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REVIEW ARTICLE

Ionizable lipids as a promising platform for creating mRNA vaccines

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Abstract

Objectives. Gene therapy involves the administration of various types of therapeutic nucleic acids into the organism, in order to treat severe hereditary diseases, as well as cancer. Furthermore, the COVID-19 pandemic demonstrated the possibility of rapid development and the effectiveness of both DNA and mRNA vaccines for the prevention of viral diseases. Numerous studies in the field of gene therapy have revealed that in most cases successful delivery of nucleic acids requires a special delivery system which protects nucleic acids from the effects of external and internal biological factors. Among the various types of such tools, non-viral delivery systems have proven to be the most versatile and safe ones. In the case of mRNA delivery, such systems are usually called mRNA vaccines, consisting of cationic or ionizable lipids. The purpose of this review is to justify the choice of the optimal structure of lipid components of mRNA vaccines and highlight the current prospects for their clinical use.

Results. In this review, we have considered the evolution of lipid structures, from cationic to ionizable, as the main components of mRNA delivery systems. Furthermore, the study demonstrated the necessity to use other types of lipids in mRNA vaccines. It also presents a review of clinical trials of mRNA vaccines against viral and oncological diseases, and provides recommendations for the design of the optimal structure of both cationic and ionizable lipids.

Conclusions. The most promising lipids for the development of mRNA vaccines are ionizable. They do not have a permanent positive charge which reduces their cytotoxicity and undesirable binding to components of the immune system. In general, mRNA vaccines can be universal and effective means for treating various types of diseases. However, their composition needs to be careful optimized.

Keywords

gene therapy, mRNA vaccines, cationic liposomes, lipid nanoparticles, cationic lipids, ionizable lipids

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ОБЗОРНАЯ СТАТЬЯ

Ионизируемые липиды как перспективная платформа для создания мРНК-вакцин

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Аннотация

Цели. Генная терапия подразумевает введение в организм различных типов терапевтических нуклеиновых кислот для лечения тяжелых наследственных, а также онкологических заболеваний. Кроме того, пандемия COVID-19 показала возможность быстрой разработки и эффективность использования ДНК- и мРНК-вакцин для профилактики вирусных заболеваний. Многочисленные исследования в области генной терапии выявили, что в большинстве случаев успешная доставка нуклеиновых кислот требует наличия специальной системы доставки, защищающей нуклеиновые кислоты от действия внешних и внутренних биологических факторов. Среди различных типов таких инструментов наиболее универсальными и безопасными зарекомендовали себя невирусные системы доставки, такие как катионные липосомы и липидные наночастицы, формируемые из катионных или ионизируемых липидов соответственно. В случае доставки мРНК такие системы обычно называют мРНК-вакцинами. Целью данного обзора являлось обоснование выбора оптимальной структуры липидных компонентов мРНК-вакцин и освещение текущих перспектив их клинического применения.

Результаты. В данном обзоре мы рассмотрели эволюцию структур липидов, начиная с катионных и заканчивая ионизируемыми, как основных компонентов систем доставки мРНК. Кроме того, показана необходимость использования в составе мРНК-вакцин других типов липидов, проведен обзор клинических испытаний мРНК-вакцин против вирусных и онкологических заболеваний, даны рекомендации по дизайну оптимальной структуры катионных и ионизируемых липидов.

Выводы. Наиболее перспективными липидами для разработки мРНК-вакцин являются ионизируемые. Они не обладают постоянным положительным зарядом, что снижает их цитотоксичность и нежелательное связывание с компонентами иммунной системы. В целом, мРНК-вакцины могут стать универсальным и эффективным средством лечения различных типов заболеваний, но требуют тщательной оптимизации их состава.

Ключевые слова

генная терапия, мРНК-вакцины, катионные липосомы, липидные наночастицы, катионные липиды, ионизируемые липиды

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INTRODUCTION

The first clinical applications of gene therapy approaches show that this field has enormous potential for treating serious diseases [1, 2], including hereditary and tumor diseases.[1] However, it also carries potential risks. Various types of nucleic acids are used as therapeutic agents in gene therapy. Plasmid DNA (pDNA) and messenger RNA (mRNA) ensure the expression of proteins which are absent in the body, while small interfering RNA (siRNA) and antisense oligonucleotides block the expression of unwanted proteins [3]. CRISPR-Cas technologies occupy a special place as the most promising tool for direct genome editing available today [4].

Regardless of type, therapeutic nucleic acids are unable independently to overcome the biological barriers of the body, such as blood plasma proteins, nucleases, cell membranes and others [5]. Special delivery systems must be used to compact and protect nucleic acids on their way to the site of therapeutic effect. These can be divided into two types. The first type, viral, is highly effective in delivering nucleic acids. However, despite the progress made in the design of viral particles, they are highly immunogenic, toxic, and potentially mutagenic [6]. Less effective but safer are non-viral nucleic acid delivery systems based on lipids [7], polymers [8, 9] or inorganic materials [10].

The development of gene therapy and improvements in its effectiveness and safety have made it possible for a number of gene therapy drugs to be approved for use. However, gene therapy only became widely used during the COVID-19 pandemic as a new generation of vaccines. The most well-known are the Russian DNA vaccine *Sputnik V* [11] and the Western mRNA vaccines *Pfizer/BioNTech* (USA/Germany) [12] and *Moderna* (USA) [13]. This was followed by a significant increase in the research and development of mRNA vaccines not only against viral infections but also against cancer. Unlike pDNA, mRNA is easier to deliver to cells because it does not need to penetrate the nucleus, in order to exert its therapeutic effect. It is sufficient for it to penetrate the cytosol of the cell.

This review will analyze the pros and cons of using different lipids as components of mRNA vaccines, including the structure and properties of these compounds. It will also mention the specifics of creating a finished vaccine, current clinical trials, and the prospects for further development of mRNA technology.

EVOLUTION OF LIPID STRUCTURES

In order to replace vaccines based on inactivated viral particles, mRNA vaccines are increasingly being developed and used. Their mechanism of action is to teach the body to produce antibodies to viruses or damaged cells. Unlike conventional vaccines, mRNA vaccines have a number of advantages:

- They do not cause a severe immune response;
- They are easily modified for mutating strains or other diseases;
- Production can be easily scaled up;
- They are purer (do not contain impurities from microorganisms);
- They are simpler and safer to produce.

Since the administration of "naked" mRNA into the body is ineffective and has no therapeutic effect, special delivery systems are required for the development of mRNA vaccines. The most biocompatible and safe systems are nanoparticles based on polymers or lipids. For example, lipid nanoparticles (LNPs) show a low level of toxicity and a high level of efficiency in delivering mRNA to the body's cells, including helping to release mRNA into the cell cytosol. Despite a huge amount of development and research in the field of lipid-based delivery systems, there is no single universal compound or nanoparticle composition which always allows for the effective delivery of different types of mRNA. The effectiveness of delivery is influenced not only by the nature of the compounds, but also by their quantity and ratio in the composition, as well as the method of nanoparticle preparation. Thus, cationic liposomes are usually obtained from cationic lipids by hydrating the lipid film followed by ultrasonic treatment and/or extrusion. Complexes of cationic liposomes and nucleic acids are then obtained by simple mixing. In the case of ionizable lipids, ready-made LNPs are usually obtained immediately by mixing the lipid with nucleic acids using microfluidic technologies [3].

There are three types of lipids most commonly used in liposome formulation: cationic or ionizable lipids responsible for packaging and delivering nucleic acids; helper lipids which assist in releasing nucleic acids into cells; and PEG lipids (polyethylene glycol derivatives) which protect the particle from interacting with blood proteins on its way into the cell.

Cationic and ionizable lipids consist of four parts: a hydrophilic part, usually represented by amines; a hydrophobic part, including a spacer which may include both alkyl groups and various steroid derivatives;

[1] The Biotech Death of Jesse Gelsinger. The New York Times. https://www.nytimes.com/1999/11/28/magazine/the-biotech-death-of-jesse-gelsinger.html. Accessed November 23, 2025.

and a linker connecting the two domains and affecting biocompatibility [14].

