caco-2/HT29-MTX 3D tissue-engineered system to validate and screen gene-editing functions [371]. Similarly, Chen et al. used LNPs loaded with siRNA in a cartilage scaffold composed of GelMA hydrogel, chondrocytes, and adipose-derived stem cells to suppress microvascular infiltration and promote new cartilage formation in mice [372]. In another study, LNP was utilized in the determination of NIH-3T3 fibroblast growth on a polylactic acid-based 3D-printed human auricular model [373]. LNPs containing cyclosporine A and coenzyme Q10 were also used to evaluate whether cell proliferation on scaffolds could be induced. Rizvi et al. developed an efficient, safe method to transiently express growth factors in hepatocytes using mRNA-LNP delivery, promoting liver regeneration. In mice, this approach reversed liver damage and accelerated restoration, offering a promising therapeutic intervention for conditions like non-alcoholic fatty liver disease [109]. Therefore, LNPs are a valuable toolbox for bioengineering and fundamental life science research.

Current challenges and strategies for in vivo RNA-LNP delivery

Safety concerns

RNA-based therapeutics are generally well-tolerated but can cause adverse effects, including inflammation, liver and kidney toxicity, and thrombocytopenia, as observed with free siRNAs [374]. The safety of mRNA as a therapeutic or vaccine modality has been the subject of extensive research and scrutiny. Naked mRNA can induce innate immune responses upon delivery into cells. The innate immune system recognizes foreign RNA molecules, such as viral RNA or exogenous mRNA, through PRRs. This recognition may trigger inflammatory cascades and the production of cytokines and chemokines. Unmodified mRNA molecules may have off-target effects, leading to unintended gene expression or interference with endogenous cellular processes. These off-target effects can contribute to toxicity or alter the normal function of cells. Modified mRNA, which incorporates various chemical modifications, enhances stability and translational efficiency and reduces immunogenicity.

Non-viral delivery systems are recognized as safer alternatives to viral vectors, which carry risks such as producing neutralizing antibodies and the potential for hepatitis and leukemia. However, non-viral systems still raise safety concerns [375]. mRNA delivery theoretically avoids oncogenesis by viral vectors and plasmid DNA transfer by electroporation [376]. In the context of non-viral vectors, cytotoxic effects are often observed in cell

culture studies, possibly due to the polycationic characteristics of the vectors. For instance, cationic liposomes have been shown to induce apoptosis [377]. Additionally, issues such as immunogenicity, hemagglutination, and inflammatory conditions like hepatitis pose challenges in live organisms [378]. Cationic vectors can induce capillary embolization by aggregating erythrocytes [379]. The likelihood of aggregation is influenced by the ratio of pseudo blood to cationic vectors, with a 1:1 ratio typically resulting in agglutination, reflecting the post-dosage period, while ratios exceeding 10:1 are less prone to aggregation [380]. PEGylation inhibits agglutination even at a 1:1 ratio [116]. Additionally, cationic non-viral vectors can elicit innate immune responses upon administration, leading to localized inflammation and tissue damage at the administration site [381].

ICLs, crucial for the efficacy of LNPs in delivering RNAs, can induce immune responses and cause toxicity by activating inflammatory pathways [382]. Their metabolites can induce inflammation by activating PPARs and cause liver damage [383]. PEGylated lipids can provoke immune responses, leading to the production of anti-PEG antibodies [383, 384]. Except for lipids, lysosomal cysteine proteases are released into the cytosol after LNP endocytosis, causing inflammation due to the activation of the NLRP3 inflammasome and release of pro-inflammatory cytokines [385]. Cathepsins also lead to cellular toxicity by promoting cell death and necroptosis by activating apoptosis pathways and rupturing the plasma membrane [386]. To mitigate toxicity, it is possible to substitute ICLs with biodegradable materials such as trehalose glycolipids or to alter the lipid head by incorporating 6,6′-trehalose dimycolate. This modification allows the LNPs to break down into non-toxic byproducts following the successful delivery of their cargo [387]. Another solution is to replace PEGylated lipids with pSar, which improves safety and maintains effectiveness [236].

Vaccine reactogenicity, a physical manifestation of the inflammatory response, includes local side effects like swelling and pain, and systemic side effects like fatigue, fever, and anaphylaxis, especially prevalent after mRNA vaccines [388, 389]. PEGylated lipids and NK cells contribute to these responses, which can sometimes lead to severe adverse events [389, 390]. Solutions to reduce reactogenicity include using a needle-free, pyro-drive liquid jet injector (PYRO) to deliver naked mRNA directly to APCs ensuring localized mRNA distribution and reducing systemic inflammation [391]. Another approach is using noncationic thiourea LNPs, which avoid high cationic charge density, demonstrating higher transfection efficiency and fewer inflammatory effects [392].

LNPs can activate the immune system through TLRs, NLRP3 inflammasome, IL-6 receptor, and MyD88, leading to beneficial immune responses but also potential adverse effects like severe allergic reactions and autoimmune phenomena [393, 394]; so, comprehensive long-term studies, careful preclinical evaluation, and clinical monitoring of immune responses are essential to ensure safety and efficacy. Strategies to enhance immunogenicity for vaccines include using adjuvant lipidoids and modifying lipid formulations with intrinsic adjuvant properties, such as incorporating TLR agonists [359, 395, 396]. Conversely, to reduce immunogenicity for repeated administrations, researchers suggest adjusting LNP compositions, using cleavable PEG variants or biodegradable polymers, and optimizing nanoparticle size and surface charge [397, 398]. For instance, smaller, neutrally charged particles exhibit reduced immunogenicity and superior lymph node targeting [398]. Additionally, incorporating immunomodulatory agents like dexamethasone and modifying vector surfaces can help minimize interactions with host immune cells, promoting immune tolerance [399]. Alternative administration routes such as IV and IN can refine immunogenic profiles [400]. More details about the immunogenicity of LNP-RNA therapeutics are mentioned in the following section.

Immunogenicity

Immunogenicity poses a significant challenge for LNPs in RNA therapy, affecting their therapeutic efficacy. Repeated administration may activate T and B cells, prompting the release of antibodies and cytokines, potentially reducing therapeutic efficacy. T cell activation can induce inflammation through proinflammatory cytokines. B cell activation may result in antibodies that recognize and neutralize LNPs, potentially compromising their function and eliciting adverse reactions. Chronic diseases requiring prolonged treatment could intensify these issues, potentially leading to CARPA [401]. The following sections address the immune recognition of RNA therapeutics and LNPs, highlighting the importance of meticulous monitoring and regulation of LNP immunogenicity through their structural, physicochemical, and biological attributes to ensure clinical effectiveness and safety.

Immune recognition of RNA therapeutics

Synthetic mRNA is sensed by innate immune cells through three major categories: (1) uridine-based RNA identification [402], (2) dsRNA recognition [403], and (3) sensing of mRNA's uncapped 5′ terminus [404]. Vaccines using nucleoside-modified mRNA, notably those with Ψ or m1Ψ, have achieved greater clinical use and success, attributed to enhanced protein synthesis and reduced detection by RNA-specific intracellular sensors [405]. As shown in Fig. 11, innate immune cells detect

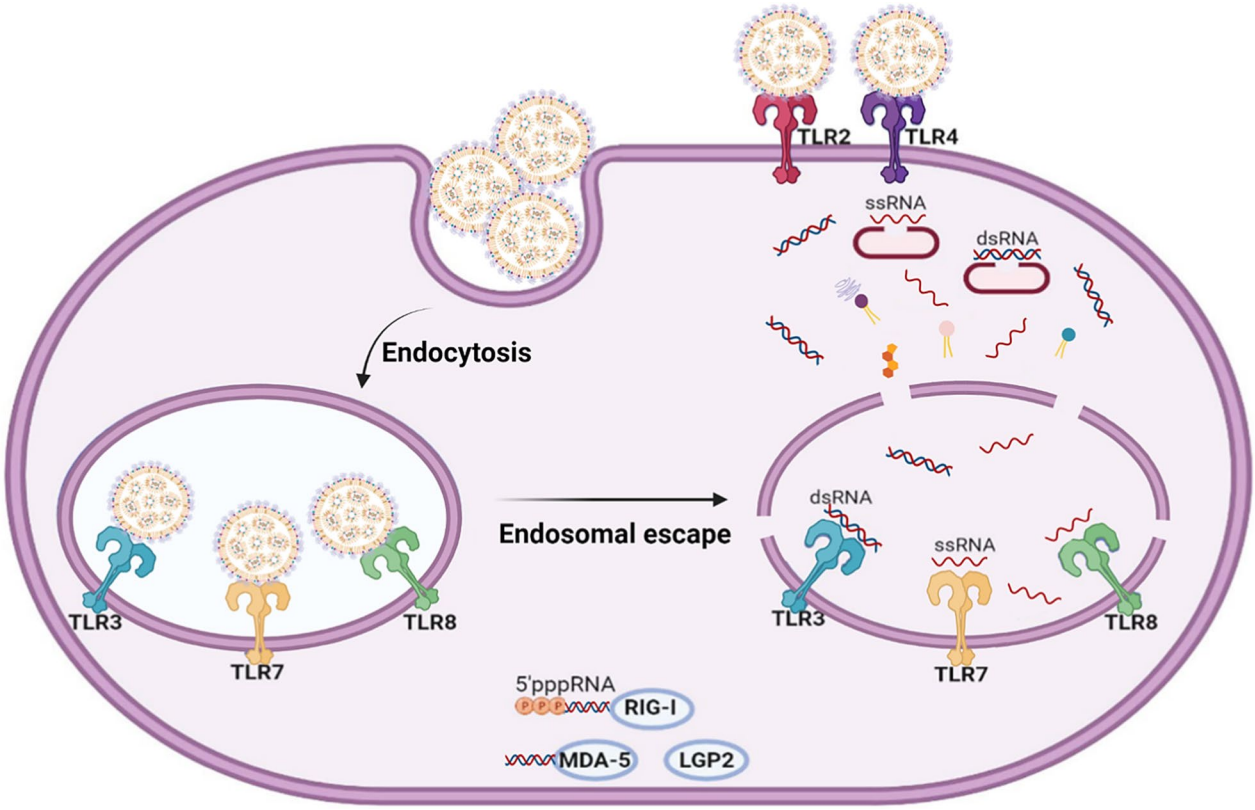

Fig. 11 Pattern recognition receptors (PRRs) identify both mRNA and lipid nanoparticles (LNPs) extracellularly and intracellularly via toll-like receptors (TLRs), RIG-I-like receptors (RLRs), and NOD-like receptors (NLRs)

RNA through endosomal PRRs (like TLRs and NLRs) and cytoplasmic RLRs. TLR7, which attaches to guanosine and UUU, and TLR8, which binds uridine and UG, are key sensors for unmodified ssRNA [402, 406]. TLR3 is responsive to dsRNA, a byproduct of mRNA. RLRs, including RIG-I, MDA-5, and LGP2, identify dsRNA based on structure and length. NLRP3 and NOD2 are specific for dsRNA and ssRNA, respectively. Introducing Ψ modifications can mitigate the response of dsRNA sensors like TLR3, RIG-I, MDA5, PKR, and OAS, thereby reducing the release of pro-inflammatory cytokines such as IL-1, IL-6, TNF, and type I IFN α/β [407]. Additionally, methods to remove dsRNA contaminants from unmodified IVT mRNA, such as HPLC purification [408], cellulose adsorption [409], and RNase III digestion [409] or adjusting reaction conditions, are crucial for enhancing protein production and minimizing immune responses. Modified nucleosides like Ψ, $m^1Ψ$, and m^5C can decrease the formation of dsRNA byproducts in mRNA synthesized using T7 RNA polymerase [407]. Lowering the Mg^{2+} concentration and using a thermostable T7 RNA polymerase during IVT can reduce dsRNA formation [410]. The choice of capping strategy also impacts mRNA performance, with cap1 structures preferred to avoid recognition by innate immune sensors like RIG-I [411]. These strategies minimize innate immune activation and enhance protein expression via mRNA therapeutics.

Immune recognition of LNPs

Both the innate and adaptive immune systems play crucial roles in detecting LNPs through various components, including (1) the entire particle, (2) individual lipids such as ICLs and cholesterol [412–414], and (3) modified or metabolized lipid products such as oxidative impurities of ICLs or oxidized phospholipids [415, 416]. For instance, single-cell RNA-sequencing analysis of draining lymph nodes from BNT162b2-vaccinated mice revealed spiked mRNA predominantly in myeloid DCs, monocytes, and macrophage clusters [417]. The following immune cells play an important role in LNP recognition:

(a) Neutrophils, vital in acute inflammation and tissue entry, respond differently to cationic and neutral lipid NPs. Cationic variants induce LDH release, a cytotoxicity marker, and degranulate neutrophils, triggering elastase and superoxide anion release.

They also stimulate NETosis, an activation marker, worsening inflammation.

(b) Macrophages, APCs originating from fetal myeloid progenitors or circulating monocytes, migrate to inflamed tissue. Resident classical macrophages are professional phagocytes expressing PRRs, such as PAMP and DAMP receptors. NPs are recognized as ligands for NAMP receptors, and their interaction with PRRs induces inflammatory mediators. The route of administration, as well as the size and charge of NPs, affect their clearance by macrophages, with larger NPs being phagocytosed by liver Kupffer cells and splenic red pulp macrophages. In addition, cationic LNPs can induce inflammatory cytokines from macrophages. Strategies to mitigate LNP immunotoxicity on macrophages include targeting NPs to specific subsets of macrophages to reduce inflammation by avoiding cationic lipids or ICLs.

(c) DCs, professional APCs found on epithelial surfaces such as the skin (Langerhans cells) and mucosal surfaces of the gastrointestinal, respiratory, and urogenital tracts, present antigens to naive T cells in lymph nodes, eliciting T cell responses. DCs are among the first immune cells to encounter NPs administered through intradermal, subcutaneous, and intra-lymphatic routes. The immune response induced by mRNA-LNP depends on the PRR pathways and DCs involved. Targeting NPs without cationic lipids or ICLs to a subset of DCs could reduce inflammation caused by mRNA-LNP.

(d) The complement system, comprising over 30 proteins synthesized by the liver, plays a vital role in innate immunity by cooperating with the adaptive immune system. The system encompasses three pathways: classical, alternative, and lectin, all culminating in the splitting of C3 into C3b (phagocytosis stimulant) and C3a (anaphylatoxins recruiting phagocytes and triggering CARPA). Therefore, intravenous NPs must avoid C3b interaction and alternative pathway activation. Sabnis et al. developed a novel amino lipid series for mRNA-LNPs, demonstrating their tolerability of up to 1 mg/kg without inducing complement activation in rats and primates [160].

(e) The adaptive immune system recognizes antigens through specific T- or B-cell receptors. Antigens are presented to T cells via MHC molecules by APCs such as DCs, macrophages, and B cells [418]. Antigen identification by T and B lymphocytes initiates the clonal proliferation of active and memory cells, which may result in hypersensitivity responses. Figure 12 represents the recognition of mRNA-LNP by innate and adaptive immune cells and their activation mechanism leading to immunogenicity.

LNPs can induce immunogenicity by binding to biological proteins and forming corona proteins like ApoE and complement proteins. These proteins activate T and B lymphocytes through proinflammatory cytokines such as IL-1, TNF, and IL-12 [420]. Lipid components in LNPs can unexpectedly amplify IL-1 to enhance TLR activation, whereas cationic lipid compositions are recognized by extracellular TLR2 and TLR4, as well as intracellular NLRP3 and STING [421]. To address these challenges, ICLs have been developed for mRNA delivery to reduce positive charge and mitigate immune and inflammatory responses [422]. Unlike common lipid materials, phospholipids in LNPs do not elicit reactive T cells [423]. Helper lipids, such as DSPC, may induce systemic inflammation by stimulating liver Kupffer cells and releasing eicosanoids and allergens that trigger inflammatory cytokines [424].

The immunogenicity induced by LNPs can be harnessed to enhance therapeutic effects, including acting as adjuvants, activating complement pathways, and facilitating mRNA delivery to specific cell types for immune recognition and presentation [425]. LNPs incorporating mRNA vaccines can evoke robust immune responses, engendering the production of diverse cell types such as Tfh cells, $CD4^+$ Th1 cells, $CD8^+$ T cells, and GC B cells, along with the generation of neutralizing antibodies [37]. Moreover, LNPs provoke the secretion of IL-12, IL-6, TNF, IFN-γ, and GM-CSF, thereby contributing to immune activation and enhancing vaccine efficacy [414]. The induction

(See figure on next page.)

Fig. 12 Activation of innate and adaptive immune cells upon mRNA-LNP administration: **A** intramuscular injection of mRNA-LNP vaccines leads to local inflammation, recruiting neutrophils, monocytes, and dendritic cells (DCs) from the bloodstream to the site of injection through the release of chemokines and cytokines, facilitating the migration of other immune cells; **B** mRNA-LNPs alone, or along with antigen-presenting cells (APCs), are localized in the nearby lymph nodes; **C** DCs, monocytes or macrophages present antigens and initiate the activation of T cells; **D** Tfh cells assist B cells during germinal center (GC) reactions, alongside follicular DCs, to refine antibody affinity. In mouse models, LNP-induced IL-6 is crucial for developing T follicular helper (Tfh) and GC B cell responses, while type I IFNs are known to enhance cytotoxic T lymphocyte (CTL) reactions Adopted from [419]

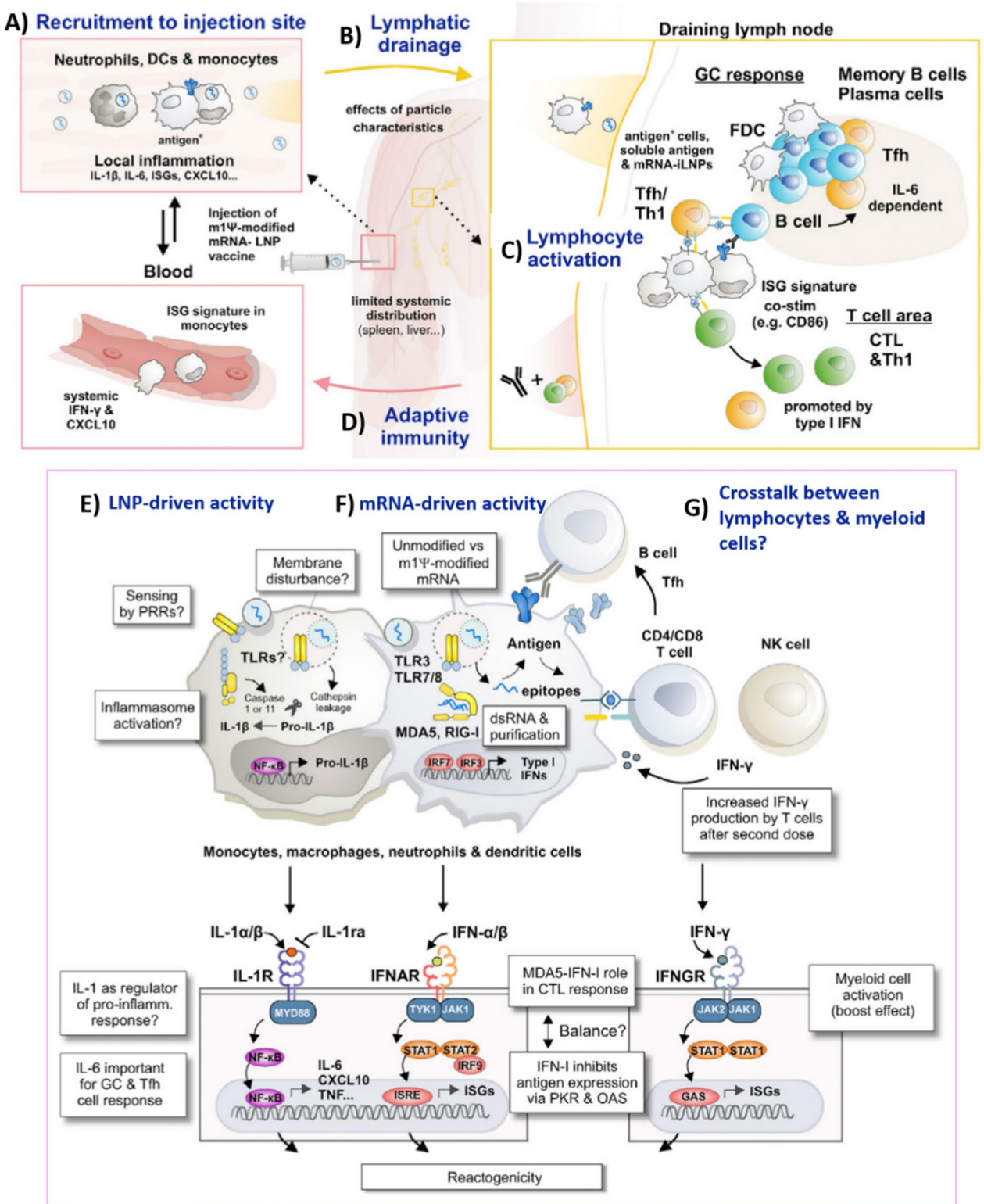

Fig. 12 (See legend on previous page.)

of IL-12, a key mediator of innate immunity, occurs through TLRs, PAMPs, or DAMPs, indicating diverse pathways for mRNA-LNP adjuvanticity, possibly independent of inflammasome components like NLRP3 and ASC [417]. IL-6 is potentially significant in enhancing B-cell responses driven by $CD8^+$ Tfh cells, including the maturation of germinal center B cells, memory B cells, and long-lived plasma cells [426].

Few researchers have examined the impact of LNP characteristics on immune responses. Hassett et al. found that LNP size significantly influenced the CMV mRNA-LNP vaccine immunogenicity. Larger LNPs (up to 100 nm) induced higher Ab titers in mice, whereas in non-human primates, immunogenicity is independent of size [398]. Another study found that liposome size influenced the Th1 and Th2 ratio, with vesicles ranging from 250 to 750 nm inducing stronger Th1 responses. However, multilamellar vesicles tended to favor Th2 responses. The surface charge of LNPs also plays a role in their immunostimulatory potential. Positive or negative vesicles generated higher Ab-neutralizing responses than neutral vesicles [427]. The functionalization of LNPs also influences their immunogenicity. For example, lipid-anchored gadolinium chelates on the LNP surface activate the complement system by IgM antibodies [428]. Mannosylated LNPs carrying SAM vaccines elicit stronger Ab production and antigen-specific $CD8^+$ T cell responses than their non-glycosylated counterparts [281].

Furthermore, the vaccination route influences the LNP immune response. Nebulized LNPs containing mRNA for an anti-hemagglutinin Ab protected mice against influenza [69]. Subcutaneous immunization with BNT162b2 resulted in reduced weight loss and potentially enhanced cytotoxic $CD8^+$ T-cell activity, along with increased neutralizing antibodies compared to intramuscular administration [429, 430]. However, intradermal or intramuscular routes were more effective in eliciting polyfunctional $CD8^+$ T-cell responses [430]. Therefore, the choice of vaccination route should align with the intended immunological outcome.

Repeated-dose pharmacokinetics and ABC phenomenon

Non-viral vectors typically result in transient gene expression dynamics, where gene activity in the liver, for instance, decreases exponentially by a factor of 10 each day [431]. In contrast, intramuscular administration exhibits a more sustained gene expression over time although the underlying mechanisms remain unclear [432]. Transcription factors such as NF-κB and AP-1 intricately regulate gene expression [433], with their transient activation correlated with the rapid decline in gene expression. To extend therapeutic efficacy, alternative strategies like administering multiple smaller doses of mRNA-LNP therapeutics have been explored. This approach can enhance treatment effectiveness and duration while mitigating adverse effects and immune reactions [6]. Notably, multiple dosing is crucial for the treatment of chronic diseases such as rheumatoid arthritis, cancer, multiple sclerosis, and genetic disorders, as it maintains steady drug levels to manage symptoms and halt disease advancement [434]. Pharmacokinetic challenges in repeat dosing stem from uncertainties about drug absorption, distribution, metabolism, and elimination [435]. Upon administration, LNPs are primarily distributed into the bloodstream and various tissues, with clearance involving the degradation of the mRNA payload and lipid components. Although clearance mechanisms may vary, LNPs are typically eliminated via renal and biliary excretion and tissue degradation. The half-life of LNPs in circulation varies based on factors such as particle size, surface modification, and administration route. In addition, patient physiology and immune responses can intensify these challenges [436].

PEG coating can induce anti-PEG antibodies upon repeated dosing [437], significantly impacting the efficacy of multiple doses [438]. PEG-specific antibodies produced in spleen B cells play a key role in the ABC phenomenon and complement activation [439]. Once injected, PEGylated NPs reach the spleen, where they bind and activate B cells, producing anti-PEG IgM. Subsequent doses may trigger complement activation if anti-PEG IgM is still circulating, resulting in CARPA [440]. For example, PEG has been associated with some cases of anaphylaxis to BioNTech/Pfizer Comirnaty and Moderna Spikevax COVID-19 vaccines in individuals with a known allergy to PEG [441]. In addition, the ABC phenomenon, driven by anti-PEG antibodies, causes opsonization and phagocytosis [442]. Opsonization by C3 fragments and accelerated absorption by Kupffer cells via complement receptor-mediated endocytosis contribute to the rapid clearance of LNPs from the body into the MPS of the lymphoid and other tissues [443].

