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Review

Lipid Nanoparticle-Based Delivery System—A Competing Place for mRNA Vaccines

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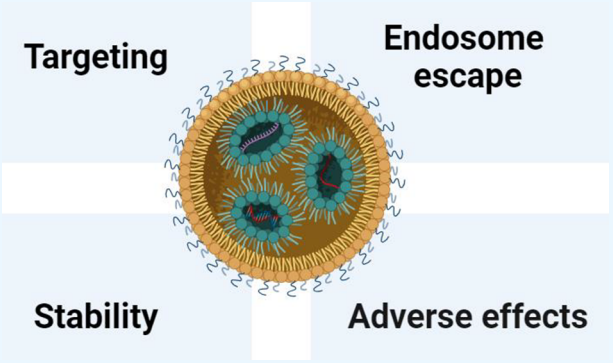
ABSTRACT: mRNA, as one of the foci of biomedical research in the past decade, has become a candidate vaccine solution for various infectious diseases and tumors and for regenerative medicine and immunotherapy due to its high efficiency, safety, and effectiveness. A stable and effective delivery system is needed to protect mRNAs from nuclease degradation while also enhancing immunogenicity. The success of mRNA lipid nanoparticles in treating COVID-19, to a certain extent, marks a milestone for mRNA vaccines and also promotes further research on mRNA delivery systems. Here, we explore mRNA vaccine delivery systems, especially lipid nanoparticles (LNPs), considering the current research status, prospects, and challenges of lipid nanoparticles, and explore other mRNA delivery systems.



1. INTRODUCTION

When mRNA is used for vaccines, it can encode target antigens, which are usually produced by DNA transcription in vitro[1] and subsequently inoculated into the body. mRNA is translated into an antigenic protein in the body, triggering an adaptive immune response.[2] The whole process does not involve cell culture, so it is simple, fast and attractive and is a candidate form of vaccine suitable for responding to outbreaks of new infectious diseases, such as COVID-19.[3] Researchers' efforts in mRNA purification, sequence optimization and nucleoside chemistry have endowed mRNAs with stability and strong immunogenicity.[4−7] Research based on mRNA itself has paved the way for its application. Although the optimization and modification of mRNAs are key to their successful translation into antigenic proteins, we must admit that the delivery system makes the same important contribution to the performance of mRNA vaccines. Naked mRNA is easily degraded in systemic circulation, whether it is modified, and the degradation products are small enough to be excreted by the kidney. These degraded small molecules are not ingested or displayed by antigen-presenting cells, nor will they trigger an adaptive immune response through the immune organs. In the past decade, a large number of new nucleic acid nanoparticle delivery systems have emerged[8−11] that aim to encapsulate mRNAs effectively to protect them from degradation by serum nucleases and promote endocytosis and endosome escape to successfully trigger the immune response. Among them, LNPs have become a highly concerning delivery system because of their efficient delivery characteristics.

In the 1960s, the first generation of LNPs, which were called liposomes, appeared.[12] They are produced in water and have a closed phospholipid bilayer.[13] Lipids can enhance the water solubility of drugs; therefore, they are considered to have potential as drug delivery systems.[12] In recent years, nanotechnology has been developed, and the function of nanoliposomes has further developed to realize simple targeting or other simple functions.[14] LNPs developed for mRNA delivery in recent years consist of ionizable cationic lipids, phospholipids, cholesterol, polyethylene glycol (PEG) lipids, and mRNAs (Figure 1). LNPs have been studied as drug delivery systems that encapsulate small molecules, nucleic acids, small interfering RNAs (siRNAs) and mRNAs.[15−20] Ionizable cationic lipids contain ionizable amine groups, self-assembled hydrophobic tails, and connectors. After entering the body, the peptide is ionized in an acidic environment, which helps to form a hexagonal phase, facilitating the escape of mRNA from degradation in the body and release into the cytoplasm.[21−24] The choice of PEG lipid depends on the molar mass and length of the PEG, which can affect the overall outcome, including targeted delivery and cell uptake efficiency.[25,26] Auxiliary lipids and cholesterol also contribute to the formation

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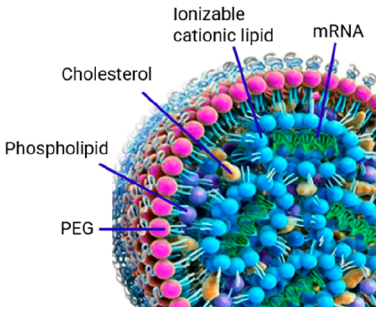

Figure 1. Composition of the mRNA-LNPs. mRNA: core active ingredient; ionizable cationic lipids: electrostatically adsorbed nucleic acids and encapsulated nucleic acids; phospholipids: accelerated structural transformation of LNPs in cells; cholesterol: stabilized structure of LNPs such that LNPs can enter cells via low-density lipoprotein (LDL); PEG: improved LNP stability, prolonged circulation time in vivo, and, to a certain extent, reduced liver enrichment.

of LNPs, which contributes to the fluidity (or rigidity) of LNPs.[27] In addition, the presence of the cold-terol complex affects the delivery and distribution of LNPs, and specific modifications enhance the targeting of certain cell types.[28,29]

The success of mRNA in treating COVID-19 has laid the foundation for its application in other places and established the confidence of researchers. The authors believe that the next successful use of mRNA vaccines is to be expected because LNPs are widely used in nucleic acid delivery research and clinical applications, and nearly 80 kinds of nucleic acid drugs based on LNPs have entered the clinical stage.[30] Although some clinical data are still available after approval and emergency use authorization (EUA) are obtained from the U.S. Food and Drug Administration (FDA), LNPs still have many problems that cannot be bypassed. There are several obstacles inside and outside the cells that prevent the application of mRNA LNPs within a wide range. Liver enrichment is a common phenomenon in the mouths of LNP patients. LNPs require some effort to pass through the liver system, and nanoparticles must first be isolated from Kupffer cells (which are macrophages resident in the liver). Kupffer cells in the liver can absorb LNPs of different sizes. In addition, LNPs also interact with hepatic sinusoidal endothelial cells (LSECs) to prevent LNPs from passing through the liver system (Figure 2).[31] A total of 30−90% of these drugs are concentrated in the liver, which leads to drug deviation during nonliver-targeted administration,[32] thus affecting the therapeutic efficacy. In addition, even for LNPs approved by the FDA for sale, only 1−4% of nucleic acids have the opportunity to escape from endosomes and reach the cytoplasm,[33] which is the main barrier for LNPs to play their role after entering cells. These limitations are the main obstacles to organ and cell targeting of mRNA vaccines. Although mRNA vaccines can induce an immune response by targeting antigen proteins, more in-depth research and additional attention need to be given to the stability and toxicity of these agents. This review discusses the research status of mRNA vaccine delivery systems.

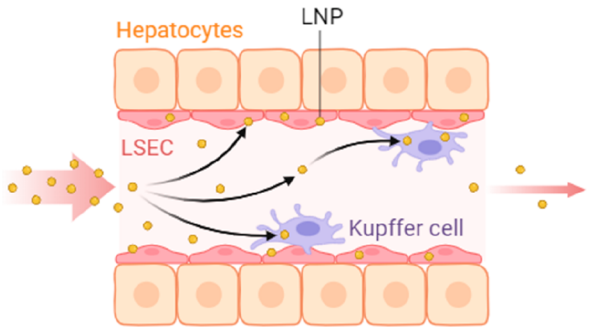

Figure 2. mRNA-LNPs are taken up by Kupffer cells with LSECs as they pass through the hepatic tract, leading to unwanted hepatic enrichment.

2. MRNA VACCINES

2.1. Mechanisms of Action of mRNA Vaccines. When the concept of mRNA vaccines was introduced, researchers first attempted to use in vitro transcribed (IVT) mRNA as a protective vaccine.[34,35] However, bare IVT mRNA is not sufficient to drive full protective immunity in most cases,[36] and although complexation of mRNA with fish sperm proteins effectively enhances the immunostimulatory potential of nucleic acids, it is still associated with the risk of reducing its translatability, thus reducing the preventive effect of the vaccine.[37] Another challenge for mRNA vaccines is the inability to exercise the correct function in dendritic cells (DCs), which may require special means to direct DC activation.[37] In addition, the poor in vivo stability of mRNAs, their susceptibility to degradation by nucleases, and their high molecular weight (MW) and high negative charge, which prevent them from entering cells by passive diffusion, are also issues that need to be addressed. LNPs, commonly used nonviral vectors for delivering mRNA vaccines, show great potential for protecting mRNAs from nuclease degradation and facilitating antigen-presenting cell (APC) uptake as well as for carrying their own adjuvant effect to enhance the immunogenicity of endogenous proteins encoded by mRNAs.

The mRNA vaccine relies on the interaction of LNPs with the cell membrane to molecularly deliver the mRNA encoding the target antigen to the cytoplasm of inoculated individual cells, where the mRNA can be translated into endogenous immunogenic proteins to elicit an immune response (Figure 3A). When a pathogen invades, the host can then rely on its immune memory to respond rapidly to clear the antigen. mRNA-LNPs enter the cell through endocytosis, where they can form endosomes that enter the cytoplasm. The acidic environment inside the endosome causes the headgroup of ionizable cationic lipids, one of the components of LNPs, to protonate and carry a positive charge, which attracts and binds the anionic headgroup of phospholipids in the endosomome. Upon binding, the hydrophobic tails of cationic lipids and phospholipids expand, and the endosomal membrane is disrupted, thereby allowing the mRNA cytoplasm to undergo translation and preventing lysosomal degradation of the endosome.[38,39] Activation of mRNA for humoral immunity is similar to that of most conventional vaccines, and although the exact process of activation of CD8+ T cells is currently not fully understood, endogenous antigens are considered potent conditions and antigenic peptides need to be presented to CD8+ T cells via the major histocompatibility complex class I (MHC I) molecular pathway, which mRNA vaccines can do very well, exerting both humoral and cellular immunity at the same time (Figure 3B).

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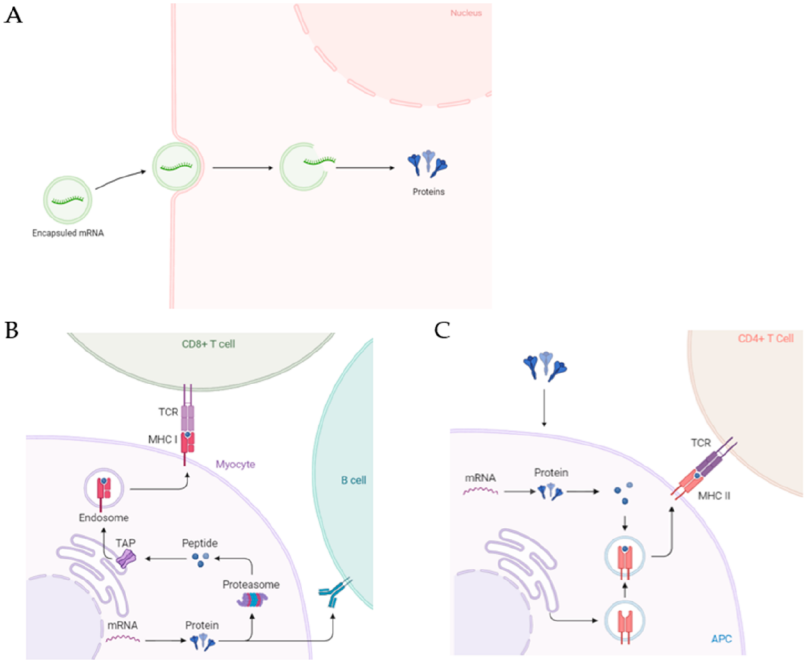

Figure 3. Process by which mRNA-LNPs are taken up and the mechanism by which adaptive immunity is induced. (A) mRNA-LNPs enter the cytoplasm via the cytosol, release mRNAs and are translated into proteins that act as immunogens. (B) Endogenous proteins are degraded by the proteasome to become peptides and are presented by MHC I to CD8+ T cells for activation, while proteins expressed on membranes or secreted can also be recognized by antigen-specific B cells as signals for B-cell activation. (C) Proteins translated from mRNAs can also activate CD4+ T cells.

2.2. mRNA-LNPs against Viruses. mRNA vaccines have emerged as promising alternative platforms to traditional vaccines. They are easy to produce, low cost, safe, and highly effective, making them ideal candidates for the prevention and treatment of infectious diseases. The development of an mRNA vaccine against COVID-19 was the first successful introduction of the entire mRNA platform. Pfizer/Biontech and Moderna are two COVID-19 mRNA vaccines (BNT162b2 and mRNA-1273) that were approved and showed 94.1%[40] and 95%[41] efficacy, respectively. The success of mRNAs in fighting COVID-19 has given confidence. Researchers have studied the effectiveness of mRNA vaccines against other viruses to improve the use of these new technologies for protecting human health.

mRNA-LNP was also applied to the Zika virus and influenza virus (H7N9 and H10N8). Mice and nonhuman primates produced high neutralizing antibody titers (1/100,000)[42] against Zika virus after administration of two or more single doses of the vaccine; in mice and ferrets, a single dose of the vaccine produced HA-inhibitory and potent neutralizing antibodies against H7N9 and H10N8.[43] LNP-embedded modified mRNA vaccines encoding viral glycoproteins have been investigated in preclinical studies against a variety of viruses, including Ebola,[44] HIV-1,[45] and Nipah virus.[46] These studies demonstrated that the vaccines were able to induce high glycoprotein-specific neutralizing antibody titers as well as antibody-dependent cytotoxicity, thereby protecting animals from lethal viral attacks. The mRNA-LNPs also showed superior immune strength to other vaccine forms when glycoproteins from cytomegalovirus (CMV),[47] vzv,[48] RSV,[49] and the rabies virus[50] were used as immunogens.