Cationic lipids (Fig. 1), such as classic DOTMA (*N*-[1-(2,3-dioleoyloxy)propyl]-*N*,*N*,*N*-trimethylammonium chloride), DOTAP (*N*-[1-(2,3-dioleoyloxy)propyl]-*N*,*N*,*N*-trimethylammonium chloride), DOSPA (*N*-(2-(2,5-bis((3-aminopropyl)amino)pentanamido)-ethyl)-*N*,*N*-dimethyl-2,3-dioleoyloxypropane-1-aminium pentahydrochloride), and EDOPC (2-(2,3-bis(oleoyloxy-propoxyethoxyphosphoryl)oxyethyl trimethylazanium) retain a positive charge independent of pH because they contain a quaternary ammonium group in the polar part of the molecule. The first cationic lipids capable of effectively delivering mRNA were DOTMA and its analogue DOTAP. Several mRNA vaccines were developed based thereon. Thus, mRNA and liposome complexes based on DOTMA and the helper lipid DOPE (1,2-dioleoyl-*sn*-glycero-3-phosphatidylethanolamine, Fig. 2) ensured delivery to dendritic cells, thereby inducing an antigen-specific adaptive immune response and an associated innate immune response in immunogenic cancer therapy [15]. At a later stage, Phase I clinical trials of a drug based on DOTAP and mRNA against glioblastoma were initiated in the United States [16]. In 2019, an mRNA vaccine against HIV was developed using DOTAP and passed preclinical trials. It also included DSPC (1,2-distearoyl-*sn*-glycero-3-phosphatidylcholine), DSPE-PEG$_{2000}$ (PEGylated 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine),

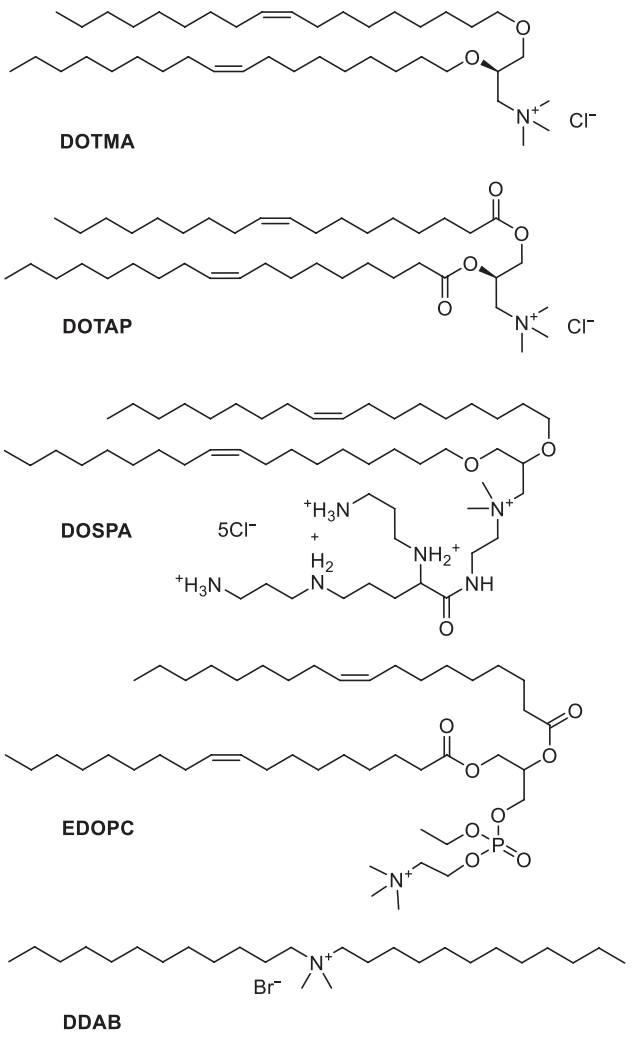

Fig. 1. Cationic lipids with quaternary ammonium groups

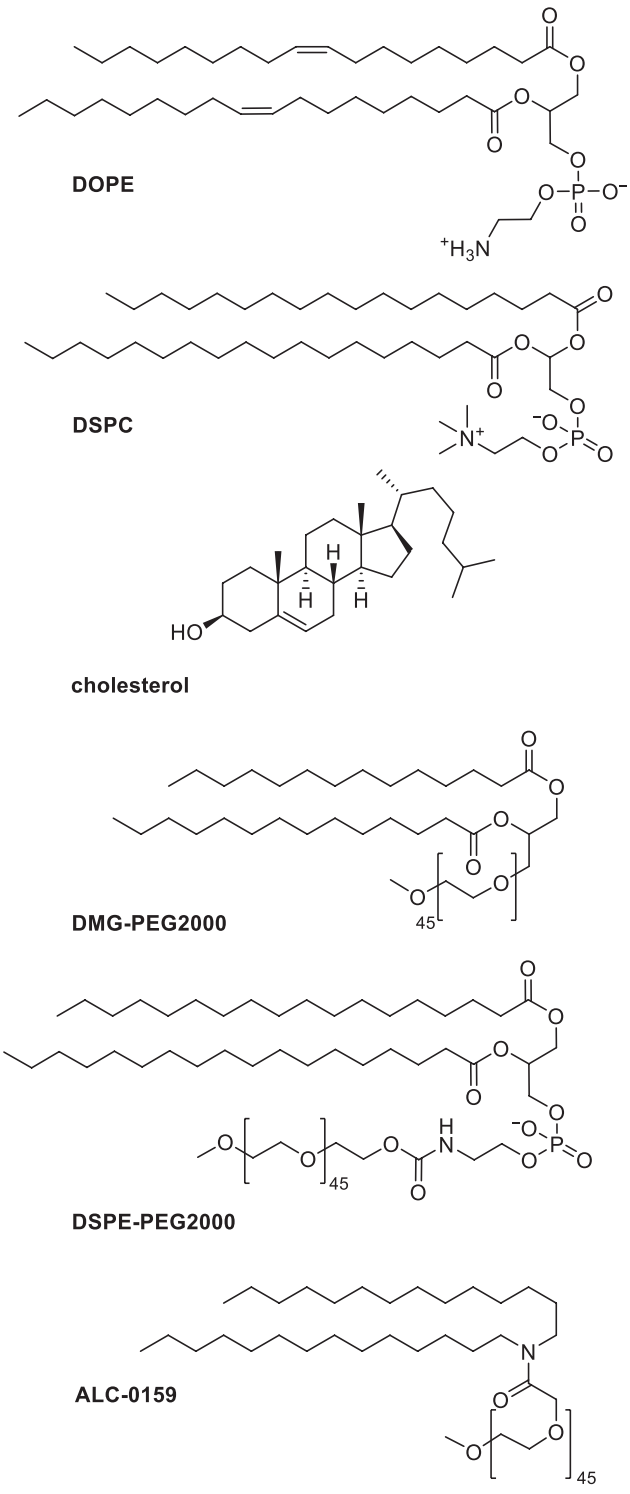

Fig. 2. Additional components of liposomal compositions

and cholesterol (Fig. 2) [17]. In 2021, complexes based on DOTAP and DOPE showed a good level of efficacy of mRNA vaccines for the treatment of autoimmune encephalomyelitis [18]. Cationic lipid-based nanoparticles have shown great promise for mRNA vaccines. This is not only because of their ability to package mRNA molecules, but also because of their ability to elicit an innate immune response, thus acting as immune adjuvants to enhance immunogenicity [19]. For example, DDAB (didodecyldimethylammonium bromide), being a cationic lipid, is capable of stimulating an immune response by activating macrophages and binding to antigens [20].

A number of cationic amphiphiles based on polyamines were developed under the guidance of M.A. Maslov. Spermine or triethylenetetramine was used as the cationic domain. By changing the design of the structures, a study was conducted on the influence of structural parts on transfection efficiency. Thus, the leading compounds were selected: cationic amphiphiles 2X3 (1,26-bis(cholest-5-en-3β-yloxycarbonylamino)-7,11,16,20-tetraazahexacosane tetrahydrochloride); and 2X7 (1,30-bis(cholest-5-en-3β-yloxycarbonylamino)-9,13,18,22-tetraaza-3,6,25,28-tetraoxatriacontane tetrahydrochloride) (Fig. 3). When delivering mRNA *in vitro* and *in vivo*, they outperformed commercially available analogues in terms of efficacy [21].

However, there still remains the problem of cytotoxicity of cationic lipids due to the presence of a permanent positive charge. In order to expand the application of non-viral vectors based on LNPs as mRNA delivery systems, second-generation cationic lipids—ionizable lipids—have been developed. They contain tertiary nitrogen atoms or other functional groups which can acquire a positive charge due to changes in pH. Since the pH in the endosome is about 4–5, protonation of the ionizable lipid occurs in it. This contributes to the disruption of membrane stability and facilitates the release of mRNA before the onset of lysosomal degradation. In this case, the acid dissociation constant (pK_a) of the lipid should be in the range of 4.5–7.0. Biocompatible drugs have been developed by changing the structural domains of cationic lipids. Lipids with a pK_a in the range of 6.2 to 6.4 showed the best results [22, 23], Therefore, ionizable lipids do not carry a permanent positive charge and have a lower level of cytotoxicity compared to cationic lipids.