Understanding the properties of PEGylated NPs is crucial for overcoming ABC challenges, as these characteristics significantly influence the stability, circulation duration, biocompatibility, biodistribution, and cellular uptake of LNPs. The following factors determine the efficacy of PEGylation [444]:

(a) PEG density: molecular weight and chain length of PEG are critical for reducing immune reactions [445]. Longer PEG chains lead to thicker surface coating on LNPs, limiting immune interaction. Extended chains prolong circulation, while shorter

chains enhance cellular internalization. Higher PEG density reduces immunogenicity by concealing antigenic features and decreasing immune detection. The proportion of PEG influences the LNP clearance rate, with higher content accelerating the elimination [446].

(b) PEG structure: conformation of PEG affects immune system accessibility. NPs with branched PEGs reduce anti-PEG Ab production and complement activation [447].

(c) PEG shedding: extended PEG chains provide "stealth" characteristics but may shed, reducing immunogenicity [149]. Rapid shedding minimizes immune interactions, while slow shedding maintains effectiveness. LNPs with short-chain PEG-lipids like C14 are preferred for hepatic gene silencing. Regulating the PEG shedding rate is crucial for repeated dosing success [448].

To circumvent the ABC phenomenon, hydrophilic polymers like PVP [449], polyglycerol [450], and pSar [451] may serve as alternatives to PEG, although their tolerability and efficacy require further investigation. In addition, the dosing interval significantly affects the ABC phenomenon, with longer intervals resulting in reduced clearance [437, 443]. Although intravenous administration is less potent in inducing ABC compared with slow infusion, subcutaneous injection enhances ABC upon repeated administration [437]. Further investigation is warranted for LNPs administered via alternative routes of administration.

Prospects

LNPs are promising delivery vectors that encapsulate and deliver RNA therapeutics to target cells and tissues while protecting them from degradation and immune reactions. Chemical modifications can reduce the immunogenicity of RNA therapeutics. In addition, RNA stability and biological function, such as gene expression, can be improved by chemically modified RNA sequences. The market for RNA-based treatments is expanding rapidly, but the sustainability of these opportunities hinges on developing effective delivery mechanisms, notably LNPs. The success of mRNA LNP COVID-19 vaccines has spurred increased interest and investment in mRNA technology, driving the commercial potential of LNP-based delivery systems (Table 4). LNPs enable gene therapy for various medical conditions, such as administering siRNA to reduce cholesterol levels, using the liver as a bioreactor to produce therapeutic proteins, and developing mRNA vaccines for cancer and infectious diseases. This includes innovative mRNA vaccines, personalized cancer immunotherapies, and CAR T cell therapy. Recent clinical trials are investigating their ability to target and destroy cancer cells by immune cells. These trials are showing promising results in terms of both safety and efficacy [452]. Beyond COVID-19 vaccines, LNPs are being utilized in the development of vaccines for other infectious diseases and genetic disorders. Innovations in this area are focused on improving the immune response and ensuring long-term protection [453].

Microfluidic technologies facilitate the high-precision, reproducible, and scalable synthesis of LNPs, overcoming some technical challenges in the field, and a QbD approach can assist in the development of optimized formulations. However, LNPs face challenges in delivering genetic materials to target tissues, such as tissue specificity and immune reactions. LNPs accumulate in the liver, which is beneficial for treating liver disease but problematic for extrahepatic delivery. To passively or actively target other tissues, LNPs can be modified by varying their physicochemical characteristics or introducing targeting ligands. The protein corona formed on LNPs after their administration affects in-vivo fate by altering their stability, integrity, release, biodistribution, excretion, and accumulation in specific organs. The protein corona composition is also specific to each disease and individual, which calls for a personalized nanomedicine approach that can predict optimal treatment for each patient. Recent research has focused on improving the safety of LNPs for widespread long-term clinical uses [454]. Strategies such as modifying the lipid composition and optimizing the particle size are being explored to enhance their biocompatibility [455]. Repeated administration of RNA therapeutics via LNPs is hindered by the immune and complement systems' recognition of these particles. Future studies aim to optimize LNP formulations to reduce the production of anti-PEG antibodies and minimize complement system activation triggered by LNPs. The design of LNPs involves careful consideration of their physicochemical properties, such as size, charge, and lipid composition. These properties influence the biodistribution, cellular uptake, and safety of the NPs [456]. The ongoing research and clinical trials are paving the way for the development of innovative treatments that have the potential to more safely and effectively tackle a diverse array of diseases.

Conclusion

The advancement of RNA therapeutics follows a cyclical process, starting with designing a delivery vector such as LNPs based on existing knowledge. These particles are manufactured using validated fabrication methods, followed by assessing formulation properties and in vivo behavior. Additionally, addressing safety issues, immunogenicity, repeated-dose effectiveness, and vector

Table 4 Recent LNP-RNA therapeutics in clinical trials

Product name	Trial number	Phase	Condition	Start date
Lipo-MERIT	NCT02410733	I	Melanoma	03/2015
VAL-506440	NCT03076385	I	Influenza	12/2015
VAL-339851	NCT03345043	I	Influenza	05/2016
IVAC_W_bre1_uID	NCT02316457	I	TNBC	10/2016
mRNA-1325	NCT03014089	I	zika virus	12/2016
mRNA-4157	NCT03313778	I	Solid tumors	08/2017
mRNA-2416	NCT03323398	I/II	Relapsed/refractory solid tumor malignancies or lymphoma/ovarian cancer	08/2017
CTX001	NCT03655678	III	TDT	09/2018
Rabipur®	NCT03713086	I	Rabies	10/2018
mRNA-2752	NCT03739931	I	Relapsed/refractory solid tumor malignancies or lymphoma	11/2018
mRNA-4157	NCT03897881	IIb	Stage III/IV melanoma	07/2019
PCV	NCT03908671	I	Esophageal cancer/non-small cell lung cancer	10/2019
PCV	NCT04161755	I	Pancreatic cancer	12/2019
mRNA-1273	NCT04283461	I	COVID-19	03/2020
NR	NCT04426669	I/II	GI cancer	05/2020
ARCT-810	NCT04416126	I	OTCD	06/2020
ARCT-021	NCT04480957	I/II	COVID-19	08/2020
NTLA-2001	NCT04601051	I	TTR-FAP/TTR-CM/wild-type transthyretin cardiac amyloidosis	11/2020
ARCT-810	NCT04442347	I	OTCD	11/2020
1 µg CoV2 SAM (LNP)	NCT04758962	I	Virus diseases	02/2021
mRNA-1273	NCT04785144	I	COVID-19	03/2021
Moderna COVID-19 Vaccine	NCT04811664	III	COVID-19	03/2021
BNT162b2	NCT04824638	II	COVID-19	03/2021
ChAdV68-S	NCT04776317	I	COVID-19	03/2021
mRNA-1273	NCT04805125	III	COVID-19	04/2021
BNT162b2/mRNA-1273	NCT04900467	NR	COVID-19	05/2021
NR	NCT05171959	NR	COVID-19	05/2021
mRNA-1273 SARS-CoV-2 vaccine	NCT04894435	II	COVID-19	05/2021
ChulaCov19 vaccine	NCT04566276	I/II	COVID-19	05/2021
mRNA-1893	NCT04917861	II	Zika Virus	06/2021
SARS-CoV-2 mRNA vaccine	NCT04847102	III	COVID-19	07/2021
mRNA-1647	NCT05085366	III	CMV	10/2021
Amaretto	NCT04981691	I	Refractory malignant solid neoplasm	10/2021
BNT162b2	NCT04961229	IV	COVID-19	10/2021
mRNA-1345	NCT05127434	II/III	RSV-LRTD	11/2021
LNP-nCOV saRNA-02 Vaccine	NCT04934111	I	COVID-19	12/2021
BNT162b2	NCT05124171	III	COVID-19	12/2021
Moderna mRNA-1273	NCT05168813	II/III	HIV/COVID-19	12/2021
NTLA-2002	NCT05120830	I/II	HAE	12/2021
mRNA-1189	NCT05164094	I	EBV	12/2021
BNT141	NCT04683939	I/IIa	Multiple solid tumors	01/2022
WU-CART-007	NCT04984356	I/II	T-cell acute lymphoblastic leukemia/lymphoblastic lymphoma	01/2022
mRNA -1574	NCT05217641	I	HIV	02/2022
AS03	NCT05289037	II	COVID-19	03/2022
mRNA-1273	NCT05315362	II	COVID-19	05/2022
mRNA-1644	NCT05414786	I	AIDS	05/2022
mRNA-3745	NCT05095727	I/II	GSD	06/2022
mRNA-1010	NCT05415462	III	Seasonal influenza	06/2022
ARCT-810	NCT05526066	II	OTCD	07/2022

Table 4 (continued)

Product name	Trial number	Phase	Condition	Start date
mRNA -1215	NCT05398796	I	Nipah virus	07/2022
BEAM-101	NCT05456880	I/II	SCD	08/2022
OTX-2002	NCT05497453	I/II	HCC/solid tumor	08/2022
mRNA-1468	NCT05701800	I/II	Herpes zoster	01/2023
LVRNA009	NCT05682638	III	COVID-19	01/2023
ARCT-032	NCT05712538	I	CF	02/2023
PTX-COVID19-B	NCT05534035	III	COVID-19	02/2023
PTX-COVID19-B	NCT05534048	III	COVID-19	02/2023
HBV vaccine	NCT05738447	I	Liver cancer/ HCC	02/2023
mRNA-1345/mRNA-1365	NCT05743881	I	RSV/HMPV	02/2023
LVRNA010	NCT05599802	I	COVID-19	02/2023
NR	NCT05745545	NR	COVID-19	02/2023
GLB-COV2-043	NCT05602961	I/II	COVID-19	02/2023
Moderna/Novavax	NCT05658523	III	COVID-19	02/2023
mRNA-1011.1/mRNA-1011.2/mRNA-1012.1	NCT05827068	I/II	Seasonal influenza	03/2023
JCXH-221	NCT05743335	I/II	COVID-19	03/2023
RVM-V001/RVM-V002	NCT05788185	I/II	COVID-19	03/2023
LVRNA021	NCT05812014	III	COVID-19	03/2023
mRNA-1283.222	NCT05815498	III	COVID-19	03/2023
Novavax	NCT05875701	III	COVID-19	03/2023
mRNA-1083	NCT05827926	I/II	COVID-19/influenza	04/2023
H3 mRNA / LNP Vaccine	NCT05829356	I	Influenza	04/2023
NR	NCT05761717	NR	Hepatocellular carcinoma	04/2023
mRNA-1195	NCT05831111	I	EBV	04/2023
mRNA-1010	NCT05827978	III	Seasonal influenza	04/2023
GSK4382276A	NCT05823974	I/II	Influenza	04/2023
H1ssF-3928	NCT05755620	I	Influenza	04/2023
mRNA-1647	NCT05683457	II	CMV	04/2023
LVRNA009	NCT05428592	III	COVID-19	04/2023
LVRNA012	NCT05549206	NR	COVID-19	04/2023
RQ3027/RQ3025	NCT05907044	NR	COVID-19	05/2023
BNT162b2/Sanofi	NCT05749926	III	COVID-19	05/2023
SWIM816	NCT05911087	II/III	COVID-19	06/2023
RH109	NCT05609045	I	COVID-19	06/2023
mRNA-1010	NCT05868382	II	Influenza	05/2023
TCR-T	NCT05905731	I	Chronic hepatitis B	06/2023
TI-0010	NCT06205524	I	COVID-19	07/2023
V3G CH848 Pr-NP1 60mcg	NCT05903339	I	HIV	08/2023
MT-302 (A)	NCT05969041	I	Epithelial tumors, malignant	08/2023
DCVC H1 HA mRNA vaccine	NCT05945485	I	Influenza	10/2023
RSV/hMPV mRNA LNP	NCT06237296	I	RSV/HMPV	01/2024
NR	NCT05387317	III	COVID-19	04/2024

functionality for tissue-specific gene delivery are crucial steps. Ongoing research and development efforts are focused on optimizing LNP formulations to boost their stability, efficacy, and tissue-specific targeting. Advances in microfluidic technologies and formulation strategies are expected to improve the performance of RNA-LNP systems, making them more effective and versatile for clinical applications. Furthermore, continued research into the biodistribution and pharmacokinetics of RNA-LNPs will provide valuable insights into their in vivo behavior and tissue targeting capabilities. This knowledge will inform the design of more efficient and targeted

delivery strategies that optimize therapeutic efficacy while minimizing off-target effects.

Abbreviations

2′-F	2′-Fluoro
2′-MOE	2′-Methoxyethyl
2′-O-Me	2′-*O*-Methyl
5hmC	5-Hydroxymethylcytosine
14PA	1,2-Dimyristoyl-*sn*-glycero-3-phosphate (sodium salt)
18BMP	Bis (monoacylglycerol)phosphate
18PA	1,2-Dioleoyl-*sn*-glycero-3-phosphate
Ψ	Pseudouridine
Ab	Antibody
ABC	Accelerated blood clearance
aEGFR	Activated epidermal growth factor receptor
AFM	Atomic force microscopy
AHP	Acute hepatic porphyria
ALAS1	5-Aminolevulinic acid synthase 1
ALC-0159	Methoxypolyethyleneglycol oxy(2000)-*N*,*N*-ditetradecylacetamide
Angptl3	Angiopoietin-like 3
AP-1	Activator protein 1
APC	Antigen-presenting cells
APNs	Antigen-presenting nanoparticles
ApoE	Apolipoprotein E
ASC	Apoptosis-associated speck-like protein containing a caspase recruitment domain
ASGPR	Asialoglycoprotein receptor
ASOs	Antisense oligonucleotides
ASSET	Anchored secondary scFv enabling targeting
ATP	Adenosine triphosphate
ATTR	Transthyretin amyloidosis
ATTR-CM	Transthyretin amyloid cardiomyopathy
b2-GPI	Beta-2 glycoprotein 1
Bcl-2	B-cell lymphoma 2
BLI	Bioluminescence imaging
BMECs	Brain microvascular endothelial cells
BP	Bisphosphonate
C12-200	1,1′-[[2-[4-[2-[2-[Bis(2-hydroxydodecyl)amino]ethylamino]ethyl]-1-piperazinyl]ethyl]imino]bis-2-dodecanol
CAR	Chimeric antigen receptor
CARPA	Complement activation-related pseudo allergy
CF	Cystic fibrosis
CHEMS	Cholesteryl hemisuccinate
CMA	Critical material attribute
CMV	Cytomegalovirus
CPP	Critical process parameter
CQA	Critical quality attribute
CRISPR	Clustered regularly interspaced palindromic repeats
Cryo-TEM	Cryogenic transmission electron microscopy
CTL	Cytotoxic T lymphocyte
DAMP	Damage-associated molecular pattern
DC	Dendritic cell
DDAB	Dimethyldioctadecylammonium bromide
ddPCR	Digital droplet polymerase chain reaction
ddRLuc	Deglycosylation-dependent renilla luciferase
DLinDAP	1,2-Dilinoleoyl-3- dimethylaminopropane
DLinDMA	1,2-Dilinoleyloxy-*N*,*N*-dimethylaminopropane
DLinKDMA	1,2-Dilinoleyloxy-ketal-*N*,*N*-dimethyl-3-aminopropane
DLinKC2DMA	2,2-Dilinoleyl-4-(2-dimethylaminoethyl)-[1, 3]-dioxolane
DLinMC3DMA	*O*-(Z,Z,Z,Z-Heptatriaconta-6,9,26,29-tetraen-19-yl)-4-(*N*,*N*-dimethylamino)
DLS	Dynamic light scattering
DMG	Dimyristoyl glycerol
DMPE	1,2-Dimyristoyl-*sn*-glycero-3-phosphorylethanolamine
DODAP	1,2-Dioleoyl-3-dimethylammonium-propane
DODMA	1,2-Dioleyloxy-3-dimethylaminopropane
DoE	Design of experiments
DOP-DEDA	Dioleoylglycerophosphate-diethylenediamine conjugate
DOPE	1,2-Dioleoyl-*sn*-glycero-3-phosphoethanolamine
DOTAP	1,2-Dioleoyl-3-trimethylammonium propane
DOTMA	1,2-Di-*O*-octadecenyl-3-trimethylammonium propane (chloride salt)
DSG	1,2-Distearoyl-*sn*-glycerol
DSPC	1,2-Distearoyl-*sn*-glycero-3-phosphocholine
DSPE	1,2-Distearoyl-*sn*-glycero-3-phosphoethanolamine
dsRNA	Double-stranded RNA
DSS	Dextran sulfate sodium
DUF5	Domain of unknown function in the fifth position
EBV	Epstein Barr virus
EE	Encapsulation efficiency
EPC	1,2-Dioleoyl-*sn*-glycero-3-ethylphosphocholine (chloride salt)
EPR	Enhanced permeability and retention
FAPs	Familial amyloid polyneuropathies
FDC	Follicular dendritic cell
FISH	Fluorescence in situ hybridization
Fluc	Firefly luciferase
FRET	Fluorescence resonance energy transfer
FRR	Flow rate ratio
GALA	Glutamic acid-alanine-leucine-alanine
GalNAc	*N*-Acetyl galactosamine
GAS	Gene activation sequence
GBM	Glioblastoma multiforme
GC	Germinal center
GelMA	Gelatin methacryloyl
GM-CSF	Granulocyte–macrophage colony-stimulating factor
Gp60	Glycoprotein 60
GSD	Glycogen storage disease
GSH	Glutathione
HA	Hyaluronic acid
HAE	Hereditary angioedema
HAO1	Hydroxyacid oxidase 1
hATTR	Hereditary transthyretin amyloidosis
HCC	Hepatocellular carcinoma
HDL	High-density lipoprotein cholesterol
HFF	Hydrodynamic flow-focusing
hFGF21	Human fibroblast growth factor 21
HIV	Human immunodeficiency virus
HMPV	Human metapneumovirus
HPLC	High-performance liquid chromatography
HSCs	Hepatic stellate cells
HSP47	Heat shock protein 47
HVJ	Hemagglutinating virus of Japan
ICH	International council for harmonization of technical requirements for pharmaceuticals for human use
ICL	Ionizable cationic lipids
IFN	Interferon
IFNAR	Interferon-alpha/beta receptor
IFNGR	Interferon-gamma receptor
IHC	Immunohistochemistry
IL	Interleukin
IN	Intranasal
iPhos	Ionizable phospholipids
Irf5	Interferon regulatory factor 5
IRF8	Interferon regulatory factor 8
IRF9	Interferon regulatory factor 9
ISGs	Interferon-stimulated genes
ISRE	Interferon stimulated response element
IVT	In vitro transcription
JAK	Janus kinase
LAMP1	Lysosome associated membrane protein-1
LASER	Light-activated siRNA endosomal release
LC–MS/MS	Liquid chromatography with tandem mass spectrometry
LDH	Lactate dehydrogenase
LDHA	Lactate dehydrogenase A
LDLR	Low-density lipoprotein receptor
LECs	Lymphatic endothelial cells
LGP2	Laboratory of genetics and physiology 2
LNA	Locked nucleic acid
LNPs	Lipid nanoparticles

LRP1	Low-density lipoprotein receptor-related protein 1
LRP	Lipoprotein receptor-related protein
LSEC	Liver sinusoidal endothelial cell
LYVE1	Lymphatic vessel endothelial hyaluronan receptor
$m^1\Psi$	*N*1-Methyl pseudouridine
m^5C	5-Methylcytidine
m^5U	5-Methyluridine
MART1	Melanoma-associated antigen recognized by T cells 1
MDA-5	Melanoma differentiation-associated protein 5
MHC	Major histocompatibility complex
miRNAs	MicroRNAs
MPS	Mononuclear phagocyte system
mRNAs	Messenger RNAs
MYD88	Myeloid differentiation primary response 88
NA	Nucleic acid
nAChR	Nicotinic acetylcholine receptor
NAMP	Nanoparticle-associated molecular pattern
ncRNAs	Non-coding RNAs
NF-κB	Nuclear factor kappa-light-chain-enhancer of activated B cells
NGLY1	*N*-Glycanase-1
NK	Natural killer
NLD1	Nebulized lung delivery 1
NLR	NOD-like receptor
NLRP3	NLR proteins, pyrin domain containing 3
NLS	Nuclear localization signal
NOD2	Nucleotide-binding oligomerization domain-containing protein 2
NPs	Nanoparticles
NTA	Nanoparticle tracking analysis
OAS	2′-5′-Oligoadenylate synthetase
ORF	Open reading frame
OTCD	Ornithine transcarbamylase deficiency
OVA	Ovalbumin
PAMP	Pathogen-associated molecular pattern
PCSK9	Proprotein convertase subtilisin/kexin-9
PDGFR β	Platelet-derived growth factor receptor beta
PDI	Polydispersity index
PD-L1	Programmed death-ligand 1
pDNA	Plasmid DNA
PEG	Polyethylene glycol
PEI	Polyethylenimine
PH1	Primary hyperoxaluria type 1
PI3K	Phosphoinositide 3-kinase
PK	Pharmacokinetic
PKR	Protein kinase R
PKU	Phenylketonuria
PLK1	Polo-like kinase 1
PMO	Phosphorodiamidate morpholino oligonucleotide
PNA	Peptide nucleic acid
PPARs	Peroxisome proliferator-activated receptors
PRR	Pattern recognition receptor
PRs	Photoreceptors
PS	Phosphorothioate
pSar	Polysarcosine
PSMA	Prostate-specific membrane antigen
PTEN	Phosphatase and tensin homolog
PV1	Plasmalemma vesicle-associated protein
QbD	Quality-by-design
QTPP	Quality target product profile
QWBA	Quantitative whole-body autoradiography
RAS	Rat sarcoma
RES	Reticuloendothelial system
RGC	Retinal ganglion cell
RGD	Arginylglycylaspartic acid
RIG-I	Retinoic acid-inducible gene-I
RISC	RNA-induced silencing complex
RLR	RIG-I-like receptor
ROS	Reactive oxygen species
RPE	Retinal pigmented epithelium
RSM	Response surface
RSV	Respiratory syncytial virus
RSV-LRTD	Respiratory syncytial virus-lower respiratory tract disease
RT-qPCR	Reverse transcription-quantitative polymerase chain reaction
s^2U	2-Thiouridine
SAM	Self-amplifying mRNA
SANS	Small-angle neutron scattering
SAXS	Small-angle X-ray scattering
SCD	Sickle cell disease
SEM	Scanning electron microscopy
sgRNAs	Single-guide RNAs
SHM	Staggered herringbone micromixer
siRNAs	Small interfering RNAs
SORT	Selective organ targeting
SPARC	Secreted protein acidic and rich in cysteine
SR-BI	Scavenger receptor class B type 1
ssRNAs	Single-stranded RNAs
STAT	Signal transducer and activator of transcription
STING	Stimulator of interferon genes
TAMs	Tumor-associated macrophages
TDT	Transfusion-dependent β-thalassemia
TEM	Transmission electron microscopy
Tfh	T follicular helper
TFR	Total flow rate
Th	T helper
TLR	Toll-like receptor
TNBC	Triple negative breast cancer
TNF	Tumor necrosis factor
TTR	Transthyretin
TTR-FAP	Transthyretin-related familial amyloid polyneuropathy
TYK1	Tyrosine kinase 1
UTRs	Untranslated regions
VCAM1	Vascular cell adhesion molecule 1
VLDLR	Very low-density lipoprotein receptor
XRD	X-ray diffraction

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Not applicable.