2.3. mRNA-LNPs for Cancer Immunotherapy. mRNA vaccines have emerged as promising platforms for the development of cancer vaccines. mRNA vaccines work by introducing single-stranded molecules that encode tumor neoantigens. mRNA-based cancer vaccines encode key components of the immune response process, such as tumor-specific antigen (TSA), tumor-associated antigen (TAA), and immunomodulatory factors, which promote immune activation to carry out antitumor functions.[51,52] Upon injection, the mRNA cancer vaccine is delivered to the cytoplasm of the APC, where it is subsequently translated into neoantigenic proteins and stimulates TLR7 and TLR8.[53,54] Subsequently, mature APCs are translocated to the lymph nodes, where they generate significant humoral and cellular immunity.[55,56] Compared with conventional vaccines, mRNA vaccines induce a more potent type I interferon response and efficiently trigger human CD8+ T-cell responses,[54] which play a key role in tumor eradication. In a previous study, Oberli et al. designed and optimized an LNP library for the delivery of an mRNA vaccine encoding the model antigen ovalbumin (OVA). The vaccine successfully induced potent CD8+ T-cell activation and immune responses. CD8+ T-cell activation was successfully induced, and the overall survival of B16F10 melanoma patients was prolonged. prolonged overall survival in a B16F10 melanoma model.[57] In another study, Bevers et al. optimized an mRNA-LNP formulation by adjusting the molar concentration of the four components of the mRNA-LNP formulation to optimize the delivery of the mRNA-LNP vaccine encoding HPV16.[58] The optimal mRNA-LNP vaccine induced a strong CD8+ T-cell response and enhanced uptake of the mRNA into the cells. These results demonstrated the feasibility of using mRNA-LNPs as a cancer vaccine.[58]

A TSA-based vaccine, mRNA-4157, which is capable of encoding up to 34 antigens, is currently being evaluated for its efficacy in the treatment of melanoma (NCT03897881). The results of the phase IIb KEYNOTE-942 trial suggest that mRNA-4157/V940 in combination with pembrolizumab may

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be a potential adjuvant therapy for melanoma because patients receiving this combination have a significantly lower risk of disease recurrence than patients receiving only the PD-1 inhibitor alone. Patients treated with this combination have a significantly lower risk of disease recurrence.[59] The ability of the autologous gene cevumeran (BNT 122), which contains up to 20 neoantigens, to stimulate immunity to neoantigens in patients with resected pancreatic ductal adenocarcinoma (PDAC) was tested in a phase 1 clinical trial. The BioNT111 vaccine developed by BioNTech is an mRNA cancer vaccine that encodes four TAAs, namely, New York Esophageal Squamous Cell Carcinoma 1 (NY-ESO-1), Melanoma-Associated Antigen A3 (MAGE-A3), tyrosinase, and tensor protein homologous transmembrane phosphatase (TPTE).[60] These mRNAs were encapsulated in liposomes and administered intravenously to patients. In a phase I trial, a vaccine alone or in combination with immune checkpoint inhibitors (ICIs) induced durable responses and had a favorable safety profile in patients with advanced melanoma.[60] These findings suggest that the BNT111 vaccine has potential as a candidate for melanoma treatment. The success of mRNA cancer vaccines in combination with immune checkpoint inhibitors has inspired further ideas on the use of mRNAs encoding immunostimulants that enhance the efficacy of immune checkpoint inhibitors. Trials of cytokine-encoded mRNA products by BioNTech and Moderna have yielded satisfactory results.[61,62] For example, Moderna-generated intratumorally administered mRNA-2416, which encodes OX40L, demonstrated safety and tolerability in a phase I trial and triggered broad proinflammatory activity and an altered TME.[62] In addition, BioNTech's BNT131 (SAR441000) (NCT03871348), which encodes IL-12sc, IL-15 sushi, and IFN-α for intratumoral administration, is being tested as a monotherapy as well as in combination with cemiplimab in patients with advanced solid tumors to alter the TME.

3. COMPOSITION OF LNPS

When the in vivo delivery of mRNA was first investigated, viruses as natural nucleic acid delivery systems first attracted the attention of researchers. However, most viruses are still dangerous because of their self-integrating DNA and pathogenicity. Risk-reducing viral vectors still do not fully address these shortcomings and are difficult to produce.[63] Nonviral vectors, which are negatively charged themselves, interact with negatively charged mRNAs, bind mRNAs and enter the cell via endocytosis. This approach circumvents the drawbacks of viral vectors but results in a decreased transfection efficiency. In the 1990s, researchers experimented with the use of cationic liposomes[64] and fish sperm proteins,[65] among others, as delivery systems, followed by further attempts with biodegradable, ionizable polymers.[66] However, the complex structure and high toxicity of these materials as well as their lower transfection efficiency prevent them from entering the clinic. LNPs solve these problems to some extent with good biocompatibility and high transfection efficiency, as well as greatly reduced toxicity. As a result, LNPs have become the most widely used mRNA delivery system[67] and have advantages over other delivery systems.

3.1. Ionizable Cationic Lipids. Ionizable cationic lipids are pH sensitive, and their head groups can transfer charge, changing from a positively charged state at low pH to a neutral state at physiological pH.[68,69] This allows the formation of mRNA−LNP complexes, and LNPs provide a safe and stable environment for mRNA in a pH-dependent manner.[70,71] Ionizable cationic lipids can remain neutral in the blood, so the pH of the mRNA released by LNPs can be adjusted to reduce toxicity.[72] In addition, in the low-pH environment of the inner body, ionizable cationic lipids can obtain a positive charge, which makes the inner body envelope unstable and allows the nanoparticles to escape from the inner body compartment.[73]

3.2. Polyethylene Glycol. Another key component of lipid nanoparticles is PEG, which has a significant effect on their properties. The most commonly used PEG in LNP structures is PEG2000 (PEG, MW = 2000), which was shown by Oberli et al. to contribute to smaller LNP particle sizes as well as stronger T-cell responses.[74] Rondine et al. explored the effect of the structure−activity relationship of PEG-peptide complexes on the uptake of Kupffer cells and showed that the smaller the MW of PEG was, the more pronounced the hepatic escape effect of the nanoparticles was when the peptide's structure was fixed.[75] PEG can increase the ability of mRNA LNPs to escape conditioning and phagocytosis by macrophages. When PEG2000 was used, modulation of the PEG density and chain length allowed it to interfere with the binding of modulator molecules (e.g., C3b and iC3b) to their corresponding receptors expressed on macrophages by means of spatial site barriers, enhancing the mobility of the PEG chains, and softness and stretching have been shown to have similar significant effects.[76] PEG on the surface of LNPs can stabilize the spatial structure of LNPs and prevent nonspecific binding with some proteins.[77] Due to its hydrophilicity, the addition of PEG chains results in a hydration cloud with a large excluded volume of LNPs, which reduces the interaction of LNPs with neighboring LNPs or blood constituents. The flexibility of the PEG conformation, which has a large degree of freedom, renders the thermodynamic interpenetration of the PEG corona unfavorable for other substances.[78] Therefore, the addition of PEG can lead to a significant prolongation of the in vivo circulation time of LNPs. The PEG content in LNPs is usually positively correlated with the blood circulation time.[79]

3.3. Other Lipids. Cholesterol, phospholipids, and PEG are the components of LNPs, except for ionizable cationic lipids. Cholesterol is key to the stability of LNPs, and its role in cell transfection experiments has been proven.[80] When the transition temperature is low, the cholesterol content in the LNPs must increase, which can cause the LNPs to transition from the lamellar phase to the hexagonal phase.[81] These processes are necessary for LNPs to release mRNA and interact with the endosomal membrane.[82] The role of phospholipids in LNPs is to stabilize the structure, which is related to the formation and destruction of the phospholipid bilayer to promote endosome escape. In addition, some phospholipids exhibit polymorphism, which can cause the inner body membrane to change from a layered to a hexagonal phase.[83,84] Moreover, the negatively charged phosphoric acid group may participate in cationic charge neutralization, which also contributes to phase transition and internal body escape.[85,86]

3.4. Clinical Application of LNPs. Malone et al. reported that the cationic lipid *n*-[1-(2,3-dioleoyloxy)propyl]-*N*,*N*,*n*-trimethylammonium chloride (DOTMA) can be used as an mRNA transfection reagent to transport mRNAs into cells.[87] However, it can cause proinflammatory and proapoptotic toxicity in vivo, so it can be quickly removed during recycling.[88] Therefore, since ionizable lipids can promote the

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escape of mRNAs into endosomes and are neutralized at physiological pH, they can be more stable, safer, and more suitable for mRNA vaccine delivery systems.[89] Ampatro Dlin-MC3-DMA (Figure 4A)[90] in the LNP formula is the first

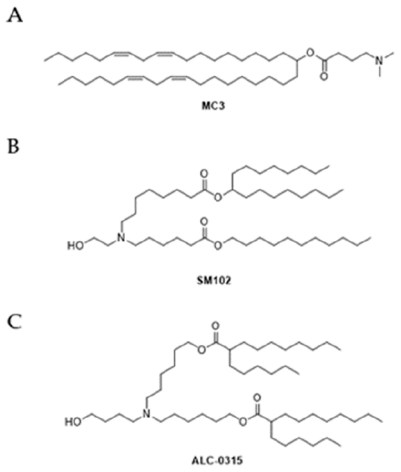

Figure 4. Molecular formulas of Dlin-MC3-DMA (A), SM102 (B), and ALC-0315 (C).

siRNA drug approved by the FDA worldwide. Moderna used Dlin-MC3-DMA to test an mRNA vaccine based on Zika virus and influenza virus and further conducted preclinical and clinical studies.[42,43] Research has shown that the vaccine form of mRNA-LNPs can generate strong protective immune responses in mice, ferrets, and nonhuman primates, and preliminary data from the first human trial of mRNA-LNPs using influenza virus as the immunogen has shown some success.[42,43] However, the alkyl tail of linoleic acid in Dlin-MC3-DMA is not easily degraded, which may lead to toxicity when the amount of this compound is too high.[91] The novel Pfizer/Biontech and Moderna vaccines, two COVID-19 mRNA vaccines (BNT162b2 and mRNA-1273) have recently been approved by the FDA and symbolize the successful use of LNPs in the field of mRNA vaccines. These two LNPs were rapidly developed to prevent the spread of COVID-19. The lipids produced by Moderna are called SM-102 (also known as lipid H; Figure 4B). Its tail has more branches, which can increase its efficacy, and the ester bond introduced by this tail improves its biodegradability in vivo and safety.[22,92] Almost at the same time, Biontech used ALC-0315 (Figure 4C), which has a chemical structure similar to that of SM-102, as the ionizable lipid in its COVID-19 mRNA vaccine LNP formula.[91,93] Both mRNA vaccines have a protective effect of more than 90%.[40,94] Monitoring of populations vaccinated with these two types of mRNA vaccines showed good protective efficacy, although the protection against mutations may be relatively poor.[41] The molar ratio of LNPs developed by Moderna was SM-102: PEG: cholesterol: phospholipids (DSPC) = 50: 1.5: 38.5: 10. The molar ratio of the LNP formula developed by Pfizer/Biontech was ALC-0315: PEG: cholesterol: phospholipids (DSPC) = 46.3: 1.6: 42.7: 9.4.[95] The particle size of the LNPs in these two formulations is approximately 80−100 nm,[22] and the LNPs contain approximately 100 mRNA molecules;[96] additionally, the LNPs perform well in the prevention and control of COVID-19.

In addition to the three approved LNPs mentioned above, a considerable number of LNP-based products are in clinical trials (Table 1). These studies demonstrated that LNPs are effective in vivo delivery vehicles, highlighting the need for a deeper understanding of LNP delivery systems.

4. CHALLENGES IN LNP DESIGN

As the most widespread and prominent mRNA vaccine delivery vector, LNPs not only demonstrate potential but also present problems that need to be solved. The resolution of these problems will expand the application of mRNA vaccines to a greater extent.

4.1. LNP Targeting. When LNPs are injected intravenously (IV) or intramuscularly (IM), a majority of the LNPs accumulate in the liver, which contradicts the original intention of achieving the function of mRNA vaccines. Researchers are attempting to modify LNPs to alter organ targeting preferences. The charge, composition, and size of LNPs can affect their trafficking, absorption, and organ distribution.[97−99] Research on LNP targeting has become a global research hotspot. The progress made in these studies has helped us gain a deeper understanding of the adaptability of LNPs to immune organs.[100−102]

4.1.1. Modifications Made to Achieve Immune Organ Targeting. Recent studies have shown that changing the apparent acid dissociation constant (pK_a) of LNPs can enable LNPs to target extrahepatic tissues (such as the spleen or lungs).[103] The pK_a of LNPs enriched in the liver is generally between 6 and 7; when the pK_a is between 2 and 6, LNPs exhibit good spleen enrichment ability.[104] The surface $pK_a \approx 5.7$ of spleen-targeted OF-Deg-Lin FLuc mRNA LNPs also provides a good example.[105] The above phenomenon may be due to the different head groups of different ionizable cationic lipids determining the pK_a, which determines the surface charge difference of LNPs, affects their ability to interact with different types of serum proteins, and ultimately leads to their different distributions in the body. The negative surface charge of LNPs is related to their uptake in the spleen.[106] Cheng et al. recently found that the presence of a fifth component, called "selective organ targeting (SORT)", can alter the organ targeting effect of LNPs in vivo.[107] Studies have shown that SORT lipids can affect the apparent pK_a values of LNPs and the interaction between LNPs and serum proteins. Changing the quantity and type of SORT lipids can endow LNPs with different targeting selectivities. Interestingly, when the lipid 18PA, which has a negative surface charge, is added to LNPs, spleen targeting can be achieved. Next, 18PA was replaced with 14PA and 18BMP, which also had negative surface charges. The results showed that the ability of the LNPs to target cells was independent of the structure of the fifth component. These findings are undoubtedly exciting and can be foreseen for the further development of mRNA vaccines. Currently, the LNP-SORT platform has been established.[108]

The spleen is an immune organ that contains a large number of lymphocytes. A library of ionizable amino polyesters (APEs) was synthesized by the ring-opening polymerization of lactones and tertiary amino alcohols. Fusion of APE to LNP (i.e., APE-LNP) resulted in the observation of an increased preference of LNP for splenic antigen-presenting cells.[109] In another study, Anderson et al. synthesized a series of alkenyl amino alcohol (AAA) lipids, named OF-XX, via a ring-opening reaction between an alkenyl epoxide and a polyamine core. One of them, OF-02 LNPs, showed notable hepatic targeting.[110] To achieve splenic targeting, they later designed OF-Deg-Lin lipids based on the lipids OF-02, which have a diketopiperazine

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Table 1. Current Clinical Trials That Are Ongoing[a]