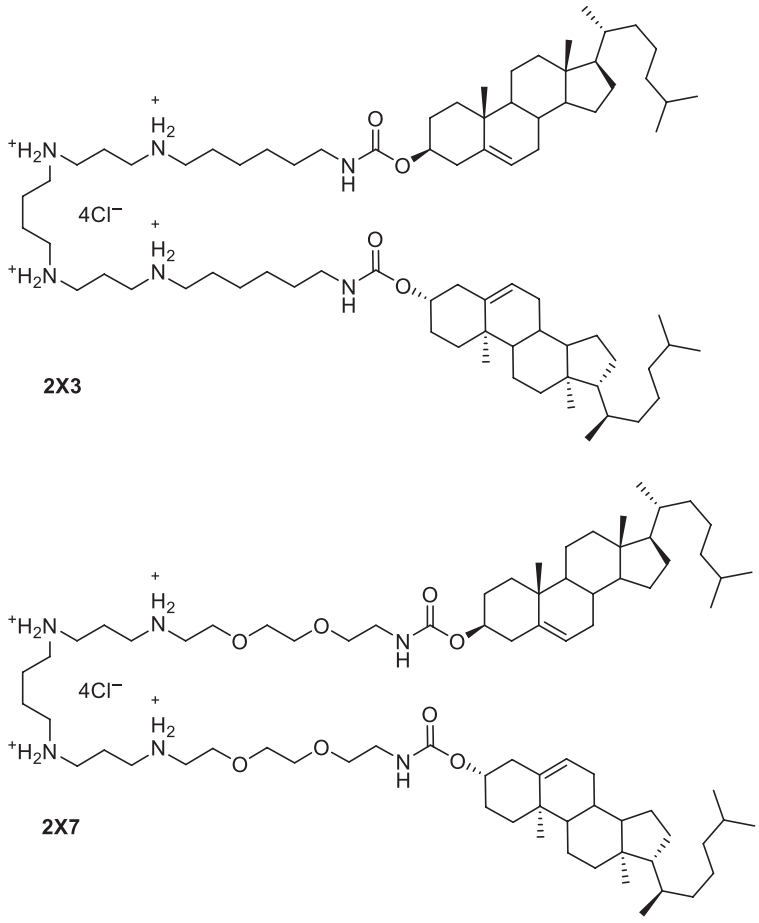

Fig. 3. Dimeric polycationic lipids

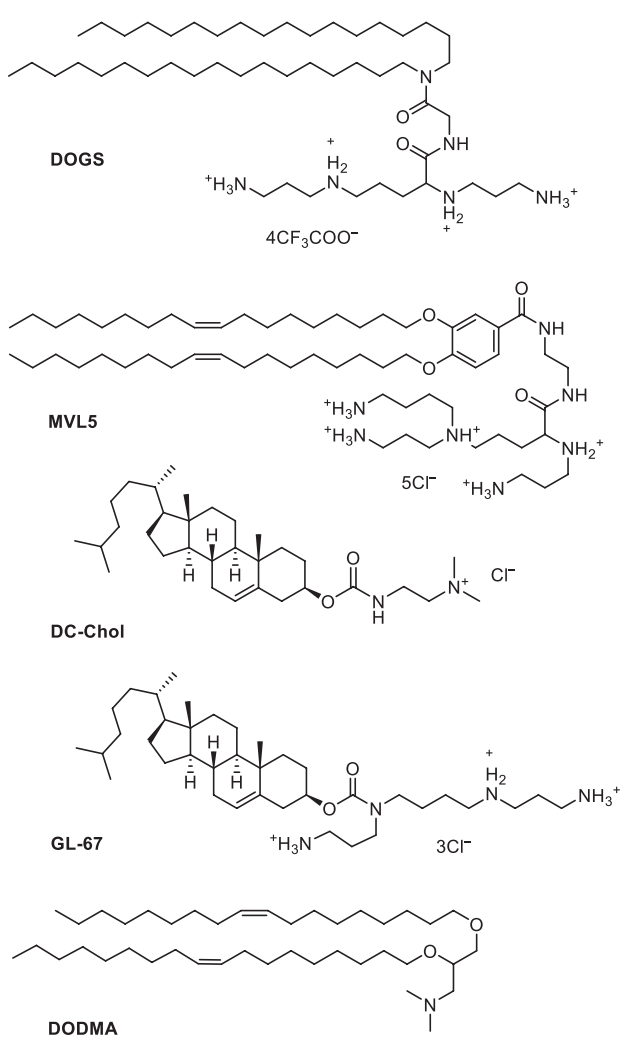

Fig. 4. Polycationic lipids

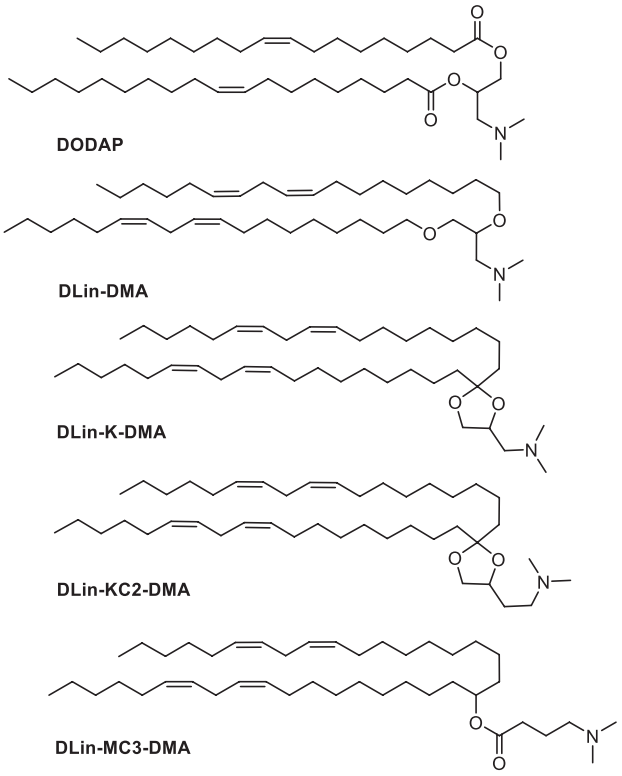

Fig. 5. Ionizable lipids-analogues of DODAP

On the other hand, the absence of a permanent charge reduces the effectiveness of LNP interaction with target cells.

The transition stage from cationic lipids to ionizable lipids was marked by the emergence of DOGS (dioctadecylamidoglycylspermine) lipids, MVL5 (N^1-[2-((1S)-1-[(3-aminopropyl)amino]-4-[di(3-aminopropyl)amino]butylcarboxamido)ethoxy]-3,4-di[oleoyloxy]-benzamide), DC-Chol (cholesteryl *N*-(2-dimethylaminoethyl)-carbamate), and GL67 (N^4-cholesteryl-spermine hydrochloride) (Fig. 4). They were originally developed for DNA delivery, but later some of them were also investigated for mRNA therapy and showed promising results. Heyes and his colleagues synthesized DODMA (1,2-dioleoyloxy-3-dimethylaminopropane) by modifying DOTMA (1,2-di-*O*-octadecenyl-3-trimethylammonium propane) and further replacing the oleoyl tails with linoleyl chains to form DLinDMA (1,2-linoleoyloxy-*N*,*N*-dimethyl-3-aminopropane) (Fig. 5). They then compared the effect of different degrees of saturation on gene suppression ability [24]. DLinDMA-based LNPs delivered miRNA and mRNA better than their analogues. For example, after delivery of mRNA aimed at enhancing expression in the retinal pigment epithelium, an improvement in the condition was noted in monogenic degenerative retinal disease against the background of retinal pigment epithelium. It also showed promising results in mRNA-based drugs against cystic fibrosis, Crohn's disease and Friedreich's ataxia [25, 26]. In another study, DLinDMA demonstrated a higher capacity than DODMA for protective immunity against respiratory syncytial virus *in vivo* [27].

The results of the above studies showed that DLin-DMA-based LNPs and their analogues (Fig. 5) are a promising platform for the creation of mRNA vaccines. Thus, by replacing ether bonds in DLin-DMA with an orthoester linker, the lipid DLin-KC2-DMA (2,2-dilinolenyl-4-dimethylaminoethyl-[1,3]-dioxolane) with pK_a values ranging from 6.2 to 6.7 can be obtained, surpassing its predecessor DLin-DMA in terms of mRNA delivery efficiency *in vivo* [28]. This lipid was the main component of the British COVAC1 mRNA vaccine against COVID-19. It also included DSPC, cholesterol, and ALC-0159 (Fig. 2). However, Phase I of clinical trials showed disappointing results: there was virtually no immune response [29].