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References

1. Paunovska K, Loughrey D, Dahlman JE. Drug delivery systems for RNA therapeutics. Nat Rev Genet. 2022;23:265–80. https://doi.org/10.1038/s41576-021-00439-4.
2. Feng R, Patil S, Zhao X, Miao Z, Qian A. RNA therapeutics—research and clinical advancements. Front Mol Biosci. 2021;8:1–13. https://doi.org/10.3389/fmolb.2021.710738.
3. Zhang C, Zhang B. RNA therapeutics: updates and future potential. Sci China Life Sci. 2023;66:12–30. https://doi.org/10.1007/S11427-022-2171-2.
4. Burrows C, Helm M, Zhou X. Modulating RNA with chemical modifications. Acc Chem Res. 2024;57:175–6. https://doi.org/10.1021/acs.accounts.3c00770.
5. Jiao L, Sun Z, Sun Z, Liu J, Deng G, Wang X. Nanotechnology-based non-viral vectors for gene delivery in cardiovascular diseases. Front Bioeng Biotechnol. 2024;12:1349077. https://doi.org/10.3389/fbioe.2024.1349077.
6. Hou X, Zaks T, Langer R, Dong Y. Lipid nanoparticles for mRNA delivery. Nat Rev Mater. 2021;6:1078–94. https://doi.org/10.1038/s41578-021-00358-0.
7. Han J, Lim J, Wang CPJ, Han JH, Shin HE, Kim SN, Jeong D, Lee SH, Chun BH, Park CG, Park W. Lipid nanoparticle-based mRNA delivery systems for cancer immunotherapy. Nano Converg. 2023;10:1–13. https://doi.org/10.1186/s40580-023-00385-3.
8. Nakamura T, Sato Y, Yamada Y, Abd Elwakil MM, Kimura S, Younis MA, Harashima H. Extrahepatic targeting of lipid nanoparticles in vivo with intracellular targeting for future nanomedicines. Adv Drug Deliv Rev. 2022;188: 114417. https://doi.org/10.1016/j.addr.2022.114417.
9. Xu X, Xia T. Recent advances in site-specific lipid nanoparticles for mRNA delivery. ACS Nanosci Au. 2023;3:192–203. https://doi.org/10.1021/acsnanoscienceau.2c00062.
10. Sahin U, Oehm P, Derhovanessian E, Jabulowsky RA, Vormehr M, Gold M, Maurus D, Schwarck-Kokarakis D, Kuhn AN, Omokoko T, Kranz LM, Diken M, Kreiter S, Haas H, Attig S, Rae R, Cuk K, Kemmer-Brück A, Breitkreuz A, Tolliver C, Caspar J, Quinkhardt J, Hebich L, Stein M, Hohberger A, Vogler I, Liebig I, Renken S, Sikorski J, Leierer M, Müller V, Mitzel-Rink H, Miederer M, Huber C, Grabbe S, Utikal J, Pinter A, Kaufmann R, Hassel JC, Loquai C, Türeci Ö. An RNA vaccine drives immunity in checkpoint-inhibitor-treated melanoma. Nature. 2020;585:107–12. https://doi.org/10.1038/s41586-020-2537-9.
11. Nanomedicine and the COVID-19 vaccines. Nat Nanotechnol. 2020;15:963. https://doi.org/10.1038/s41565-020-00820-0.
12. Crooke ST, Witztum JL, Bennett CF, Baker BF. RNA-targeted therapeutics. Cell Metab. 2018;27:714–39. https://doi.org/10.1016/j.cmet.2018.03.004.
13. Damase TR, Sukhovershin R, Boada C, Taraballi F, Pettigrew RI, Cooke JP. The limitless future of RNA therapeutics. Front Bioeng Biotechnol. 2021;9:1–24. https://doi.org/10.3389/fbioe.2021.628137.
14. Sahin U, Karikó K, Türeci Ö. mRNA-based therapeutics—developing a new class of drugs. Nat Rev Drug Discov. 2014;13:759–80. https://doi.org/10.1038/nrd4278.
15. Kaufmann KB, Büning H, Galy A, Schambach A, Grez M. Gene therapy on the move. EMBO Mol Med. 2013;5:1642–61. https://doi.org/10.1002/emmm.201202287.
16. Wang F, Wang L, Zou X, Duan S, Li Z, Deng Z, Luo J, Lee SY, Chen S. Advances in CRISPR–Cas systems for RNA targeting, tracking and editing. Biotechnol Adv. 2019;37:708–29. https://doi.org/10.1016/j.biotechadv.2019.03.016.
17. Chen X, Mangala LS, Rodriguez-Aguayo C, Kong X, Lopez-Berestein G, Sood AK. RNA interference-based therapy and its delivery systems. Cancer Metastasis Rev. 2018;37:107–24. https://doi.org/10.1007/s10555-017-9717-6.
18. Brad Wan W, Seth PP. The medicinal chemistry of therapeutic oligonucleotides. J Med Chem. 2016;59:9645–67. https://doi.org/10.1021/acs.jmedchem.6b00551.
19. Eckstein F. Phosphorothioates, essential components of therapeutic oligonucleotides. Nucleic Acid Ther. 2014;24:374–87. https://doi.org/10.1089/nat.2014.0506.
20. Prakash TP. An overview of sugar-modified oligonucleotides for antisense therapeutics. Chem Biodivers. 2011;8:1616–41. https://doi.org/10.1002/cbdv.201100081.
21. Dirin M, Winkler J. Influence of diverse chemical modifications on the ADME characteristics and toxicology of antisense oligonucleotides. Expert Opin Biol Ther. 2013;13:875–88. https://doi.org/10.1517/14712598.2013.774366.
22. Setten RL, Rossi JJ, Han S. The current state and future directions of RNAi-based therapeutics. Nat Rev Drug Discov. 2019;18:421–46. https://doi.org/10.1038/s41573-019-0017-4.
23. Winkler J. Oligonucleotide conjugates for therapeutic applications. Ther Deliv. 2013;4:791–809. https://doi.org/10.4155/tde.13.47.
24. Iversen PL. Phosphorodiamidate morpholino oligomers: favorable properties for sequence-specific gene inactivation. Curr Opin Mol Ther. 2001;3:235–8.
25. Nielsen PE. Gene targeting using peptide nucleic acid. Methods Mol Biol. 2005;288:343–58. https://doi.org/10.1385/1-59259-823-4:343.
26. Chen Q, Zhang Y, Yin H. Recent advances in chemical modifications of guide RNA, mRNA and donor template for CRISPR-mediated genome editing. Adv Drug Deliv Rev. 2021;168:246–58. https://doi.org/10.1016/j.addr.2020.10.014.
27. Hendel A, Bak RO, Clark JT, Kennedy AB, Ryan DE, Roy S, Steinfeld I, Lunstad BD, Kaiser RJ, Wilkens AB, Bacchetta R, Tsalenko A, Dellinger D, Bruhn L, Porteus MH. Chemically modified guide RNAs enhance CRISPR–Cas genome editing in human primary cells. Nat Biotechnol. 2015;33:985–9. https://doi.org/10.1038/nbt.3290.
28. Ryan DE, Taussig D, Steinfeld I, Phadnis SM, Lunstad BD, Singh M, Vuong X, Okochi KD, McCaffrey R, Olesiak M, Roy S, Yung CW, Curry B, Sampson JR, Bruhn L, Dellinger DJ. Improving CRISPR–Cas specificity with chemical modifications in single-guide RNAs. Nucleic Acids Res. 2018;46:792–803. https://doi.org/10.1093/nar/gkx1199.
29. Kim S, Koo T, Jee HG, Cho HY, Lee G, Lim DG, Shin HS, Kim JS. CRISPR RNAs trigger innate immune responses in human cells. Genome Res. 2018;28:367–73. https://doi.org/10.1101/gr.231936.117.
30. Cromwell CR, Sung K, Park J, Krysler AR, Jovel J, Kim SK, Hubbard BP. Incorporation of bridged nucleic acids into CRISPR RNAs improves Cas9 endonuclease specificity. Nat Commun. 2018;9:1–11. https://doi.org/10.1038/s41467-018-03927-0.
31. Ni S, Yao H, Wang L, Lu J, Jiang F, Lu A, Zhang G. Chemical modifications of nucleic acid aptamers for therapeutic purposes. Int J Mol Sci. 2017;18:1683. https://doi.org/10.3390/ijms18081683.
32. Hoffmann S, Hoos J, Klussmann S, Vonhoff S. RNA aptamers and spiegelmers: synthesis, purification, and post-synthetic PEG conjugation. Curr Protoc Nucleic Acid Chem. 2011;46:4.46.1-4.46.30. https://doi.org/10.1002/0471142700.NC0446S46.
33. Shi H, He X, Cui W, Wang K, Deng K, Li D, Xu F. Locked nucleic acid/DNA chimeric aptamer probe for tumor diagnosis with improved serum stability and extended imaging window in vivo. Anal Chim Acta. 2014;812:138–44. https://doi.org/10.1016/j.aca.2013.12.023.
34. Maier KE, Levy M. From selection hits to clinical leads: progress in aptamer discovery. Mol Ther Methods Clin Dev. 2016;3:16014. https://doi.org/10.1038/mtm.2016.14.
35. Melamed JR, Hajj KA, Chaudhary N, Strelkova D, Arral ML, Pardi N, Alameh M-G, Miller JB, Farbiak L, Siegwart DJ, Weissman D, Whitehead KA. Lipid nanoparticle chemistry determines how nucleoside base modifications alter mRNA delivery. J Control Release. 2022;341:206–14. https://doi.org/10.1016/j.jconrel.2021.11.022.

36. Warminski M, Mamot A, Depaix A, Kowalska J, Jemielity J. Chemical modifications of mRNA ends for therapeutic applications. Acc Chem Res. 2023;56:2814–26. https://doi.org/10.1021/acs.accounts.3c00442.
37. Samaridou E, Heyes J, Lutwyche P. Lipid nanoparticles for nucleic acid delivery: current perspectives. Adv Drug Deliv Rev. 2020;154–155:37–63. https://doi.org/10.1016/J.ADDR.2020.06.002.
38. Mendonça MCP, Kont A, Kowalski PS, O'Driscoll CM. Design of lipid-based nanoparticles for delivery of therapeutic nucleic acids. Drug Discov Today. 2023;28: 103505. https://doi.org/10.1016/j.drudis.2023.103505.
39. Zhang Y, Sun C, Wang C, Jankovic KE, Dong Y. Lipids and lipid derivatives for RNA delivery. Chem Rev. 2021;121:12181–277. https://doi.org/10.1021/acs.chemrev.1c00244.
40. Eygeris Y, Gupta M, Kim J, Sahay G. Chemistry of lipid nanoparticles for RNA delivery. Acc Chem Res. 2022;55:2–12. https://doi.org/10.1021/acs.accounts.1c00544.
41. Hassett KJ, Benenato KE, Jacquinet E, Lee A, Woods A, Yuzhakov O, Himansu S, Deterling J, Geilich BM, Ketova T, Mihai C, Lynn A, McFadyen I, Moore MJ, Senn JJ, Stanton MG, Almarsson Ö, Ciaramella G, Brito LA. Optimization of lipid nanoparticles for intramuscular administration of mRNA vaccines. Mol Ther Nucleic Acids. 2019;15:1–11. https://doi.org/10.1016/J.OMTN.2019.01.013.
42. Lamb YN. Inclisiran: first approval. Drugs. 2021;81:389–95. https://doi.org/10.1007/s40265-021-01473-6.
43. Hoy SM. Patisiran: first global approval. Drugs. 2018;78:1625–31. https://doi.org/10.1007/s40265-018-0983-6.
44. Maier MA, Jayaraman M, Matsuda S, Liu J, Barros S, Querbes W, Tam YK, Ansell SM, Kumar V, Qin J, Zhang X, Wang Q, Panesar S, Hutabarat R, Carioto M, Hettinger J, Kandasamy P, Butler D, Rajeev KG, Pang B, Charisse K, Fitzgerald K, Mui BL, Du X, Cullis P, Madden TD, Hope MJ, Manoharan M, Akinc A. Biodegradable lipids enabling rapidly eliminated lipid nanoparticles for systemic delivery of RNAi therapeutics. Mol Ther. 2013;21:1570–8. https://doi.org/10.1038/MT.2013.124.
45. Ponti F, Campolungo M, Melchiori C, Bono N, Candiani G. Cationic lipids for gene delivery: many players, one goal. Chem Phys Lipids. 2021;235:105032. https://doi.org/10.1016/j.chemphyslip.2020.105032.
46. Shirazi RS, Ewert KK, Leal C, Majzoub RN, Bouxsein NF, Safinya CR. Synthesis and characterization of degradable multivalent cationic lipids with disulfide-bond spacers for gene delivery. Biochim Biophys Acta Biomembr. 1808;2011:2156–66. https://doi.org/10.1016/j.bbamem.2011.04.020.
47. Buck J, Grossen P, Cullis PR, Huwyler J, Witzigmann D. Lipid-based DNA therapeutics: hallmarks of non-viral gene delivery. ACS Nano. 2019;13:3754–82. https://doi.org/10.1021/acsnano.8b07858.
48. Heyes J, Palmer L, Bremner K, MacLachlan I. Cationic lipid saturation influences intracellular delivery of encapsulated nucleic acids. J Control Release. 2005;107:276–87. https://doi.org/10.1016/j.jconrel.2005.06.014.
49. Witzigmann D, Kulkarni JA, Leung J, Chen S, Cullis PR, van der Meel R. Lipid nanoparticle technology for therapeutic gene regulation in the liver. Adv Drug Deliv Rev. 2020;159:344–63. https://doi.org/10.1016/j.addr.2020.06.026.
50. Schoenmaker L, Witzigmann D, Kulkarni JA, Verbeke R, Kersten G, Jiskoot W, Crommelin DJA. mRNA-lipid nanoparticle COVID-19 vaccines: structure and stability. Int J Pharm. 2021;601: 120586. https://doi.org/10.1016/j.ijpharm.2021.120586.
51. Ma T, Chen X, Wang M. Intracellular delivery of mRNA for cell-selective CRISPR/Cas9 genome editing using lipid nanoparticles. ChemBioChem. 2023;24: e202200801. https://doi.org/10.1002/cbic.202200801.
52. Cai W, Luo T, Chen X, Mao L, Wang M. A combinatorial library of biodegradable lipid nanoparticles preferentially deliver mRNA into tumor cells to block mutant RAS signaling. Adv Funct Mater. 2022;32:2204947. https://doi.org/10.1002/adfm.202204947.
53. Miao L, Lin J, Huang Y, Li L, Delcassian D, Ge Y, Shi Y, Anderson DG. Synergistic lipid compositions for albumin receptor mediated delivery of mRNA to the liver. Nat Commun. 2020;11:2424. https://doi.org/10.1038/s41467-020-16248-y.
54. Chen J, Ye Z, Huang C, Qiu M, Song D, Li Y, Xu Q. Lipid nanoparticle-mediated lymph node–targeting delivery of mRNA cancer vaccine elicits robust CD8+ T cell response. Proc Natl Acad Sci. 2022;119:e2207841119. https://doi.org/10.1073/pnas.2207841119.
55. Cheng X, Lee RJ. The role of helper lipids in lipid nanoparticles (LNPs) designed for oligonucleotide delivery. Adv Drug Deliv Rev. 2016;99:129–37. https://doi.org/10.1016/j.addr.2016.01.022.
56. Kulkarni JA, Witzigmann D, Leung J, Tam YYC, Cullis PR. On the role of helper lipids in lipid nanoparticle formulations of siRNA. Nanoscale. 2019;11:21733–9. https://doi.org/10.1039/C9NR09347H.
57. Kulkarni JA, Myhre JL, Chen S, Tam YYC, Danescu A, Richman JM, Cullis PR. Design of lipid nanoparticles for in vitro and in vivo delivery of plasmid DNA. Nanomed Nanotechnol Biol Med. 2017;13:1377–87. https://doi.org/10.1016/j.nano.2016.12.014.
58. LoPresti ST, Arral ML, Chaudhary N, Whitehead KA. The replacement of helper lipids with charged alternatives in lipid nanoparticles facilitates targeted mRNA delivery to the spleen and lungs. J Control Release. 2022;345:819–31. https://doi.org/10.1016/J.JCONREL.2022.03.046.
59. Anindita J, Tanaka H, Yamakawa T, Sato Y, Matsumoto C, Ishizaki K, Oyama T, Suzuki S, Ueda K, Higashi K, Moribe K, Sasaki K, Ogura Y, Yonemochi E, Sakurai Y, Hatakeyama H, Akita H. The effect of cholesterol content on the adjuvant activity of nucleic-acid-free lipid nanoparticles. Pharmaceutics. 2024;16:181. https://doi.org/10.3390/pharmaceutics16020181.
60. Tenchov BG, MacDonald RC, Siegel DP. Cubic phases in phosphatidylcholine-cholesterol mixtures: cholesterol as membrane "fusogen." Biophys J. 2006;91:2508–16. https://doi.org/10.1529/biophysj.106.083766.
61. Roces CB, Lou G, Jain N, Abraham S, Thomas A, Halbert GW, Perrie Y. Manufacturing considerations for the development of lipid nanoparticles using microfluidics. Pharmaceutics. 2020;12:1095. https://doi.org/10.3390/pharmaceutics12111095.
62. Semple SC, Chonn A, Cullis PR. Influence of cholesterol on the association of plasma proteins with liposomes. Biochemistry. 1996;35:2521–5. https://doi.org/10.1021/BI950414I.
63. Hald Albertsen C, Kulkarni JA, Witzigmann D, Lind M, Petersson K, Simonsen JB. The role of lipid components in lipid nanoparticles for vaccines and gene therapy. Adv Drug Deliv Rev. 2022;188: 114416. https://doi.org/10.1016/j.addr.2022.114416.
64. Paunovska K, Da Silva Sanchez AJ, Sago CD, Gan Z, Lokugamage MP, Islam FZ, Kalathoor S, Krupczak BR, Dahlman JE. Nanoparticles containing oxidized cholesterol deliver mRNA to the liver microenvironment at clinically relevant doses. Adv Mater. 2019;31:1807748. https://doi.org/10.1002/adma.201807748.
65. Zeng Y, Escalona-Rayo O, Knol R, Kros A, Slütter B. Lipid nanoparticle-based mRNA candidates elicit potent T cell responses. Biomater Sci. 2022;11:964–74. https://doi.org/10.1039/d2bm01581a.
66. Eygeris Y, Patel S, Jozic A, Sahay G, Sahay G. Deconvoluting lipid nanoparticle structure for messenger RNA delivery. Nano Lett. 2020;20:4543–9. https://doi.org/10.1021/acs.nanolett.0c01386.
67. Hafez I, Maurer N, Cullis P. On the mechanism whereby cationic lipids promote intracellular delivery of polynucleic acids. Gene Ther. 2001;8:1188–96. https://doi.org/10.1038/sj.gt.3301506.
68. Ge X, Chen L, Zhao B, Yuan W. Rationale and application of PEGylated lipid-based system for advanced target delivery of siRNA. Front Pharmacol. 2021;11: 598175. https://doi.org/10.3389/fphar.2020.598175.
69. Lokugamage MP, Vanover D, Beyersdorf J, Hatit MZCC, Rotolo L, Echeverri ES, Peck HE, Ni H, Yoon J-KK, Kim YT, Santangelo PJ, Dahlman JE. Optimization of lipid nanoparticles for the delivery of nebulized therapeutic mRNA to the lungs. Nat Biomed Eng. 2021;5:1059–68. https://doi.org/10.1038/s41551-021-00786-x.
70. Logan A, Howard CB, Huda P, Kimpton K, Ma Z, Thurecht KJ, McCarroll JA, Moles E, Kavallaris M. Targeted delivery of polo-like kinase 1 siRNA nanoparticles using an EGFR-PEG bispecific antibody inhibits proliferation of high-risk neuroblastoma. J Control Release. 2024;367:806–20. https://doi.org/10.1016/j.jconrel.2024.02.007.
71. Verbeke R, Lentacker I, De Smedt SC, Dewitte H. The dawn of mRNA vaccines: the COVID-19 case. J Control Release. 2021;333:511–20. https://doi.org/10.1016/j.jconrel.2021.03.043.
72. Chen S, Tam YYC, Lin PJC, Sung MMH, Tam YK, Cullis PR. Influence of particle size on the in vivo potency of lipid nanoparticle formulations of siRNA. J Control Release. 2016;235:236–44. https://doi.org/10.1016/j.jconrel.2016.05.059.

73. Seo H, Jeon L, Kwon J, Lee H. High-precision synthesis of RNA-loaded lipid nanoparticles for biomedical applications. Adv Healthc Mater. 2023;12:2203033. https://doi.org/10.1002/adhm.202203033.
74. Mehta M, Bui TA, Yang X, Aksoy Y, Goldys EM, Deng W. Lipid-based nanoparticles for drug/gene delivery: an overview of the production techniques and difficulties encountered in their industrial development. ACS Mater Au. 2023;3:600–19. https://doi.org/10.1021/acsmaterialsau.3c00032.
75. Carvalho BG, Ceccato BT, Michelon M, Han SW, de la Torre LG. Advanced microfluidic technologies for lipid nano-microsystems from synthesis to biological application. Pharmaceutics. 2022;14:141. https://doi.org/10.3390/pharmaceutics14010141.
76. Pattni BS, Chupin VV, Torchilin VP. New developments in liposomal drug delivery. Chem Rev. 2015;115:10938–66. https://doi.org/10.1021/acs.chemrev.5b00046.
77. Maeki M, Uno S, Niwa A, Okada Y, Tokeshi M. Microfluidic technologies and devices for lipid nanoparticle-based RNA delivery. J Control Release. 2022;344:80–96. https://doi.org/10.1016/j.jconrel.2022.02.017.
78. Amreen K, Goel S. Review—Miniaturized and microfluidic devices for automated nanoparticle synthesis. ECS J Solid State Sci Technol. 2021;10: 017002. https://doi.org/10.1149/2162-8777/abdb19.
79. Hanna AR, Shepherd SJ, Issadore D, Mitchell MJ. Microfluidic generation of diverse lipid nanoparticle libraries. Nanomedicine. 2024;19:455–7. https://doi.org/10.2217/nnm-2023-0345.
80. Zöller K, Haddadzadegan S, Lindner S, Veider F, Bernkop-Schnürch A. Design of charge converting lipid nanoparticles via a microfluidic coating technique. Drug Deliv Transl Res. 2024;14:3173–85. https://doi.org/10.1007/s13346-024-01538-5.
81. Ma Z, Tong H, Lin S, Zhou L, Sun D, Li B, Tian C, Chu J. Scalable synthesis of lipid nanoparticles for nucleic acid drug delivery using an isometric channel-size enlarging strategy. Nano Res. 2024;17:2899–907. https://doi.org/10.1007/s12274-023-6031-1.
82. Hallan SS, Sguizzato M, Esposito E, Cortesi R. Challenges in the physical characterization of lipid nanoparticles. Pharmaceutics. 2021;13:549. https://doi.org/10.3390/pharmaceutics13040549.
83. Leung AKK, Tam YYC, Chen S, Hafez IM, Cullis PR. Microfluidic mixing: a general method for encapsulating macromolecules in lipid nanoparticle systems. J Phys Chem B. 2015;119:8698–706. https://doi.org/10.1021/acs.jpcb.5b02891.
84. Leung AKK, Hafez IM, Baoukina S, Belliveau NM, Zhigaltsev IV, Afshinmanesh E, Tieleman DP, Hansen CL, Hope MJ, Cullis PR. Lipid nanoparticles containing siRNA synthesized by microfluidic mixing exhibit an electron-dense nanostructured core. J Phys Chem C. 2012;116:18440–50. https://doi.org/10.1021/jp303267y.
85. Lee SM, Cheng Q, Yu X, Liu S, Johnson LT, Siegwart DJ. A systematic study of unsaturation in lipid nanoparticles leads to improved mRNA transfection in vivo. Angew Chemie Int Ed. 2021;60:5848–53. https://doi.org/10.1002/anie.202013927.
86. Zhao P, Hou X, Yan J, Du S, Xue Y, Li W, Xiang G, Dong Y. Long-term storage of lipid-like nanoparticles for mRNA delivery. Bioact Mater. 2020;5:358–63. https://doi.org/10.1016/j.bioactmat.2020.03.001.
87. Crommelin DJA, Anchordoquy TJ, Volkin DB, Jiskoot W, Mastrobattista E. Addressing the cold reality of mRNA vaccine stability. J Pharm Sci. 2021;110:997–1001. https://doi.org/10.1016/j.xphs.2020.12.006.
88. Gurba-Bryśkiewicz L, Maruszak W, Smuga DA, Dubiel K, Wieczorek M. Quality by design (QbD) and design of experiments (DOE) as a strategy for tuning lipid nanoparticle formulations for RNA delivery. Biomedicines. 2023;11:2752. https://doi.org/10.3390/biomedicines11102752.
89. Rawal M, Singh A, Amiji MM. Quality-by-design concepts to improve nanotechnology-based drug development. Pharm Res. 2019;36:1–20. https://doi.org/10.1007/s11095-019-2692-6.
90. Cunha S, Costa CP, Moreira JN, SousaLobo JM, Silva AC. Using the quality by design (QbD) approach to optimize formulations of lipid nanoparticles and nanoemulsions: a review. Nanomed Nanotechnol Biol Med. 2020;28: 102206. https://doi.org/10.1016/j.nano.2020.102206.
91. Anderson MJ, Whitcomb PJ. RSM simplified: optimizing processes using response surface methods for design of experiments. New York: Taylor and Francis; 2016. https://doi.org/10.1201/9781315382326.
92. Blakney AK, McKay PF, Ibarzo Yus B, Hunter JE, Dex EA, Shattock RJ. The skin you are in: design-of-experiments optimization of lipid nanoparticle self-amplifying RNA formulations in human skin explants. ACS Nano. 2019;13:5920–30. https://doi.org/10.1021/acsnano.9b01774.
93. Kauffman KJ, Dorkin JR, Yang JH, Heartlein MW, Derosa F, Mir FF, Fenton OS, Anderson DG. Optimization of lipid nanoparticle formulations for mRNA delivery in vivo with fractional factorial and definitive screening designs. Nano Lett. 2015;15:7300–6. https://doi.org/10.1021/ACS.NANOLETT.5B02497.
94. Hashiba A, Toyooka M, Sato Y, Maeki M, Tokeshi M, Harashima H. The use of design of experiments with multiple responses to determine optimal formulations for in vivo hepatic mRNA delivery. J Control Release. 2020;327:467–76. https://doi.org/10.1016/J.JCONREL.2020.08.031.
95. Thanki K, Zeng X, Justesen S, Tejlmann S, Falkenberg E, Van Driessche E, Mørck Nielsen H, Franzyk H, Foged C. Engineering of small interfering RNA-loaded lipid-poly(DL-lactic-co-glycolic acid) hybrid nanoparticles for highly efficient and safe gene silencing: a quality by design-based approach. Eur J Pharm Biopharm. 2017;120:22–33. https://doi.org/10.1016/J.EJPB.2017.07.014.
96. Reklaitis GV, Seymour C, García-Munoz S. Comprehensive quality by design for pharmaceutical product development and manufacture. New York: American Institute of Chemical Engineers; 2017. https://doi.org/10.1002/9781119356189.
97. Breitkreitz MC, Goicoechea H. Introduction to quality by design in pharmaceutical manufacturing and analytical development, vol. 10. Cham: Springer; 2023. https://doi.org/10.1007/978-3-031-31505-3.
98. Ly HH, Daniel S, Soriano SKV, Kis Z, Blakney AK. Optimization of lipid nanoparticles for saRNA expression and cellular activation using a design-of-experiment approach. Mol Pharm. 2022;19:1892–905. https://doi.org/10.1021/ACS.MOLPHARMACEUT.2C00032.
99. Terada T, Kulkarni JA, Huynh A, Chen S, Van Der Meel R, Tam YYC, Cullis PR. Characterization of lipid nanoparticles containing ionizable cationic lipids using design-of-experiments approach. Langmuir. 2021;37:1120–8. https://doi.org/10.1021/ACS.LANGMUIR.0C03039.
100. Rapalli VK, Khosa A, Singhvi G, Girdhar V, Jain R, Dubey SK. Application of QbD principles in nanocarrier-based drug delivery systems. In: Pharmaceutical quality by design. London: Academic Press; 2019. p. 255–96. https://doi.org/10.1016/B978-0-12-815799-2.00014-9.
101. Bastogne T, Hassler L, Bruniaux J, Thomassin M, Gidrol X, Sulpice E, Navarro FP. A Bayesian implementation of quality-by-design for the development of cationic nano-lipid for siRNA transfection. IEEE Trans Nanobiosci. 2023;22:455–66. https://doi.org/10.1109/TNB.2022.3213412.
102. Young RE, Nelson KM, Hofbauer SI, Vijayakumar T, Alameh M-G, Weissman D, Papachristou C, Gleghorn JP, Riley RS. Lipid nanoparticle composition drives mRNA delivery to the placenta. BioRxiv. 2022. https://doi.org/10.1101/2022.12.22.521490.
103. Beg S, Rahman M, Kohli K. Quality-by-design approach as a systematic tool for the development of nanopharmaceutical products. Drug Discov Today. 2019;24:717–25. https://doi.org/10.1016/j.drudis.2018.12.002.
104. Fumoto S, Yamamoto T, Okami K, Maemura Y, Terada C, Yamayoshi A, Nishida K. Understanding in vivo fate of nucleic acid and gene medicines for the rational design of drugs. Pharmaceutics. 2021;13:159. https://doi.org/10.3390/pharmaceutics13020159.
105. Kawakami S, Ito Y, Fumoto S, Yamashita F, Hashida M. Enhanced gene expression in lung by a stabilized lipoplex using sodium chloride for complex formation. J Gene Med. 2005;7:1526–33. https://doi.org/10.1002/jgm.813.
106. Yusuf A, Almotairy ARZ, Henidi H, Alshehri OY, Aldughaim MS. Nanoparticles as drug delivery systems: a review of the implication of nanoparticles' physicochemical properties on responses in biological systems. Polymers. 2023;15:1596. https://doi.org/10.3390/polym15071596.
107. Ci L, Hard M, Zhang H, Gandham S, Hua S, Wickwire J, Wehrman T, Slauter R, Auerbach A, Kenney M, Mercer G, Hendrick T, Almarsson OE, Cheung E, Burdette D. Biodistribution of lipid 5, mRNA, and its translated protein following intravenous administration of mRNA-encapsulated lipid nanoparticles in rats. Drug Metab Dispos. 2023;51:813–23. https://doi.org/10.1124/dmd.122.000980.
108. Shimizu K, Higuchi Y, Kozu Y, Hashida M, Konishi S. Development of a suction device for stabilizing in vivo real-time imaging of murine tissues. J Biosci Bioeng. 2011;112:508–10. https://doi.org/10.1016/j.jbiosc.2011.07.015.