Trial	Condition	Intervention	Sponsor	Phase
NCT04283461	COVID-19 Immunization	mRNA-1273	NIAID	I
			ModernaTX, Inc.	
NCT04785144	COVID-19 Immunization	mRNA-1273	NIAID	I
		mRNA-1273.351	ModernaTX, Inc.	
NCT05057182	COVID-19 Immunization	BNT162b2	The University of Hong Kong	IV
NCT05658523	COVID-19	Bivalent Moderna	MCRI, CEPI and PDIII	III
		Novavax		
NCT04816669	COVID-19	BNT162b2	BioNTech SE Pfizer	III
NCT05231369	COVID-19	ChulaCov19 BNA159 mRNA vaccine	Chula CRC, ACRO	I
NCT04847102	COVID-19	SARS-CoV-2 mRNA Vaccine	Walvax Biotechnology, Abogen Biosciences, Yuxi Walvax Biotechnology	III
NCT04776317	COVID-19	ChAdV68-S; ChAdV68-S-TCE	NIAID	I
		SAM-LNP-S; SAM-LNP-S-TCE	Gritstone bio, Inc.	
NCT04821674	COVID-19	DS-5670a	Daiichi Sankyo	I/II
NCT04480957	SARS-CoV-2	ARCT-021	Arcturus Therapeutics, Inc.	I/II
NCT04844268	SARS-CoV-2	VACCINE RNA MCTI	SENAI CIMATEC	I
		CIMATEC HDT (HDT-301)		
NCT04811664	SARS-CoV-2 Infection	Moderna	NIAID	III
		COVID-19 Vaccine		
NCT05534048	SARS-CoV-2 Infection	PTX-COVID19-B	Everest Medicines (Singapore) Pte. Ltd.	III
		Comirnaty		
NCT05534035	SARS-CoV-2 Infection	PTX-COVID19-B	Everest Medicines (Singapore) Pte. Ltd.	III
		Vaxzevria		
NCT05057169	COVID-19 Vaccination	BNT162b2	The University of Hong Kong	IV
		CoronaVac		
NCT05639894	Respiratory Syncytial Virus Infection	RSV mRNA LNP CL-0059	Sanofi	I/II
		RSV mRNA LNP CL-0137		
		LNP CL-0137 High Dose		
NCT05516459	Coronavirus Infections	Pfizer BNT162b2 Vaccine	SUMC Negev	
NCT05755620	Influenza	Influenza Virus Quadrivalent	NIAID	I
		Inactivated Vaccine		
		VRC- FLUNPF099–00-VP		
NCT04758962	Virus Diseases	1 μg CoV-2 SAM (LNP)	GlaxoSmithKline	I
NCT03897881	Melanoma	mRNA-4157	ModernaTX, Inc.	II
		Pembrolizumab		
NCT03323398	Refractory Solid Tumor	mRNA-2416	ModernaTX, Inc.	I/II
	Malignancies or Lymphoma	Durvalumab		
	Ovarian Cancer			
NCT02314052	Hepatocellular Carcinoma	DCR-MYC	Dicerna Pharmaceuticals, Inc.	I/II
NCT01437007	Hepatic Metastases involving	TKM-080301	National Cancer Institute (NCI)	I
	Pancreas Cancer, Gastric Cancer, Breast Cancer, Ovarian Cancer, Colorectal Cancer		National Institutes of Health Clinical Center (CC)	
NCT02110563	Solid Tumors, Multiple Myeloma, Non-Hodgkin Lymphoma, Pancreatic Neuroendocrine Tumors, PNET,NHL	DCR-MYC	Dicerna Pharmaceuticals, Inc.	I
NCT04675996	Solid Tumor	INT-1B3	InteRNA	I
NCT05703971	Small Cell Lung Cancer	OTX-2002	Genprex Inc.	I/II
	Extensive Stage	quaratusugene		
		ozeplasmid		
		atezolizumab		

[a]From clinicaltrials.gov. Abbreviations: NIAID = National Institute of Allergy and Infectious Diseases; MCRI = Murdoch Childrens Research Institute; CEPI = Coalition for Epidemic Preparedness Innovations; PDIII = The Peter Doherty Institute for Infection and Immunity; ChulaVRC = Chulalongkorn University Chula Vaccine Research Center, Bangkok, Thailan; ACRO = Academic Clinical Research Office, Faculty of Medicine, Khon Kaen University BioNet-Asia; SUMCs = Soroka University Medical Center Ben-Gurion University of the Negev.

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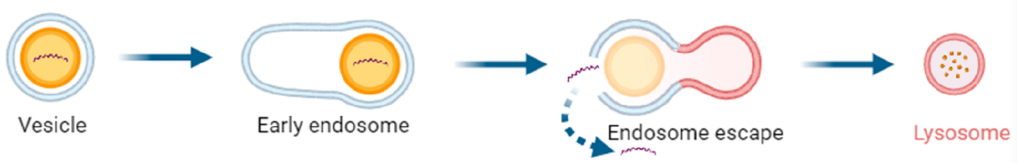

Figure 5. Ionizable cationic lipids carry a positive charge in an acidic environment and fuse with endosomal membranes to release mRNA, with the remainder being degraded by lysosomes.

core and four esterified unsaturated tails. A specific preference for B lymphocytes in the spleen was achieved.[111] In addition, by increasing the length of the carbon chain to 4, the lipid named OF-C4-Deg-Lin was further modified, and this lipid possessed even better mRNA delivery capacity.[112] Gomi et al. prepared a series of LNPs using alcohol-soluble phosphatidylserine (PS) molecules to achieve splenic targeting, which is a potential direction for the development of vac-cine LNPs.[113] Dong et al. designed TNT-b10, optimized on the basis of the siRNA carrier TNT-a 10, which has altered functional group positions and a significantly improved ability to target the spleen.[114] Ni et al. also studied several ionizable lipids (Pi lipids) with piperazine, where Pi-A10 can preferentially deliver mRNA to immune cells in the liver and spleen, with 50% expressed in macrophages of the spleen and 30% expressed in DCs of the spleen.[115] Targeted delivery of DCs is also necessary for cancer vaccines to function. Veiga et al. designed an unmodified RNA lipid complex (RNA-LPX) that targets spleen DCs to activate immune responses by reducing the content of ionizable cationic lipids. Significant T-cell responses were observed in clinical trials, proving that this method can work.[116] Furthermore, Tomácz et al. combined CD4 antibodies with LNPs and compared with unmodified LNPs with CD4, the accumulation rate of radiolabeled mRNA in the spleen was greater during systemic administration. Cre-based reporter gene expression experiments also showed stronger spleen- and lymph node-targeting ability.[117] Epstein et al. coupled CD5 antibodies to the surface of LNPs and found a trend toward targeting lymph node T cells after systemic administration, enabling T cells to be reprogrammed in vivo.[118] DC205 is highly expressed in some DCs, and Katakowski et al. reported that modifying LNPs with a single-chain antibody specific to DEC205 can lead to DC205+ DC uptake in the spleen.[119] These studies demonstrated that LNPs can target specific immune cells by coupling certain antibodies or ligands, achieving immune organ targeting, and stimulating immune response activation in specific viral infection backgrounds.

In addition to the above research, Okuda et al. reported that the particle size is also one of the factors affecting the targeting preference of LNPs. They found that adding salt to mRNA buffer can increase the particle size to approximately 200 nm, allowing the particles to travel more to mouse spleen lymphocytes and DCs without changing the LNP composition or effectively activating immune cells.[100]

4.1.2. Administration Routes. In addition to modifying the LNP itself, local administration, such as via lymph node intramuscular injection, can also increase the targeting potential of LNPs, which prevents them from reaching specific organs after systemic administration and helps in the selective delivery of LNPs.

In preclinical or clinical trials, LNPs are mainly administered through intradermal, subcutaneous, or intramuscular injection, allowing them to target nearby draining lymph nodes and present antigens to T cells, exposing antigens to B cells and activating the immune response.[120−122] The two COVID-19 mRNA vaccines currently on the market are administered through intramuscular injection. In regard to tumor vaccines, in addition to the above three pathways, colon administration, nasal drip administration, or intratumoral administration can also improve the targeting and efficacy of LNPs.[123−125] The method of needle-free injection can alleviate patient pain while also allowing LNPs to reach immune cells under the skin under the influence of high-pressure sources.

4.2. Endosome Escape. When LNPs enter the target cell, the mRNA inside must escape from the endomes to the cytoplasm to be translated into the target protein for function. However, most mRNAs are transported by the endosome to the lysosome for degradation,[126,127] and only a small portion of the proteins can escape into the cytoplasm.[128,129] This situation has been the main barrier for accounting for drug delivery for more than 50 years[130] and is another major problem that needs to be solved in the application of LNPs. A better understanding of the interactions between LNPs and in vivo pathways can guide the design of better treatment methods. It is necessary to improve the effectiveness of mRNA vaccines and allow more mRNAs to escape from endosomes. Currently, it is possible to advance this field by changing the LNP structure to address the mechanism of endosome escape, but there is no perfect method for achieving endosome escape. To ultimately achieve the goal of internal escape, any feasible opportunity is worth trying.

4.2.1. Mechanism of Endosomal Escape. In a series of processes from early endosomes to late endosomes and then to lysosomal degradation, the pH steadily decreases from physiological 7.4, early endosomes decrease to 6.5, late endosomes decrease to 6, and lysosomal degradation decreases to 5.[131] Then, LNPs can escape endophagy through a membrane phase transition related to pH. The highest proportion of ionizable cationic lipids in the LNP structure determines the basic properties of LNPs, which are undoubtedly closely related to the function of endosome escape.[132] When the hydrophobic tail of ionizable cationic lipids is equivalent in size to the head, LNPs are dispersed in a bilayer form in water.[133] Early endosomes are vesicles that engulf LNPs. As endosomes mature, the pH gradually decreases and the head of the ionizable cationic lipids gradually acquires a positive charge, allowing them to interact with negatively charged endosomes. During this process, endosomes completely fuse with LNPs, allowing mRNA to be released into the cytoplasm (Figure 5).

4.2.2. Modification of Ionizable Cationic Lipids. Because ionizable cationic lipids fuse with endosomal membranes under acidic conditions and because the hydrophobic tail expands, it is more advantageous for them to insert into the phospholipid bilayer to form the HII phase if they have a

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conical structure. The size of the hydrophobic tail of ionizable cationic lipids strongly influences the efficiency of endosomal membrane inversion. When the hydrophobic tail has an unsaturated bond, the spatial hindrance created by the rigid structure of the cis-double bond facilitates the formation of the HII phase and promotes the escape of endosomes.[134] Yusuke Sato et al. developed a library of ionizable cationic lipids that can respond to pH changes in 2019.[135] Among them, the lipid CL1H6, which has a high endosomal escape efficiency, consists of a hydrophilic head with a tertiary amine and two tails with double bonds. The hydrophilic head of CL1H6 affects the apparent pK_a of the LNP to control endosomal escape and organ preference, a process that is achieved by membrane fusion rather than disruption of the endosomal membrane (analyses showed an intracellular translocation ED_{50} of 0.0025 mg/kg). Tanaka et al.[136] developed a self-degrading lipid molecule (ssPalmO Phe) based on a double-bonded hydrophobic tail that can accelerate the breakdown of LNPs and promote the release of RNA into the cytoplasm, in which the inserted aromatic ring can alter the structure of LNPs to enhance endosomal escape.[136] The above evidence suggests that hydrophobic tails carrying unsaturated bonds can indeed increase endosomal escape, and it is speculated that the rate of endosomal escape increases with an increasing number of unsaturated bonds. Based on these results, Heyes et al. speculated that short-branched trialkyl hydrophobic structural domains may also improve the endosomal escape efficiency of LNPs.[137]

4.2.3. Modification of Other Lipids. As mentioned earlier, LNPs are composed of four components, each of which plays its own role, and the formation of LNPs is indispensable. In addition to increasing the escape rate of endosomes by altering ionizable cationic lipids, optimization based on cholesterol and phospholipids can also contribute to this process. Cholesterol is related to the structural stability of LNPs and can regulate membrane fluidity[138] while directly disrupting the stability of endosomes and promoting mRNA release to the cytoplasm.[139] Researchers have shown that the length of the alkyl tail, the flexibility of the sterol ring, and the polarity of −OH are related to improvement of transfection efficiency in cholesterol research. Siddharth et al. prepared a series of cholesterol analogs to replace LNPs and found that when cholesterol analogs with alkyl substituents at the C-24 position were used in the LNP structure, the LNPs showed increased transfection efficiency.[140] The mRNA release of LNPs formed by replacing cholesterol with sitosterol increased by more than 10-fold during transfection.[140] Gaurav et al. also discovered that the use of β, the assembly of LNPs with sitosterol instead of cholesterol, can enhance the transfection ability of LNPs. It is speculated that β-sitosterol provides a polyhedral shape to LNPs, thereby promoting endosome escape.[141] Increasing the proportion of phospholipids in LNPs can improve the delivery of mRNAs. The formation of LNPs requires phospholipids to regulate the electrostatic interaction among ionizable cationic lipids, mRNAs, and solvents. The LNP membrane is rich in phospholipids, while the inner membrane is rich in a phospholipid bilayer. This membrane is rich in bis-(monoacyl-glycerol)phosphate (BMP). Unlike phospholipids containing PC and PS groups (which can increase the stability of the LNP bilayer structure), phospholipids containing PE can guide the bending of the LNP membrane to promote fusion with the inner membrane. Daniel et al. attempted to explain the role of phospholipids in endosome escape based on the influence of chain length and the unsaturation of phospholipids when the PE and PC head groups remain unchanged.[142] The results showed that LNPs with PE as the head base had the highest efficiency in delivering mRNA. Currently, an increasing number of LNPs are using sterol lipids to replace cholesterol to increase internal body escape, and on this basis, sterol lipids with PE heads can further improve the internal body escape efficiency of LNPs.

4.2.4. Adding Exogenous Substances to Promote Endosome Escape. In addition to the modification of the LNPs themselves, the addition of other components is another method for improving endosome escape. Like for most proteins, Yusuke et al.'s research showed that protamine itself carries a large amount of negative charge, and when mixed with LNPs, it can effectively control the positive charge toxicity of LNPs.[143] YSK05 is a cationic lipid designed specifically for endosomal escape. The amount of fluorescent protein expressed in cells incubated with protamine and LNPs containing YSK05 was three times greater than that in cells without protamine, proving that the addition of exogenous protamine can increase the efficiency of LNP endosome escape.[143] Several other methods for endosomal escape using siRNA as a delivery target have also been reported. Cell-penetrating peptides (CPPs) are one of the techniques used to increase the level of intracellular escape. After the addition of CPP, the first step in the cell cycle is binding to the sulfated heparin proteoglycan on the cell surface, which allows siRNA to enter the cell through nonendocytosis.[144] Kim et al. recently attempted to add biocompatible polyhistidine (pHis) to traditional LNPs to enhance endosome escape and observed good results in siRNA delivery.[145] This solution is achieved not by promoting membrane phase transition but by the proton sponge effect generated by the aggregation of a large number of nitrogen atoms through histidine polymerization.

4.3. mRNA-LNP Stability. Encapsulation of LNPs prevents premature degradation of mRNAs in vivo and enhances the in vivo stability of mRNAs.[146] Storage stability is also a necessary consideration in the design of mRNA vaccines due to the time required for transport, delivery, and community distribution. To distribute the vaccine efficiently worldwide, it should have a sufficiently long shelf life, preferably at temperatures consistent with those of conventional vaccines (2−8 °C) or higher. While most conventional vaccines can be stored in a refrigerator at 4 °C for more than 6 months, mRNA-LNP vaccines require refrigerated cold-chain transport. mRNA-1273 can be stored at temperatures between −15 and −25 °C, whereas BNT162b2 can be stored at temperatures between −60 and −90 °C.[147,148] Very low storage temperatures have become a major obstacle for vaccine distribution, especially in countries with poor infrastructure. However, the mRNA-LNP stability at nonrefrigerated temperatures needs to be further improved.