With further modification of the linker group to an ester, the lipid DLin-MC3-DMA (dilinoleyl methyl 4-dimethylaminobutyrate) was developed (Fig. 5).

It formed the basis of the ONPATTRO® drug (*Patisiran*, USA), approved for use in the European Union and the United States for the treatment of hereditary amyloid polyneuropathy (ATTR) by delivering siRNA [30]. The drug is not without side effects. These are prevented by the additional administration of immunosuppressants, antihistamines, and other agents [30]. Later, it was shown that this ionizable lipid is effective for the combined delivery of mRNA together with siRNA [27]. It also showed a high level of efficacy in an mRNA vaccine against dengue fever [31]. In 2017 clinical trials were conducted of an mRNA vaccine against ATTR. Studies proved its efficacy and safety for use with minimal side effects, and two years later it was subsequently approved by the U.S. Food and Drug Administration (FDA). DLin-MC3-DMA served as an important precursor and starting point for the development of biodegradable ester-linked ionizable lipids which reduce cytotoxicity and increase mRNA delivery efficiency [32].

By changing the position of the ester bond, the leader compound—lipid L319—was discovered [33]. Similarly, the biodegradable lipids ATX-100, Lipid 5 [34], Lipid H (SM-102), ALC-0315, Acuitas A9 [35], and LP-01 (Fig. 6) showed improved pharmacokinetics for *in vivo* delivery when compared to cationic lipids [36]. It was found that branched alkyl tails can improve the release of mRNA molecules in the endosome. Furthermore, studies of SM-102-based liposomes helped to identify a relationship between particle size and immunogenicity in mice when using different drugs [37]. The SM-102 lipid was included in *Moderna*'s COVID-19 mRNA vaccine. During 2020, three phases of clinical trials were conducted, proving the high efficacy of the drug (94.1%). At the same time, no serious inflammatory reactions were identified in response to vaccination. Therefore, the FDA approved this composition for use. In 2024, based on clinical trials of *Moderna*'s COVID-19 vaccine, the FDA approved the use of the same composition for an mRNA vaccine against respiratory syncytial virus for people over 60 years of age, which was given the commercial name mRESVIA®.

Biodegradable ionizable lipids based on esters demonstrated higher efficiency in mRNA delivery compared to the ionizable lipid DLin-MC3-DMA (Fig. 5). Lipid 5 was found to be three times more active, while the Acuitas ALC-0315 lipid used in the *Pfizer*/*BioNTech* mRNA vaccine against COVID-19 [38], has a six times higher level of activity compared to the DLin-MC3-DMA lipid in the delivery of luciferase mRNA *in vivo*. The ALC-0315 lipid became the platform for mRNA delivery in the COVID-19 vaccine. In 2019, the Comirnaty® vaccine, developed by *Pfizer*/*BioNTech*, underwent clinical trials and was approved by the FDA for use in 2021, as it had no serious side effects and provided immunity in 90% of cases. However, its effectiveness lasted only six months. In 2023, using a similar composition, D.Yu. Logunov and his research group delivered antibodies against botulinum toxin A [39]. *In vivo* studies have yielded results showing the promise of this treatment method in emergency cases of botulism. In 2024, based on ALC-0315, in combination with ALC-0159, DSPC, and cholesterol, a Russian mRNA vaccine was created. It is a combined vaccine against influenza and COVID-19 [40], as well as a trivalent vaccine against influenza [41] which elicits a cross-specific humoral immune response. Furthermore, Lipid A6, synthesized under the guidance of Lei Miao, the hydrophobic tails of which contain a triple bond (Fig. 6), enhances the release of mRNA from endosomes due to its structure [42, 43].

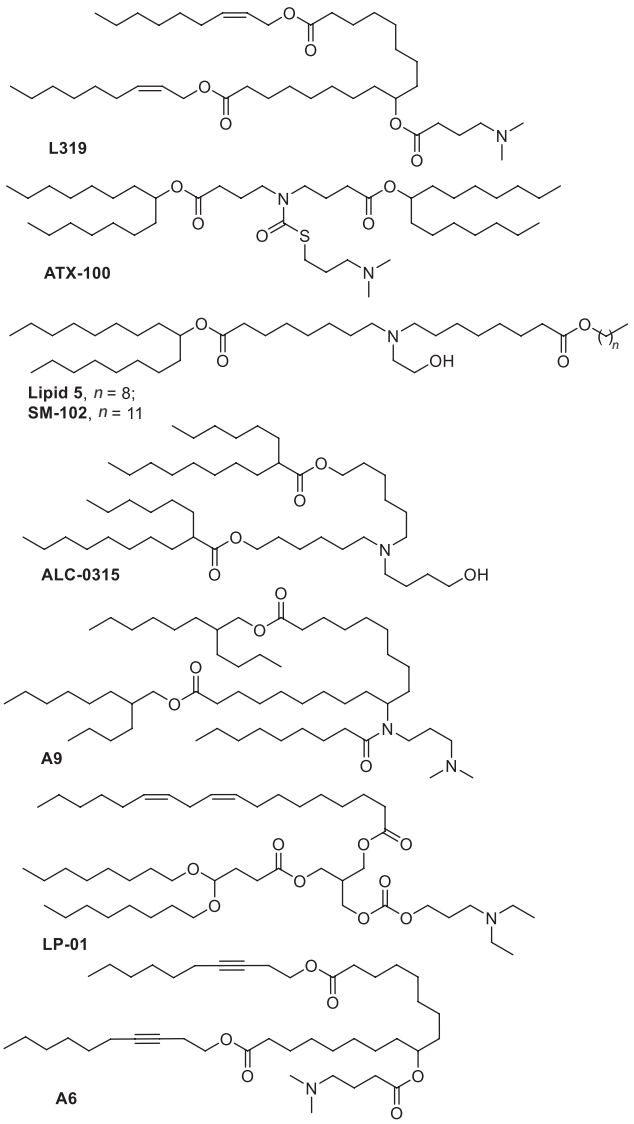

Fig. 6. Ionizable lipids with branched tails

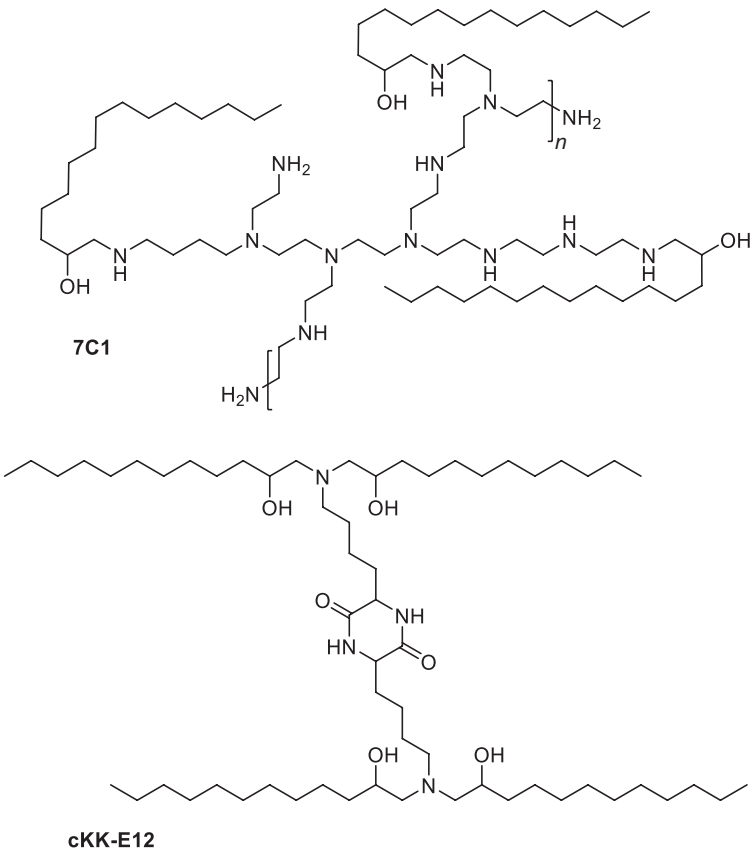

Fig. 7. Ionizable lipids 7C1 and cKK-E12

In 2019, Melissa Lokugamage and her colleagues from the US synthesized a library of various ionizable lipids. The leading compound was 7C1 which has a polymer structure based on ethylenediamine and branched hydrophobic tails. Based on this, LNPs of various compositions were studied *in vivo*. The most effective delivery of mRNA encoding an antibody targeting hemagglutinin was demonstrated by LNPs consisting of 7C1, cKK-E12, $C_{14}PEG_{2000}$, and cholesterol which protected mice from fatal infection with the H1N1 influenza virus [44].