109. Rizvi F, Everton E, Smith AR, Liu H, Osota E, Beattie M, Tam Y, Pardi N, Weissman D, Gouon-Evans V. Murine liver repair via transient activation of regenerative pathways in hepatocytes using lipid nanoparticle-complexed nucleoside-modified mRNA. Nat Commun. 2021;12:613. https://doi.org/10.1038/s41467-021-20903-3.
110. Givlaari | European Medicines Agency (n.d.). https://www.ema.europa.eu/en/medicines/human/EPAR/givlaari. Accessed 17 Dec 2023.
111. Onpattro | European Medicines Agency (n.d.). https://www.ema.europa.eu/en/medicines/human/EPAR/onpattro. Accessed 17 Dec 2023.
112. Comirnaty | European Medicines Agency (n.d.). https://www.ema.europa.eu/en/medicines/human/EPAR/comirnaty. Accessed 19 Dec 2023.
113. Onpattro | European Medicines Agency (n.d.). https://www.ema.europa.eu/en/medicines/human/EPAR/onpattro. Accessed 19 Dec 2023.
114. Vervaeke P, Borgos SE, Sanders NN, Combes F. Regulatory guidelines and preclinical tools to study the biodistribution of RNA therapeutics. Adv Drug Deliv Rev. 2022;184: 114236. https://doi.org/10.1016/j.addr.2022.114236.
115. Alp G, Aydogan N. Lipid-based mucus penetrating nanoparticles and their biophysical interactions with pulmonary mucus layer. Eur J Pharm Biopharm. 2020;149:45–57. https://doi.org/10.1016/j.ejpb.2020.01.017.
116. Eliyahu H, Servel N, Domb A, Barenholz Y. Lipoplex-induced hemagglutination: potential involvement in intravenous gene delivery. Gene Ther. 2002;9:850–8. https://doi.org/10.1038/sj.gt.3301705.
117. Francia V, Schiffelers RM, Cullis PR, Witzigmann D. The biomolecular corona of lipid nanoparticles for gene therapy. Bioconjug Chem. 2020;31:2046–59. https://doi.org/10.1021/ACS.BIOCONJCHEM.0C00366.
118. Zhang R, El-Mayta R, Murdoch TJ, Warzecha CC, Billingsley MM, Shepherd SJ, Gong N, Wang L, Wilson JM, Lee D, Mitchell MJ. Helper lipid structure influences protein adsorption and delivery of lipid nanoparticles to spleen and liver. Biomater Sci. 2021;9:1449–63. https://doi.org/10.1039/D0BM01609H.
119. Francia V, Yang K, Deville S, Reker-Smit C, Nelissen I, Salvati A. Corona composition can affect the mechanisms cells use to internalize nanoparticles. ACS Nano. 2019;13:11107–21. https://doi.org/10.1021/acsnano.9b03824.
120. Francia V, Montizaan D, Salvati A. Interactions at the cell membrane and pathways of internalization of nano-sized materials for nanomedicine. Beilstein J Nanotechnol. 2020;11:338–53. https://doi.org/10.3762/bjnano.11.25.
121. Yoshikawa N, Fumoto S, Yoshikawa K, Hu D, Okami K, Kato R, Nakashima M, Miyamoto H, Nishida K. Interaction of lipoplex with albumin enhances gene expression in hepatitis mice. Pharmaceutics. 2020;12:341. https://doi.org/10.3390/pharmaceutics12040341.
122. Wisse E, Jacobs F, Topal B, Frederik P, De Geest B. The size of endothelial fenestrae in human liver sinusoids: implications for hepatocyte-directed gene transfer. Gene Ther. 2008;15:1193–9. https://doi.org/10.1038/gt.2008.60.
123. Danhier F. To exploit the tumor microenvironment: since the EPR effect fails in the clinic, what is the future of nanomedicine? J Control Release. 2016;244:108–21. https://doi.org/10.1016/j.jconrel.2016.11.015.
124. Fumoto S, Kawakami S, Ito Y, Shigeta K, Yamashita F, Hashida M. Enhanced hepatocyte-selective in vivo gene expression by stabilized galactosylated liposome/plasmid DNA complex using sodium chloride for complex formation. Mol Ther. 2004;10:719–29. https://doi.org/10.1016/j.ymthe.2004.07.015.
125. Boado RJ. Blood–brain barrier transport of non-viral gene and RNAi therapeutics. Pharm Res. 2007;24:1772–87. https://doi.org/10.1007/s11095-007-9321-5.
126. Song K-H, Fan AC, Hinkle JJ, Newman J, Borden MA, Harvey BK. Microbubble gas volume: a unifying dose parameter in blood–brain barrier opening by focused ultrasound. Theranostics. 2017;7:144–52. https://doi.org/10.7150/thno.15987.
127. Li X, Guo X, Hu M, Cai R, Chen C. Optimal delivery strategies for nanoparticle-mediated mRNA delivery. J Mater Chem B. 2023;11:2063–77. https://doi.org/10.1039/d2tb02455a.
128. Cheng Q, Wei T, Farbiak L, Johnson LT, Dilliard SA, Siegwart DJ. Selective organ targeting (SORT) nanoparticles for tissue-specific mRNA delivery and CRISPR–Cas gene editing. Nat Nanotechnol. 2020;15:313–20. https://doi.org/10.1038/s41565-020-0669-6.
129. Wang S, Zhang J, Zhou H, Lu YC, Jin X, Luo L, You J. The role of protein corona on nanodrugs for organ-targeting and its prospects of application. J Control Release. 2023;360:15–43. https://doi.org/10.1016/j.jconrel.2023.06.014.
130. Simonsen JB. Lipid nanoparticle-based strategies for extrahepatic delivery of nucleic acid therapies—challenges and opportunities. J Control Release. 2024;370:763–72. https://doi.org/10.1016/j.jconrel.2024.04.022.
131. Liu M, Feng Y, Lu Y, Huang R, Zhang Y, Zhao Y, Mo R. Lymph-targeted high-density lipoprotein-mimetic nanovaccine for multi-antigenic personalized cancer immunotherapy. Sci Adv. 2024;10: eadk2444. https://doi.org/10.1126/SCIADV.ADK2444.
132. Chen D, Ganesh S, Wang W, Lupieri A, Amiji M. Role of vitronectin-rich protein corona on tumor-specific siRNA delivery and transfection with lipid nanoparticles. Nanomedicine. 2021;16:535–51. https://doi.org/10.2217/nnm-2020-0428.
133. Chen D, Parayath N, Ganesh S, Wang W, Amiji M. The role of apolipoprotein- and vitronectin-enriched protein corona on lipid nanoparticles for- and vivo targeted delivery and transfection of oligonucleotides in murine tumor models. Nanoscale. 2019;11:18806–24. https://doi.org/10.1039/c9nr05788a.
134. Cai R, Chen C. The crown and the scepter: roles of the protein corona in nanomedicine. Adv Mater. 2019;31:1805740. https://doi.org/10.1002/adma.201805740.
135. Ji Q, Zhu H, Qin Y, Zhang R, Wang L, Zhang E, Zhou X, Meng R. GP60 and SPARC as albumin receptors: key targeted sites for the delivery of antitumor drugs. Front Pharmacol. 2024;15:1329636. https://doi.org/10.3389/FPHAR.2024.1329636/BIBTEX.
136. Qiu M, Tang Y, Chen J, Muriph R, Ye Z, Huang C, Evans J, Henske EP, Xu Q. Lung-selective mRNA delivery of synthetic lipid nanoparticles for the treatment of pulmonary lymphangioleiomyomatosis. Proc Natl Acad Sci. 2022;119: e2116271119. https://doi.org/10.1073/pnas.2116271119.
137. Paunovska K, Da Silva Sanchez AJ, Lokugamage MP, Loughrey D, Echeverri ES, Cristian A, Hatit MZC, Santangelo PJ, Zhao K, Dahlman JE. The extent to which lipid nanoparticles require apolipoprotein e and low-density lipoprotein receptor for delivery changes with ionizable lipid structure. Nano Lett. 2022;22:10025–33. https://doi.org/10.1021/acs.nanolett.2c03741.
138. Zhang Y, Wu JLY, Lazarovits J, Chan WCW. An analysis of the binding function and structural organization of the protein corona. J Am Chem Soc. 2020;142:8827–36. https://doi.org/10.1021/jacs.0c01853.
139. Wilhelm S, Tavares AJ, Dai Q, Ohta S, Audet J, Dvorak HF, Chan WCW. Analysis of nanoparticle delivery to tumours. Nat Rev Mater. 2016;1:16014. https://doi.org/10.1038/natrevmats.2016.14.
140. Giulimondi F, Digiacomo L, Pozzi D, Palchetti S, Vulpis E, Capriotti AL, Chiozzi RZ, Laganà A, Amenitsch H, Masuelli L, Peruzzi G, Mahmoudi M, Screpanti I, Zingoni A, Caracciolo G. Interplay of protein corona and immune cells controls blood residency of liposomes. Nat Commun. 2019;10:3686. https://doi.org/10.1038/s41467-019-11642-7.
141. Sato Y, Okabe N, Note Y, Hashiba K, Maeki M, Tokeshi M, Harashima H. Hydrophobic scaffolds of pH-sensitive cationic lipids contribute to miscibility with phospholipids and improve the efficiency of delivering short interfering RNA by small-sized lipid nanoparticles. Acta Biomater. 2020;102:341–50. https://doi.org/10.1016/j.actbio.2019.11.022.
142. Hayashi Y, Takamiya M, Jensen PB, Ojea-Jiménez I, Claude H, Antony C, Kjaer-Sorensen K, Grabher C, Boesen T, Gilliland D, Oxvig C, Strähle U, Weiss C. Differential nanoparticle sequestration by macrophages and scavenger endothelial cells visualized in vivo in real-time and at ultrastructural resolution. ACS Nano. 2020;14:1665–81. https://doi.org/10.1021/acsnano.9b07233.
143. Abstiens K, Maslanka Figueroa S, Gregoritza M, Goepferich AM. Interaction of functionalized nanoparticles with serum proteins and its impact on colloidal stability and cargo leaching. Soft Matter. 2019;15:709–20. https://doi.org/10.1039/C8SM02189A.
144. Ezzat K, Pernemalm M, Pålsson S, Roberts TC, Järver P, Dondalska A, Bestas B, Sobkowiak MJ, Levänen B, Sköld M, Thompson EA, Saher O, Kari OK, Lajunen T, Sverremark Ekström E, Nilsson C, Ishchenko Y, Malm T, Wood MJA, Power UF, Masich S, Lindén A, Sandberg JK, Lehtiö J, Spetz A-L, EL Andaloussi S. The viral protein corona directs viral pathogenesis

and amyloid aggregation. Nat Commun. 2019;10:2331. https://doi.org/10.1038/s41467-019-10192-2.
145. Saha K, Rahimi M, Yazdani M, Kim ST, Moyano DF, Hou S, Das R, Mout R, Rezaee F, Mahmoudi M, Rotello VM. Regulation of macrophage recognition through the interplay of nanoparticle surface functionality and protein corona. ACS Nano. 2016;10:4421–30. https://doi.org/10.1021/acsnano.6b00053.
146. Sieber S, Grossen P, Detampel P, Siegfried S, Witzigmann D, Huwyler J. Zebrafish as an early stage screening tool to study the systemic circulation of nanoparticulate drug delivery systems in vivo. J Control Release. 2017;264:180–91. https://doi.org/10.1016/j.jconrel.2017.08.023.
147. Kulkarni JA, Witzigmann D, Chen S, Cullis PR, Van Der Meel R. Lipid nanoparticle technology for clinical translation of sirna therapeutics. Acc Chem Res. 2019;52:2435–44. https://doi.org/10.1021/acs.accounts.9b00368.
148. Chen D, Ganesh S, Wang W, Amiji M. The role of surface chemistry in serum protein corona-mediated cellular delivery and gene silencing with lipid nanoparticles. Nanoscale. 2019;11:8760–75. https://doi.org/10.1039/c8nr09855g.
149. Zhu X, Tao W, Liu D, Wu J, Guo Z, Ji X, Bharwani Z, Zhao L, Zhao X, Farokhzad OC, Shi J. Surface de-PEGylation controls nanoparticle-mediated siRNA delivery in vitro and in vivo. Theranostics. 2017;7:1990–2002. https://doi.org/10.7150/thno.18136.
150. El-Sayed A, Harashima H. Endocytosis of gene delivery vectors: from clathrin-dependent to lipid raft-mediated endocytosis. Mol Ther. 2013;21:1118–30. https://doi.org/10.1038/mt.2013.54.
151. Hu J, Liu J, Yang D, Lu M, Yin J. Physiological roles of asialoglycoprotein receptors (ASGPRs) variants and recent advances in hepatic-targeted delivery of therapeutic molecules via ASGPRs. Protein Pept Lett. 2014;21:1025–30. https://doi.org/10.2174/09298665216661406261 02429.
152. Kaneda Y, Nakajima T, Nishikawa T, Yamamoto S, Ikegami H, Suzuki N, Nakamura H, Morishita R, Kotani H. Hemagglutinating virus of Japan (HVJ) envelope vector as a versatile gene delivery system. Mol Ther. 2002;6:219–26. https://doi.org/10.1006/mthe.2002.0647.
153. Akita H, Ito R, Khalil I, Futaki S, Harashima H. Quantitative three-dimensional analysis of the intracellular trafficking of plasmid DNA transfected by a nonviral gene delivery system using confocal laser scanning microscopy. Mol Ther. 2004;9:443–51. https://doi.org/10.1016/j.ymthe.2004.01.005.
154. Lambert TJ, Waters JC. Navigating challenges in the application of superresolution microscopy. J Cell Biol. 2017;216:53–63. https://doi.org/10.1083/jcb.201610011.
155. Wittrup A, Ai A, Liu X, Hamar P, Trifonova R, Charisse K, Manoharan M, Kirchhausen T, Lieberman J. Visualizing lipid-formulated siRNA release from endosomes and target gene knockdown. Nat Biotechnol. 2015;33:870–6. https://doi.org/10.1038/nbt.3298.
156. Meng C, Chen Z, Li G, Welte T, Shen H. Nanoplatforms for mRNA therapeutics. Adv Ther. 2021;4:2000099. https://doi.org/10.1002/adtp.202000099.
157. Jiang Y, Lu Q, Wang Y, Xu E, Ho A, Singh P, Wang Y, Jiang Z, Yang F, Tietjen GT, Cresswell P, Saltzman WM. Quantitating endosomal escape of a library of polymers for mRNA delivery. Nano Lett. 2020;20:1117–23. https://doi.org/10.1021/acs.nanolett.9b04426.
158. Tanaka H, Takahashi T, Konishi M, Takata N, Gomi M, Shirane D, Miyama R, Hagiwara S, Yamasaki Y, Sakurai Y, Ueda K, Higashi K, Moribe K, Shinsho E, Nishida R, Fukuzawa K, Yonemochi E, Okuwaki K, Mochizuki Y, Nakai Y, Tange K, Yoshioka H, Tamagawa S, Akita H. Self-degradable lipid-like materials based on "hydrolysis accelerated by the intra-particle enrichment of reactant (HyPER)" for messenger RNA delivery. Adv Funct Mater. 2020;30:1910575. https://doi.org/10.1002/ADFM.201910575.
159. Khalil IA, Kimura S, Sato Y, Harashima H. Synergism between a cell penetrating peptide and a pH-sensitive cationic lipid in efficient gene delivery based on double-coated nanoparticles. J Control Release. 2018;275:107–16. https://doi.org/10.1016/j.jconrel.2018.02.016.
160. Sabnis S, Kumarasinghe ES, Salerno T, Mihai C, Ketova T, Senn JJ, Lynn A, Bulychev A, McFadyen I, Chan J, Almarsson Ö, Stanton MG, Benenato KE. A novel amino lipid series for mRNA delivery: improved endosomal escape and sustained pharmacology and safety in non-human primates. Mol Ther. 2018;26:1509–19. https://doi.org/10.1016/J.YMTHE.2018.03.010.
161. Hajj KA, Ball RL, Deluty SB, Singh SR, Strelkova D, Knapp CM, Whitehead KA. Branched-tail lipid nanoparticles potently deliver mRNA in vivo due to enhanced ionization at endosomal pH. Small. 2019;15:1805097. https://doi.org/10.1002/smll.201805097.
162. Kim J, Jozic A, Sahay G. Naturally derived membrane lipids impact nanoparticle-based messenger RNA delivery. Cell Mol Bioeng. 2020;13:463–74. https://doi.org/10.1007/s12195-020-00619-y.
163. Sakurai F, Nishioka T, Saito H, Baba T, Okuda A, Matsumoto O, Taga T, Yamashita F, Takakura Y, Hashida M. Interaction between DNA–cationic liposome complexes and erythrocytes is an important factor in systemic gene transfer via the intravenous route in mice: the role of the neutral helper lipid. Gene Ther. 2001;8:677–86. https://doi.org/10.1038/sj.gt.3301460.
164. Akinc A, Thomas M, Klibanov AM, Langer R. Exploring polyethylenimine-mediated DNA transfection and the proton sponge hypothesis. J Gene Med. 2005;7:657–63. https://doi.org/10.1002/jgm.696.
165. Hu Y, Haynes MT, Wang Y, Liu F, Huang L. A highly efficient synthetic vector: nonhydrodynamic delivery of DNA to hepatocyte nuclei in vivo. ACS Nano. 2013;7:5376–84. https://doi.org/10.1021/nn4012384.
166. Fumoto S, Nakajima S, Mine T, Yoshikawa N, Kitahara T, Sasaki H, Miyamoto H, Nishida K. Efficient in vivo gene transfer by intraperitoneal injection of plasmid DNA and calcium carbonate microflowers in mice. Mol Pharm. 2012;9:1962–70. https://doi.org/10.1021/mp2006592.
167. Hu Y, Wan JMF, Yu ACH. Membrane perforation and recovery dynamics in microbubble-mediated sonoporation. Ultrasound Med Biol. 2013;39:2393–405. https://doi.org/10.1016/j.ultrasmedbio.2013.08.003.
168. Chang DC, Reese TS. Changes in membrane structure induced by electroporation as revealed by rapid-freezing electron microscopy. Biophys J. 1990;58:1–12. https://doi.org/10.1016/S0006-3495(90)82348-1.
169. Döhner K, Sodeik B. The role of the cytoskeleton during viral infection. Membr Traffick Viral Replication. 2004;285:67–108. https://doi.org/10.1007/3-540-26764-6_3.
170. Ondrej V, Lukásová E, Falk M, Kozubek S. The role of actin and microtubule networks in plasmid DNA intracellular trafficking. Acta Biochim Pol. 2007;54:657–63. https://doi.org/10.18388/abp.2007_3239.
171. Wang R, Brattain MG. The maximal size of protein to diffuse through the nuclear pore is larger than 60 kDa. FEBS Lett. 2007;581:3164–70. https://doi.org/10.1016/j.febslet.2007.05.082.
172. Tammam SN, Azzazy HME, Breitinger HG, Lamprecht A. Chitosan nanoparticles for nuclear targeting: the effect of nanoparticle size and nuclear localization sequence density. Mol Pharm. 2015;12:4277–89. https://doi.org/10.1021/acs.molpharmaceut.5b00478.
173. Lin M, Qi X. Advances and challenges of stimuli-responsive nucleic acids delivery system in gene therapy. Pharmaceutics. 2023;15:1450. https://doi.org/10.3390/pharmaceutics15051450.
174. Yan Y, Liu X-Y, Lu A, Wang X-Y, Jiang L-X, Wang J-C. Non-viral vectors for RNA delivery. J Control Release. 2022;342:241–79. https://doi.org/10.1016/j.jconrel.2022.01.008.
175. Torchilin VP. Multifunctional, stimuli-sensitive nanoparticulate systems for drug delivery. Nat Rev Drug Discov. 2014;13:813–27. https://doi.org/10.1038/nrd4333.
176. Liu S, Liu J, Li H, Mao K, Wang H, Meng X, Wang J, Wu C, Chen H, Wang X, Cong X, Hou Y, Wang Y, Wang M, Yang YG, Sun T. An optimized ionizable cationic lipid for brain tumor-targeted siRNA delivery and glioblastoma immunotherapy. Biomaterials. 2022;287: 121645. https://doi.org/10.1016/J.BIOMATERIALS.2022.121645.
177. Hirai Y, Saeki R, Song F, Koide H, Fukata N, Tomita K, Maeda N, Oku N, Asai T. Charge-reversible lipid derivative: a novel type of pH-responsive lipid for nanoparticle-mediated siRNA delivery. Int J Pharm. 2020;585:119479. https://doi.org/10.1016/j.ijpharm.2020.119479.
178. Xiong H, Liu S, Wei T, Cheng Q, Siegwart DJ. Theranostic dendrimer-based lipid nanoparticles containing PEGylated BODIPY dyes for tumor imaging and systemic mRNA delivery in vivo. J Control Release. 2020;325:198–205. https://doi.org/10.1016/J.JCONREL.2020.06.030.
179. Chen Z, Tian Y, Yang J, Wu F, Liu S, Cao W, Xu W, Hu T, Siegwart DJ, Xiong H. Modular design of biodegradable ionizable lipids for improved mRNA delivery and precise cancer metastasis delineation in vivo. J Am Chem Soc. 2023;145:24302–14. https://doi.org/10.1021/JACS.3C09143.