4.3.1. Adding Protective Agents. In contrast to the harsh storage conditions used for mRNA-1273 and BNT162b2, Onpattro (Patisaran), a siRNA-LNP drug product, can be stored for up to 3 years between 2 and 8 °C.[149] The ionizable cationic lipid that makes up the LNPs is DLin-MC3-DMA. dLin-MC3-DMA: DSPC: cholesterol: PEG2000-C-DMG = 50: 10: 38.5: 1.5; the composition of this LNP is similar to that of the LNPs of the two neo-crowned mRNA vaccines. Based on these data, it was hypothesized that mRNA is the destabilizing factor in overall mRNA-LNPs and determines the storage conditions of mRNA vaccines. Moderna uses Tris-

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HCL as a stabilizer of mRNA-1273 to stabilize the mRNA,[148] and other nonreducing free radical scavengers could perhaps be used as well, but the extent to which this approach alters the storage stability of the mRNA-LNP above 0 °C has not yet been determined.

4.3.2. Lyophilization. Since mRNA-LNPs are readily degraded in water, lyophilization may be an effective way to store mRNAs at relatively high temperatures. The lyophilized form of Pfizer's mRNA-based cytomegalovirus vaccine (mRNA-1647) was tested in phase 2 clinical trials. It has a shelf life of up to 18 months at 5 °C.[148] Freeze-drying is widely used for live attenuated virus vaccines.[150] Its use in naked mRNA preparations has also been investigated, demonstrating its applicability and beneficial effects on the mRNA stability. Hiromi et al. demonstrated that the use of freeze-drying to store mRNA vaccines resulted in essentially unchanged vaccine potency after 24 weeks of storage at 4 °C.[151] Thus, lyophilization may be a possible method for increasing the stability of mRNA−LNP combinations, thus allowing higher storage temperatures of mRNA vaccines. However, lyophilization is also a time-consuming and labor-intensive process, and alternative drying techniques should be considered.

4.4. Adverse Effects. Anaphylactic reactions triggered by vaccination with the COVID-19 mRNA are rare but not unknown. Between 14 and 23 December 2020, 175 patients with severe anaphylactic reactions were reported to the Vaccine Adverse Event Reporting System (VAERS) after 893,360,19 people received the first dose of BNT162b2.[152] The first dose of mRNA-1273 was initiated on 21 December 2020, and between 21 December 2020 and 10 January 2021, 10 anaphylactic reactions were identified out of 4,041,396 vaccines.[153] However, neither the mRNA nor the excipients used in both vaccines (BNT162b2: sucrose, sodium chloride, potassium chloride, disodium phosphate dihydrate, potassium dihydrogen phosphate, and water) were used for injection. mRNA-1273: aminoglutethimide, aminoglutethimide hydrochloride, acetic acid, sodium acetate, and sucrose) constitute allergens. When using polyethylene glycolated nanomedicines (e.g., polyethylene glycol), individuals experiencing allergic reactions had higher titers of anti-PEG IgG, while individuals with high titers of anti-PEG IgG did not all experience allergic reactions.[154] It is hypothesized that the nature of the anti-PEG IgG agent may have some influence on this phenomenon as well as individual differences in antibody sensitivity. It has also been reported that anaphylactic reactions may be associated with the preexistence of anti-PEG IgE,[155] but PEG is widely available for use in everyday hygiene and cosmetics, and these products do not cause anaphylactic reactions. Overall, the causes of allergic reactions caused by mRNA vaccines are unclear, and further studies are needed to understand the causes of allergic reactions, which are also necessary for the establishment of mRNA vaccines.

5. OTHER MRNA DELIVERY SYSTEMS

While working on improving LNPs, scientists have also invested their efforts in other delivery systems. Different chemical and biological materials based on polymers, nucleic acids, peptides, proteins, biofilms, metals, and inorganic materials have been developed for mRNA delivery. These delivery systems were either developed at the same time as the LNPs or were designed to overcome some of the limitations of the LNPs.

5.1. Polymer Delivery Systems. Polymer delivery systems, although far less clinically advanced than LNPs, are also an important class of mRNA carriers. systems are also an important class of mRNA carriers. Cationic/ionizable polymers with different chemical structures, including polyethyleneimine (PEI), polyesters, poly(amino acids), polyolefins, polysaccharides, cationic/ionizable polymers with different chemical structures, and polysaccharides), and cationic/ionizable polymers with different topologies, including linear polymers, dendrimers, hyperbranched polymers, and polysaccharides, were studied for mRNA delivery.[156,157] In our recent study, we synthesized a set of alternating hydroxy-tertiary amine (PHTA)-based copolymers, PHTA-Cn, and identified the lead polymer nanocarrier PHTA-C18, which successfully delivered mRNA-based cancer vaccines in vivo and induced effective antitumor cellular immune responses.[158] Notably, unlike conventional LNPs, which induce an inflammatory response to a different extent, PHTA-C18-based polymeric nanocarriers trigger only negligible inflammatory side effects in vivo, which provides a promising approach for constructing nanocarriers.

5.2. Inorganic Material-Based Delivery Systems. Inorganic materials have been used to synthesize nanostructured materials for nucleic acid delivery applications. These inorganic NPs have been precisely formulated and can be designed to have a variety of sizes, structures, and geometries. Common inorganic NPs include calcium phosphate and mesoporous silica. Huang et al. designed tumor-targeted lipid-dendritic polymer-calcium phosphate (TT-LDCP) nanoparticles (NPs) and delivered siRNA targeting plasmid DNA encoding the immune checkpoint ligand PD-L1 and immunostimulatory IL-2 to hepatocellular carcinoma (HCC) cells, which increased tumor infiltration of CD8 T cells and inhibited HCC progression.[159] Xu et al. synthesized biodegradable mesoporous silica nanoparticles (MSNs) and coloaded multiple neoantigenic peptides, CpG oligodeoxynucleotide adjuvants, and the photosensitizer chlorin e6 into MSNs, which triggered neoantigen specific, tumor-infiltrating cytotoxic T-cell lymphocytes after IV administration, with strong antitumor efficacy.[160] Zhang et al. applied an mRNA-MSN vaccine preparation consisting of naked mRNAs encoding OVA and granulocyte macrophage colony-stimulating factor, as well as MSNs, to a xenografted E.G7-OVA prophylactic tumor model and showed a very strong inhibitory effect on the tumor.[161]

5.3. Peptide-Based Delivery Systems. CPPs can also cross cell membranes and efficiently deliver nucleic acids. Lou et al. developed a method for formulating mRNA polymers functionalized with GALA that showed a high transfection efficiency and no cytotoxicity. An arginine-rich CPP variant, RALA, has been developed for mRNA vaccine delivery.[162]

5.4. Biological-Membrane-Based Delivery Systems. Various biofilm-based delivery systems, such as extracellular vesicles[163] (EVs) (e.g., exosomes), have been used for mRNA delivery. The most commonly used EVs are exosomes 30−100 nm in length.[144] The initial discovery of nucleic acids in exosomes[164] suggested that EVs have the potential to be loaded with nucleic acids. Transmembrane proteins and lipids on the surface of EVs from host cells can release signals to evade phagocytosis[165] and cross cellular barriers, which is an advantage that makes EVs unique to EVs compared to other delivery systems, including LNPs.[166] You et al. produced human dermal fibroblast-based EVs by cell nanocutting for the

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delivery of mRNAs encoding extracellular matrix α1 type I collagen and successfully replenished dermal collagen in mice. Popowski et al. developed inhalable lung-derived exosomes that showed better tissue distribution than LNPs when delivering mRNA to the lungs of rodents and nonhuman primates.[167,168] Ma et al. utilized a mixture of EV-loaded mRNAs of human vascular endothelial growth factor A (VEGF-A) and human bone morphogenetic protein 2 (BMP-2) to achieve efficient bone regeneration in rats.[169] Exosomes are well tolerated after repeated administration and have been widely used as nanocarriers for the delivery of various therapeutic agents, including mRNAs.[170]

Moreover, EVs show great potential for mRNA delivery due to their excellent biocompatibility and stability and ability to cross biological barriers. Bioengineered exosomes show greater potential than natural exosomes for disease treatment due to their improved in vivo performance. However, unmodified EVs tend to accumulate in the liver, kidney, and spleen, possibly leading to insufficient mRNA delivery to the target site.[171,172] To address this problem, scientists have generated bioengineered natural EVs by inserting tissue-specific coding sequences or combining them with peptides, but the production of bioengineered EVs is expensive and time-consuming. This may also explain why few clinical applications of EV-based mRNA products have been reported thus far.

5.5. Hybrid Delivery Systems. Lipid−polymer hybrid carriers, also known as lipid polymers (LPPs), are a promising class of organic−organic hybrid carriers in which the lipid component can be cationic/ionizable lipids, phospholipids, cholesterol, or PEG−lipids and the polymer component can be polylactic−coglycolic acid (PLGA) or dendritic polymers. Supe et al. used LPP-loaded human antigen R (HuR) siRNA to effectively alleviate diabetic retinopathy (DR) in rats.[173] Persano et al. used LPP loaded with OVA mRNA to reduce B16-OVA-based tumor nodules by more than 90% in mice after injection.[174] Biochemical hybrid supports allow for the relatively easy and precise synthesis of chemical carriers with good biocompatibility and modification targeting ability. One hybridization strategy is to prepare synthetic NPs for the encapsulation of mRNA vectors; for example, Liu et al. prepared ABNP-mRNA biomimetic NPs by fusing mRNA-PEI/poly(L-lysine) complexes with erythrocyte membranes with apolipoprotein E (ApoE), enhancing blood−brain barrier (BBB) penetration.[175] In addition, Wu et al. designed folate-modified exosome-liposome hybrid nanoparticles loaded with ALKBH5 mRNA to inhibit disease progression in a preclinical colorectal cancer (CRC) model by regulating the ALKBH5/JMJD8/PKM2 axis and inhibiting glycolysis.[176] The use of an mRNA hybrid delivery system has gradually emerged, and this approach has become a promising mRNA delivery method.

6. CONCLUSION

The successful application of mRNA vaccines (against COVID-19) has led to the emergence of a new intersection between nanotechnology and the infectious disease vaccine industry for the treatment of many different diseases, including infections and cancers, among others, opening up a potential avenue for the protection of human health. However, as a new research direction, there are still many challenges to overcome. The properties, of LNPs, such as particle size, morphology, and surface properties, are strongly influenced by lipid structure and composition, which can affect the in vivo distribution, release efficiency, and storage stability of mRNA-LNPs. Scientists from different fields around the world are working together to address the issues of hepatic enrichment and insufficient in vivo escape in the application of LNPs. Scientists have used a number of strategies to achieve mRNA-LNP enrichment in target tissues and target cells, including (1) altering the structure or ratio of lipids, including ionizable lipids, cholesterol, and polyethylene glycolated lipids; (2) adding components other than the traditional four components to alter LNP properties; and (3) altering the mode of inoculation. All of these factors are altered to increase the interaction with different target organs or cells to some extent, and additional studies are needed to achieve complete organ or cell targeting. Regarding endosomal escape, altering the structure of lipids to allow better fusion of LNPs with endosomal membranes and utilizing a charge-based pH-sensitive approach to facilitate endosomal escape will be the focus of future LNP research and design. The finished mRNA-LNPs are currently licensed with specific protectants to increase their stability at common temperatures, which could be a more promising storage option if the time-consuming and expensive issue of lyophilization can be resolved. In addition, some discussions on other delivery systems, especially EVs, which have their own advantages in terms of biocompatibility and permeability and have the potential to achieve specific cellular targeting through surface-modified ligands, are also presented in this paper; however, if these systems are combined with the advantages of LNPs, then these systems could lead to a significant boost in the development of nucleic acid and drug delivery. Research on LNPs and other delivery systems will continue, and these steadily advancing research advances have led to a steady increase in the use of mRNA delivery systems.

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■ REFERENCES

(1) Wolff, J. A.; Malone, R. W.; Williams, P.; Chong, W.; Acsadi, G.; Jani, A.; Felgner, P. L. Direct gene transfer into mouse muscle in vivo. *Science* **1990**, *247* (4949), 1465−1468.

(2) Martinon, F.; Krishnan, S.; Lenzen, G.; Magné, R.; Gomard, E.; Guillet, J. G.; Lévy, J. P.; Meulien, P. Induction of virus-specific cytotoxic T lymphocytes in vivo by liposome-entrapped mRNA. *Eur. J. Immunol.* **1993**, *23* (7), 1719−22.

(3) Thi, T. T. H.; Suys, E. J. A.; Lee, J. S.; Nguyen, D. H.; Park, K. D.; Truong, N. P. Lipid-Based Nanoparticles in the Clinic and Clinical Trials: From Cancer Nanomedicine to COVID-19 Vaccines. *Vaccines (Basel)* **2021**, *9* (4), 359.

(4) Aviv, H.; Voloch, Z.; Bastos, R.; Levy, S. Biosynthesis and stability of globin mRNA in cultured erythroleukemic Friend cells. *Cell* **1976**, *8* (4), 495−503.

(5) Luo, Y.; Na, Z.; Slavoff, S. A. P-Bodies: Composition, Properties, and Functions. *Biochemistry* **2018**, *57* (17), 2424−2431.

(6) Ross, J.; Sullivan, T. D. Half-lives of beta and gamma globin messenger RNAs and of protein synthetic capacity in cultured human reticulocytes. *Blood* **1985**, *66* (5), 1149−1154.

(7) Karikó, K.; Buckstein, M.; Ni, H.; Weissman, D. Suppression of RNA recognition by Toll-like receptors: the impact of nucleoside modification and the evolutionary origin of RNA. *Immunity* **2005**, *23* (2), 165−75.

(8) Chou, L. Y.; Ming, K.; Chan, W. C. Strategies for the intracellular delivery of nanoparticles. *Chem. Soc. Rev.* **2011**, *40* (1), 233−45.

(9) Lakhal, S.; Wood, M. J. Exosome nanotechnology: an emerging paradigm shift in drug delivery: exploitation of exosome nanovesicles for systemic in vivo delivery of RNAi heralds new horizons for drug delivery across biological barriers. *Bioessays.* **2011**, *33* (10), 737−41.

(10) Hočevar, S.; Milošević, A.; Rodriguez-Lorenzo, L.; Ackermann-Hirschi, L.; Mottas, I.; Petri-Fink, A.; Rothen-Rutishauser, B.; Bourquin, C.; Clift, M. J. D. Polymer-Coated Gold Nanospheres Do Not Impair the Innate Immune Function of Human B Lymphocytes in Vitro. *ACS Nano* **2019**, *13* (6), 6790−6800.

(11) Ibba, M. L.; Ciccone, G.; Esposito, C. L.; Catuogno, S.; Giangrande, P. H. Advances in mRNA nonviral delivery approaches. *Adv. Drug Deliv Rev.* **2021**, *177*, No. 113930.

(12) Tenchov, R.; Bird, R.; Curtze, A. E.; Zhou, Q. Lipid Nanoparticles—From Liposomes to mRNA Vaccine Delivery, a Landscape of Research Diversity and Advancement. *ACS Nano* **2021**, *15* (11), 16982−17015.

(13) Bally, M.; Bailey, K.; Sugihara, K.; Grieshaber, D.; Vörös, J.; Städler, B. Liposome and lipid bilayer arrays toward biosensing applications. *Small* **2010**, *6* (22), 2481−97.