It is known that cells contain reducing enzymes such as glutaredoxin and glutathione [45–47], enzymes of the thioredoxin family [48, 49], as well as gamma interferon-induced lysosomal thiol reductase (GILT) [50, 51]. When an additional disulfide bond linker is included in the composition of ionizable lipids, the effect of reducing enzymes can reduce cytotoxicity and increase transfection efficiency due to a better release of mRNA in the cell [52]. Thus, the disulfide lipid ssPalmE (Fig. 8) based on alpha-tocopherol contributed to the effective suppression of tumor growth [53, 54]. Its analogue ssPalmO-Phe (Fig. 8) showed excellent mRNA delivery efficiency due to the inclusion of an aromatic ring that facilitates the release of nucleic acid from endosomes [35, 53].

Miyabe and her team used the pH-sensitive lipid YSK05 (Fig. 9) to deliver adjuvants to vaccines. They found that it has a high level of adhesion to the cell membrane, increasing its effectiveness [55]. Its analogue YSK12-C4, based on aliphatic tertiary amine (Fig. 9), is more effective in knocking out genes in mouse dendritic cells [56, 57]. It was also found that the combination of YSK12-C4 and YSK05 with the addition of PEG_{2000}-DMG and cholesterol to form liposomes increases the efficiency of mRNA delivery [25].

Using the CL4H6 lipid (Fig. 9), which has a lower degree of unsaturation compared to the above compounds, siRNA was delivered to hepatocytes, as well as to mice cells carrying the OS-RC-2 gene [25, 58]. CL4H6-based LNPs demonstrated a high level of stability in the bloodstream, tumor specificity, and strong gene suppression in the test groups. When delivering mRNA, LNPs based on this lipid showed the best biocompatibility. They also showed a high level of delivery efficiency, slightly surpassing the ALC-0315 lipid. Also, compared to ALC-0315 and SM-102, the stability of LNPs with CL4H6 was significantly higher [59].

Another representative of ionizable lipids is 98N12-5 based on triethylenetetramine and laurylamine

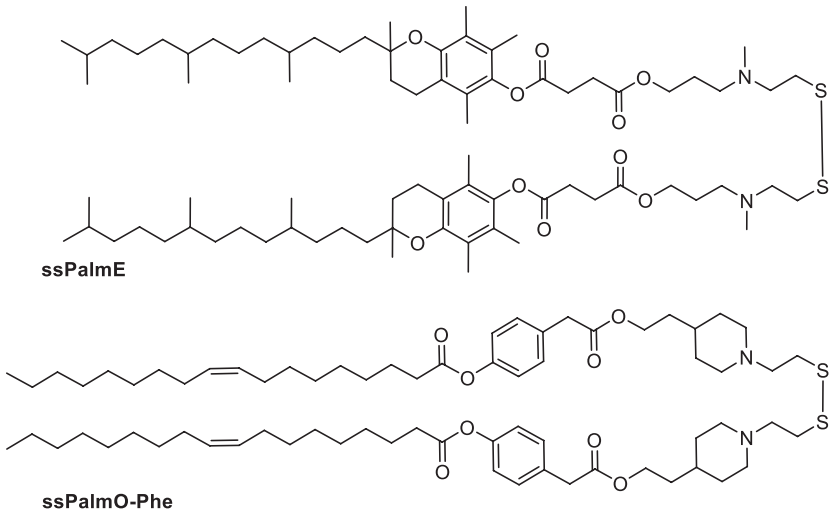

Fig. 8. Disulfide ionizable lipids

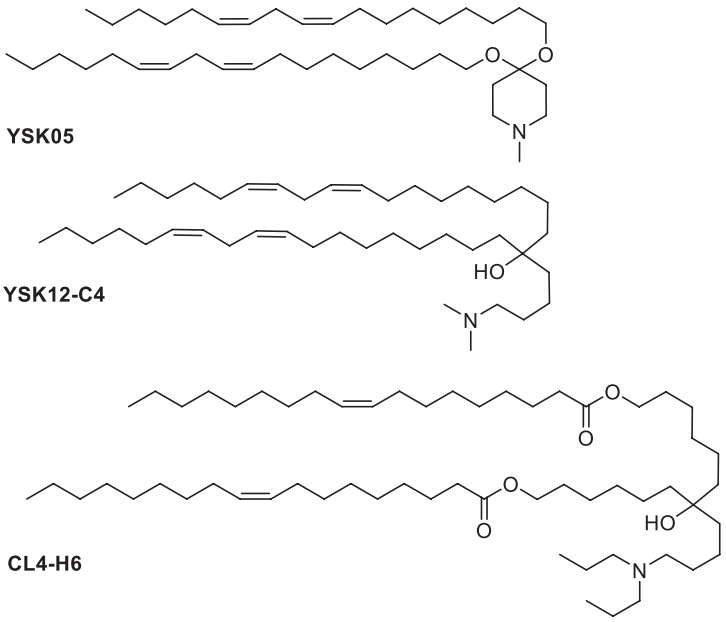

Fig. 9. Ionizable lipids with unsaturated hydrophobic tails

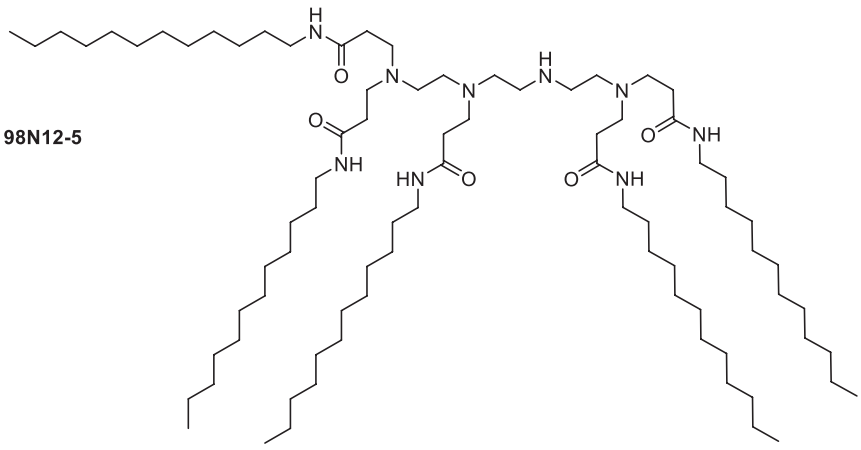

Fig. 10. Ionizable lipid 98N12-5

residues (Fig. 10) [50, 51]. This lipid showed the same transfection efficiency *in vitro* as Lipofectamine 2000. Unlike commercial transfection agents, its efficiency remained the same even when the lipid concentration was reduced. LNPs consisting of 98N12-5, cholesterol, and PEG lipid effectively delivered various siRNAs and mRNAs *in vivo* [60, 61].

Hydroxylated analogues of lipid 98N12-5, including C12-200 and C14-113 (Fig. 11), showed a greater level of efficiency in mRNA delivery and also ensured targeting of hepatocytes [62, 63]. Liu and colleagues reported that an analogue of the aforementioned lipids, TT3, enables effective delivery of mRNA molecules encoding CRISPR/Cas9 [64, 65], factor IX [66], and SARS-CoV-2 [67].

Based on studies conducted in 2014 [68], Suzuki and his research team synthesized a library of ionizable lipids with two asymmetric hydrocarbon tails, such as L021 (Fig. 12) [69]. Further replacement of the cyclopropane fragment in the hydrophobic part with an ester bond led to the production of L101, a biodegradable lipid (Fig. 12) with high gene suppression efficiency in mouse hepatocytes and rapid clearance [70].

Further development of ionizable lipids is moving towards targeted delivery. The targeted delivery of vaccines and immunotherapeutic drugs to immune cells, as well as to primary and secondary lymphoid organs, avoids side effects in other cells and tissues. For example, lipids containing polycyclic tails and/or cyclic imidazole head groups, such as 93-O17S (Fig. 13) [71, 72], target T cells. It has been demonstrated that the presence of a cyclic amine in the polar part of the lipid A18-Iso5-2DC18 (Fig. 13) ensures binding to the stimulator of interferon genes (STING) protein, leading to the maturation of dendritic cells and exerts an antitumor effect through immune stimulation [73].