180. Kamath D, Iwakuma T, Bossmann SH. Therapeutic potential of combating cancer by restoring wild-type p53 through mRNA nanodelivery. Nanomedicine. 2024;56: 102732. https://doi.org/10.1016/J.NANO.2024.102732.
181. Sies H, Jones DP. Reactive oxygen species (ROS) as pleiotropic physiological signalling agents. Nat Rev Mol Cell Biol. 2020;21:363–83. https://doi.org/10.1038/s41580-020-0230-3.
182. Liu XX, Xiang J, Zhu D, Jiang L, Zhou Z, Tang J, Liu XX, Huang Y, Shen Y, Liu XX, Xiang J, Zhu D, Zhou Z, Tang J, Shen Y, Jiang L, Huang Y. Fusogenic reactive oxygen species triggered charge-reversal vector for effective gene delivery. Adv Mater. 2016;28:1743–52. https://doi.org/10.1002/ADMA.201504288.
183. Wang QY, Xu YS, Zhang NX, Dong ZP, Zhao BN, Liu LC, Lu T, Wang Y. Phenylboronic ester-modified anionic micelles for ROS-stimuli response in HeLa cell. Drug Deliv. 2020;27:681–90. https://doi.org/10.1080/10717544.2020.1748761.
184. Ruan C, Liu L, Wang Q, Chen X, Chen Q, Lu Y, Zhang Y, He X, Zhang Y, Guo Q, Sun T, Jiang C. Reactive oxygen species-biodegradable gene carrier for the targeting therapy of breast cancer. ACS Appl Mater Interfaces. 2018;10:10398–408. https://doi.org/10.1021/acsami.8b01712.
185. Zheng N, Luo X, Zhang Z, Wang A, Song W. Cationic polyporphyrins as siRNA delivery vectors for photodynamic and gene synergistic anticancer therapy. ACS Appl Mater Interfaces. 2021;13:27513–21. https://doi.org/10.1021/acsami.1c07662.
186. Huang P, Deng H, Wang C, Zhou Y, Chen X. Cellular trafficking of nanotechnology-mediated mRNA delivery. Adv Mater. 2024;36:2307822. https://doi.org/10.1002/adma.202307822.
187. Qiu N, Gao J, Liu Q, Wang J, Shen Y. Enzyme-responsive charge-reversal polymer-mediated effective gene therapy for intraperitoneal tumors. Biomacromolecules. 2018;19:2308–19. https://doi.org/10.1021/acs.biomac.8b00440.
188. Klassen LMB, Chequin A, Manica GCM, Biembengut IV, Toledo MB, Baura VA, Pedrosa FDO, Ramos EAS, Costa FF, de Souza EM, Klassen G. MMP9 gene expression regulation by intragenic epigenetic modifications in breast cancer. Gene. 2018;642:461–6. https://doi.org/10.1016/J.GENE.2017.11.054.
189. Yoshinaga N, Ishii T, Naito M, Endo T, Uchida S, Cabral H, Osada K, Kataoka K. Polyplex micelles with phenylboronate/gluconamide cross-linking in the core exerting promoted gene transfection through spatiotemporal responsivity to intracellular pH and ATP concentration. J Am Chem Soc. 2017;139:18567–75. https://doi.org/10.1021/jacs.7b08816.
190. Tang X, Fan X, Zhao X, Su W. Triton X-100-modified adenosine triphosphate-responsive siRNA delivery agent for antitumor therapy. Mol Pharm. 2020;17:3696–708. https://doi.org/10.1021/ACS.MOLPHARMACEUT.0C00291.
191. Zhou Z, Zhang Q, Zhang M, Li H, Chen G, Qian C, Oupicky D, Sun M. ATP-activated decrosslinking and charge-reversal vectors for siRNA delivery and cancer therapy. Theranostics. 2018;8:4604–19. https://doi.org/10.7150/THNO.26889.
192. Gao Y, Jia L, Wang Q, Hu H, Zhao X, Chen D, Qiao M. PH/Redox dual-responsive polyplex with effective endosomal escape for codelivery of siRNA and doxorubicin against drug-resistant cancer cells. ACS Appl Mater Interfaces. 2019;11:16296–310. https://doi.org/10.1021/acsami.9b02016.
193. Zhang Z, Wang Q, Liu Q, Zheng Y, Zheng C, Yi K, Zhao Y, Gu Y, Wang Y, Wang C, Zhao X, Shi L, Kang C, Liu Y. Dual-locking nanoparticles disrupt the PD-1/PD-L1 pathway for efficient cancer immunotherapy. Adv Mater. 2019;31:1905751. https://doi.org/10.1002/ADMA.201905751.
194. Wang Z, Chang Z, Lu M, Shao D, Yue J, Yang D, Zheng X, Li M, He K, Zhang M, Chen L, Fei Dong W. Shape-controlled magnetic mesoporous silica nanoparticles for magnetically-mediated suicide gene therapy of hepatocellular carcinoma. Biomaterials. 2018;154:147–57. https://doi.org/10.1016/J.BIOMATERIALS.2017.10.047.
195. Wang J, Chen P, Dong Y, Xie H, Wang Y, Soto F, Ma P, Feng X, Du W, Liu BF. Designer exosomes enabling tumor targeted efficient chemo/gene/photothermal therapy. Biomaterials. 2021;276: 121056. https://doi.org/10.1016/J.BIOMATERIALS.2021.121056.
196. Wang H, Miao W, Wang F, Cheng Y. A self-assembled coumarin-anchored dendrimer for efficient gene delivery and light-responsive drug delivery. Biomacromolecules. 2018;19:2194–201. https://doi.org/10.1021/acs.biomac.8b00246.
197. Mo Y, Cheng MHY, D'Elia A, Doran K, Ding L, Chen J, Cullis PR, Zheng G. Light-activated siRNA endosomal release (LASER) by porphyrin lipid nanoparticles. ACS Nano. 2023;17:4688–703. https://doi.org/10.1021/ACSNANO.2C10936.
198. Wang J, He H, Xu X, Wang X, Chen Y, Yin L. Far-red light-mediated programmable anti-cancer gene delivery in cooperation with photodynamic therapy. Biomaterials. 2018;171:72–82. https://doi.org/10.1016/J.BIOMATERIALS.2018.04.020.
199. Chen L, Li G, Wang X, Li J, Zhang Y. Spherical nucleic acids for near-infrared light-responsive self-delivery of small-interfering RNA and antisense oligonucleotide. ACS Nano. 2021;15:11929–39. https://doi.org/10.1021/ACSNANO.1C03072.
200. Un K, Kawakami S, Suzuki R, Maruyama K, Yamashita F, Hashida M. Suppression of melanoma growth and metastasis by DNA vaccination using an ultrasound-responsive and mannose-modified gene carrier. Mol Pharm. 2011;8:543–54. https://doi.org/10.1021/mp100369n.
201. Shapiro MG, Goodwill PW, Neogy A, Yin M, Foster FS, Schaffer DV, Conolly SM. Biogenic gas nanostructures as ultrasonic molecular reporters. Nat Nanotechnol. 2014;9(4):311–6. https://doi.org/10.1038/nnano.2014.32.
202. Zhang C, Chen J, Song Y, Luo J, Jin P, Wang X, Xin L, Qiu F, Yao J, Wang G, Huang P. Ultrasound-enhanced reactive oxygen species responsive charge-reversal polymeric nanocarriers for efficient pancreatic cancer gene delivery. ACS Appl Mater Interfaces. 2022;14:2587–96. https://doi.org/10.1021/acsami.1c20030.
203. Lipids and lipid nanoparticle formulations for delivery of nucleic acids. 2016.
204. Kim J, Eygeris Y, Gupta M, Sahay G. Self-assembled mRNA vaccines. Adv Drug Deliv Rev. 2021;170:83–112. https://doi.org/10.1016/J.ADDR.2020.12.014.
205. Zhao X, Glass Z, Chen J, Yang L, Kaplan DL, Xu Q. mRNA delivery using bioreducible lipidoid nanoparticles facilitates neural differentiation of human mesenchymal stem cells. Adv Healthc Mater. 2021;10:2000938. https://doi.org/10.1002/ADHM.202000938.
206. Li Y, Jarvis R, Zhu K, Glass Z, Ogurlu R, Gao P, Li P, Chen J, Yu Y, Yang Y, Xu Q. Protein and mRNA delivery enabled by cholesteryl-based biodegradable lipidoid nanoparticles. Angew Chemie. 2020;132:15067–74. https://doi.org/10.1002/ANGE.202004994.
207. Whitehead KA, Dorkin JR, Vegas AJ, Chang PH, Veiseh O, Matthews J, Fenton OS, Zhang Y, Olejnik KT, Yesilyurt V, Chen D, Barros S, Klebanov B, Novobrantseva T, Langer R, Anderson DG. Degradable lipid nanoparticles with predictable in vivo siRNA delivery activity. Nat Commun. 2014;5:1–10. https://doi.org/10.1038/NCOMMS5277.
208. Guan X, Guo Z, Wang T, Lin L, Chen J, Tian H, Chen X. A pH-responsive detachable PEG shielding strategy for gene delivery system in cancer therapy. Biomacromolecules. 2017;18:1342–9. https://doi.org/10.1021/acs.biomac.7b00080.
209. Chen L, Chen C, Chen W, Li K, Chen X, Tang X, Xie G, Luo X, Wang X, Liang H, Yu S. Biodegradable black phosphorus nanosheets mediate specific delivery of hTERT siRNA for synergistic cancer therapy. ACS Appl Mater Interfaces. 2018;10:21137–48. https://doi.org/10.1021/ACSAMI.8B04807.
210. Zhao Y, He Z, Gao H, Tang H, He J, Guo Q, Zhang W, Liu J. Fine tuning of core-shell structure of hyaluronic acid/cell-penetrating peptides/siRNA nanoparticles for enhanced gene delivery to macrophages in antiatherosclerotic therapy. Biomacromolecules. 2018;19:2944–56. https://doi.org/10.1021/acs.biomac.8b00501.
211. Zhang X, Hong K, Sun Q, Zhu Y, Du J. Bioreducible, arginine-rich polydisulfide-based siRNA nanocomplexes with excellent tumor penetration for efficient gene silencing. Biomater Sci. 2021;9:5275–92. https://doi.org/10.1039/D1BM00643F.
212. Wang B, Shen B, Xiang W, Shen H. Advances in the study of LNPs for mRNA delivery and clinical applications. Virus Genes. 2024. https://doi.org/10.1007/s11262-024-02102-6.
213. Kawabata K, Takakura Y, Hashida M. The fate of plasmid DNA after intravenous injection in mice: involvement of scavenger receptors in its hepatic uptake. Pharm Res. 1995;12:825–30. https://doi.org/10.1023/A:1016248701505.

214. Yang J. Intratumoral injection of naked DNA. In: Gene therapy for cancer. New Jersey: Humana Press; 2000. p. 313–21. https://doi.org/10.1385/1-59259-086-1:313.
215. Byrne BJ, Falk DJ, Pacak CA, Nayak S, Herzog RW, Elder ME, Collins SW, Conlon TJ, Clement N, Cleaver BD, Cloutier DA, Porvasnik SL, Islam S, Elmallah MK, Martin A, Smith BK, Fuller DD, Lawson LA, Mah CS. Pompe disease gene therapy. Hum Mol Genet. 2011;20:R61–8. https://doi.org/10.1093/hmg/ddr174.
216. Lorenzer C, Dirin M, Winkler A-M, Baumann V, Winkler J. Going beyond the liver: progress and challenges of targeted delivery of siRNA therapeutics. J Control Release. 2015;203:1–15. https://doi.org/10.1016/j.jconrel.2015.02.003.
217. Melamed JR, Yerneni SS, Arral ML, LoPresti ST, Chaudhary N, Sehrawat A, Muramatsu H, Alameh M-G, Pardi N, Weissman D, Gittes GK, Whitehead KA. Ionizable lipid nanoparticles deliver mRNA to pancreatic β cells via macrophage-mediated gene transfer. Sci Adv. 2023;9: eade1444. https://doi.org/10.1126/sciadv.ade1444.
218. Röltgen K, Nielsen SCA, Silva O, Younes SF, Zaslavsky M, Costales C, Yang F, Wirz OF, Solis D, Hoh RA, Wang A, Arunachalam PS, Colburg D, Zhao S, Haraguchi E, Lee AS, Shah MM, Manohar M, Chang I, Gao F, Mallajosyula V, Li C, Liu J, Shoura MJ, Sindher SB, Parsons E, Dashdorj NJ, Dashdorj ND, Monroe R, Serrano GE, Beach TG, Chinthrajah RS, Charville GW, Wilbur JL, Wohlstadter JN, Davis MM, Pulendran B, Troxell ML, Sigal GB, Natkunam Y, Pinsky BA, Nadeau KC, Boyd SD. Immune imprinting, breadth of variant recognition, and germinal center response in human SARS-CoV-2 infection and vaccination. Cell. 2022;185:1025-1040.e14. https://doi.org/10.1016/j.cell.2022.01.018.
219. Kreiter S, Selmi A, Diken M, Koslowski M, Britten CM, Huber C, Türeci Ö, Sahin U. Intranodal vaccination with naked antigen-encoding RNA elicits potent prophylactic and therapeutic antitumoral immunity. Cancer Res. 2010;70:9031–40. https://doi.org/10.1158/0008-5472.CAN-10-0699.
220. Janoria KG, Gunda S, Boddu SHS, Mitra AK. Novel approaches to retinal drug delivery. Expert Opin Drug Deliv. 2007;4:371–88. https://doi.org/10.1517/17425247.4.4.371.
221. Patel S, Ryals RC, Weller KK, Pennesi ME, Sahay G. Lipid nanoparticles for delivery of messenger RNA to the back of the eye. J Control Release. 2019;303:91–100. https://doi.org/10.1016/j.jconrel.2019.04.015.
222. Herrera-Barrera M, Ryals RC, Gautam M, Jozic A, Landry M, Korzun T, Gupta M, Acosta C, Stoddard J, Reynaga R, Tschetter W, Jacomino N, Taratula O, Sun C, Lauer AK, Neuringer M, Sahay G. Peptide-guided lipid nanoparticles deliver mRNA to the neural retina of rodents and nonhuman primates. Sci Adv. 2023;9: eadd4623. https://doi.org/10.1126/sciadv.add4623.
223. Sadauskas E, Wallin H, Stoltenberg M, Vogel U, Doering P, Larsen A, Danscher G. Kupffer cells are central in the removal of nanoparticles from the organism. Part Fibre Toxicol. 2007;4:1–7. https://doi.org/10.1186/1743-8977-4-10.
224. Jin H, Jeong M, Lee G, Kim M, Yoo Y, Kim HJ, Cho J, Lee Y, Lee H. Engineered lipid nanoparticles for the treatment of pulmonary fibrosis by regulating epithelial–mesenchymal transition in the lungs. Adv Funct Mater. 2023;33:2209432. https://doi.org/10.1002/adfm.202209432.
225. Yang T, Huang D, Li C, Zhao D, Li J, Zhang M, Chen Y, Wang Q, Liang Z, Liang X-J, Li Z, Huang Y. Rolling microneedle electrode array (RoMEA) empowered nucleic acid delivery and cancer immunotherapy. Nano Today. 2021;36: 101017. https://doi.org/10.1016/j.nantod.2020.101017.
226. Busignies V, Charrueau C, Tchoreloff P, Escriou V. Nanostructures for oral delivery of therapeutic nucleic acids. In: Nanostructures for oral medicine. Amsterdam: Elsevier; 2017. p. 147–72. https://doi.org/10.1016/B978-0-323-47720-8.00007-9.
227. El-Mayta R, Zhang R, Shepherd SJ, Wang F, Billingsley MM, Dudkin V, Klein D, Lu HD, Mitchell MJ. A nanoparticle platform for accelerated in vivo oral delivery screening of nucleic acids. Adv Ther. 2021;4:2000111. https://doi.org/10.1002/adtp.202000111.
228. Gennemark P, Walter K, Clemmensen N, Rekic D, Nilsson CAM, Knoechel J, Holtta M, Wang Y, Yu RZ, Riney SJ, Monia BP, Andersson U, Jansson-Lofmark R, Rocha CSJ, Linden D, Ryden-Bergsten K, Hurt-Camejo E, Crooke RM, Tillman L, Carlsson BB, Elebring M, Tivesten A, Davies N, Rekić D, Nilsson CAM, Knöchel J, Hölttä M, Wernevik L, Rosengren B, Kakol-Palm D, Wang Y, Yu RZ, Geary RS, Riney SJ, Monia BP, Isaksson R, Jansson-Löfmark R, Rocha CSJ, Lindén D, Hurt-Camejo E, Crooke RM, Tillman L, Rydén-Bergsten T, Carlsson BB, Andersson U, Elebring M, Tivesten A, Davies N. Abstract 13307: an oral antisense oligonucleotide for PCSK9 inhibition in humans. Circulation. 2020;142:A13307. https://doi.org/10.1161/circ.142.suppl_3.13307.
229. Poon W, Zhang YN, Ouyang B, Kingston BR, Wu JLY, Wilhelm S, Chan WCW. Elimination pathways of nanoparticles. ACS Nano. 2019;13:5785–98. https://doi.org/10.1021/acsnano.9b01383.
230. Lin Y, Cheng Q, Wei T. Surface engineering of lipid nanoparticles: targeted nucleic acid delivery and beyond. Biophys Rep. 2023;9:255. https://doi.org/10.52601/bpr.2023.230022.
231. Attia MF, Anton N, Wallyn J, Omran Z, Vandamme TF. An overview of active and passive targeting strategies to improve the nanocarriers efficiency to tumour sites. J Pharm Pharmacol. 2019;71:1185–98. https://doi.org/10.1111/JPHP.13098.
232. Xu J, Tian L, Qi W, Lv Q, Wang T. Advancements in NSCLC: from pathophysiological insights to targeted treatments. Am J Clin Oncol Cancer Clin Trials. 2024;47:291–303. https://doi.org/10.1097/COC.0000000000001088.
233. Liu W, Liu L, Li H, Xie Y, Bai J, Guan J, Qi H, Sun J. Targeted pathophysiological treatment of ischemic stroke using nanoparticle-based drug delivery system. J Nanobiotechnol. 2024;22(1):499. https://doi.org/10.1186/S12951-024-02772-2.
234. Nakamura T, Kawai M, Sato Y, Maeki M, Tokeshi M, Harashima H. The effect of size and charge of lipid nanoparticles prepared by microfluidic mixing on their lymph node transitivity and distribution. Mol Pharm. 2020;17:944–53. https://doi.org/10.1021/acs.molpharmaceut.9b01182.
235. Di J, Du Z, Wu K, Jin S, Wang X, Li T, Xu Y. Biodistribution and non-linear gene expression of mRNA LNPs affected by delivery route and particle size. Pharm Res. 2022;39:105–14. https://doi.org/10.1007/s11095-022-03166-5.
236. Nogueira SS, Schlegel A, Maxeiner K, Weber B, Barz M, Schroer MA, Blanchet CE, Svergun DI, Ramishetti S, Peer D, Langguth P, Sahin U, Haas H. Polysarcosine-functionalized lipid nanoparticles for therapeutic mRNA delivery. ACS Appl Nano Mater. 2020;3:10634–45. https://doi.org/10.1021/acsanm.0c01834.
237. Fang J, Nakamura H, Maeda H. The EPR effect: unique features of tumor blood vessels for drug delivery, factors involved, and limitations and augmentation of the effect. Adv Drug Deliv Rev. 2011;63:136–51. https://doi.org/10.1016/j.addr.2010.04.009.
238. Kranz LM, Diken M, Haas H, Kreiter S, Loquai C, Reuter KC, Meng M, Fritz D, Vascotto F, Hefesha H, Grunwitz C, Vormehr M, Hüsemann Y, Selmi A, Kuhn AN, Buck J, Derhovanessian E, Rae R, Attig S, Diekmann J, Jabulowsky RA, Heesch S, Hassel J, Langguth P, Grabbe S, Huber C, Türeci Ö, Sahin U. Systemic RNA delivery to dendritic cells exploits antiviral defence for cancer immunotherapy. Nature. 2016;534:396–401. https://doi.org/10.1038/nature18300.
239. Fenton OS, Kauffman KJ, Kaczmarek JC, McClellan RL, Jhunjhunwala S, Tibbitt MW, Zeng MD, Appel EA, Dorkin JR, Mir FF, Yang JH, Oberli MA, Heartlein MW, DeRosa F, Langer R, Anderson DG. Synthesis and biological evaluation of ionizable lipid materials for the in vivo delivery of messenger RNA to B lymphocytes. Adv Mater. 2017;29:1606944. https://doi.org/10.1002/adma.201606944.
240. Liu S, Cheng Q, Wei T, Yu X, Johnson LT, Farbiak L, Siegwart DJ. Membrane-destabilizing ionizable phospholipids for organ-selective mRNA delivery and CRISPR–Cas gene editing. Nat Mater. 2021;20:701–10. https://doi.org/10.1038/s41563-020-00886-0.
241. Lokugamage MP, Sago CD, Gan Z, Krupczak BR, Dahlman JE. Constrained nanoparticles deliver siRNA and sgRNA to T cells in vivo without targeting ligands. Adv Mater. 2019;31:1902251. https://doi.org/10.1002/adma.201902251.
242. Ding F, Zhang H, Cui J, Li Q, Yang C. Boosting ionizable lipid nanoparticle-mediated in vivo mRNA delivery through optimization of lipid amine-head groups. Biomater Sci. 2021;9:7534–46. https://doi.org/10.1039/D1BM00866H.
243. Xue L, Zhao G, Gong N, Han X, Shepherd SJ, Xiong X, Xiao Z, Palanki R, Xu J, Swingle KL, Warzecha CC, El-Mayta R, Chowdhary V, Yoon IC, Xu J, Cui J, Shi Y, Alameh MG, Wang K, Wang L, Pochan DJ, Weissman D, Vaughan AE, Wilson JM, Mitchell MJ. Combinatorial design of siloxane-incorporated lipid nanoparticles augments intracellular processing for tissue-specific mRNA therapeutic delivery. Nat Nanotechnol. 2024. https://doi.org/10.1038/s41565-024-01747-6.