(14) Das, S. S.; Bharadwaj, P.; Bilal, M.; Barani, M.; Rahdar, A.; Taboada, P.; Bungau, S.; Kyzas, G. Z. Stimuli-Responsive Polymeric Nanocarriers for Drug Delivery, Imaging, and Theragnosis. *Polymers (Basel)* **2020**, *12* (6), 1397.

(15) Choi, B.; Choudhary, M. C.; Regan, J.; Sparks, J. A.; Padera, R. F.; Qiu, X.; Solomon, I. H.; Kuo, H. H.; Boucau, J.; Bowman, K.; Adhikari, U. D.; Winkler, M. L.; Mueller, A. A.; Hsu, T. Y.; Desjardins, M.; Baden, L. R.; Chan, B. T.; Walker, B. D.; Lichterfeld, M.; Brigl, M.; Kwon, D. S.; Kanjilal, S.; Richardson, E. T.; Jonsson, A. H.; Alter, G.; Barczak, A. K.; Hanage, W. P.; Yu, X. G.; Gaiha, G. D.; Seaman, M. S.; Cernadas, M.; Li, J. Z. Persistence and Evolution of SARS-CoV-2 in an Immunocompromised Host. *N Engl J. Med.* **2020**, *383* (23), 2291−2293.

(16) Anderson, E. J.; Rouphael, N. G.; Widge, A. T.; Jackson, L. A.; Roberts, P. C.; Makhene, M.; Chappell, J. D.; Denison, M. R.; Stevens, L. J.; Pruijssers, A. J.; McDermott, A. B.; Flach, B.; Lin, B. C.; Doria-Rose, N. A.; O'Dell, S.; Schmidt, S. D.; Corbett, K. S.; Swanson, P. A.; Padilla, M.; Neuzil, K. M.; Bennett, H.; Leav, B.; Makowski, M.; Albert, J.; Cross, K.; Edara, V. V.; Floyd, K.; Suthar, M. S.; Martinez, D. R.; Baric, R.; Buchanan, W.; Luke, C. J.; Phadke, V. K.; Rostad, C. A.; Ledgerwood, J. E.; Graham, B. S.; Beigel, J. H. Safety and Immunogenicity of SARS-CoV-2 mRNA-1273 Vaccine in Older Adults. *N Engl J. Med.* **2020**, *383* (25), 2427−2438.

(17) Sahin, U.; Karikó, K.; Türeci, Ö. mRNA-based therapeutics−developing a new class of drugs. *Nat. Rev. Drug Discovery* **2014**, *13* (10), 759−80.

(18) Akinc, A.; Maier, M. A.; Manoharan, M.; Fitzgerald, K.; Jayaraman, M.; Barros, S.; Ansell, S.; Du, X.; Hope, M. J.; Madden, T. D.; Mui, B. L.; Semple, S. C.; Tam, Y. K.; Ciufolini, M.; Witzigmann, D.; Kulkarni, J. A.; van der Meel, R.; Cullis, P. R. The Onpattro story and the clinical translation of nanomedicines containing nucleic acid-based drugs. *Nat. Nanotechnol.* **2019**, *14* (12), 1084−1087.

(19) Polack, F. P.; Thomas, S. J.; Kitchin, N.; Absalon, J.; Gurtman, A.; Lockhart, S.; Perez, J. L.; Perez Marc, G.; Moreira, E. D.; Zerbini, C.; Bailey, R.; Swanson, K. A.; Roychoudhury, S.; Koury, K.; Li, P.; Kalina, W. V.; Cooper, D.; Frenck, R. W.; Hammitt, L. L.; Tureci, O.; Nell, H.; Schaefer, A.; Unal, S.; Tresnan, D. B.; Mather, S.; Dormitzer, P. R.; Sahin, U.; Jansen, K. U.; Gruber, W. C. Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine. *N Engl J. Med.* **2020**, *383* (27), 2603−2615.

(20) Hou, X.; Zaks, T.; Langer, R.; Dong, Y. Lipid nanoparticles for mRNA delivery. *Nat. Rev. Mater.* **2021**, *6* (12), 1078−1094.

(21) Ramachandran, S.; Satapathy, S. R.; Dutta, T. Delivery Strategies for mRNA Vaccines. *Pharmaceut Med.* **2022**, *36* (1), 11−20.

(22) Sabnis, S.; Kumarasinghe, E. S.; Salerno, T.; Mihai, C.; Ketova, T.; Senn, J. J.; Lynn, A.; Bulychev, A.; McFadyen, I.; Chan, J.; Almarsson, Ö; Stanton, M. G.; Benenato, K. E. A Novel Amino Lipid Series for mRNA Delivery: Improved Endosomal Escape and Sustained Pharmacology and Safety in Nonhuman Primates. *Mol. Ther.* **2018**, *26* (6), 1509−1519.

(23) Pardi, N.; Hogan, M. J.; Porter, F. W.; Weissman, D. mRNA vaccines − a new era in vaccinology. *Nat. Rev. Drug Discovery* **2018**, *17* (4), 261−279.

(24) Leung, A. K.; Hafez, I. M.; Baoukina, S.; Belliveau, N. M.; Zhigaltsev, I. V.; Afshinmanesh, E.; Tieleman, D. P.; Hansen, C. L.; Hope, M. J.; Cullis, P. R. Lipid Nanoparticles Containing siRNA Synthesized by Microfluidic Mixing Exhibit an Electron-Dense Nanostructured Core. *J. Phys. Chem. C Nanomater Interfaces.* **2012**, *116* (34), 18440−18450.

(25) Knop, K.; Hoogenboom, R.; Fischer, D.; Schubert, U. S. Poly(ethylene glycol) in drug delivery: pros and cons as well as potential alternatives. *Angew. Chem., Int. Ed. Engl.* **2010**, *49* (36), 6288−308.

(26) Zhu, X.; Tao, W.; Liu, D.; Wu, J.; Guo, Z.; Ji, X.; Bharwani, Z.; Zhao, L.; Zhao, X.; Farokhzad, O. C.; Shi, J. Surface De-PEGylation Controls Nanoparticle-Mediated siRNA Delivery In Vitro and In Vivo. *Theranostics* **2017**, *7* (7), 1990−2002.

(27) Cheng, X.; Lee, R. J. The role of helper lipids in lipid nanoparticles (LNPs) designed for oligonucleotide delivery. *Adv. Drug Deliv Rev.* **2016**, *99* (Pt A), 129−137.

(28) Eygeris, Y.; Patel, S.; Jozic, A.; Sahay, G. Deconvoluting Lipid Nanoparticle Structure for Messenger RNA Delivery. *Nano Lett.* **2020**, *20* (6), 4543−4549.

(29) Nature Communications at Ten. *Nat. Commun.* **2020**, *11*(1), 1812. DOI: 10.1038/s41467-020-15592-3.

(30) Verma, M.; Ozer, I.; Xie, W.; Gallagher, R.; Teixeira, A.; Choy, M. The landscape for lipid-nanoparticle-based genomic medicines. *Nat. Rev. Drug Discovery* **2023**, *22* (5), 349−350.
(31) Zhang, Y. N.; Poon, W.; Tavares, A. J.; McGilvray, I. D.; Chan, W. C. W. Nanoparticle-liver interactions: Cellular uptake and hepatobiliary elimination. *J. Controlled Release* **2016**, *240*, 332−348.
(32) Soutschek, J.; Akinc, A.; Bramlage, B.; Charisse, K.; Constien, R.; Donoghue, M.; Elbashir, S.; Geick, A.; Hadwiger, P.; Harborth, J.; John, M.; Kesavan, V.; Lavine, G.; Pandey, R. K.; Racie, T.; Rajeev, K. G.; Röhl, I.; Toudjarska, I.; Wang, G.; Wuschko, S.; Bumcrot, D.; Koteliansky, V.; Limmer, S.; Manoharan, M.; Vornlocher, H. P. Therapeutic silencing of an endogenous gene by systemic administration of modified siRNAs. *Nature* **2004**, *432* (7014), 173−8.
(33) Schlich, M.; Palomba, R.; Costabile, G.; Mizrahy, S.; Pannuzzo, M.; Peer, D.; Decuzzi, P. Cytosolic delivery of nucleic acids: The case of ionizable lipid nanoparticles. *Bioeng Transl Med.* **2021**, *6* (2), No. e10213.
(34) Weissman, D.; Ni, H.; Scales, D.; Dude, A.; Capodici, J.; McGibney, K.; Abdool, A.; Isaacs, S. N.; Cannon, G.; Karikó, K. HIV gag mRNA transfection of dendritic cells (DC) delivers encoded antigen to MHC class I and II molecules, causes DC maturation, and induces a potent human in vitro primary immune response. *J. Immunol.* **2000**, *165* (8), 4710−7.
(35) Van Gulck, E.; Vlieghe, E.; Vekemans, M.; Van Tendeloo, V. F.; Van De Velde, A.; Smits, E.; Anguille, S.; Cools, N.; Goossens, H.; Mertens, L.; De Haes, W.; Wong, J.; Florence, E.; Vanham, G.; Berneman, Z. N. mRNA-based dendritic cell vaccination induces potent antiviral T-cell responses in HIV-1-infected patients. *AIDS.* **2012**, *26* (4), F1−12.
(36) Aligholipour Farzani, T.; Földes, K.; Ergünay, K.; Gurdal, H.; Bastug, A.; Ozkul, A. Immunological Analysis of a CCHFV mRNA Vaccine Candidate in Mouse Models. *Vaccines (Basel)* **2019**, *7* (3), 115.
(37) Karam, M.; Daoud, G. mRNA vaccines: Past, present, future. *Asian J. Pharm. Sci.* **2022**, *17* (4), 491−522.
(38) Selby, L. I.; Cortez-Jugo, C. M.; Such, G. K.; Johnston, A. P. R. Nanoescapology: progress toward understanding the endosomal escape of polymeric nanoparticles. *Wiley Interdiscip Rev. Nanomed Nanobiotechnol* **2017**, *9* (5), No. e1452.
(39) Kim, J.; Eygeris, Y.; Gupta, M.; Sahay, G. Self-assembled mRNA vaccines. *Adv. Drug Deliv Rev.* **2021**, *170*, 83−112.
(40) Baden, L. R.; El Sahly, H. M.; Essink, B.; Kotloff, K.; Frey, S.; Novak, R.; Diemert, D.; Spector, S. A.; Rouphael, N.; Creech, C. B.; 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, B. S.; 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* (5), 403−416.
(41) Nanduri, S.; Pilishvili, T.; Derado, G.; Soe, M. M.; Dollard, P.; Wu, H.; Li, Q.; Bagchi, S.; Dubendris, H.; Link-Gelles, R.; Jernigan, J. A.; Budnitz, D.; Bell, J.; Benin, A.; Shang, N.; Edwards, J. R.; Verani, J. R.; Schrag, S. J. Effectiveness of Pfizer-BioNTech and Moderna Vaccines in Preventing SARS-CoV-2 Infection Among Nursing Home Residents Before and During Widespread Circulation of the SARS-CoV-2 B.1.617.2 (Delta) Variant − National Healthcare Safety Network, March 1-August 1, 2021. *MMWR Morb Mortal Wkly Rep.* **2021**, *70* (34), 1163−1166.
(42) Richner, J. M.; Himansu, S.; Dowd, K. A.; Butler, S. L.; Salazar, V.; Fox, J. M.; Julander, J. G.; Tang, W. W.; Shresta, S.; Pierson, T. C.; Ciaramella, G.; Diamond, M. S. Modified mRNA Vaccines Protect against Zika Virus Infection. *Cell.* **2017**, *168* (6), 1114−1125.e10; Erratum. *Cell* **2017**, *169* (1), 176.
(43) Bahl, K.; Senn, J. J.; Yuzhakov, O.; Bulychev, A.; Brito, L. A.; Hassett, K. J.; Laska, M. E.; Smith, M.; Almarsson, Ö; Thompson, J.; Ribeiro, A. M.; Watson, M.; Zaks, T.; Ciaramella, G. Preclinical and Clinical Demonstration of Immunogenicity by mRNA Vaccines against H10N8 and H7N9 Influenza Viruses. *Mol. Ther.* **2017**, *25* (6), 1316−1327.
(44) Meyer, M.; Huang, E.; Yuzhakov, O.; Ramanathan, P.; Ciaramella, G.; Bukreyev, A. Modified mRNA-Based Vaccines Elicit Robust Immune Responses and Protect Guinea Pigs From Ebola Virus Disease. *J. Infect Dis.* **2018**, *217* (3), 451−455.
(45) Pardi, N.; LaBranche, C. C.; Ferrari, G.; Cain, D. W.; Tombácz, I.; Parks, R. J.; Muramatsu, H.; Mui, B. L.; Tam, Y. K.; Karikó, K.; Polacino, P.; Barbosa, C. J.; Madden, T. D.; Hope, M. J.; Haynes, B. F.; Montefiori, D. C.; Hu, S. L.; Weissman, D. Characterization of HIV-1 Nucleoside-Modified mRNA Vaccines in Rabbits and Rhesus Macaques. *Mol. Ther Nucleic Acids.* **2019**, *15*, 36−47.
(46) Lo, M. K.; Spengler, J. R.; Welch, S. R.; Harmon, J. R.; Coleman-McCray, J. D.; Scholte, F. E. M.; Shrivastava-Ranjan, P.; Montgomery, J. M.; Nichol, S. T.; Weissman, D.; Spiropoulou, C. F. Evaluation of a Single-Dose Nucleoside-Modified Messenger RNA Vaccine Encoding Hendra Virus-Soluble Glycoprotein Against Lethal Nipah virus Challenge in Syrian Hamsters. *J. Infect Dis.* **2020**, *221* (Suppl 4), S493−S498.
(47) Nelson, C. S.; Jenks, J. A.; Pardi, N.; Goodwin, M.; Roark, H.; Edwards, W.; McLellan, J. S.; Pollara, J.; Weissman, D.; Permar, S. R. Human Cytomegalovirus Glycoprotein B Nucleoside-Modified mRNA Vaccine Elicits Antibody Responses with Greater Durability and Breadth than MF59-Adjuvanted gB Protein Immunization. *J. Virol.* **2020**, *94* (9), e00186−20.
(48) Aliprantis, A. O.; Shaw, C. A.; Griffin, P.; Farinola, N.; Railkar, R. A.; Cao, X.; Liu, W.; Sachs, J. R.; Swenson, C. J.; Lee, H.; Cox, K. S.; Spellman, D. S.; Winstead, C. J.; Smolenov, I.; Lai, E.; Zaks, T.; Espeseth, A. S.; Panther, L. A phase 1, randomized, placebo-controlled study to evaluate the safety and immunogenicity of an mRNA-based RSV prefusion F protein vaccine in healthy younger and older adults. *Hum Vaccin Immunother.* **2021**, *17* (5), 1248−1261.
(49) Espeseth, A. S.; Cejas, P. J.; Citron, M. P.; Wang, D.; DiStefano, D. J.; Callahan, C.; Donnell, G. O.; Galli, J. D.; Swoyer, R.; Touch, S.; Wen, Z.; Antonello, J.; Zhang, L.; Flynn, J. A.; Cox, K. S.; Freed, D. C.; Vora, K. A.; Bahl, K.; Latham, A. H.; Smith, J. S.; Gindy, M. E.; Ciaramella, G.; Hazuda, D.; Shaw, C. A.; Bett, A. J. Modified mRNA/lipid nanoparticle-based vaccines expressing respiratory syncytial virus F protein variants are immunogenic and protective in rodent models of RSV infection. *NPJ. Vaccines.* **2020**, *5* (1), 16.
(50) Aldrich, C.; Leroux-Roels, I.; Huang, K. B.; Bica, M. A.; Loeliger, E.; Schoenborn-Kellenberger, O.; Walz, L.; Leroux-Roels, G.; von Sonnenburg, F.; Oostvogels, L. Proof-of-concept of a low-dose unmodified mRNA-based rabies vaccine formulated with lipid nanoparticles in human volunteers: A phase 1 trial. *Vaccine* **2021**, *39* (8), 1310−1318.
(51) Laumont, C. M.; Vincent, K.; Hesnard, L.; Audemard, É; Bonneil, É; Laverdure, J. P.; Gendron, P.; Courcelles, M.; Hardy, M. P.; Côté, C.; Durette, C.; St-Pierre, C.; Benhammadi, M.; Lanoix, J.; Vobecky, S.; Haddad, E.; Lemieux, S.; Thibault, P.; Perreault, C. Noncoding regions are the main source of targetable tumor-specific antigens. *Sci. Transl Med.* **2018**, *10* (470), No. eaau5516.
(52) Alarcon, N. O.; Jaramillo, M.; Mansour, H. M.; Sun, B. Therapeutic Cancer Vaccines-Antigen Discovery and Adjuvant Delivery Platforms. *Pharmaceutics* **2022**, *14* (7), 1448.
(53) Tatematsu, M.; Funami, K.; Seya, T.; Matsumoto, M. Extracellular RNA Sensing by Pattern Recognition Receptors. *J. Innate Immun.* **2018**, *10*, 398−406.
(54) Zhang, C.; Maruggi, G.; Shan, H.; Li, J. Advances in mRNA Vaccines for Infectious Diseases. *Front. Immunol.* **2019**, *10*, 594.
(55) Iavarone, C.; O'Hagan, D. T.; Yu, D.; Delahaye, N. F.; Ulmer, J. B. Mechanism of action of mRNA-based vaccines. *Expert. Rev. Vaccines.* **2017**, *16*, 871−881.
(56) Edwards, D. K.; Jasny, E.; Yoon, H.; Horscroft, N.; Schanen, B.; Geter, T.; Fotin-Mleczek, M.; Petsch, B.; Wittman, V. Adjuvant effects of a sequence-engineered mRNA vaccine: Translational profiling demonstrates similar human and murine innate response. *J. Transl. Med.* **2017**, *15*, 1.