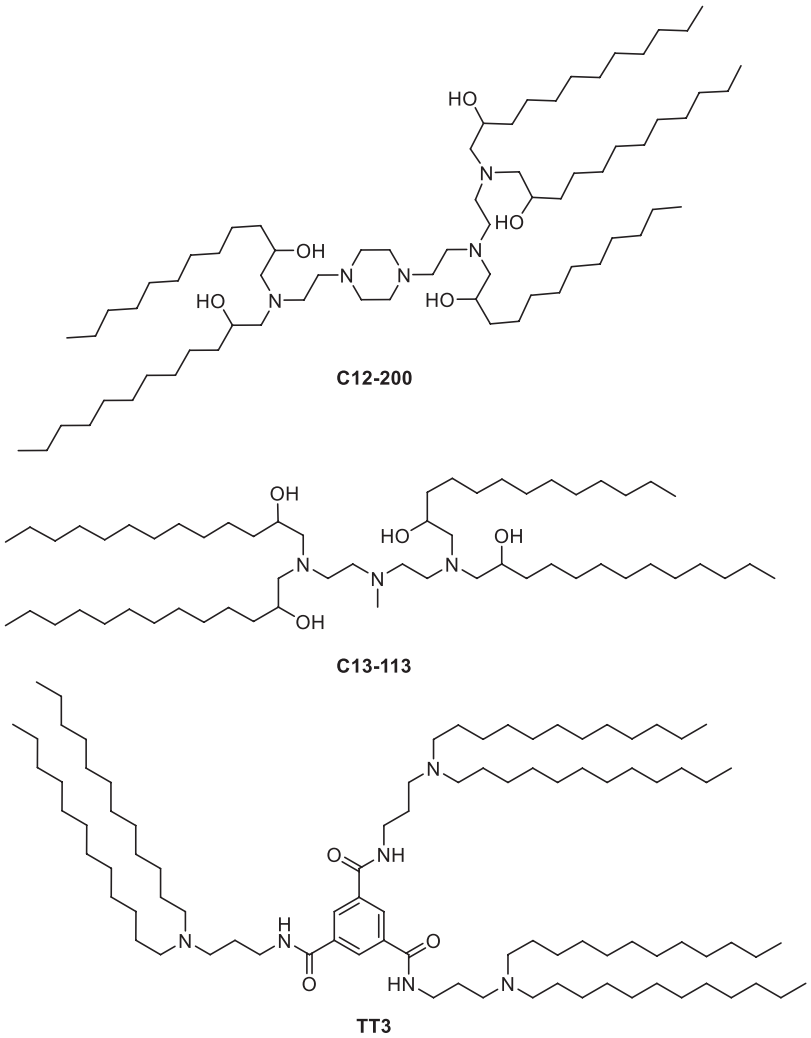

Fig. 11. Symmetric ionizable lipids with branched tails

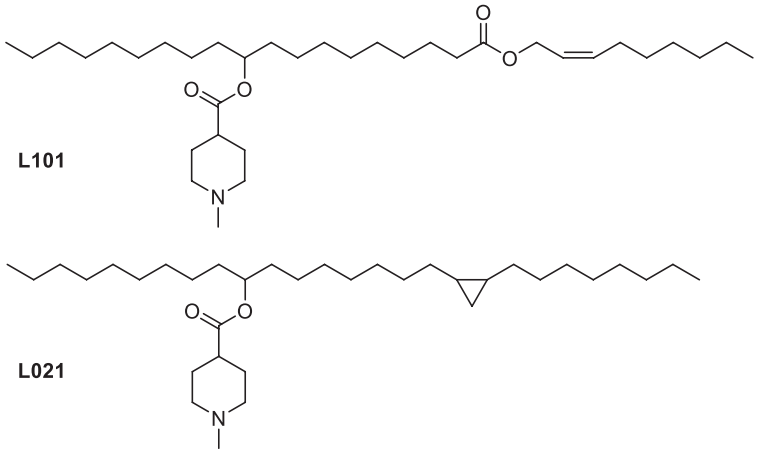

Fig. 12. Methylpiperidine-based ionizable lipids

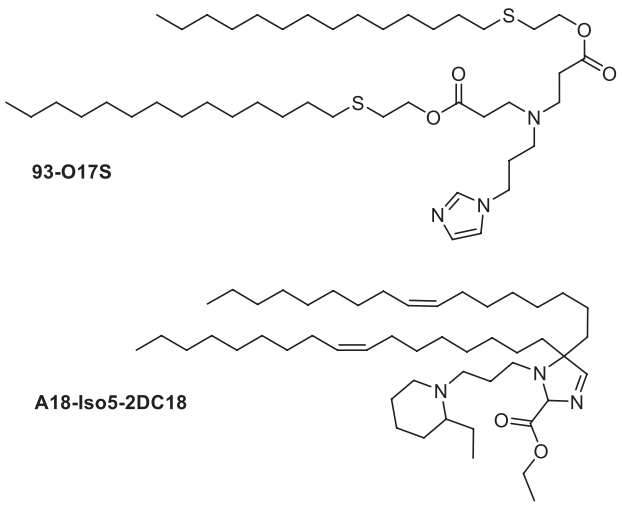

Fig. 13. Imidazole-containing lipids

Gene therapy using ionizable lipids also shows promise for use in fighting drug-resistant bacteria. For example, vitamin-based ionizable lipids (Fig. 14) deliver mRNA encoding antimicrobial peptides and cathepsin B, the accumulation of which in macrophages suppresses the growth and development of bacterial infections resistant to multiple antibiotics. The most effective of these were LNPs obtained from a lipophilic derivative of vitamin C, in combination with DOPE and cholesterol which protected mice from bacterial-induced sepsis [74].

When comparing cationic and ionizable lipids (Table 1), the main difference is the nature of their positive charge which determines the advantages and disadvantages of the two types of lipids. The constant positive charge of cationic lipids ensures the colloidal stability of nanoparticles and effective fusion with

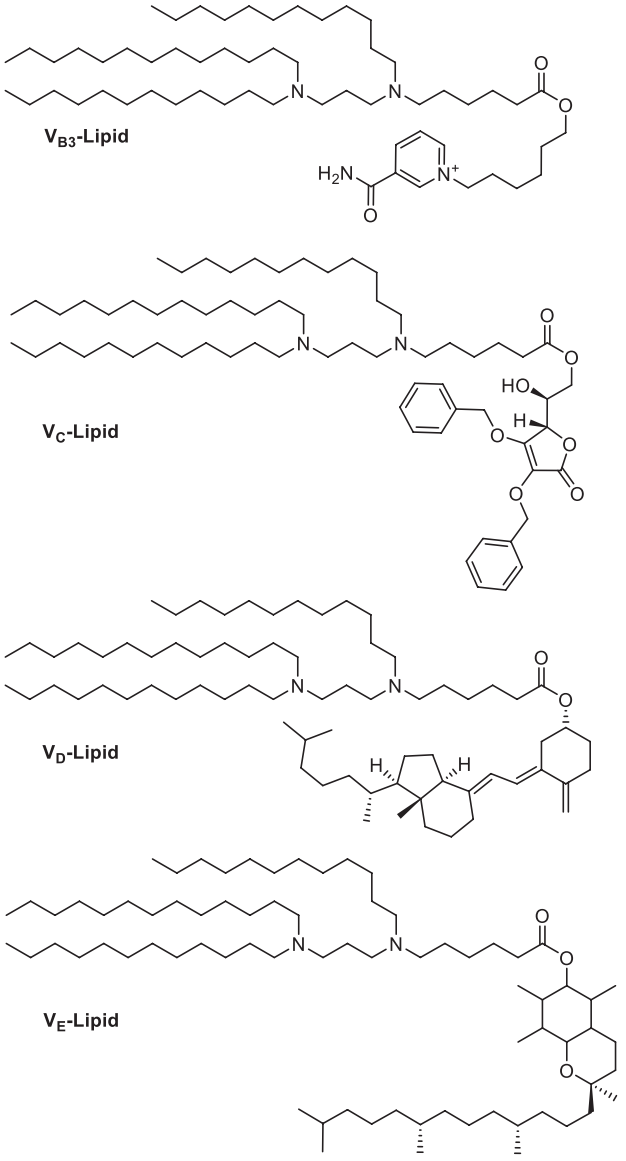

Fig. 14. Vitamin-based ionizable lipids

Table 1. Comparison of cationic and ionizable lipids

Parameter	Cationic lipids	Ionizable lipids
Lipid charge	Constant positive	Neutral, positive at slightly acidic pH
Colloidal stability	High	Average
Cytotoxicity	High	Low
Efficiency of formation of complexes with therapeutic NA	High	High, but complexation requires a slightly acidic buffer
Efficiency cell membrane fusion	High	Average
The most commonly used method of preparation	Lipid film hydration method	Microfluidic technologies
Capital and operating costs for nanoparticle formation	Low	High

the cell membrane. However, cytotoxicity increases proportionally to the charge [44]. In this regard, the application of ionizable lipids, without permanent charge, appears to be safer. An important aspect is the technology used to produce the finished product. As mentioned above, the main method for obtaining LNPs based on ionizable lipids is microfluidic technology which ensures greater reproducibility of nanoparticle characteristics and easy automation. However, it imposes strict requirements on the solubility of lipids in ethanol and increases capital and operating costs [20].