244. Zhang X, Zhao W, Nguyen GN, Zhang C, Zeng C, Yan J, Du S, Hou X, Li W, Jiang J, Deng B, McComb DW, Dorkin R, Shah A, Barrera L, Gregoire F, Singh M, Chen D, Sabatino DE, Dong Y. Functionalized lipid-like nanoparticles for in vivo mRNA delivery and base editing. Sci Adv. 2020;6: eabc2315. https://doi.org/10.1126/sciadv.abc2315.
245. Kowalski PS, Capasso Palmiero U, Huang Y, Rudra A, Langer R, Anderson DG. Ionizable amino-polyesters synthesized via ring opening polymerization of tertiary amino-alcohols for tissue selective mRNA delivery. Adv Mater. 2018;30:1801151. https://doi.org/10.1002/adma.201801151.
246. Paunovska K, Gil CJ, Lokugamage MP, Sago CD, Sato M, Lando GN, Gamboa Castro M, Bryksin AV, Dahlman JE. Analyzing 2000 in vivo drug delivery data points reveals cholesterol structure impacts nanoparticle delivery. ACS Nano. 2018;12:8341–9. https://doi.org/10.1021/acsnano.8b03640.
247. Jung O, Jung H, Thuy LT, Choi M, Kim S, Jeon H, Yang J, Kim S, Kim T, Lee E, Kim Y, Choi JS. Modulating lipid nanoparticles with histidinamide-conjugated cholesterol for improved intracellular delivery of mRNA. Adv Healthc Mater. 2024;13:2303857. https://doi.org/10.1002/adhm.202303857.
248. Gan Z, Lokugamage MP, Hatit MZCC, Loughrey D, Paunovska K, Sato M, Cristian A, Dahlman JE. Nanoparticles containing constrained phospholipids deliver mRNA to liver immune cells in vivo without targeting ligands. Bioeng Transl Med. 2020;5: e10161. https://doi.org/10.1002/btm2.10161.
249. Álvarez-Benedicto E, Farbiak L, Márquez Ramírez M, Wang X, Johnson LT, Mian O, Guerrero ED, Siegwart DJ. Optimization of phospholipid chemistry for improved lipid nanoparticle (LNP) delivery of messenger RNA (mRNA). Biomater Sci. 2022;10:549–59. https://doi.org/10.1039/D1BM01454D.
250. Sago CD, Lokugamage MP, Islam FZ, Krupczak BR, Sato M, Dahlman JE. Nanoparticles that deliver RNA to bone marrow identified by in vivo directed evolution. J Am Chem Soc. 2018;140:17095–105. https://doi.org/10.1021/jacs.8b08976.
251. Conte G, Costabile G, Baldassi D, Rondelli V, Bassi R, Colombo D, Linardos G, Fiscarelli EV, Sorrentino R, Miro A, Quaglia F, Brocca P, D'Angelo I, Merkel OM, Ungaro F. Hybrid lipid/polymer nanoparticles to tackle the cystic fibrosis mucus barrier in siRNA delivery to the lungs: does PEGylation make the difference? ACS Appl Mater Interfaces. 2022;14:7565–78. https://doi.org/10.1021/acsami.1c14975.
252. SalahpourAnarjan F. Active targeting drug delivery nanocarriers: ligands. Nano-Struct Nano-Objects. 2019;19: 100370. https://doi.org/10.1016/j.nanoso.2019.100370.
253. Kedmi R, Veiga N, Ramishetti S, Goldsmith M, Rosenblum D, Dammes N, Hazan-Halevy I, Nahary L, Leviatan-Ben-Arye S, Harlev M, Behlke M, Benhar I, Lieberman J, Peer D. A modular platform for targeted RNAi therapeutics. Nat Nanotechnol. 2018;13:214–9. https://doi.org/10.1038/s41565-017-0043-5.
254. Zhao Z, Ukidve A, Kim J, Mitragotri S. Targeting strategies for tissue-specific drug delivery. Cell. 2020;181:151–67. https://doi.org/10.1016/j.cell.2020.02.001.
255. Yamamoto T, Sawamura M, Wada F, Harada-Shiba M, Obika S. Serial incorporation of a monovalent GalNAc phosphoramidite unit into hepatocyte-targeting antisense oligonucleotides. Bioorg Med Chem. 2016;24:26–32. https://doi.org/10.1016/j.bmc.2015.11.036.
256. Kim M, Jeong M, Hur S, Cho Y, Park J, Jung H, Seo Y, Woo HA, Nam KT, Lee K, Lee H. Engineered ionizable lipid nanoparticles for targeted delivery of RNA therapeutics into different types of cells in the liver. Sci Adv. 2021;7: eabf4398. https://doi.org/10.1126/SCIADV.ABF4398.
257. Kono Y, Kawakami S, Higuchi Y, Maruyama K, Yamashita F, Hashida M. Antitumor effect of nuclear factor-κB decoy transfer by mannose-modified bubble lipoplex into macrophages in mouse malignant ascites. Cancer Sci. 2014;105:1049–55. https://doi.org/10.1111/cas.12452.
258. Bhattacharya S, Ghosh A, Maiti S, Ahir M, Debnath GH, Gupta P, Bhattacharjee M, Ghosh S, Chattopadhyay S, Mukherjee P, Adhikary A. Delivery of thymoquinone through hyaluronic acid-decorated mixed Pluronic® nanoparticles to attenuate angiogenesis and metastasis of triple-negative breast cancer. J Control Release. 2020;322:357–74. https://doi.org/10.1016/j.jconrel.2020.03.033.
259. Lee D, Amirthalingam S, Lee C, Rajendran AK, Ahn Y-H, Hwang NS. Strategies for targeted gene delivery using lipid nanoparticles and cell-derived nanovesicles. Nanoscale Adv. 2023;5:3834–56. https://doi.org/10.1039/D3NA00198A.
260. Steffens RC, Wagner E. Directing the way—receptor and chemical targeting strategies for nucleic acid delivery. Pharm Res. 2023;40:47–76. https://doi.org/10.1007/s11095-022-03385-w.
261. Rurik JG, Tombácz I, Yadegari A, Méndez Fernández PO, Shewale SV, Li L, Kimura T, Soliman OY, Papp TE, Tam YK, Mui BL, Albelda SM, Puré E, June CH, Aghajanian H, Weissman D, Parhiz H, Epstein JA. CAR T cells produced in vivo to treat cardiac injury. Science (80-). 2022;375:91–6. https://doi.org/10.1126/science.abm0594.
262. Weinstein S, Toker IA, Emmanuel R, Ramishetti S, Hazan-Halevy I, Rosenblum D, Goldsmith M, Abraham A, Benjamini O, Bairey O, Raanani P, Nagler A, Lieberman J, Peer D. Harnessing RNAi-based nanomedicines for therapeutic gene silencing in B-cell malignancies. Proc Natl Acad Sci USA. 2016;113:E16–22. https://doi.org/10.1073/pnas.1519273113.
263. Parhiz H, Shuvaev VV, Pardi N, Khoshnejad M, Kiseleva RY, Brenner JS, Uhler T, Tuyishime S, Mui BL, Tam YK, Madden TD, Hope MJ, Weissman D, Muzykantov VR. PECAM-1 directed re-targeting of exogenous mRNA providing two orders of magnitude enhancement of vascular delivery and expression in lungs independent of apolipoprotein E-mediated uptake. J Control Release. 2018;291:106–15. https://doi.org/10.1016/j.jconrel.2018.10.015.
264. Takeuchi T, Futaki S. Current understanding of direct translocation of arginine-rich cell-penetrating peptides and its internalization mechanisms. Chem Pharm Bull (Tokyo). 2016;64:1431–7. https://doi.org/10.1248/cpb.c16-00505.
265. Su F-Y, Zhao QH, Dahotre SN, Gamboa L, Bawage SS, Silva Trenkle AD, Zamat A, Phuengkham H, Ahmed R, Santangelo PJ, Kwong GA. In vivo mRNA delivery to virus-specific T cells by light-induced ligand exchange of MHC class I antigen-presenting nanoparticles. Sci Adv. 2022;8:7950. https://doi.org/10.1126/sciadv.abm7950.
266. Peng JQ, Fumoto S, Suga T, Miyamoto H, Kuroda N, Kawakami S, Nishida K. Targeted co-delivery of protein and drug to a tumor in vivo by sophisticated RGD-modified lipid-calcium carbonate nanoparticles. J Control Release. 2019;302:42–53. https://doi.org/10.1016/j.jconrel.2019.03.021.
267. Suga T, Fuchigami Y, Hagimori M, Kawakami S. Ligand peptide-grafted PEGylated liposomes using HER2 targeted peptide-lipid derivatives for targeted delivery in breast cancer cells: the effect of serine-glycine repeated peptides as a spacer. Int J Pharm. 2017;521:361–4. https://doi.org/10.1016/j.ijpharm.2017.02.041.
268. Lou B, De Koker S, Lau CYJ, Hennink WE, Mastrobattista E. MRNA polyplexes with post-conjugated GALA peptides efficiently target, transfect, and activate antigen presenting cells. Bioconjug Chem. 2018;30:461–75. https://doi.org/10.1021/acs.bioconjchem.8b00524.
269. Liang C, Guo B, Wu H, Shao N, Li D, Liu J, Dang L, Wang C, Li H, Li S, Lau WK, Cao Y, Yang Z, Lu C, He X, Au DWT, Pan X, Zhang B-T, Lu C, Zhang H, Yue K, Qian A, Shang P, Xu J, Xiao L, Bian Z, Tan W, Liang Z, He F, Zhang L, Lu A, Zhang G. Aptamer-functionalized lipid nanoparticles targeting osteoblasts as a novel RNA interference-based bone anabolic strategy. Nat Med. 2015;21:288–94. https://doi.org/10.1038/nm.3791.
270. Lee JB, Zhang K, Tam YYC, Quick J, Tam YK, Lin PJ, Chen S, Liu Y, Nair JK, Zlatev I, Rajeev KG, Manoharan M, Rennie PS, Cullis PR. A Glu-urea-Lys ligand-conjugated lipid nanoparticle/siRNA system inhibits androgen receptor expression in vivo. Mol Ther Nucleic Acids. 2016;5: e348. https://doi.org/10.1038/mtna.2016.43.
271. Dammes N, Goldsmith M, Ramishetti S, Dearling JLJ, Veiga N, Packard AB, Peer D. Conformation-sensitive targeting of lipid nanoparticles for RNA therapeutics. Nat Nanotechnol. 2021;16:1030–8. https://doi.org/10.1038/s41565-021-00928-x.
272. Kheirolomoom A, Kare AJ, Ingham ES, Paulmurugan R, Robinson ER, Baikoghli M, Inayathullah M, Seo JW, Wang J, Fite BZ, Wu B, Tumbale SK, Raie MN, Cheng RH, Nichols L, Borowsky AD, Ferrara KW. In situ T-cell transfection by anti-CD3-conjugated lipid nanoparticles leads to T-cell activation, migration, and phenotypic shift. Biomaterials. 2022;281: 121339. https://doi.org/10.1016/j.biomaterials.2021.121339.
273. Li Q, Chan C, Peterson N, Hanna RN, Alfaro A, Allen KL, Wu H, Dall'Acqua WF, Borrok MJ, Santos JL. Engineering caveolae-targeted lipid nanoparticles to deliver mRNA to the lungs. ACS Chem Biol. 2020;15:830–6. https://doi.org/10.1021/acschembio.0c00003.

274. Katakowski JA, Mukherjee G, Wilner SE, Maier KE, Harrison MT, DiLorenzo TP, Levy M, Palliser D. Delivery of siRNAs to dendritic cells using DEC205-targeted lipid nanoparticles to inhibit immune responses. Mol Ther. 2016;24:146–55. https://doi.org/10.1038/mt.2015.175.
275. Tombácz I, Laczkó D, Shahnawaz H, Muramatsu H, Natesan A, Yadegari A, Papp TE, Alameh M-G, Shuvaev V, Mui BL, Tam YK, Muzykantov V, Pardi N, Weissman D, Parhiz H. Highly efficient CD4+ T cell targeting and genetic recombination using engineered CD4+ cell-homing mRNA-LNPs. Mol Ther. 2021;29:3293–304. https://doi.org/10.1016/j.ymthe.2021.06.004.
276. Marcos-Contreras OA, Greineder CF, Kiseleva RY, Parhiz H, Walsh LR, Zuluaga-Ramirez V, Myerson JW, Hood ED, Villa CH, Tombacz I, Pardi N, Seliga A, Mui BL, Tam YK, Glassman PM, Shuvaev VV, Nong J, Brenner JS, Khoshnejad M, Madden T, Weissmann D, Persidsky Y, Muzykantov VR. Selective targeting of nanomedicine to inflamed cerebral vasculature to enhance the blood–brain barrier. PNAS. 2020;117:3405–14. https://doi.org/10.1073/pnas.1912012117.
277. Miettinen HM, Gripentrog JM, Lord CI, Nagy JO. CD177-mediated nanoparticle targeting of human and mouse neutrophils. PLoS ONE. 2018;13:e0200444. https://doi.org/10.1371/journal.pone.0200444.
278. Veiga N, Goldsmith M, Diesendruck Y, Ramishetti S, Rosenblum D, Elinav E, Behlke MA, Benhar I, Peer D. Leukocyte-specific siRNA delivery revealing IRF8 as a potential anti-inflammatory target. J Control Release. 2019;313:33–41. https://doi.org/10.1016/j.jconrel.2019.10.001.
279. Rosenblum D, Gutkin A, Kedmi R, Ramishetti S, Veiga N, Jacobi AM, Schubert MS, Friedmann-Morvinski D, Cohen ZR, Behlke MA, Lieberman J, Peer D. CRISPR–Cas9 genome editing using targeted lipid nanoparticles for cancer therapy. Sci Adv. 2020;6:9450–68. https://doi.org/10.1126/sciadv.abc9450.
280. Xue L, Gong N, Shepherd SJ, Xiong X, Liao X, Han X, Zhao G, Song C, Huang X, Zhang H, Padilla MS, Qin J, Shi Y, Alameh M-G, Pochan DJ, Wang K, Long F, Weissman D, Mitchell MJ. Rational design of bisphosphonate lipid-like materials for mRNA delivery to the bone microenvironment. J Am Chem Soc. 2022;144:9926–37. https://doi.org/10.1021/jacs.2c02706.
281. Goswami R, Chatzikleanthous D, Lou G, Giusti F, Bonci A, Taccone M, Brazzoli M, Gallorini S, Ferlenghi I, Berti F, O'Hagan DT, Pergola C, Baudner BC, Adamo R. Mannosylation of LNP results in improved potency for self-amplifying RNA (SAM) vaccines. ACS Infect Dis. 2019;5:1546–58. https://doi.org/10.1021/acsinfecdis.9b00084.
282. Verbeke R, Lentacker I, Breckpot K, Janssens J, Van Calenbergh S, De Smedt SC, Dewitte H. Broadening the message: a nanovaccine co-loaded with messenger RNA and α-GalCer induces antitumor immunity through conventional and natural killer T cells. ACS Nano. 2019;13:1655–69. https://doi.org/10.1021/acsnano.8b07660.
283. Wang Y, Zhang L, Xu Z, Miao L, Huang L. mRNA vaccine with antigen-specific checkpoint blockade induces an enhanced immune response against established melanoma. Mol Ther. 2018;26:420–34. https://doi.org/10.1016/j.ymthe.2017.11.009.
284. Akinc A, Querbes W, De S, Qin J, Frank-Kamenetsky M, Jayaprakash KN, Jayaraman M, Rajeev KG, Cantley WL, Dorkin JR, Butler JS, Qin L, Racie T, Sprague A, Fava E, Zeigerer A, Hope MJ, Zerial M, Sah DW, Fitzgerald K, Tracy MA, Manoharan M, Koteliansky V, De Fougerolles A, Maier MA. Targeted delivery of RNAi therapeutics with endogenous and exogenous ligand-based mechanisms. Mol Ther. 2010;18:1357–64. https://doi.org/10.1038/mt.2010.85.
285. Jia Z, Gong Y, Pi Y, Liu X, Gao L, Kang L, Wang J, Yang F, Tang J, Lu W, Li Q, Zhang W, Yan Z, Yu L. pPB peptide-mediated siRNA-loaded stable nucleic acid lipid nanoparticles on targeting therapy of hepatic fibrosis. Mol Pharm. 2018;15:53–62. https://doi.org/10.1021/acs.molpharmaceut.7b00709.
286. Goswami R, O'Hagan DT, Adamo R, Baudner BC, O'Hagan DT, Adamo R, Baudner BC. Conjugation of mannans to enhance the potency of liposome nanoparticles for the delivery of RNA vaccines. Pharmaceutics. 2021;13:240. https://doi.org/10.3390/pharmaceutics13020240.
287. Ramishetti S, Hazan-Halevy I, Palakuri R, Chatterjee S, NaiduGonna S, Dammes N, Freilich I, KolikShmuel L, Danino D, Peer D. A combinatorial library of lipid nanoparticles for RNA delivery to leukocytes. Adv Mater. 2020;32:1906128. https://doi.org/10.1002/adma.201906128.
288. Andresen T, Bruun J, Larsen TB, Jølck RI, Eliasen R, Holm R, Gjetting T. Investigation of enzyme-sensitive lipid nanoparticles for delivery of siRNA to blood–brain barrier and glioma cells. Int J Nanomed. 2015;10:5995. https://doi.org/10.2147/IJN.S87334.
289. Conceição M, Mendonça L, Nóbrega C, Gomes C, Costa P, Hirai H, Moreira JN, Lima MC, Manjunath N, Pereira de Almeida L. Intravenous administration of brain-targeted stable nucleic acid lipid particles alleviates Machado-Joseph disease neurological phenotype. Biomaterials. 2016;82:124–37. https://doi.org/10.1016/j.biomaterials.2015.12.021.
290. Sakurai Y, Abe N, Yoshikawa K, Oyama R, Ogasawara S, Murata T, Nakai Y, Tange K, Tanaka H, Akita H. Targeted delivery of lipid nanoparticle to lymphatic endothelial cells via anti-podoplanin antibody. J Control Release. 2022;349:379–87. https://doi.org/10.1016/j.jconrel.2022.06.052.
291. Butowska K, Han X, Gong N, El-Mayta R, Haley RM, Xue L, Zhong W, Guo W, Wang K, Mitchell MJ. Doxorubicin-conjugated siRNA lipid nanoparticles for combination cancer therapy. Acta Pharm Sin B. 2023;13:1429–37. https://doi.org/10.1016/j.apsb.2022.07.011.
292. Swart LE, Koekman CA, Seinen CW, Issa H, Rasouli M, Schiffelers RM, Heidenreich O. A robust post-insertion method for the preparation of targeted siRNA LNPs. Int J Pharm. 2022;620: 121741. https://doi.org/10.1016/j.ijpharm.2022.121741.
293. Cepparulo P, Cuomo O, Campani V, Vinciguerra A, Sisalli MJ, Nele V, Anzilotti S, Valsecchi V, Casamassa A, Brancaccio P, Scorziello A, De Rosa G, Annunziato L, Pignataro G. Anti-miRNA103/107 encapsulated in transferrin-conjugated lipid nanoparticles crosses blood–brain barrier and reduces brain ischemic damage. Mol Ther Nucleic Acids. 2024;35:102131. https://doi.org/10.1016/j.omtn.2024.102131.
294. Kim Y, Choi J, Kim EH, Park W, Jang H, Jang Y, Chi S, Kweon D, Lee K, Kim SH, Yang Y. Design of PD-L1-targeted lipid nanoparticles to turn on PTEN for efficient cancer therapy. Adv Sci. 2024;11:2309917. https://doi.org/10.1002/advs.202309917.
295. Han X, Gong N, Xue L, Billingsley MM, El-Mayta R, Shepherd SJ, Alameh MG, Weissman D, Mitchell MJ. Ligand-tethered lipid nanoparticles for targeted RNA delivery to treat liver fibrosis. Nat Commun. 2023;14(1):1–12. https://doi.org/10.1038/s41467-022-35637-z.
296. Cohen ZR, Ramishetti S, Peshes-Yaloz N, Goldsmith M, Wohl A, Zibly Z, Peer D. Localized RNAi therapeutics of chemoresistant grade IV glioma using hyaluronan-grafted lipid-based nanoparticles. ACS Nano. 2015;9:1581–91. https://doi.org/10.1021/nn506248s.
297. Tang Q, Liu J, Jiang Y, Zhang M, Mao L, Wang M. Cell-selective messenger RNA delivery and CRISPR/Cas9 genome editing by modulating the interface of phenylboronic acid-derived lipid nanoparticles and cellular surface sialic acid. ACS Appl Mater Interfaces. 2019;11:46585–90. https://doi.org/10.1021/acsami.9b17749.
298. Zhao X, Chen J, Qiu M, Li Y, Glass Z, Xu Q. Imidazole-based synthetic lipidoids for in vivo mRNA delivery into primary T lymphocytes. Angew Chemie Int Ed. 2020;59:20083–9. https://doi.org/10.1002/anie.202008082.
299. Ni H, Hatit MZC, Zhao K, Loughrey D, Lokugamage MP, Peck HE, Del Cid A, Muralidharan A, Kim Y, Santangelo PJ, Dahlman JE. Piperazine-derived lipid nanoparticles deliver mRNA to immune cells in vivo. Nat Commun. 2022;13:4766. https://doi.org/10.1038/s41467-022-32281-5.
300. Miao L, Li L, Huang Y, Delcassian D, Chahal J, Han J, Shi Y, Sadtler K, Gao W, Lin J, Doloff JC, Langer R, Anderson DG. Delivery of mRNA vaccines with heterocyclic lipids increases anti-tumor efficacy by STING-mediated immune cell activation. Nat Biotechnol. 2019;37:1174–85. https://doi.org/10.1038/s41587-019-0247-3.
301. Hou X, Zhang X, Zhao W, Zeng C, Deng B, McComb DW, Du S, Zhang C, Li W, Dong Y. Vitamin lipid nanoparticles enable adoptive macrophage transfer for the treatment of multidrug-resistant bacterial sepsis. Nat Nanotechnol. 2020;15:41–6. https://doi.org/10.1038/s41565-019-0600-1.
302. Gomi M, Sakurai Y, Sato M, Tanaka H, Miyatake Y, Fujiwara K, Watanabe M, Shuto S, Nakai Y, Tange K, Hatakeyama H, Akita H. Delivering mRNA to secondary lymphoid tissues by phosphatidylserine-loaded lipid nanoparticles. Adv Healthc Mater. 2023;12:2202528. https://doi.org/10.1002/adhm.202202528.
303. Vavilis T, Stamoula E, Ainatzoglou A, Sachinidis A, Lamprinou M, Dardalas I, Vizirianakis IS. mRNA in the context of protein replacement therapy. Pharmaceutics. 2023;15:166. https://doi.org/10.3390/pharmaceutics15010166.
304. Lian X, Chatterjee S, Sun Y, Dilliard SA, Moore S, Xiao Y, Bian X, Yamada K, Sung YC, Levine RM, Mayberry K, John S, Liu X, Smith C, Johnson