https://doi.org/10.1021/acsomega.3c08353
ACS Omega 2024, 9, 6219−6234

(57) Oberli, M. A.; Reichmuth, A. M.; Dorkin, J. R.; Mitchell, M. J.; Fenton, O. S.; Jaklenec, A.; Anderson, D. G.; Langer, R.; Blankschtein, D. Lipid Nanoparticle Assisted mRNA Delivery for Potent Cancer Immunotherapy. *Nano Lett.* **2017**, *17* (3), 1326−1335.
(58) Bevers, S.; Kooijmans, S. A.A.; Van de Velde, E.; Evers, M. J.W.; Seghers, S.; Gitz-Francois, J. J. J. M.; van Kronenburg, N. C.H.; Fens, M. H.A.M.; Mastrobattista, E.; Hassler, L.; Sork, H.; Lehto, T.; Ahmed, K. E.; El Andaloussi, S.; Fiedler, K.; Breckpot, K.; Maes, M.; Van Hoorick, D.; Bastogne, T.; Schiffelers, R. M.; De Koker, S. mRNA-LNP vaccines tuned for systemic immunization induce strong antitumor immunity by engaging splenic immune cells. *Mol. Ther.* **2022**, *30* (9), 3078−3094.
(59) American Association for Cancer Research. mRNA Vaccine Slows Melanoma Recurrence. *Cancer Discov.* **2023**, *13* (6), 1278.
(60) Sahin, U.; Oehm, P.; Derhovanessian, E.; Jabulowsky, R. A.; Vormehr, M.; Gold, M.; Maurus, D.; Schwarck-Kokarakis, D.; Kuhn, A. N.; Omokoko, T.; Kranz, L. M.; Diken, M.; Kreiter, S.; Haas, H.; Attig, S.; Rae, R.; Cuk, K.; Kemmer-Brück, A.; Breitkreuz, A.; Tolliver, C.; Caspar, J.; Quinkhardt, J.; Hebich, L.; Stein, M.; Hohberger, A.; Vogler, I.; Liebig, I.; Renken, S.; Sikorski, J.; Leierer, M.; Müller, V.; Mitzel-Rink, H.; Miederer, M.; Huber, C.; Grabbe, S.; Utikal, J.; Pinter, A.; Kaufmann, R.; Hassel, J. C.; Loquai, C.; Türeci, Ö. An RNA vaccine drives immunity in checkpoint-inhibitor-treated melanoma. *Nature* **2020**, *585* (7823), 107−12.
(61) Deng, Z.; Tian, Y.; Song, J.; An, G.; Yang, P. mRNA vaccines: the dawn of a new era of cancer immunotherapy. *Front Immunol* **2022**, *13*, No. 887125.
(62) Porciuncula, A.; Morgado, M.; Gupta, R.; Syrigos, K.; Meehan, R.; Zacharek, S. J.; Frederick, J. P.; Schalper, K. A. Spatial Mapping and Immunomodulatory Role of the OX40/OX40L Pathway in Human Non-Small Cell Lung Cancer. *Clin. Cancer Res.* **2021**, *27* (22), 6174−6183.
(63) Yin, H.; Kanasty, R. L.; Eltoukhy, A. A.; Vegas, A. J.; Dorkin, J. R.; Anderson, D. G. Nonviral vectors for gene-based therapy. *Nat. Rev. Genet.* **2014**, *15* (8), 541−55.
(64) Dhaliwal, H. K.; Fan, Y.; Kim, J.; Amiji, M. M. Intranasal Delivery and Transfection of mRNA Therapeutics in the Brain Using Cationic Liposomes. *Mol. Pharmaceutics* **2020**, *17* (6), 1996−2005.
(65) Petsch, B.; Schnee, M.; Vogel, A. B.; Lange, E.; Hoffmann, B.; Voss, D.; Schlake, T.; Thess, A.; Kallen, K. J.; Stitz, L.; Kramps, T. Protective efficacy of in vitro synthesized, specific mRNA vaccines against influenza A virus infection. *Nat. Biotechnol.* **2012**, *30* (12), 1210−6.
(66) Chen, R.; Zhang, H.; Yan, J.; Bryers, J. D. Scaffold-mediated delivery for nonviral mRNA vaccines. *Gene Ther.* **2018**, *25* (8), 556−567.
(67) John, S.; Yuzhakov, O.; Woods, A.; Deterling, J.; Hassett, K.; Shaw, C. A.; Ciaramella, G. Multiantigenic human cytomegalovirus mRNA vaccines that elicit potent humoral and cell-mediated immunity. *Vaccine.* **2018**, *36* (12), 1689−1699.
(68) Samaridou, E.; Heyes, J.; Lutwyche, P. Lipid nanoparticles for nucleic acid delivery: Current perspectives. *Adv. Drug Deliv Rev.* **2020**, *154−155*, 37−63.
(69) Hajj, K.; Whitehead, K. Tools for translation: nonviral materials for therapeutic mRNA delivery. *Nat. Rev. Mater.* **2017**, *2*, 17056.
(70) Kowalski, P. S.; Rudra, A.; Miao, L.; Anderson, D. G. Delivering the Messenger: Advances in Technologies for Therapeutic mRNA Delivery. *Mol. Ther.* **2019**, *27* (4), 710−728.
(71) Ferhan, A. R.; Park, S.; Park, H.; Tae, H.; Jackman, J. A.; Cho, N.-J. Lipid nanoparticle technologies for nucleic acid delivery: A nanoarchitectonics perspective. *Adv. Funct. Mater.* **2022**, *32*, No. 2203669.
(72) Chaudhary, N.; Weissman, D.; Whitehead, K. A. mRNA vaccines for infectious diseases: principles, delivery and clinical translation [published correction appears in Nat Rev Drug Discov. 2021 Sep 21;:]. *Nat. Rev. Drug Discovery* **2021**, *20* (11), 817−838.
(73) Meng, C.; Chen, Z.; Mai, J.; Shi, Q.; Tian, S.; Hinkle, L.; Li, J.; Zhang, Z.; Ramirez, M.; Zhang, L.; Xu, Y.; Zhang, J.; Pan, P. Y.; Chen, S. H.; Li, H.; Shen, H. Virus-Mimic mRNA Vaccine for Cancer Treatment. *Adv. Ther (Weinh).* **2021**, *4* (11), No. 2100144.
(74) Oberli, M. A.; Reichmuth, A. M.; Dorkin, J. R.; Mitchell, M. J.; Fenton, O. S.; Jaklenec, A.; Anderson, D. G.; Langer, R.; Blankschtein, D. Lipid Nanoparticle Assisted mRNA Delivery for Potent Cancer Immunotherapy. *Nano Lett.* **2017**, *17* (3), 1326−1335.
(75) Allen, R. J.; Mathew, B.; Rice, K. G. PEG-Peptide Inhibition of Scavenger Receptor Uptake of Nanoparticles by the Liver. *Mol. Pharmaceutics* **2018**, *15* (9), 3881−3891.
(76) Haroon, H. B.; Hunter, A. C.; Farhangrazi, Z. S.; Moghimi, S. M. A brief history of long circulating nanoparticles. *Adv. Drug Deliv Rev.* **2022**, *188*, No. 114396.
(77) Woodle, M. C. Sterically stabilized liposome therapeutics. *Adv. Drug Delivery Rev.* **1995**, *16* (2), 249−265.
(78) Suk, J. S.; Xu, Q.; Kim, N.; Hanes, J.; Ensign, L. M. PEGylation as a strategy for improving nanoparticle-based drug and gene delivery. *Adv. Drug Deliv Rev.* **2016**, *99* (Pt A), 28−51.
(79) Li, S. D.; Huang, L. Stealth nanoparticles: high density but sheddable PEG is a key for tumor targeting. *J. Controlled Release* **2010**, *145* (3), 178−81.
(80) De, A.; Ko, Y. T. A tale of nucleic acid-ionizable lipid nanoparticles: Design and manufacturing technology and advancement. *Expert Opin Drug Delivery* **2023**, *20* (1), 75−91.
(81) Takahashi, H.; Sinoda, K.; Hatta, I. Effects of cholesterol on the lamellar and the inverted hexagonal phases of dielaidoylphosphatidylethanolamine. *Biochim. Biophys. Acta* **1996**, *1289* (2), 209−16.
(82) Zuhorn, I. S.; Bakowsky, U.; Polushkin, E.; Visser, W. H.; Stuart, M. C.; Engberts, J. B.; Hoekstra, D. Nonbilayer phase of lipoplex-membrane mixture determines endosomal escape of genetic cargo and transfection efficiency. *Mol. Ther.* **2005**, *11* (5), 801−10.
(83) Dobbs, W.; Heinrich, B.; Bourgogne, C.; Donnio, B.; Terazzi, E.; Bonnet, M. E.; Stock, F.; Erbacher, P.; Bolcato-Bellemin, A. L.; Douce, L. Mesomorphic imidazolium salts: new vectors for efficient siRNA transfection. *J. Am. Chem. Soc.* **2009**, *131* (37), 13338−46.
(84) Cui, L.; Chen, D.; Zhu, L. Conformation transformation determined by different self-assembled phases in a DNA complex with cationic polyhedral oligomeric silsesquioxane lipid. *ACS Nano* **2008**, *2* (5), 921−927.
(85) Koiwai, K.; Tokuhisa, K.; Karinaga, R.; Kudo, Y.; Kusuki, S.; Takeda, Y.; Sakurai, K. Transition from a normal to inverted cylinder for an amidine-bearing lipid/pDNA complex and its excellent transfection. *Bioconjug Chem.* **2005**, *16* (6), 1349−51.
(86) Smisterová, J.; Wagenaar, A.; Stuart, M. C.; Polushkin, E.; ten Brinke, G.; Hulst, R.; Engberts, J. B.; Hoekstra, D. Molecular shape of the cationic lipid controls the structure of cationic lipid/dioleylphosphatidylethanolamine-DNA complexes and the efficiency of gene delivery. *J. Biol. Chem.* **2001**, *276* (50), 47615−22.
(87) Malone, R. W.; Felgner, P. L.; Verma, I. M. Cationic liposome-mediated RNA transfection. *Proc. Natl. Acad. Sci. U. S. A.* **1989**, *86* (16), 6077−81.
(88) Cui, S.; Wang, Y.; Gong, Y.; Lin, X.; Zhao, Y.; Zhi, D.; Zhou, Q.; Zhang, S. Correlation of the cytotoxic effects of cationic lipids with their headgroups. *Toxicol Res. (Camb)* **2018**, *7* (3), 473−479.
(89) Cullis, P. R.; Hope, M. J. Lipid Nanoparticle Systems for Enabling Gene Therapies. *Mol. Ther.* **2017**, *25* (7), 1467−1475.
(90) Jayaraman, M.; Ansell, S. M.; Mui, B. L.; Tam, Y. K.; Chen, J.; Du, X.; Butler, D.; Eltepu, L.; Matsuda, S.; Narayanannair, J. K.; Rajeev, K. G.; Hafez, I. M.; Akinc, A.; Maier, M. A.; Tracy, M. A.; Cullis, P. R.; Madden, T. D.; Manoharan, M.; Hope, M. J. Maximizing the potency of siRNA lipid nanoparticles for hepatic gene silencing in vivo. *Angew. Chem., Int. Ed. Engl.* **2012**, *51* (34), 8529−33.
(91) Buschmann, M. D.; Carrasco, M. J.; Alishetty, S.; Paige, M.; Alameh, M. G.; Weissman, D. Nanomaterial Delivery Systems for mRNA Vaccines. *Vaccines (Basel)* **2021**, *9* (1), 65.
(92) Hassett, K. J.; Benenato, K. E.; Jacquinet, E.; Lee, A.; Woods, A.; Yuzhakov, O.; Himansu, S.; Deterling, J.; Geilich, B. M.; Ketova, T.; Mihai, C.; Lynn, A.; McFadyen, I.; Moore, M. J.; Senn, J. J.; Stanton, M. G.; Almarsson, Ö; Ciaramella, G.; Brito, L. A.