Thus, ionizable lipid-based LNPs are increasingly being used as mRNA delivery systems. Over the past five years, there has been an increase in positive clinical trial results. This in turn has expanded the range of clinically approved drugs for gene and immunotherapy for various diseases, from drug-resistant infections to severe orphan diseases [75].

CLINICAL TRIALS OF MRNA VACCINES

Clinical trials are a mandatory part of drug efficacy and safety studies, necessary for registering a drug and obtaining permission for its use in medical practice. There are four phases of clinical trials [76].

Phase I studies are usually conducted on a small number of healthy volunteers. In the case of highly toxic drugs used to treat seriously or terminally ill patients, studies have been conducted with such patients, for example, those with cancer or acquired immunodeficiency syndrome (AIDS). During Phase I, parameters such as absorption, distribution, metabolism, excretion, as well as the preferred form of administration and safe dosage levels were evaluated. The duration of such studies varies from several weeks to several years.

Several hundred people participate in Phase II clinical trials. An important goal of these studies is to determine the dosage and administration regimen for the next phase of testing. Sometimes Phases I and II are combined, allowing not only the effectiveness of the drug to be determined immediately, but also its safe doses.

Phase III trials are randomized controlled trials involving a large group of patients, up to several thousand people. The purpose of these trials is to confirm the safety and efficacy of the drug as previously assessed in the two previous phases. Phase III trials may also examine the relationship between the effect and the dose of the drug, or the effect of the drug when used in patients with varying degrees of severity or in combination with other drugs.

The final phase of clinical trials is Phase IV, also known as the post-registration phase. Its main purpose is to gather additional information on the safety of the drug in a sufficiently large group over a long period of time.

The percentage of drugs approved for use after clinical trials depends on the requirements of the country's regulatory authority and the field of medicine. The lowest percentage is in oncology. The strictest rules are in the US, where the FDA allows no more than 25–30% of drugs to be sold. In recent years the proportion of approved drugs has been declining, since the requirements for evidence of their efficacy and safety have become more stringent.

To sum up, clinical trials are a very complex and demanding part of the process. They are nevertheless necessary for the clinical application of drugs. In this regard, drugs which have successfully passed them, or

individual phases of research, deserve special attention. Unlike standard drugs, mRNA- and LNP-based drugs undergo more complex clinical trials, since they are two independent compositions brought together. If one of these compositions is unable to confirm its efficacy and safety in any phase, then the trials have to be started all over again. Therefore, the most convenient approach is to test the drug delivery system (LNP, cationic liposomes) and, after confirming its efficacy and safety, proceed to study the therapeutic activity of the nucleic acids loaded into this system.

Despite the existence of a huge number of lipids which have shown excellent efficacy *in vitro* and even *in vivo*, only a few of them have reached clinical trials. Only exceptional compounds have achieved success after Phase I clinical trials. Some have shown such impressive results that they are participating in several trials for mRNA vaccines against various diseases.

In Table 2, we have compiled a list of clinical trials of mRNA vaccines, mainly targeting various viral infections and tumors of different types. Furthermore, research is being conducted on a number of bacterial and parasitic infections, such as malaria.

Some of the mRNA vaccines listed in the table are in the final stages of testing. For example, mRNA-1283 (NCT05815498), an updated vaccine against COVID-19, is currently completing a randomized, double-blind Phase III study to evaluate safety, immunogenicity, and relative efficacy. Preliminary results show that vaccinated individuals of all age groups (12 years and older) have higher antibody titers against both the BA.4/5 strain and earlier strains of the coronavirus, when compared to the mRNA-1273 mRNA vaccine already in use.

Phase III of a randomized, double-blind, placebo-controlled clinical trial of an mRNA vaccine against norovirus infection (mRNA-1403, NCT06592794) has begun. Norovirus causes acute intestinal infection and is highly heterogeneous, Therefore, a multivalent vaccine against the two main genetic groups of the virus, including several strains, is used. Previous phases of the study demonstrated an increase in HBGA-blocking antibody titers on the 29th day after a single vaccination, especially for the genetic group II of norovirus. As a result of truncating the interim analysis data, no risk was identified in the use of this vaccine.

mRNA vaccines have great potential for cancer immunotherapy. Delivery of mRNA encoding a tumor antigen activates the patient's immune system to fight the tumor. For example, mRNA-4157 in combination with pembrolizumab has shown a high level of immunogenicity against various types of solid tumors [77]. Phase III clinical trials (NCT05933577) have begun for melanoma and squamous cell carcinoma.

For the most part, the composition of mRNA vaccines undergoing clinical trials is confidential. The most well-known exceptions, where the components of the drug have been disclosed, are the *Pfizer*/*BioNTech* and *Moderna* vaccines against COVID-19. They used ionizable lipids ALC-0315 and SM-102, respectively (Fig. 6). The lipid ALC-0315 was also used in the mRNA vaccine from *CureVac* (Germany) which was expected to provide longer-lasting immunity compared to Comirnaty® from *Pfizer*/*BioNTech*. However, Phase III clinical trials showed an extremely low level of vaccine efficacy (47%)[2]. Later, Phase III trials began for another COVID-19 vaccine using the same liposomal composition but with a different mRNA design (NCT04860258). As for the SM-102 lipid, it is known to be used in vaccines under development against cytomegalovirus (NCT05085366), Zika virus (NCT04917861), and seasonal influenza virus (A/H1N1, A/H3N2, B/Victoria, and B/Yamagata) for people over 50 [78] (NCT05566639, Table 2).

In 2020, Phase I clinical trials were completed of an mRNA vaccine against Chikungunya virus (CHIKV) based on the DLin-MC3-DMA (MC3) lipid (Fig. 5) developed by *Moderna* (NCT03325075). Furthermore, its mRNA vaccines against Zika virus and H10N8 influenza successfully completed Phase I clinical trials in 2021 [79, 80]. *Patisiran*'s (USA) ALN-TTR02 vaccine against ATTR successfully completed Phase II trials in 2024 (NCT01617967).

Arcturus Therapeutics (USA) chose the lipid ATX-100 (Fig. 6) to deliver mRNA in its vaccines. In 2021, Phase II clinical trials of the COVID-19 vaccine (NCT04480957) were completed. In 2023, Phase I trials for ornithine transcarbamylase deficiency (NCT04442347) and Phase Ib trials for cystic fibrosis (NCT05712538) were successfully completed. In 2024, clinical trials of mRNA vaccines against influenza (NCT06602531, Phase I) and cystic fibrosis (NCT06747858, Phase II) began.

In 2023, Phase I clinical trials of an mRNA vaccine against ATTR were completed. The preliminary results obtained indicate its safety and efficacy. For example, mRNA and CRISPR-Cas9 delivery systems (NCT04601051) targeting transthyretin (TTR) achieved 87% efficacy in reducing serum TTR levels in patients with ATTR [81]. This process was not accompanied by serious side effects, so Phase II trials are planned to start soon [82].

[2] CureVac Provides Update on Phase 2b/3 Trial of First-Generation COVID-19 Vaccine Candidate, CVnCoV – CureVac n.d. https://www.curevac.com/en/curevac-provides-update-on-phase-2b-3-trial-of-first-generation-covid-19-vaccine-candidate-cvncov/. Accessed April 13, 2025.