LT, Wang X, Zhang CC, Liu DR, Newby GA, Weiss MJ, Yen JS, Siegwart DJ. Bone-marrow-homing lipid nanoparticles for genome editing in diseased and malignant haematopoietic stem cells. Nat Nanotechnol. 2024;19:1409–17. https://doi.org/10.1038/s41565-024-01680-8.
305. Vaidya A, Moore S, Chatterjee S, Guerrero E, Kim M, Farbiak L, Dilliard SA, Siegwart DJ, Vaidya A, Moore S, Chatterjee S, Guerrero E, Kim M, Farbiak L, Dilliard SA, Siegwart DJ. Expanding RNAi to kidneys, lungs, and spleen via selective ORgan targeting (SORT) siRNA lipid nanoparticles. Adv Mater. 2024. https://doi.org/10.1002/ADMA.202313791.
306. Sun Y, Chatterjee S, Lian X, Traylor Z, Sattiraju SR, Xiao Y, Dilliard SA, Sung YC, Kim M, Lee SM, Moore S, Wang X, Zhang D, Wu S, Basak P, Wang J, Liu J, Mann RJ, LePage DF, Jiang W, Abid S, Hennig M, Martinez A, Wustman BA, Lockhart DJ, Jain R, Conlon RA, Drumm ML, Hodges CA, Siegwart DJ. In vivo editing of lung stem cells for durable gene correction in mice. Science. 2024;384:1196–202. https://doi.org/10.1126/SCIENCE.ADK9428.
307. Álvarez-Benedicto E, Tian Z, Chatterjee S, Orlando D, Kim M, Guerrero ED, Wang X, Siegwart DJ. Spleen SORT LNP generated in situ CAR T cells extend survival in a mouse model of lymphoreplete B cell. Lymphoma. 2023;135: e202310395. https://doi.org/10.1002/anie.202310395.
308. Zhao Y, Ye Z, Song D, Wich D, Gao S, Khirallah J, Xu Q. Nanomechanical action opens endo-lysosomal compartments. Nat Commun. 2023;14(1):1–11. https://doi.org/10.1038/s41467-023-42280-9.
309. Yang T, Poenisch M, Khanal R, Hu Q, Dai Z, Li R, Song G, Yuan Q, Yao Q, Shen X, Taubert R, Engel B, Jaeckel E, Vogel A, Falk CS, Schambach A, Gerovska D, Araúzo-Bravo MJ, Vondran FWR, Cantz T, Horscroft N, Balakrishnan A, Chevessier F, Ott M, Sharma AD. Corrigendum to 'Therapeutic HNF4A mRNA attenuates liver fibrosis in a preclinical model' [J Hepatol (2021) 1420–1433]. J Hepatol. 2022;77:270. https://doi.org/10.1016/j.jhep.2022.03.023.
310. Han JP, Kim MJ, Choi BS, Lee JH, Lee GS, Jeong M, Lee Y, Kim EA, Oh HK, Go N, Lee H, Lee KJ, Kim UG, Lee JY, Kim S, Chang J, Lee H, Song DW, Yeom SC. In vivo delivery of CRISPR–Cas9 using lipid nanoparticles enables antithrombin gene editing for sustainable hemophilia A and B therapy. Sci Adv. 2022;8: eabj6901. https://doi.org/10.1126/SCIADV.ABJ6901.
311. Jiang L, Berraondo P, Jericó D, Guey LT, Sampedro A, Frassetto A, Benenato KE, Burke K, Santamaría E, Alegre M, Pejenaute Á, Kalariya M, Butcher W, Park J-S, Zhu X, Sabnis S, Kumarasinghe ES, Salerno T, Kenney M, Lukacs CM, Ávila MA, Martini PGV, Fontanellas A. Systemic messenger RNA as an etiological treatment for acute intermittent porphyria. Nat Med. 2018;24:1899–909. https://doi.org/10.1038/s41591-018-0199-z.
312. Wang Y, Tiruthani K, Li S, Hu M, Zhong G, Tang Y, Roy S, Zhang L, Tan J, Liao C, Liu R. mRNA delivery of a bispecific single-domain antibody to polarize tumor-associated macrophages and synergize immunotherapy against liver malignancies. Adv Mater. 2021;33:2007603. https://doi.org/10.1002/ADMA.202007603.
313. Kim J, Jozic A, Mukherjee A, Nelson D, Chiem K, Khan MSR, Torrelles JB, Martinez-Sobrido L, Sahay G. Rapid generation of circulating and mucosal decoy human ACE2 using mRNA nanotherapeutics for the potential treatment of SARS-CoV-2. Adv Sci. 2022. https://doi.org/10.1002/ADVS.202202556.
314. Zhang D, Wang G, Yu X, Wei T, Farbiak L, Johnson LT, Taylor AM, Xu J, Hong Y, Zhu H, Siegwart DJ. Enhancing CRISPR/Cas gene editing through modulating cellular mechanical properties for cancer therapy. Nat Nanotechnol. 2022;17(7):777–87. https://doi.org/10.1038/s41565-022-01122-3.
315. Kong N, Tao W, Ling X, Wang J, Xiao Y, Shi S, Ji X, Shajii A, Gan ST, Kim NY, Duda DG, Xie T, Farokhzad OC, Shi J. Synthetic mRNA nanoparticle-mediated restoration of p53 tumor suppressor sensitizes p53-deficient cancers to mTOR inhibition. Sci Transl Med. 2019;11: eaaw1565. https://doi.org/10.1126/SCITRANSLMED.AAW1565.
316. Sun H, Zhang Y, Wang J, Su J, Zhou D, Yu X, Xu Y, Yang W. Application of lung-targeted lipid nanoparticle-delivered mRNA of soluble PD-L1 via SORT technology in acute respiratory distress syndrome. Theranostics. 2023;13:4974. https://doi.org/10.7150/THNO.86466.
317. Massaro M, Wu S, Baudo G, Liu H, Collum S, Lee H, Stigliano C, Segura-Ibarra V, Karmouty-Quintana H, Blanco E. Lipid nanoparticle-mediated mRNA delivery in lung fibrosis. Eur J Pharm Sci. 2023;183: 106370. https://doi.org/10.1016/J.EJPS.2023.106370.
318. Wei T, Sun Y, Cheng Q, Chatterjee S, Traylor Z, Johnson LT, Coquelin ML, Wang J, Torres MJ, Lian X, Wang X, Xiao Y, Hodges CA, Siegwart DJ. Lung SORT LNPs enable precise homology-directed repair mediated CRISPR/Cas genome correction in cystic fibrosis models. Nat Commun. 2023;14(1):1–14. https://doi.org/10.1038/s41467-023-42948-2.
319. Liu J-QQ, Zhang C, Zhang X, Yan J, Zeng C, Talebian F, Lynch K, Zhao W, Hou X, Du S, Kang DD, Deng B, McComb DW, Bai X-FF, Dong Y. Intratumoral delivery of IL-12 and IL-27 mRNA using lipid nanoparticles for cancer immunotherapy. J Control Release. 2022;345:306–13. https://doi.org/10.1016/j.jconrel.2022.03.021.
320. Thran M, Mukherjee J, Pönisch M, Fiedler K, Thess A, Mui BL, Hope MJ, Tam YK, Horscroft N, Heidenreich R, Fotin-Mleczek M, Shoemaker CB, Schlake T. mRNA mediates passive vaccination against infectious agents, toxins, and tumors. EMBO Mol Med. 2017;9:1434–47. https://doi.org/10.15252/emmm.201707678.
321. Rybakova Y, Kowalski PS, Huang Y, Gonzalez JT, Heartlein MW, DeRosa F, Delcassian D, Anderson DG. mRNA delivery for therapeutic anti-HER2 antibody expression in vivo. Mol Ther. 2019;27:1415–23. https://doi.org/10.1016/j.ymthe.2019.05.012.
322. Veiga N, Goldsmith M, Granot Y, Rosenblum D, Dammes N, Kedmi R, Ramishetti S, Peer D. Cell specific delivery of modified mRNA expressing therapeutic proteins to leukocytes. Nat Commun. 2018;91(9):1–9. https://doi.org/10.1038/s41467-018-06936-1.
323. Xu X, Wang X, Liao Y-P, Luo L, Xia T, Nel AE. Use of a liver-targeting immune-tolerogenic mRNA lipid nanoparticle platform to treat peanut-induced anaphylaxis by single- and multiple-epitope nucleotide sequence delivery. ACS Nano. 2023;17:4942–57. https://doi.org/10.1021/acsnano.2c12420.
324. Qin S, Tang X, Chen Y, Chen K, Fan N, Xiao W, Zheng Q, Li G, Teng Y, Wu M, Song X. mRNA-based therapeutics: powerful and versatile tools to combat diseases. Signal Transduct Target Ther. 2022;7:166. https://doi.org/10.1038/s41392-022-01007-w.
325. Chen C-Y, Tran DM, Cavedon A, Cai X, Rajendran R, Lyle MJ, Martini PGV, Miao CH. Treatment of hemophilia A using factor VIII messenger RNA lipid nanoparticles. Mol Ther Nucleic Acids. 2020;20:534–44. https://doi.org/10.1016/j.omtn.2020.03.015.
326. DeRosa F, Guild B, Karve S, Smith L, Love K, Dorkin JR, Kauffman KJ, Zhang J, Yahalom B, Anderson DG, Heartlein MW. Therapeutic efficacy in a hemophilia B model using a biosynthetic mRNA liver depot system. Gene Ther. 2016;23:699–707. https://doi.org/10.1038/gt.2016.46.
327. Cheng Q, Wei T, Jia Y, Farbiak L, Zhou K, Zhang S, Wei Y, Zhu H, Siegwart DJ. Dendrimer-based lipid nanoparticles deliver therapeutic FAH mRNA to normalize liver function and extend survival in a mouse model of hepatorenal tyrosinemia type I. Adv Mater. 2018;30:1805308. https://doi.org/10.1002/adma.201805308.
328. DeRosa F, Smith L, Shen Y, Huang Y, Pan J, Xie H, Yahalom B, Heartlein MW. Improved efficacy in a Fabry disease model using a systemic mRNA liver depot system as compared to enzyme replacement therapy. Mol Ther. 2019;27:878–89. https://doi.org/10.1016/j.ymthe.2019.03.001.
329. Swingle KL, Safford HC, Geisler HC, Hamilton AG, Thatte AS, Billingsley MM, Joseph RA, Mrksich K, Padilla MS, Ghalsasi AA, Alameh M-G, Weissman D, Mitchell MJ. Ionizable lipid nanoparticles for in vivo mRNA delivery to the placenta during pregnancy. J Am Chem Soc. 2023;145:4691–706. https://doi.org/10.1021/jacs.2c12893.
330. Nabhan JF, Wood KM, Rao VP, Morin J, Bhamidipaty S, LaBranche TP, Gooch RL, Bozal F, Bulawa CE, Guild BC. Intrathecal delivery of frataxin mRNA encapsulated in lipid nanoparticles to dorsal root ganglia as a potential therapeutic for Friedreich's ataxia. Sci Rep. 2016;6:20019. https://doi.org/10.1038/srep20019.
331. Sahu I, Haque AKMA, Weidensee B, Weinmann P, Kormann MSD. Recent developments in mRNA-based protein supplementation therapy to target lung diseases. Mol Ther. 2019;27:803–23. https://doi.org/10.1016/j.ymthe.2019.02.019.
332. Davies N, Hovdal D, Edmunds N, Nordberg P, Dahlén A, Dabkowska A, Arteta MY, Radulescu A, Kjellman T, Höijer A, Seeliger F, Holmedal E, Andihn E, Bergenhem N, Sandinge A-S, Johansson C, Hultin L, Johansson M, Lindqvist J, Björsson L, Jing Y, Bartesaghi S, Lindfors L, Andersson S. Functionalized lipid nanoparticles for subcutaneous administration of mRNA to achieve systemic exposures of a therapeutic protein. Mol Ther

Nucleic Acids. 2021;24:369–84. https://doi.org/10.1016/j.omtn.2021.03.008.
333. Kazemian P, Yu S-Y, Thomson SB, Birkenshaw A, Leavitt BR, Ross CJD. Lipid-nanoparticle-based delivery of CRISPR/Cas9 genome-editing components. Mol Pharm. 2022;19:1669–86. https://doi.org/10.1021/acs.molpharmaceut.1c00916.
334. Doudna JA, Charpentier E. The new frontier of genome engineering with CRISPR–Cas9. Science (80-). 2014;346:1258096. https://doi.org/10.1126/science.1258096.
335. Li Y, Yang T, Yu Y, Shi N, Yang L, Glass Z, Bolinger J, Finkel IJ, Li W, Xu Q. Combinatorial library of chalcogen-containing lipidoids for intracellular delivery of genome-editing proteins. Biomaterials. 2018;178:652–62. https://doi.org/10.1016/J.BIOMATERIALS.2018.03.011.
336. Fu Q, Hu L, Shen T, Yang R, Jiang L. Recent advances in gene therapy for familial hypercholesterolemia: an update review. J Clin Med. 2022;11:6773. https://doi.org/10.3390/jcm11226773.
337. Qiu M, Glass Z, Chen J, Haas M, Jin X, Zhao X, Rui X, Ye Z, Li Y, Zhang F, Xu Q. Lipid nanoparticle-mediated codelivery of Cas9 mRNA and single-guide RNA achieves liver-specific in vivo genome editing of Angptl3. Proc Natl Acad Sci USA. 2021;118: e2020401118. https://doi.org/10.1073/pnas.2020401118.
338. Villiger L, Rothgangl T, Witzigmann D, Oka R, Lin PJC, Qi W, Janjuha S, Berk C, Ringnalda F, Beattie MB, Stoffel M, Thöny B, Hall J, Rehrauer H, van Boxtel R, Tam YK, Schwank G. In vivo cytidine base editing of hepatocytes without detectable off-target mutations in RNA and DNA. Nat Biomed Eng. 2021;5:179–89. https://doi.org/10.1038/s41551-020-00671-z.
339. Kenjo E, Hozumi H, Makita Y, Iwabuchi KA, Fujimoto N, Matsumoto S, Kimura M, Amano Y, Ifuku M, Naoe Y, Inukai N, Hotta A. Low immunogenicity of LNP allows repeated administrations of CRISPR–Cas9 mRNA into skeletal muscle in mice. Nat Commun. 2021;12:7101. https://doi.org/10.1038/s41467-021-26714-w.
340. Im SH, Jang M, Park JH, Chung HJ. Finely tuned ionizable lipid nanoparticles for CRISPR/Cas9 ribonucleoprotein delivery and gene editing. J Nanobiotechnol. 2024;22:1–13. https://doi.org/10.1186/s12951-024-02427-2.
341. Herrera-Barrera M, Gautam M, Lokras A, Vlasova K, Foged C, Sahay G. Lipid nanoparticle-enabled intracellular delivery of prime editors. AAPS J. 2023;25:1–10. https://doi.org/10.1208/s12248-023-00833-2.
342. Dana N, Vaseghi G, Haghjooy Javanmard S. Activation of PPARγ inhibits TLR4 signal transduction pathway in melanoma cancer in vitro. Adv Pharm Bull. 2020;10:458–63. https://doi.org/10.34172/apb.2020.056.
343. Fan Y-N, Li M, Luo Y-L, Chen Q, Wang L, Zhang H-B, Shen S, Gu Z, Wang J. Cationic lipid-assisted nanoparticles for delivery of mRNA cancer vaccine. Biomater Sci. 2018;6:3009–18. https://doi.org/10.1039/C8BM00908B.
344. Perche F, Benvegnu T, Berchel M, Lebegue L, Pichon C, Jaffrès P-A, Midoux P. Enhancement of dendritic cells transfection in vivo and of vaccination against B16F10 melanoma with mannosylated histidylated lipopolyplexes loaded with tumor antigen messenger RNA. Nanomed Nanotechnol Biol Med. 2011;7:445–53. https://doi.org/10.1016/j.nano.2010.12.010.
345. Okumura K, Nakase M, Inui M, Nakamura S, Watanabe Y, Tagawa T. Bax mRNA therapy using cationic liposomes for human malignant melanoma. J Gene Med. 2008;10:910–7. https://doi.org/10.1002/jgm.1214.
346. Oberli MA, Reichmuth AM, Dorkin JR, Mitchell MJ, Fenton OS, Jaklenec A, Anderson DG, Langer R, Blankschtein D. Lipid nanoparticle assisted mRNA delivery for potent cancer immunotherapy. Nano Lett. 2017;17:1326–35. https://doi.org/10.1021/acs.nanolett.6b03329.
347. Lai I, Swaminathan S, Baylot V, Mosley A, Dhanasekaran R, Gabay M, Felsher DW. Lipid nanoparticles that deliver IL-12 messenger RNA suppress tumorigenesis in MYC oncogene-driven hepatocellular carcinoma. J Immunother Cancer. 2018;6:125. https://doi.org/10.1186/s40425-018-0431-x.
348. Zhang R, Men K, Zhang X, Huang R, Tian Y, Zhou B, Yu C, Wang Y, Ji X, Hu Q, Yang L. Delivery of a modified mRNA encoding IL-22 binding protein (IL-22BP) for colon cancer gene therapy. J Biomed Nanotechnol. 2018;14:1239–51. https://doi.org/10.1166/jbn.2018.2577.
349. Van Hoecke L, Roose K. How mRNA therapeutics are entering the monoclonal antibody field. J Transl Med. 2019;17:54. https://doi.org/10.1186/s12967-019-1804-8.
350. Wise HM, Hermida MA, Leslie NR. Prostate cancer, PI3K, PTEN and prognosis. Clin Sci. 2017;131:197–210. https://doi.org/10.1042/CS20160026.
351. da Silva JR, Rodrigues KB, Pelegrin GF, Sales NS, Muramatsu H, de Oliveira Silva M, Porchia BFMM, Moreno ACR, Aps LRMM, Venceslau-Carvalho AA, Tombácz I, Fotoran WL, Karikó K, Lin PJC, Tam YK, de Oliveira Diniz M, Pardi N, de Souza Ferreira LC. Single immunizations of self-amplifying or non-replicating mRNA-LNP vaccines control HPV-associated tumors in mice. Sci Transl Med. 2023;15: eabn3464. https://doi.org/10.1126/scitranslmed.abn3464.
352. Wu Y, Meng L, Zhang H, Hu S, Li F, Yu Y. Lipid-based poly(I:C) adjuvants strongly enhance the immunogenicity of SARS-CoV-2 receptor-binding domain vaccine. Infect Dis Immun. 2023;3:3–12. https://doi.org/10.1097/ID9.0000000000000074.
353. Cai X, Li JJ, Liu T, Brian O, Li J. Infectious disease mRNA vaccines and a review on epitope prediction for vaccine design. Brief Funct Genom. 2021;20:289–303. https://doi.org/10.1093/bfgp/elab027.
354. Painter MM, Mathew D, Goel RR, Apostolidis SA, Pattekar A, Kuthuru O, Baxter AE, Herati RS, Oldridge DA, Gouma S, Hicks P, Dysinger S, Lundgreen KA, Kuri-Cervantes L, Adamski S, Hicks A, Korte S, Giles JR, Weirick ME, McAllister CM, Dougherty J, Long S, D'Andrea K, Hamilton JT, Betts MR, Bates P, Hensley SE, Grifoni A, Weiskopf D, Sette A, Greenplate AR, Wherry EJ. Rapid induction of antigen-specific CD4+ T cells is associated with coordinated humoral and cellular immunity to SARS-CoV-2 mRNA vaccination. Immunity. 2021;54:2133-2142.e3. https://doi.org/10.1016/j.immuni.2021.08.001.
355. Pilkington EH, Suys EJA, Trevaskis NL, Wheatley AK, Zukancic D, Algarni A, Al-Wassiti H, Davis TP, Pouton CW, Kent SJ, Truong NP. From influenza to COVID-19: lipid nanoparticle mRNA vaccines at the frontiers of infectious diseases. Acta Biomater. 2021;131:16–40. https://doi.org/10.1016/j.actbio.2021.06.023.
356. Feldman RA, Fuhr R, Smolenov I, Ribeiro A, Panther L, Watson M, Senn JJ, Smith M, Almarsson Ö, Pujar HS, Laska ME, Thompson J, Zaks T, Ciaramella G. mRNA vaccines against H10N8 and H7N9 influenza viruses of pandemic potential are immunogenic and well tolerated in healthy adults in phase 1 randomized clinical trials. Vaccine. 2019;37:3326–34. https://doi.org/10.1016/j.vaccine.2019.04.074.
357. Pardi N, Secreto AJ, Shan X, Debonera F, Glover J, Yi Y, Muramatsu H, Ni H, Mui BL, Tam YK, Shaheen F, Collman RG, Karikó K, Danet-Desnoyers GA, Madden TD, Hope MJ, Weissman D. Administration of nucleoside-modified mRNA encoding broadly neutralizing antibody protects humanized mice from HIV-1 challenge. Nat Commun. 2017;8:14630. https://doi.org/10.1038/ncomms14630.
358. Firdessa-Fite R, Creusot RJ. Nanoparticles versus dendritic cells as vehicles to deliver mRNA encoding multiple epitopes for immunotherapy. Mol Ther Methods Clin Dev. 2020;16:50–62. https://doi.org/10.1016/j.omtm.2019.10.015.
359. Li B, Jiang AY, Raji I, Atyeo C, Raimondo TM, Gordon AGR, Rhym LH, Samad T, MacIsaac C, Witten J, Mughal H, Chicz TM, Xu Y, McNamara RP, Bhatia S, Alter G, Langer R, Anderson DG. Enhancing the immunogenicity of lipid-nanoparticle mRNA vaccines by adjuvanting the ionizable lipid and the mRNA. Nat Biomed Eng. 2023. https://doi.org/10.1038/s41551-023-01082-6.
360. Maruggi G, Mallett CP, Westerbeck JW, Chen T, Lofano G, Friedrich K, Qu L, Sun JT, McAuliffe J, Kanitkar A, Arrildt KT, Wang K-FF, McBee I, McCoy D, Terry R, Rowles A, Abrahim MA, Ringenberg MA, Gains MJ, Spickler C, Xie X, Zou J, Shi P-YY, Dutt T, Henao-Tamayo M, Ragan I, Bowen RA, Johnson R, Nuti S, Luisi K, Ulmer JB, Steff A-MM, Jalah R, Bertholet S, Stokes AH, Yu D. A self-amplifying mRNA SARS-CoV-2 vaccine candidate induces safe and robust protective immunity in preclinical models. Mol Ther. 2022;30:1897–912. https://doi.org/10.1016/j.ymthe.2022.01.001.
361. Zhang H, Han X, Alameh M, Shepherd SJ, Padilla MS, Xue L, Butowska K, Weissman D, Mitchell MJ. Rational design of anti-inflammatory lipid nanoparticles for mRNA delivery. J Biomed Mater Res Part A. 2022;110:1101–8. https://doi.org/10.1002/jbm.a.37356.
362. Vijayamahantesh V, Patra T, Meyer K, Alameh M-G, Reagan EK, Weissman D, Ray R. Modified E2 glycoprotein of hepatitis C virus enhances proinflammatory cytokines and protective immune response. J Virol. 2022. https://doi.org/10.1128/jvi.00523-22.
363. Ding F, Zhang H, Li Q, Yang C. Identification of a potent ionizable lipid for efficient macrophage transfection and systemic anti-interleukin-1β

siRNA delivery against acute liver failure. J Mater Chem B. 2021;9:5136–49. https://doi.org/10.1039/D1TB00736J.
364. Verma P, Arora A, Rana K, Mehta D, Kar R, Verma V, Srikanth CV, Patil VS, Bajaj A. Gemini lipid nanoparticle (GLNP)-mediated oral delivery of TNF-α siRNA mitigates gut inflammation via inhibiting the differentiation of CD4+ T cells. Nanoscale. 2022;14:14717–31. https://doi.org/10.1039/d1nr05644a.
365. Pu Y, Wu W, Zhou B, Xiang H, Yu J, Yin H, Zhang Y, Du D, Chen Y, Xu H. Starvation therapy enabled "switch-on" NIR-II photothermal nanoagent for synergistic in situ photothermal immunotherapy. Nano Today. 2022;44: 101461. https://doi.org/10.1016/j.nantod.2022.101461.
366. Papi M, Pozzi D, Palmieri V, Caracciolo G. Principles for optimization and validation of mRNA lipid nanoparticle vaccines against COVID-19 using 3D bioprinting. Nano Today. 2022;43: 101403. https://doi.org/10.1016/j.nantod.2022.101403.
367. Kapteijn R, Shitut S, Aschmann D, Zhang L, de Beer M, Daviran D, Roverts R, Akiva A, van Wezel GP, Kros A, Claessen D. Endocytosis-like DNA uptake by cell wall-deficient bacteria. Nat Commun. 2022;13:5524. https://doi.org/10.1038/s41467-022-33054-w.
368. Gilleron J, Querbes W, Zeigerer A, Borodovsky A, Marsico G, Schubert U, Manygoats K, Seifert S, Andree C, Stöter M, Epstein-Barash H, Zhang L, Koteliansky V, Fitzgerald K, Fava E, Bickle M, Kalaidzidis Y, Akinc A, Maier M, Zerial M. Image-based analysis of lipid nanoparticle-mediated siRNA delivery, intracellular trafficking and endosomal escape. Nat Biotechnol. 2013;31:638–46. https://doi.org/10.1038/nbt.2612.
369. Ho TC, Kim HS, Chen Y, Li Y, LaMere MW, Chen C, Wang H, Gong J, Palumbo CD, Ashton JM, Kim HW, Xu Q, Becker MW, Leong KW. Scaffold-mediated CRISPR–cas9 delivery system for acute myeloid leukemia therapy. Sci Adv. 2021. https://doi.org/10.1126/sciadv.abg3217.
370. Kim MW, Jeong HY, Kang SJ, Choi MJ, You YM, Im CS, Lee TS, Song IH, Lee CG, Rhee K-J, Lee YK, Park YS. Cancer-targeted nucleic acid delivery and quantum dot imaging using EGF receptor aptamer-conjugated lipid nanoparticles. Sci Rep. 2017;7:9474. https://doi.org/10.1038/s41598-017-09555-w.
371. Yang T, Han H, Chen Y, Yang L, Parker R, Li Y, Kaplan DL, Xu Q. Study the lipidoid nanoparticle mediated genome editing protein delivery using 3D intestinal tissue model. Bioact Mater. 2021;6:3671–7. https://doi.org/10.1016/j.bioactmat.2021.03.027.
372. Chen Y, Chen W, Ren Y, Li S, Liu M, Xing J, Han Y, Chen Y, Tao R, Guo L, Sui X, Guo Q, Liu S, Han Y. Lipid nanoparticle-encapsulated VEGFa siRNA facilitates cartilage formation by suppressing angiogenesis. Int J Biol Macromol. 2022;221:1313–24. https://doi.org/10.1016/j.ijbiomac.2022.09.065.
373. Yalgın A, Köse FA, Gökçe EH. The effect of cyclosporine A and co-enzyme Q10 loaded solid lipid nanoparticles on 3D printed human auricular model: evaluation of cell growth. J Drug Deliv Sci Technol. 2023;79: 104087. https://doi.org/10.1016/j.jddst.2022.104087.
374. Frazier KS. Antisense oligonucleotide therapies: the promise and the challenges from a toxicologic pathologist's perspective. Toxicol Pathol. 2015;43:78–89. https://doi.org/10.1177/0192623314551840.
375. Nidetz NF, McGee MC, Tse LV, Li C, Cong L, Li Y, Huang W. Adeno-associated viral vector-mediated immune responses: understanding barriers to gene delivery. Pharmacol Ther. 2020;207: 107453. https://doi.org/10.1016/j.pharmthera.2019.107453.
376. Wang Z, Troilo PJ, Wang X, Griffiths TG, Pacchione SJ, Barnum AB, Harper LB, Pauley CJ, Niu Z, Denisova L, Follmer TT, Rizzuto G, Ciliberto G, Fattori E, Monica NL, Manam S, Ledwith BJ. Detection of integration of plasmid DNA into host genomic DNA following intramuscular injection and electroporation. Gene Ther. 2004;11:711–21. https://doi.org/10.1038/sj.gt.3302213.
377. Yoshida T, Oide N, Sakamoto T, Yotsumoto S, Negishi Y, Tsuchiya S, Aramaki Y. Induction of cancer cell-specific apoptosis by folate-labeled cationic liposomes. J Control Release. 2006;111:325–32. https://doi.org/10.1016/J.JCONREL.2005.12.016.
378. Man SM, Karki R, Kanneganti T. AIM2 inflammasome in infection, cancer, and autoimmunity: role in DNA sensing, inflammation, and innate immunity. Eur J Immunol. 2016;46:269–80. https://doi.org/10.1002/eji.201545839.
379. Litzinger DC, Brown JM, Wala I, Kaufman SA, Han GY, Farrell CL, Collins D. Fate of cationic liposomes and their complex with oligonucleotive in vivo. Biochim Biophys Acta Biomembr. 1996;1281:139–49. https://doi.org/10.1016/0005-2736(95)00268-5.
380. Yoshikawa N, Fumoto S, Nakashima M, Shimokawa K, Miyamoto H, Nishida K. The role of fibronectin in pulmonary gene transfer following intravenous administration of lipoplex in mice. Biol Pharm Bull. 2013;36:1807–13. https://doi.org/10.1248/bpb.b13-00500.
381. Crooke ST. Molecular mechanisms of antisense oligonucleotides. Nucleic Acid Ther. 2017;27:70–7. https://doi.org/10.1089/nat.2016.0656.
382. Parhiz H, Brenner JS, Patel PN, Papp TE, Shahnawaz H, Li Q, Shi R, Zamora ME, Yadegari A, Marcos-Contreras OA, Natesan A, Pardi N, Shuvaev VV, Kiseleva R, Myerson JW, Uhler T, Riley RS, Han X, Mitchell MJ, Lam K, Heyes J, Weissman D, Muzykantov VR. Added to pre-existing inflammation, mRNA-lipid nanoparticles induce inflammation exacerbation (IE). J Control Release. 2022;344:50–61. https://doi.org/10.1016/j.jconrel.2021.12.027.
383. Bitounis D, Jacquinet E, Rogers MA, Amiji MM. Strategies to reduce the risks of mRNA drug and vaccine toxicity. Nat Rev Drug Discov. 2024;23(4):281–300. https://doi.org/10.1038/s41573-023-00859-3.
384. Ishida T, Kiwada H. Accelerated blood clearance (ABC) phenomenon upon repeated injection of PEGylated liposomes. Int J Pharm. 2008;354:56–62. https://doi.org/10.1016/J.IJPHARM.2007.11.005.
385. Yadati T, Houben T, Bitorina A, Shiri-Sverdlov R. The ins and outs of cathepsins: physiological function and role in disease management. Cells. 2020. https://doi.org/10.3390/CELLS9071679.
386. Xie Z, Zhao M, Yan C, Kong W, Lan F, Narengaowa, Zhao S, Yang Q, Bai Z, Qing H, Ni J. Cathepsin B in programmed cell death machinery: mechanisms of execution and regulatory pathways. Cell Death Dis. 2023;14:1–18. https://doi.org/10.1038/s41419-023-05786-0.
387. Bae SH, Yoo S, Lee J, Park HJ, Kwon SP, Jin H, Park SI, Lee YS, Bang YJ, Roh G, Lee S, Youn SB, Kim IW, Oh HR, El-Damasy AK, Keum G, Kim H, Youn H, Nam JH, Bang EK. A lipid nanoparticle platform incorporating trehalose glycolipid for exceptional mRNA vaccine safety. Bioact Mater. 2024;38:486–98. https://doi.org/10.1016/J.BIOACTMAT.2024.05.012.
388. Baden LR, El Sahly HM, Essink B, Kotloff K, Frey S, Novak R, Diemert D, Spector SA, Rouphael N, Creech CB, McGettigan J, Khetan S, Segall N, Solis J, Brosz A, Fierro C, Schwartz H, Neuzil K, Corey L, Gilbert P, Janes H, Follmann D, Marovich M, Mascola J, Polakowski L, Ledgerwood J, Graham BS, Bennett H, Pajon R, Knightly C, Leav B, Deng W, Zhou H, Han S, Ivarsson M, Miller J, Zaks T. Efficacy and safety of the mRNA-1273 SARS-CoV-2 vaccine. N Engl J Med. 2021;384:403–16. https://doi.org/10.1056/nejmoa2035389.
389. Korzun T, Moses AS, Diba P, Sattler AL, Taratula OR, Sahay G, Taratula O, Marks DL. From bench to bedside: implications of lipid nanoparticle carrier reactogenicity for advancing nucleic acid therapeutics. Pharmaceuticals. 2023;16:1088. https://doi.org/10.3390/PH16081088.
390. Graydon EK, Conner TL, Dunham K, Olsen C, Goguet E, Coggins SA, Rekedal M, Samuels E, Jackson-Thompson B, Moser M, Lindrose A, Hollis-Perry M, Wang G, Maiolatesi S, Alcorta Y, Reyes A, Wong M, Ramsey K, Davies J, Parmelee E, Ortega O, Sanchez M, Moller S, Inglefield J, Tribble D, Burgess T, O'Connell R, Malloy AMW, Pollett S, Broder CC, Laing ED, Anderson SK, Mitre E. Natural killer cells and BNT162b2 mRNA vaccine reactogenicity and durability. Front Immunol. 2023. https://doi.org/10.3389/FIMMU.2023.1225025.
391. Abbasi S, Matsui-Masai M, Yasui F, Hayashi A, Tockary TA, Mochida Y, Akinaga S, Kohara M, Kataoka K, Uchida S. Carrier-free mRNA vaccine induces robust immunity against SARS-CoV-2 in mice and non-human primates without systemic reactogenicity. Mol Ther. 2024;32:1266–83. https://doi.org/10.1016/J.YMTHE.2024.03.022.
392. Wang C, Zhao C, Wang W, Liu X, Deng H. Biomimetic noncationic lipid nanoparticles for mRNA delivery. Proc Natl Acad Sci USA. 2023;120: e2311276120. https://doi.org/10.1073/pnas.2311276120.
393. Kokkinopoulos I, Jordan WJ, Ritter MA. Toll-like receptor mRNA expression patterns in human dendritic cells and monocytes. Mol Immunol. 2005;42:957–68. https://doi.org/10.1016/J.MOLIMM.2004.09.037.
394. Chen L, Deng H, Cui H, Fang J, Zuo Z, Deng J, Li Y, Wang X, Zhao L, Chen L, Deng H, Cui H, Fang J, Zuo Z, Deng J, Li Y, Wang X, Zhao L. Inflammatory responses and inflammation-associated diseases in organs. Oncotarget. 2017;9:7204–18. https://doi.org/10.18632/ONCOTARGET.23208.
395. Han X, Alameh MG, Butowska K, Knox JJ, Lundgreen K, Ghattas M, Gong N, Xue L, Xu Y, Lavertu M, Bates P, Xu J, Nie G, Zhong Y, Weissman D,