https://doi.org/10.1021/acsomega.3c08353
ACS Omega 2024, 9, 6219−6234

Optimization of Lipid Nanoparticles for Intramuscular Administration of mRNA Vaccines. *Mol. Ther Nucleic Acids.* **2019**, *15*, 1−11.
(93) Hope, M. J. et al. Lipid nanoparticle formulations. Patent WO-2018081480-A1, 2016. Lipid nanoparticle formulations. *PubChem Patent Summary*, 2018. https://pubchem.ncbi.nlm.nih.gov/patent/WO-2018081480-A1 (accessed Sep. 15, 2023).
(94) Polack, F. P.; Thomas, S. J.; Kitchin, N.; Absalon, J.; Gurtman, A.; Lockhart, S.; Perez, J. L.; Perez Marc, G.; Moreira, E. D.; Zerbini, C.; Bailey, R.; Swanson, K. A.; Roychoudhury, S.; Koury, K.; Li, P.; Kalina, W. V.; Cooper, D.; Frenck, R. W.; Hammitt, L. L.; Tureci, O.; Nell, H.; Schaefer, A.; Unal, S.; Tresnan, D. B.; Mather, S.; Dormitzer, P. R.; Sahin, U.; Jansen, K. U.; Gruber, W. C. Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine. *N Engl J. Med.* **2020**, *383* (27), 2603−2615.
(95) DeFrancesco, L. Whither COVID-19 vaccines? *Nat. Biotechnol.* **2020**, *38* (10), 1132−1145.
(96) Yanez Arteta, M.; Kjellman, T.; Bartesaghi, S.; Wallin, S.; Wu, X.; Kvist, A. J.; Dabkowska, A.; Székely, N.; Radulescu, A.; Bergenholtz, J.; Lindfors, L. Successful reprogramming of cellular protein production through mRNA delivered by functionalized lipid nanoparticles. *Proc. Natl. Acad. Sci. U. S. A.* **2018**, *115* (15), E3351−E3360.
(97) Jiang, Y.; Huo, S.; Mizuhara, T.; Das, R.; Lee, Y. W.; Hou, S.; Moyano, D. F.; Duncan, B.; Liang, X. J.; Rotello, V. M. The Interplay of Size and Surface Functionality on the Cellular Uptake of Sub10 nm Gold Nanoparticles. *ACS Nano* **2015**, *9* (10), 9986−93.
(98) Raj, S.; Khurana, S.; Choudhari, R.; Kesari, K. K.; Kamal, M. A.; Garg, N.; Ruokolainen, J.; Das, B. C.; Kumar, D. Specific targeting cancer cells with nanoparticles and drug delivery in cancer therapy. *Semin Cancer Biol.* **2021**, *69*, 166−177.
(99) De Jong, W. H.; Hagens, W. I.; Krystek, P.; Burger, M. C.; Sips, A. J.; Geertsma, R. E. Particle size-dependent organ distribution of gold nanoparticles after intravenous administration. *Biomaterials.* **2008**, *29* (12), 1912−9.
(100) 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. Pharmaceutics* **2020**, *17* (3), 944−953.
(101) Luozhong, S.; Yuan, Z.; Sarmiento, T.; Chen, Y.; Gu, W.; McCurdy, C.; Gao, W.; Li, R.; Wilkens, S.; Jiang, S. Phosphatidylserine Lipid Nanoparticles Promote Systemic RNA Delivery to Secondary Lymphoid Organs. *Nano Lett.* **2022**, *22* (20), 8304−8311.
(102) Takanashi, A.; Pouton, C. W.; Al-Wassiti, H. Delivery and Expression of mRNA in the Secondary Lymphoid Organs Drive Immune Responses to Lipid Nanoparticle-mRNA Vaccines after Intramuscular Injection. *Mol. Pharmaceutics* **2023**, *20* (8), 3876−3885.
(103) Okuda, K.; Sato, Y.; Iwakawa, K.; Sasaki, K.; Okabe, N.; Maeki, M.; Tokeshi, M.; Harashima, H. On the size-regulation of RNA-loaded lipid nanoparticles synthesized by microfluidic device. *J. Controlled Release* **2022**, *348*, 648−659.
(104) Dilliard, S. A.; Cheng, Q.; Siegwart, D. J. On the mechanism of tissue-specific mRNA delivery by selective organ targeting nanoparticles. *Proc. Natl. Acad. Sci. U. S. A.* **2021**, *118* (52), No. e2109256118.
(105) Fenton, O. S.; Kauffman, K. J.; Kaczmarek, J. C.; McClellan, R. L.; Jhunjhunwala, S.; Tibbitt, M. W.; Zeng, M. D.; Appel, E. A.; Dorkin, J. R.; Mir, F. F.; Yang, J. H.; Oberli, M. A.; Heartlein, M. W.; DeRosa, F.; Langer, R.; Anderson, D. G. Synthesis and Biological Evaluation of Ionizable Lipid Materials for the In Vivo Delivery of Messenger RNA to B Lymphocytes. *Adv. Mater.* **2017**, *29* (33), No. 1606944.
(106) Pattipeiluhu, R.; Arias-Alpizar, G.; Basha, G.; Chan, K. Y. T.; Bussmann, J.; Sharp, T. H.; Moradi, M. A.; Sommerdijk, N.; Harris, E. N.; Cullis, P. R.; Kros, A.; Witzigmann, D.; Campbell, F. Anionic Lipid Nanoparticles Preferentially Deliver mRNA to the Hepatic Reticuloendothelial System. *Adv. Mater.* **2022**, *34* (16), No. e2201095.
(107) Cheng, Q.; Wei, T.; Farbiak, L.; Johnson, L. T.; Dilliard, S. A.; Siegwart, D. J. Selective organ targeting (SORT) nanoparticles for tissue-specific mRNA delivery and CRISPR−Cas gene editing. *Nat. Nanotechnol.* **2020**, *15* (4), 313−320.
(108) Wei, T.; Cheng, Q.; Farbiak, L.; Anderson, D. G.; Langer, R.; Siegwart, D. J. Delivery of Tissue-Targeted Scalpels: Opportunities and Challenges for In Vivo CRISPR/Cas-Based Genome Editing. *ACS Nano* **2020**, *14* (8), 9243−9262.
(109) Kowalski, P. S.; Capasso Palmiero, U.; Huang, Y.; Rudra, A.; Langer, R.; Anderson, D. G. Ionizable Amino-Polyesters Synthesized via Ring Opening Polymerization of Tertiary Amino-Alcohols for Tissue Selective mRNA Delivery. *Adv. Mater.* **2018**, *30*, No. e1801151.
(110) Fenton, O. S.; Kauffman, K. J.; McClellan, R. L.; Appel, E. A.; Dorkin, J. R.; Tibbitt, M. W.; Heartlein, M. W.; DeRosa, F.; Langer, R.; Anderson, D. G. Bioinspired Alkenyl Amino Alcohol Ionizable Lipid Materials for Highly Potent In Vivo mRNA Delivery. *Adv. Mater.* **2016**, *28* (15), 2939−43.
(111) Fenton, O. S.; Kauffman, K. J.; Kaczmarek, J. C.; McClellan, R. L.; Jhunjhunwala, S.; Tibbitt, M. W.; Zeng, M. D.; Appel, E. A.; Dorkin, J. R.; Mir, F. F.; Yang, J. H.; Oberli, M. A.; Heartlein, M. W.; DeRosa, F.; Langer, R.; Anderson, D. G. Synthesis and Biological Evaluation of Ionizable Lipid Materials for the In Vivo Delivery of Messenger RNA to B Lymphocytes. *Adv. Mater.* **2017**, *29* (33), No. 1606944.
(112) Fenton, O. S.; Kauffman, K. J.; McClellan, R. L.; Kaczmarek, J. C.; Zeng, M. D.; Andresen, J. L.; Rhym, L. H.; Heartlein, M. W.; DeRosa, F.; Anderson, D. G. Customizable Lipid Nanoparticle Materials for the Delivery of siRNAs and mRNAs. Angew. *Chem. Int. Ed Engl.* **2018**, *57* (41), 13582−13586.
(113) 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* (9), No. e2202528.
(114) Li, B.; Luo, X.; Deng, B.; Giancola, J. B.; McComb, D. W.; Schmittgen, T. D.; Dong, Y. Effects of local structural transformation of lipid-like compounds on delivery of messenger RNA. *Sci. Rep.* **2016**, *6*, 22137.
(115) Ni, H.; Hatit, M. Z. C.; Zhao, K.; Loughrey, D.; Lokugamage, M. P.; Peck, H. E.; Cid, A. D.; Muralidharan, A.; Kim, Y.; Santangelo, P. J.; Dahlman, J. E. Piperazine-derived lipid nanoparticles deliver mRNA to immune cells in vivo. *Nat. Commun.* **2022**, *13* (1), 4766.
(116) 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**, *9* (1), 4493.
(117) Tombácz, I.; Laczkó, D.; Shahnawaz, H.; Muramatsu, H.; Natesan, A.; Yadegari, A.; Papp, T. E.; Alameh, M. G.; Shuvaev, V.; Mui, B. L.; Tam, Y. K.; 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* (11), 3293−3304.
(118) Rurik, J. G.; Tombácz, I.; Yadegari, A.; Méndez Fernández, P. O.; Shewale, S. V.; Li, L.; Kimura, T.; Soliman, O. Y.; Papp, T. E.; Tam, Y. K.; Mui, B. L.; Albelda, S. M.; Puré, E.; June, C. H.; Aghajanian, H.; Weissman, D.; Parhiz, H.; Epstein, J. A. CAR T cells produced in vivo to treat cardiac injury. *Science.* **2022**, *375* (6576), 91−96.
(119) Katakowski, J. A.; Mukherjee, G.; Wilner, S. E.; Maier, K. E.; Harrison, M. T.; DiLorenzo, T. P.; Levy, M.; Palliser, D. Delivery of siRNAs to Dendritic Cells Using DEC205-Targeted Lipid Nanoparticles to Inhibit Immune Responses. *Mol. Ther.* **2016**, *24* (1), 146−55.
(120) Sago, C. D.; Lokugamage, M. P.; Loughrey, D.; Lindsay, K. E.; Hincapie, R.; Krupczak, B. R.; Kalathoor, S.; Sato, M.; Echeverri, E. S.; Fitzgerald, J. P.; Gan, Z.; Gamboa, L.; Paunovska, K.; Sanhueza, C. A.; Hatit, M. Z. C.; Finn, M. G.; Santangelo, P. J.; Dahlman, J. E. Augmented lipid-nanoparticle-mediated in vivo genome editing in the