Table 2. Lipid-based mRNA vaccines on clinical trials

mRNA vaccines	Application	Sponsor	Phase of trials	Status	NCT number
ARCT-021	COVID-19	*Arcturus Therapeutics* (USA)	2	Completed	NCT04480957
ChulaCov19	COVID-19	Chulalongkorn University (Thailand)	2	Completed	NCT04566276
CVnCoV	COVID-19	*CureVac* (Germany)	3	Active	NCT04860258
mRNA-1283	COVID-19	*Moderna* (USA)	3	Completed	NCT05815498
Awcorna	COVID-19	*Walvax Biotechnology* (China)	3	Active	NCT04847102
mRNA-4157	Adjuvant treatment for melanoma	*Moderna* (USA)	3	Active	NCT05933577
mRNA-4157	Adjuvant treatment for non-small cell lung cancer	*Moderna* (USA)	3	Recruiting	NCT06077760
mRNA-1975/1982	Lyme disease	*Moderna* (USA)	1/2	Completed	NCT05975099
mRNA-1893	Zika virus	*Moderna* (USA)	2	Completed	NCT04917861
mRNA-1215	Nipah virus	*Moderna* (USA)	1	Completed	NCT05398796
BNT163	Herpes simplex virus	*BioNTech* (Germany) / University of Pennsylvania (USA)	1	Active	NCT05432583
mRNA-1608	Herpes simplex virus	*Moderna* (USA)	1/2	Completed	NCT06033261
mRNA-1189	Epstein–Barr virus infection	*Moderna* (USA)	1/2	Active	NCT05164094
mRNA-1195	Epstein–Barr virus infection	*Moderna* (USA)	2	Recruiting	NCT06735248
eOD-GT8 60mer mRNA Vaccine and Core-g28v2 60mer mRNA Vaccine	HIV	International AIDS Vaccine Initiative (USA)	1	Active	NCT05001373
G505 MD39.3, BG505 MD39.3 gp151, and BG505 MD39.3 gp151 CD4KO	HIV	National Institute of Allergy and Infectious Diseases (USA)	1	Active	NCT05217641
ARCT-2304	Influenza	*Arcturus Therapeutics* (USA)	1	Active	NCT06602531
mRNA-1010	Influenza	*Moderna* (USA)	3	Completed	NCT05827978
mRNA-1010	Influenza	*Moderna* (USA)	3	Completed	NCT05566639

Table 2. Continued

mRNA vaccines	Application	Sponsor	Phase of trials	Status	NCT number
BNT161	Influenza	*Pfizer* (USA) / *BioNTech* (Germany)	3	Completed	NCT05540522
ARCT-810	Ornithine transcarbamylase deficiency	*Arcturus Therapeutics* (USA)	2	Active	NCT06488313
BNT165	Malaria	*BioNTech* (Germany)	1/2	Active	NCT06069544
BNT113	Metastatic/Recurrent head and neck cancer	*BioNTech* (Germany)	2	Recruiting	NCT04534205
mRNA-3705	Methylmalonic acidemia	*Moderna* (USA)	1/2	Recruiting	NCT04899310
BNT142	Multiple solid tumors	*BioNTech* (Germany)	1/2	Active	NCT05262530
ARCT-032	Cystic fibrosis	*Arcturus Therapeutics* (USA)	2	Active	NCT06747858
ARCT-032	Cystic fibrosis	*Arcturus Therapeutics* (USA)	2	Recruiting	NCT06747858
mRNA-3692/VX-522	Cystic fibrosis	*Moderna* (USA)	1/2	Recruiting	NCT05668741
mRNA-3745	G6Pase Glycogen storage disorder, type 1a	*Moderna* (USA)	1/2	Recruiting	NCT05095727
mRNA-1403/1405	Norovirus	*Moderna* (USA)	3	Active	NCT06592794
BNT167	Shingles	*Pfizer* (USA)	2	Recruiting	NCT05703607
mRNA-4157	Cutaneous squamous cell carcinoma	*Moderna* (USA)	2/3	Active	NCT06295809
mRNA-4157	Renal cell carcinoma	*Moderna* (USA)	2	Active	NCT06307431
BNT111	Advanced melanoma	*BioNTech* (Germany)	2	Active	NCT04526899
mRNA-3927	Propionic acidemia	*Moderna* (USA)	1/2	Recruiting	NCT04159103 NCT05130437
mRNA-4157	Bladder cancer	*Moderna* (USA)	1/2	Recruiting	NCT06305767
ALN-TTR02	Transthyretin amyloidosis	*Alnylam Pharmaceuticals* (USA)	2	Completed	NCT01617967
NTLA-2001	Transthyretin amyloidosis	*Intellia Therapeutics* (USA)	1	Active	NCT04601051
BNT164	Tuberculosis	*Gates Foundation* (USA)	1/2	Active	NCT05547464
mRNA-1647	Cytomegalovirus infection	*Moderna* (USA)	3	Active	NCT05085366
VAL-181388	Chikungunya	*Moderna* (USA)	1	Completed	NCT03325075

In 2023, Phase I clinical trials of an mRNA vaccine against COVID-19 using lipid A9 as a delivery vehicle were completed [29], and in 2024, Phase II clinical trials of an mRNA vaccine against COVID-19 based on lipid CL1 — ChulaCov19 (NCT04566276) [83].

CONCLUSIONS AND PROSPECTS

Gene therapy has enormous potential for treating a wide range of diseases. In recent years, mRNA vaccines, the main component of which are ionizable lipids, have attracted the most interest. Unlike cationic lipids, ionizable lipids do not have a permanent positive charge, which reduces the risks of cytotoxicity and binding to blood serum proteins. Lipids are used to form cationic liposomes or LNPs which ensure effective delivery of therapeutic nucleic acids.

Both types of lipids consist of the following components: (1) a hydrophobic domain necessary for fusion with the cell membrane; (2) a polar domain for binding and protecting the drug; (3) a spacer for separating the hydrophobic and polar domains in space; as well as (4) linkers which bind these components together. Furthermore, the combination of hydrophobic and polar domains provides the amphiphilic properties necessary for the formation of nanoparticles in an aqueous environment. All these components also influence other properties of nanoparticles: size, stability, cytotoxicity, and mRNA delivery efficiency.

The hydrophobic domain consists of alkyl tails (1 to 5), but less commonly sterol residues (cholesterol, sitosterol, tocopherol) (Fig. 3). The optimal length of alkyl tails, providing a reasonable balance between cytotoxicity and nucleic acid delivery efficiency, is 14–18 carbon atoms (Fig. 4). Branched alkyl substituents (Fig. 5), including asymmetric ones (Fig. 6), have proven themselves well in mRNA delivery. In this case, the branching point can be either a carbon atom (Fig. 12) or a nitrogen atom (Fig. 14).

The polar domain can be cationic or ionizable. In the case of cationic lipids, the best results are demonstrated by structures with a distributed charge system based on polyamines, especially spermine (Fig. 3). With an optimal positive charge/cytotoxicity ratio, such lipids with a constant positive charge show the highest mRNA delivery efficiency. However, ionizable lipids are becoming more promising for clinical application. Heterocycles (e.g., imidazole or piperidine) (Figs. 12, 13) and nitrogen atoms with a closely located electron-accepting group which lowers pK_a (Figs. 5, 7, 9) act as ionizable groups. Hydroxyalkyl groups with 2 to 4 carbon atoms perform well as electron acceptors (Fig. 6). Furthermore, amide or ester linkers are electron acceptors.

Since the close proximity of hydrophobic and polar domains can interfere with nanoparticle formation, it is recommended that a hydrocarbon spacer 3–8 carbon atoms long be included in the lipid structure, especially in the presence of bulky functional groups. Individual structural elements of lipids are connected by linkers. They facilitate the synthesis of the compound and subsequently play an important role in the metabolism of lipids in the body. Therefore, it is important to maintain a balance between the stability of the compound in the bloodstream and tissues and its biocompatibility. After delivery, lipids must be broken down into simple fragments which can be easily utilized by the excretory system. The most “balanced” linkers are disulfide and carbamate linkers. Linkers based on ether bonds are more stable but can increase cytotoxicity. Ester linkers, on the other hand, do not cause side effects, but do not always provide sufficient stability.

Thus, lipids, primarily ionizable ones, form one of the main components of mRNA vaccines. Their modular structure determines the further behavior and properties of nanoparticles. Platforms for delivering mRNA in vaccines against COVID-19, influenza, and various tumors and genetic diseases have been developed based on ionizable lipids (Table 2). mRNA vaccines against COVID-19 and respiratory syncytial virus have already been approved for use. Many others are undergoing clinical trials.

Most mRNA vaccines under development use the principles of replacement gene therapy, where normally functioning mRNA is delivered, ensuring the synthesis of the desired protein for only a limited time. The administered mRNA gradually degrades. In the treatment of viral or tumor diseases, one or more injections are usually sufficient to prevent infection or achieve recovery (remission). Treatment of a hereditary disease using this approach will require lifelong administration of the drug. The solution to this problem may be a transition to corrective gene therapy, in which a malfunctioning copy of the gene is corrected at a specific point. The most promising tool for such genome editing is CRISPR-Cas technology. An important milestone are Phase I clinical trials of a drug for the treatment of transthyretin amyloidosis without the need for repeated therapy (NCT04601051). In this study, LNPs deliver mRNA encoding the Cas9 protein and single-stranded guide RNA targeting the transthyretin gene. Preliminary results show that this composition reduces the amount of amyloid in patients after a single application. It can be assumed that with the

increase in the selectivity of genome editing and the efficiency of delivery of the CRISPR-Cas nucleic acid complex, this approach will be widely introduced into the clinical practice of treating hereditary diseases.

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The authors declare no conflicts of interest.

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