Mitchell MJ. Adjuvant lipid-substituted lipid nanoparticles augment the immunogenicity of SARS-CoV-2 mRNA vaccines. Nat Nanotechnol. 2023;18(9):1105–14. https://doi.org/10.1038/s41565-023-01404-4.
396. Lee Y, Jeong M, Park J, Jung H, Lee H. Immunogenicity of lipid nanoparticles and its impact on the efficacy of mRNA vaccines and therapeutics. Exp Mol Med. 2023;55(10):2085–96. https://doi.org/10.1038/s12276-023-01086-x.
397. Xu H, Wang KQ, Deng YH, Chen DW. Effects of cleavable PEG-cholesterol derivatives on the accelerated blood clearance of PEGylated liposomes. Biomaterials. 2010;31:4757–63. https://doi.org/10.1016/J.BIOMATERIALS.2010.02.049.
398. Hassett KJ, Higgins J, Woods A, Levy B, Xia Y, Hsiao CJ, Acosta E, Almarsson Ö, Moore MJ, Brito LA, Ma J, Li M, Qi Z, Fu M, Sun T, Elsheikha HM, Cong W. Impact of lipid nanoparticle size on mRNA vaccine immunogenicity. J Control Release. 2021;335: 150562. https://doi.org/10.1016/j.jconrel.2021.05.021.
399. Chen S, Zaifman J, Kulkarni JA, Zhigaltsev IV, Tam YK, Ciufolini MA, Tam YYC, Cullis PR. Dexamethasone prodrugs as potent suppressors of the immunostimulatory effects of lipid nanoparticle formulations of nucleic acids. J Control Release. 2018;286:46–54. https://doi.org/10.1016/J.JCONREL.2018.07.026.
400. Anderluzzi G, Lou G, Woods S, Schmidt ST, Gallorini S, Brazzoli M, Johnson R, Roberts CW, O'Hagan DT, Baudner BC, Perrie Y. The role of nanoparticle format and route of administration on self-amplifying mRNA vaccine potency. J Control Release. 2022;342:388–99. https://doi.org/10.1016/J.JCONREL.2021.12.008.
401. Jeong M, Lee Y, Park J, Jung H, Lee H. Lipid nanoparticles (LNPs) for in vivo RNA delivery and their breakthrough technology for future applications. Adv Drug Deliv Rev. 2023;200: 114990. https://doi.org/10.1016/j.addr.2023.114990.
402. Zhang Z, Ohto U, Shibata T, Krayukhina E, Taoka M, Yamauchi Y, Tanji H, Isobe T, Uchiyama S, Miyake K, Shimizu T. Structural analysis reveals that toll-like receptor 7 is a dual receptor for guanosine and single-stranded RNA. Immunity. 2016;45:737–48. https://doi.org/10.1016/j.immuni.2016.09.011.
403. Alexopoulou L, Holt AC, Medzhitov R, Flavell RA. Recognition of double-stranded RNA and activation of NF-κB by Toll-like receptor 3. Nature. 2001;413:732–8. https://doi.org/10.1038/35099560.
404. Abbas YM, Laudenbach BT, Martínez-Montero S, Cencic R, Habjan M, Pichlmair A, Damha MJ, Pelletier J, Nagar B. Structure of human IFIT1 with capped RNA reveals adaptable mRNA binding and mechanisms for sensing N1 and N2 ribose 2′-*O* methylations. Proc Natl Acad Sci USA. 2017;114:E2106–15. https://doi.org/10.1073/pnas.1612444114.
405. Karikó K, Muramatsu H, Welsh FA, Ludwig J, Kato H, Akira S, Weissman D. Incorporation of pseudouridine into mRNA yields superior nonimmunogenic vector with increased translational capacity and biological stability. Mol Ther. 2008;16:1833–40. https://doi.org/10.1038/mt.2008.200.
406. Tanji H, Ohto U, Shibata T, Taoka M, Yamauchi Y, Isobe T, Miyake K, Shimizu T. Toll-like receptor 8 senses degradation products of single-stranded RNA. Nat Struct Mol Biol. 2015;22:109–15. https://doi.org/10.1038/nsmb.2943.
407. Mu X, Greenwald E, Ahmad S, Hur S. An origin of the immunogenicity of in vitro transcribed RNA. Nucleic Acids Res. 2018;46:5239–49. https://doi.org/10.1093/nar/gky177.
408. Karikó K, Muramatsu H, Ludwig J, Weissman D. Generating the optimal mRNA for therapy: HPLC purification eliminates immune activation and improves translation of nucleoside-modified, protein-encoding mRNA. Nucleic Acids Res. 2011;39:e142–e142. https://doi.org/10.1093/nar/gkr695.
409. Baiersdörfer M, Boros G, Muramatsu H, Mahiny A, Vlatkovic I, Sahin U, Karikó K. A facile method for the removal of dsRNA contaminant from in vitro-transcribed mRNA. Mol Ther Nucleic Acids. 2019;15:26–35. https://doi.org/10.1016/j.omtn.2019.02.018.
410. Wu MZ, Asahara H, Tzertzinis G, Roy B. Synthesis of low immunogenicity RNA with high-temperature in vitro transcription. RNA. 2020;26:345–60. https://doi.org/10.1261/rna.073858.119.
411. Devarkar SC, Wang C, Miller MT, Ramanathan A, Jiang F, Khan AG, Patel SS, Marcotrigiano J. Structural basis for m7G recognition and 2′-*O*-methyl discrimination in capped RNAs by the innate immune receptor RIG-I. Proc Natl Acad Sci USA. 2016;113:596–601. https://doi.org/10.1073/pnas.1515152113.
412. Ndeupen S, Bouteau A, Herbst C, Qin Z, Jacobsen S, Powers NE, Hutchins Z, Kurup D, Diba LZ, Watson M, Ramage H, Igyártó BZ. Langerhans cells and cDC1s play redundant roles in mRNA-LNP induced protective anti-influenza and anti-SARS-CoV-2 immune responses. PLOS Pathog. 2022;18: e1010255. https://doi.org/10.1371/journal.ppat.1010255.
413. Anand PK. Lipids, inflammasomes, metabolism, and disease. Immunol Rev. 2020;297:108–22. https://doi.org/10.1111/imr.12891.
414. Alameh M-G, Weissman D. Nucleoside modifications of in vitro transcribed mRNA to reduce immunogenicity and improve translation of prophylactic and therapeutic antigens. In: RNA Therapeutics. London: Elsevier; 2022. p. 141–69. https://doi.org/10.1016/B978-0-12-821595-1.00014-2.
415. Packer M, Gyawali D, Yerabolu R, Schariter J, White P. A novel mechanism for the loss of mRNA activity in lipid nanoparticle delivery systems. Nat Commun. 2021;12:6777. https://doi.org/10.1038/s41467-021-26926-0.
416. Weismann D, Binder CJ. The innate immune response to products of phospholipid peroxidation. Biochim Biophys Acta Biomembr. 1818;2012:2465–75. https://doi.org/10.1016/j.bbamem.2012.01.018.
417. Li C, Lee A, Grigoryan L, Arunachalam PS, Scott MKD, Trisal M, Wimmers F, Sanyal M, Weidenbacher PA, Feng Y, Adamska JZ, Valore E, Wang Y, Verma R, Reis N, Dunham D, O'Hara R, Park H, Luo W, Gitlin AD, Kim P, Khatri P, Nadeau KC, Pulendran B. Mechanisms of innate and adaptive immunity to the Pfizer-BioNTech BNT162b2 vaccine. Nat Immunol. 2022;23(4):543–55. https://doi.org/10.1038/s41590-022-01163-9.
418. Bianca C, Brézin L. Modeling the antigen recognition by B-cell and T-cell receptors through thermostatted kinetic theory methods. Int J Biomath. 2017;10:1750072. https://doi.org/10.1142/S1793524517500723.
419. Verbeke R, Hogan MJ, Loré K, Pardi N. Innate immune mechanisms of mRNA vaccines. Immunity. 2022;55:1993–2005. https://doi.org/10.1016/j.immuni.2022.10.014.
420. Neagu M, Piperigkou Z, Karamanou K, Engin AB, Docea AO, Constantin C, Negrei C, Nikitovic D, Tsatsakis A. Protein bio-corona: critical issue in immune nanotoxicology. Arch Toxicol. 2017;91:1031–48. https://doi.org/10.1007/s00204-016-1797-5.
421. Lonez C, Bessodes M, Scherman D, Vandenbranden M, Escriou V, Ruysschaert J-M. Cationic lipid nanocarriers activate Toll-like receptor 2 and NLRP3 inflammasome pathways. Nanomed Nanotechnol Biol Med. 2014;10:775–82. https://doi.org/10.1016/j.nano.2013.12.003.
422. Billingsley MM, Singh N, Ravikumar P, Zhang R, June CH, Mitchell MJ. Ionizable lipid nanoparticle-mediated mRNA delivery for human CAR T cell engineering. Nano Lett. 2020;20:1578–89. https://doi.org/10.1021/acs.nanolett.9b04246.
423. O'Donnell VB, Rossjohn J, Wakelam MJO. Phospholipid signaling in innate immune cells. J Clin Invest. 2018;128:2670–9. https://doi.org/10.1172/JCI97944.
424. Hatziantoniou S, Maltezou HC, Tsakris A, Poland GA, Anastassopoulou C. Anaphylactic reactions to mRNA COVID-19 vaccines: a call for further study. Vaccine. 2021;39:2605–7. https://doi.org/10.1016/j.vaccine.2021.03.073.
425. Szebeni J, Storm G, Ljubimova JY, Castells M, Phillips EJ, Turjeman K, Barenholz Y, Crommelin DJA, Dobrovolskaia MA. Applying lessons learned from nanomedicines to understand rare hypersensitivity reactions to mRNA-based SARS-CoV-2 vaccines. Nat Nanotechnol. 2022;17:337–46. https://doi.org/10.1038/s41565-022-01071-x.
426. Alameh M-G, Tombácz I, Bettini E, Lederer K, Ndeupen S, Sittplangkoon C, Wilmore JR, Gaudette BT, Soliman OY, Pine M, Hicks P, Manzoni TB, Knox JJ, Johnson JL, Laczkó D, Muramatsu H, Davis B, Meng W, Rosenfeld AM, Strohmeier S, Lin PJC, Mui BL, Tam YK, Karikó K, Jacquet A, Krammer F, Bates P, Cancro MP, Weissman D, Luning Prak ET, Allman D, Igyártó BZ, Locci M, Pardi N. Lipid nanoparticles enhance the efficacy of mRNA and protein subunit vaccines by inducing robust T follicular helper cell and humoral responses. Immunity. 2021;54:2877–28927. https://doi.org/10.1016/j.immuni.2021.11.001.
427. Agallou M, Margaroni M, Tsanaktsidou E, Badounas F, Kammona O, Kiparissides C, Karagouni E. A liposomal vaccine promotes strong adaptive immune responses via dendritic cell activation in draining lymph

nodes. J Control Release. 2023;356:386–401. https://doi.org/10.1016/j.jconrel.2023.03.006.
428. Pham CTN, Mitchell LM, Huang JL, Lubniewski CM, Schall OF, Killgore JK, Pan D, Wickline SA, Lanza GM, Hourcade DE. Variable antibody-dependent activation of complement by functionalized phospholipid nanoparticle surfaces. J Biol Chem. 2011;286:123–30. https://doi.org/10.1074/jbc.M110.180760.
429. Syenina A, Gan ES, Toh JZN, de Alwis R, Lin LZ, Tham CYL, Yee JX, Leong YS, Sam H, Cheong C, Teh YE, Wee ILE, Ng DHL, Chan KR, Sim JXY, Kalimuddin S, Ong EZ, Low JG, Ooi EE. Adverse effects following anti-COVID-19 vaccination with mRNA-based BNT162b2 are alleviated by altering the route of administration and correlate with baseline enrichment of T and NK cell genes. PLOS Biol. 2022;20: e3001643. https://doi.org/10.1371/journal.pbio.3001643.
430. Rosenbaum P, Tchitchek N, Joly C, RodriguezPozo A, Stimmer L, Langlois S, Hocini H, Gosse L, Pejoski D, Cosma A, Beignon A-S, Dereuddre-Bosquet N, Levy Y, Le Grand R, Martinon F. Vaccine inoculation route modulates early immunity and consequently antigen-specific immune response. Front Immunol. 2021;12: 645210. https://doi.org/10.3389/fimmu.2021.645210.
431. Liu F, Song YK, Liu D. Hydrodynamics-based transfection in animals by systemic administration of plasmid DNA. Gene Ther. 1999;6:1258–66. https://doi.org/10.1038/sj.gt.3300947.
432. Wolff JA, Malone RW, Williams P, Chong W, Acsadi G, Jani A, Felgner PL. Direct gene transfer into mouse muscle in vivo. Science (80-). 1990;247:1465–8. https://doi.org/10.1126/science.1690918.
433. Un K, Kawakami S, Higuchi Y, Suzuki R, Maruyama K, Yamashita F, Hashida M. Involvement of activated transcriptional process in efficient gene transfection using unmodified and mannose-modified bubble lipoplexes with ultrasound exposure. J Control Release. 2011;156:355–63. https://doi.org/10.1016/j.jconrel.2011.06.040.
434. Beck JD, Reidenbach D, Salomon N, Sahin U, Türeci Ö, Vormehr M, Kranz LM. mRNA therapeutics in cancer immunotherapy. Mol Cancer. 2021;20:69. https://doi.org/10.1186/s12943-021-01348-0.
435. Anderson EJ, Rouphael NG, Widge AT, Jackson LA, Roberts PC, Makhene M, Chappell JD, Denison MR, Stevens LJ, Pruijssers AJ, McDermott AB, Flach B, Lin BC, Doria-Rose NA, O'Dell S, Schmidt SD, Corbett KS, Swanson PA, Padilla M, Neuzil KM, Bennett H, Leav B, Makowski M, Albert J, Cross K, Edara VV, Floyd K, Suthar MS, Martinez DR, Baric R, Buchanan W, Luke CJ, Phadke VK, Rostad CA, Ledgerwood JE, Graham BS, Beigel JH. Safety and immunogenicity of SARS-CoV-2 mRNA-1273 vaccine in older adults. N Engl J Med. 2020;383:2427–38. https://doi.org/10.1056/NEJMoa2028436.
436. Allen JC, Toapanta FR, Chen W, Tennant SM. Understanding immunosenescence and its impact on vaccination of older adults. Vaccine. 2020;38:8264–72. https://doi.org/10.1016/j.vaccine.2020.11.002.
437. Saadati R, Dadashzadeh S, Abbasian Z, Soleimanjahi H. Accelerated blood clearance of PEGylated PLGA nanoparticles following repeated injections: effects of polymer dose, PEG coating, and encapsulated anticancer drug. Pharm Res. 2013;30:985–95. https://doi.org/10.1007/s11095-012-0934-y.
438. Ibrahim M, Ramadan E, Elsadek NE, Emam SE, Shimizu T, Ando H, Ishima Y, Elgarhy OH, Sarhan HA, Hussein AK, Ishida T. Polyethylene glycol (PEG): the nature, immunogenicity, and role in the hypersensitivity of PEGylated products. J Control Release. 2022;351:215–30. https://doi.org/10.1016/j.jconrel.2022.09.031.
439. Kohli AG, Kierstead PH, Venditto VJ, Walsh CL, Szoka FC. Designer lipids for drug delivery: from heads to tails. J Control Release. 2014;190:274–87. https://doi.org/10.1016/j.jconrel.2014.04.047.
440. Dézsi L, Fülöp T, Mészáros T, Szénási G, Urbanics R, Vázsonyi C, Őrfi E, Rosivall L, Nemes R, Kok RJ, Metselaar JM, Storm G, Szebeni J. Features of complement activation-related pseudoallergy to liposomes with different surface charge and PEGylation: comparison of the porcine and rat responses. J Control Release. 2014;195:2–10. https://doi.org/10.1016/j.jconrel.2014.08.009.
441. McSweeney MD, Mohan M, Commins SP, Lai SK. Anaphylaxis to Pfizer/BioNTech mRNA COVID-19 vaccine in a patient with clinically confirmed PEG allergy. Front Allergy. 2021;2: 715844. https://doi.org/10.3389/falgy.2021.715844.
442. Ishida T, Wang X, Shimizu T, Nawata K, Kiwada H. PEGylated liposomes elicit an anti-PEG IgM response in a T cell-independent manner. J Control Release. 2007;122:349–55. https://doi.org/10.1016/j.jconrel.2007.05.015.
443. Merle NS, Noe R, Halbwachs-Mecarelli L, Fremeaux-Bacchi V, Roumenina LT. Complement system part II: role in immunity. Front Immunol. 2015;6: 136998. https://doi.org/10.3389/fimmu.2015.00257.
444. Suk JS, Xu Q, Kim N, Hanes J, Ensign LM. PEGylation as a strategy for improving nanoparticle-based drug and gene delivery. Adv Drug Deliv Rev. 2016;99:28–51. https://doi.org/10.1016/j.addr.2015.09.012.
445. Mohamed M, Abu Lila AS, Shimizu T, Alaaeldin E, Hussein A, Sarhan HA, Szebeni J, Ishida T. PEGylated liposomes: immunological responses. Sci Technol Adv Mater. 2019;20:710–24. https://doi.org/10.1080/14686996.2019.1627174.
446. Hoang Thi TT, Pilkington EH, Nguyen DH, Lee JS, Park KD, Truong NP. The importance of poly(ethylene glycol) alternatives for overcoming peg immunogenicity in drug delivery and bioconjugation. Polymers. 2020;12:298. https://doi.org/10.3390/polym12020298.
447. Liu M, Li J, Zhao D, Yan N, Zhang H, Liu M, Tang X, Hu Y, Ding J, Zhang N, Liu X, Deng Y, Song Y, Zhao X. Branched PEG-modification: a new strategy for nanocarriers to evade of the accelerated blood clearance phenomenon and enhance anti-tumor efficacy. Biomaterials. 2022;283: 121415. https://doi.org/10.1016/j.biomaterials.2022.121415.
448. Suzuki T, Suzuki Y, Hihara T, Kubara K, Kondo K, Hyodo K, Yamazaki K, Ishida T, Ishihara H. PEG shedding-rate-dependent blood clearance of PEGylated lipid nanoparticles in mice: faster PEG shedding attenuates anti-PEG IgM production. Int J Pharm. 2020;588: 119792. https://doi.org/10.1016/j.ijpharm.2020.119792.
449. Ishihara T, Maeda T, Sakamoto H, Takasaki N, Shigyo M, Ishida T, Kiwada H, Mizushima Y, Mizushima T. Evasion of the accelerated blood clearance phenomenon by coating of nanoparticles with various hydrophilic polymers. Biomacromolecules. 2010;11:2700–6. https://doi.org/10.1021/bm100754e.
450. AbuLila AS, Uehara Y, Ishida T, Kiwada H. Application of polyglycerol coating to plasmid DNA Lipoplex for the evasion of the accelerated blood clearance phenomenon in nucleic acid delivery. J Pharm Sci. 2014;103:557–66. https://doi.org/10.1002/jps.23823.
451. Kang DD, Hou X, Wang L, Xue Y, Li H, Zhong Y, Wang S, Deng B, McComb DW, Dong Y. Engineering LNPs with polysarcosine lipids for mRNA delivery. Bioact Mater. 2024;37:86–93. https://doi.org/10.1016/j.bioactmat.2024.03.017.
452. Dhayalan M, Wang W, Riyaz SUM, Dinesh RA, Shanmugam J, Irudayaraj SS, Stalin A, Giri J, Mallik S, Hu R. Advances in functional lipid nanoparticles: from drug delivery platforms to clinical applications. 3 Biotech. 2024;14:1–28. https://doi.org/10.1007/s13205-023-03901-8.
453. Thi TTH, Suys EJA, Lee JS, Nguyen DH, Park KD, Truong NP. Lipid-based nanoparticles in the clinic and clinical trials: from cancer nanomedicine to COVID-19 vaccines. Vaccines. 2021;9:359. https://doi.org/10.3390/VACCINES9040359.
454. Liu Y, Huang Y, He G, Guo C, Dong J, Wu L. Development of mRNA lipid nanoparticles: targeting and therapeutic aspects. Int J Mol Sci. 2024;25:10166. https://doi.org/10.3390/ijms251810166.
455. Wang J, Ding Y, Chong K, Cui M, Cao Z, Tang C, Tian Z, Hu Y, Zhao Y, Jiang S. Recent advances in lipid nanoparticles and their safety concerns for mRNA delivery. Vaccines. 2024;2024(12):1148. https://doi.org/10.3390/VACCINES12101148.
456. Waheed I, Ali A, Tabassum H, Khatoon N, Lai WF, Zhou X. Lipid-based nanoparticles as drug delivery carriers for cancer therapy. Front Oncol. 2024;14:1296091. https://doi.org/10.3389/fonc.2024.1296091.

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