https://doi.org/10.1021/acsomega.3c08353
ACS Omega 2024, 9, 6219−6234

lungs and spleen by disrupting Cas9 activity in the liver. *Nat. Biomed Eng.* **2022**, *6* (2), 157−167.
(121) Kubiatowicz, L. J.; Mohapatra, A.; Krishnan, N.; Fang, R. H.; Zhang, L. mRNA nanomedicine: Design and recent applications. *Exploration (Beijing)* **2022**, *2* (6), No. 20210217.
(122) Lokugamage, M. P.; Vanover, D.; Beyersdorf, J.; Hatit, M. Z. C.; Rotolo, L.; Echeverri, E. S.; Peck, H. E.; Ni, H.; Yoon, J. K.; Kim, Y.; Santangelo, P. J.; Dahlman, J. E. Optimization of lipid nanoparticles for the delivery of nebulized therapeutic mRNA to the lungs. *Nat. Biomed Eng.* **2021**, *5* (9), 1059−1068.
(123) Teng, Z.; Meng, L. Y.; Yang, J. K.; He, Z.; Chen, X. G.; Liu, Y. Bridging nanoplatform and vaccine delivery, a landscape of strategy to enhance nasal immunity. *J. Controlled Release* **2022**, *351*, 456−475.
(124) Fehervari, Z. Intranasal vaccination. *Nat. Immunol.* **2021**, *22* (9), 1071.
(125) Nicolai, L.; Leunig, A.; Pekayvaz, K.; Esefeld, M.; Anjum, A.; Rath, J.; Riedlinger, E.; Ehreiser, V.; Mader, M.; Eivers, L.; Hoffknecht, M. L.; Zhang, Z.; Kugelmann, D.; Rossaro, D.; Escaig, R.; Kaiser, R.; Polewka, V.; Titova, A.; Petzold, T.; Spiekermann, K.; Iannacone, M.; Thiele, T.; Greinacher, A.; Stark, K.; Massberg, S. Thrombocytopenia and splenic platelet-directed immune responses after IV ChAdOx1 nCov-19 administration. *Blood.* **2022**, *140* (5), 478−490.
(126) Griffiths, G.; Gruenberg, J.; Marsh, M.; Wohlmann, J.; Jones, A. T.; Parton, R. G. Nanoparticle entry into cells; the cell biology weak link. *Adv. Drug Deliv Rev.* **2022**, *188*, No. 114403.
(127) Woodman, P. G.; Futter, C. E. Multivesicular bodies: co-ordinated progression to maturity. *Curr. Opin Cell Biol.* **2008**, *20* (4), 408−14.
(128) 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* (8), 870−6.
(129) 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* (7), 638−46.
(130) Yoo, Y. J.; Lee, C. H.; Park, S. H.; Lim, Y. T. Nanoparticle-based delivery strategies of multifaceted immunomodulatory RNA for cancer immunotherapy. *J. Controlled Release* **2022**, *343*, 564−583.
(131) Spadea, A.; Jackman, M.; Cui, L.; Pereira, S.; Lawrence, M. J.; Campbell, R. A.; Ashford, M. Nucleic Acid-Loaded Lipid Nanoparticle Interactions with Model Endosomal Membranes. *ACS Appl. Mater. Interfaces* **2022**, *14* (26), 30371−30384.
(132) Degors, I. M. S.; Wang, C.; Rehman, Z. U.; Zuhorn, I. S. Carriers Break Barriers in Drug Delivery: Endocytosis and Endosomal Escape of Gene Delivery Vectors. *Acc. Chem. Res.* **2019**, *52* (7), 1750−1760.
(133) Zhang, Y.; Sun, C.; Wang, C.; Jankovic, K. E.; Dong, Y. Lipids and Lipid Derivatives for RNA Delivery. *Chem. Rev.* **2021**, *121* (20), 12181−12277.
(134) Halali, M. A.; Larocque, M.; de Lannoy, C.-F.ço. Investigating the stability of electrically conductive membranes. *J. Membr. Sci.* **2021**, *627*, No. 119181.
(135) Nakamura, T.; Nakade, T.; Yamada, K.; Sato, Y.; Harashima, H. The hydrophobic tail of a pH-sensitive cationic lipid influences siRNA transfection activity and toxicity in human NK cell lines. *Int. J. Pharm.* **2021**, *609*, No. 121140.
(136) Tanaka, H.; Takata, N.; Sakurai, Y.; Yoshida, T.; Inoue, T.; Tamagawa, S.; Nakai, Y.; Tange, K.; Yoshioka, H.; Maeki, M.; Tokeshi, M.; Akita, H. Delivery of Oligonucleotides Using a Self-Degradable Lipid-Like Material. *Pharmaceutics.* **2021**, *13* (4), 544.
(137) Lam, K.; Leung, A.; Martin, A.; Wood, M.; Schreiner, P.; Palmer, L.; Daly, O.; Zhao, W.; McClintock, K.; Heyes, J. Unsaturated, Trialkyl Ionizable Lipids are Versatile Lipid-Nanoparticle Components for Therapeutic and Vaccine Applications. *Adv. Mater.* **2023**, *35* (15), No. e2209624.
(138) Hald Albertsen, C.; Kulkarni, J. A.; Witzigmann, D.; Lind, M.; Petersson, K.; Simonsen, J. B. The role of lipid components in lipid nanoparticles for vaccines and gene therapy. *Adv. Drug Deliv Rev.* **2022**, *188*, No. 114416.
(139) Beg, S.; Almalki, W. H.; Khatoon, F.; Alharbi, K. S.; Alghamdi, S.; Akhter, M. H.; Khalilullah, H.; Baothman, A. A.; Hafeez, A.; Rahman, M.; Akhter, S.; Choudhry, H. Lipid/polymer-based nanocomplexes in nucleic acid delivery as cancer vaccines. *Drug Discov Today* **2021**, *26* (8), 1891−1903.
(140) Patel, S.; Ashwanikumar, N.; Robinson, E.; Xia, Y.; Mihai, C.; Griffith, J. P., 3rd; Hou, S.; Esposito, A. A.; Ketova, T.; Welsher, K.; Joyal, J. L.; Almarsson, Ö; Sahay, G. Naturally occurring cholesterol analogs in lipid nanoparticles induce polymorphic shape and enhance intracellular delivery of mRNA. *Nat. Commun.* **2020**, *11* (1), 983; Erratum. *Nat. Commun.* **2020**, *11* (1), 3435.
(141) Kim, J.; Jozic, A.; Lin, Y.; Eygeris, Y.; Bloom, E.; Tan, X.; Acosta, C.; MacDonald, K. D.; Welsher, K. D.; Sahay, G. Engineering Lipid Nanoparticles for Enhanced Intracellular Delivery of mRNA through Inhalation. *ACS Nano* **2022**, *16* (9), 14792−14806.
(142) Álvarez-Benedicto, E.; Farbiak, L.; Márquez Ramírez, M.; Wang, X.; Johnson, L. T.; Mian, O.; Guerrero, E. D.; Siegwart, D. J. Optimization of phospholipid chemistry for improved lipid nanoparticle (LNP) delivery of messenger RNA (mRNA). *Biomater Sci.* **2022**, *10* (2), 549−559.
(143) Sato, Y.; Matsui, H.; Sato, R.; Harashima, H. Neutralization of negative charges of siRNA results in improved safety and efficient gene silencing activity of lipid nanoparticles loaded with high levels of siRNA. *J. Controlled Release* **2018**, *284*, 179−187.
(144) Elber, R. Defect Formation and Peptide Permeation across Phospholipid Membranes. *J. Phys. Chem. B* **2023**, *127* (37), 7810−7818.
(145) Kim, I. G.; Jung, W. H.; You, G.; Lee, H.; Shin, Y. J.; Lim, S. W.; Chung, B. H.; Mok, H. Efficient Delivery of Globotriaosylceramide Synthase siRNA using Polyhistidine-Incorporated Lipid Nanoparticles. *Macromol. Biosci.* **2023**, *23* (4), No. e2200423.
(146) Pardi, N.; Tuyishime, S.; Muramatsu, H.; Kariko, K.; Mui, B. L.; Tam, Y. K.; Madden, T. D.; Hope, M. J.; Weissman, D. Expression kinetics of nucleoside-modified mRNA delivered in lipid nanoparticles to mice by various routes. *J. Controlled Release* **2015**, *217*, 345−51.
(147) Committee for Medicinal Products for Human Use (CHMP). *EMA Assessment report Comirnaty Common name: COVID-19 mRNA vaccine (nucleoside-modified)*, 2020. https://www.ema.europa.eu/en/documents/assessment-report/comirnaty-epar-public-assessment-report_en.pdf (accessed Oct. 7, 2023).
(148) *EMA COVID-19 Vaccine Moderna: Summary of Product Characteristics*, 2021. https://www.ema.europa.eu/en/documents/product-information/covid-19-vaccine-moderna-epar-product-information_en.pdf (accessed Oct. 7, 2023).
(149) *EMA Onpattro: Summary of Product Characteristics*, 2018. https://www.ema.europa.eu/en/documents/product-information/onpattro-epar-product-information_en.pdf (accessed Oct. 7, 2023).
(150) Hansen, L.; Van Renterghem, J.; Daoussi, R.; Vervaet, C.; Remon, J. P.; De Beer, T. Spectroscopic evaluation of a freeze-dried vaccine during an accelerated stability study. *Eur J Pharm Biopharm.* **2016**, *104*, 89−100.
(151) Muramatsu, H.; Lam, K.; Bajusz, C.; Laczkó, D.; Karikó, K.; Schreiner, P.; Martin, A.; Lutwyche, P.; Heyes, J.; Pardi, N. Lyophilization provides long-term stability for a lipid nanoparticle-formulated, nucleoside-modified mRNA vaccine. *Mol. Ther.* **2022**, *30* (5), 1941−1951.
(152) CDC COVID-19 Response Team; Food and Drug Administration. Allergic Reactions Including Anaphylaxis After Receipt of the First Dose of Pfizer-BioNTech COVID-19 Vaccine − United States, December 14−23, 2020. *MMWR Morb Mortal Wkly Rep.* **2021**, *70* (2), 46−51.
(153) CDC COVID-19 Response Team; Food and Drug Administration. Allergic Reactions Including Anaphylaxis After

https://doi.org/10.1021/acsomega.3c08353
ACS Omega 2024, 9, 6219−6234

Receipt of the First Dose of Moderna COVID-19 Vaccine − United States, December 21, 2020-January 10, 2021. *MMWR Morb Mortal Wkly Rep.* **2021**, *70* (4), 125−129.
(154) Ganson, N. J.; Povsic, T. J.; Sullenger, B. A.; Alexander, J. H.; Zelenkofske, S. L.; Sailstad, J. M.; Rusconi, C. P.; Hershfield, M. S. Preexisting anti-polyethylene glycol antibody linked to first-exposure allergic reactions to pegnivacogin, a PEGylated RNA aptamer. *J. Allergy Clin Immunol.* **2016**, *137* (5), 1610−1613.e7.
(155) Zhou, Z. H.; Stone, C. A., Jr; Jakubovic, B.; Phillips, E. J.; Sussman, G.; Park, J.; Hoang, U.; Kirshner, S. L.; Levin, R.; Kozlowski, S. Anti-PEG IgE in anaphylaxis associated with polyethylene glycol. *J. Allergy Clin Immunol Pract.* **2021**, *9* (4), 1731−1733.e3.
(156) Huang, P.; Deng, H.; Zhou, Y.; Chen, X. The roles of polymers in mRNA delivery. *Matter* **2022**, *5*, 1670.
(157) Guo, S.; Fu, D.; Utupova, A.; Sun, D.; Zhou, M.; Jin, Z.; Zhao, K. Applications of polymer-based nanoparticles in vaccine field. *Nat. Rev.* **2019**, *8* (1), 143−155.
(158) Huang, P.; Jiang, L.; Pan, H.; Ding, L.; Zhou, B.; Zhao, M.; Zou, J.; Li, B.; Qi, M.; Deng, H.; Zhou, Y.; Chen, X. An Integrated Polymeric mRNA Vaccine without Inflammation Side Effects for Cellular Immunity Mediated Cancer Therapy. *Adv. Mater.* **2023**, *35* (3), No. e2207471.
(159) Huang, K. W.; Hsu, F. F.; Qiu, J. T.; Chern, G. J.; Lee, Y. A.; Chang, C. C.; Huang, Y. T.; Sung, Y. C.; Chiang, C. C.; Huang, R. L.; Lin, C. C.; Dinh, T. K.; Huang, H. C.; Shih, Y. C.; Alson, D.; Lin, C. Y.; Lin, Y. C.; Chang, P. C.; Lin, S. Y.; Chen, Y. Highly efficient and tumor-selective nanoparticles for dual-targeted immunogene therapy against cancer. *Sci. Adv.* **2020**, *6* (3), No. eaax5032.
(160) Xu, C.; Nam, J.; Hong, H.; Xu, Y.; Moon, J. J. Positron Emission Tomography-Guided Photodynamic Therapy with Biodegradable Mesoporous Silica Nanoparticles for Personalized Cancer Immunotherapy. *ACS Nano* **2019**, *13* (10), 12148−12161.
(161) Zhang, W.; Liu, Y.; Min Chin, J.; Phua, K. K. L. Sustained release of PKR inhibitor C16 from mesoporous silica nanoparticles significantly enhances mRNA translation and anti-tumor vaccination. *Eur. J. Pharm. Biopharm* **2021**, *163*, 179−187.
(162) Lou, B.; De Koker, S.; Lau, C. Y. J.; Hennink, W. E.; Mastrobattista, E. mRNA Polyplexes with Post-Conjugated GALA Peptides Efficiently Target, Transfect, and Activate Antigen Presenting Cells. *Bioconjug Chem.* **2019**, *30* (2), 461−475.
(163) Li, Y.; Ma, X.; Yue, Y.; Zhang, K.; Cheng, K.; Feng, Q.; Ma, N.; Liang, J.; Zhang, T.; Zhang, L.; Chen, Z.; Wang, X.; Ren, L.; Zhao, X.; Nie, G. Rapid Surface Display of mRNA Antigens by Bacteria-Derived Outer Membrane Vesicles for a Personalized Tumor Vaccine. *Adv. Mater.* **2022**, *34* (20), No. e2109984.
(164) Valadi, H.; Ekström, K.; Bossios, A.; Sjöstrand, M.; Lee, J. J.; Lötvall, J. O. Exosome-mediated transfer of mRNAs and microRNAs is a novel mechanism of genetic exchange between cells. *Nat. Cell Biol.* **2007**, *9* (6), 654−659.
(165) Koh, E.; Lee, E. J.; Nam, G. H.; Hong, Y.; Cho, E.; Yang, Y.; Kim, I. S. Exosome-SIRPα, a CD47 blockade increases cancer cell phagocytosis. *Biomaterials.* **2017**, *121*, 121−129.
(166) Kong, N.; Tao, W.; Ling, X.; Wang, J.; Xiao, Y.; Shi, S.; Ji, X.; Shajii, A.; Gan, S. T.; Kim, N. Y.; Duda, D. G.; Xie, T.; Farokhzad, O. C.; Shi, J. Synthetic mRNA nanoparticle-mediated restoration of p53 tumor suppressor sensitizes p53-deficient cancers to mTOR inhibition. *Sci. Transl Med.* **2019**, *11* (523), No. eaaw1565.
(167) Duan, L.; Xu, L.; Xu, X.; Qin, Z.; Zhou, X.; Xiao, Y.; Liang, Y.; Xia, J. Exosome-mediated delivery of gene vectors for gene therapy. *Nanoscale* **2021**, *13* (3), 1387−1397.
(168) Zhang, Y.; Liu, Q.; Zhang, X.; Huang, H.; Tang, S.; Chai, Y.; Xu, Z.; Li, M.; Chen, X.; Liu, J.; Yang, C. Recent advances in exosome-mediated nucleic acid delivery for cancer therapy. *J. Nanobiotechnology* **2022**, *20* (1), 279.
(169) Ma, Y.; Sun, L.; Zhang, J.; Chiang, C. L.; Pan, J.; Wang, X.; Kwak, K. J.; Li, H.; Zhao, R.; Rima, X. Y.; Zhang, C.; Zhang, A.; Liu, Y.; He, Z.; Hansford, D.; Reategui, E.; Liu, C.; Lee, A. S.; Yuan, Y.; Lee, L. J. Exosomal mRNAs for Angiogenic-Osteogenic Coupled Bone Repair. *Adv. Sci. (Weinh)* **2023**, *10* (33), No. e2302622.
(170) You, Y.; Tian, Y.; Yang, Z.; Shi, J.; Kwak, K. J.; Tong, Y.; Estania, A. P.; Cao, J.; Hsu, W. H.; Liu, Y.; Chiang, C. L.; Schrank, B. R.; Huntoon, K.; Lee, D.; Li, Z.; Zhao, Y.; Zhang, H.; Gallup, T. D.; Ha, J.; Dong, S.; Li, X.; Wang, Y.; Lu, W. J.; Bahrani, E.; Lee, L. J.; Teng, L.; Jiang, W.; Lan, F.; Kim, B. Y. S.; Lee, A. S. Intradermally delivered mRNA-encapsulating extracellular vesicles for collagen-replacement therapy. *Nat. Biomed Eng.* **2023**, *7* (7), 887−900.
(171) Kim, J.; Afshari, A.; Sengupta, R.; Sebastiano, V.; Gupta, A.; Kim, Y. H; Iorns, E.; Tsui, R.; Denis, A.; Perfito, N.; Errington, T. M; Iorns, E.; Tsui, R.; Denis, A.; Perfito, N.; Errington, T. M Replication study: Melanoma exosomes educate bone marrow progenitor cells toward a pro-metastatic phenotype through MET. *Elife* **2018**, *7*, No. e39944.
(172) Lai, C. P.; Mardini, O.; Ericsson, M.; Prabhakar, S.; Maguire, C.; Chen, J. W.; Tannous, B. A.; Breakefield, X. O. Dynamic biodistribution of extracellular vesicles in vivo using a multimodal imaging reporter. *ACS Nano* **2014**, *8* (1), 483−494.
(173) Supe, S.; Upadhya, A.; Tripathi, S.; Dighe, V.; Singh, K. Liposome-polyethylenimine complexes for the effective delivery of HuR siRNA in the treatment of diabetic retinopathy. *Drug Deliv Transl Res.* **2023**, *13* (6), 1675−1698.
(174) Persano, S.; Guevara, M. L.; Li, Z.; Mai, J.; Ferrari, M.; Pompa, P. P.; Shen, H. Lipopolyplex potentiates anti-tumor immunity of mRNA-based vaccination. *Biomaterials.* **2017**, *125*, 81−89.
(175) Fang, R. H.; Gao, W.; Zhang, L. Targeting drugs to tumors using cell membrane-coated nanoparticles. *Nat. Rev. Clin Oncol.* **2023**, *20* (1), 33−48.
(176) Wu, S.; Yun, J.; Tang, W.; Familiari, G.; Relucenti, M.; Wu, J.; Li, X.; Chen, H.; Chen, R. Therapeutic m6A Eraser ALKBH5 mRNA-Loaded Exosome-Liposome Hybrid Nanoparticles Inhibit Progression of Colorectal Cancer in Preclinical Tumor Models. *ACS Nano* **2023**, *17* (12), 11838−11854.

https://doi.org/10.1021/acsomega.3c08353
ACS Omega 2024, 9, 6219−6234