Review Article

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mRNA-based modalities for infectious disease management

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ABSTRACT

The novel coronavirus disease 2019 (COVID-19) is still rampant all over the world, causing incalculable losses to the world. Major pharmaceutical organizations around the globe are focusing on vaccine research and drug development to prevent further damage caused by the pandemic. The messenger RNA (mRNA) technology has got ample of attention after the success of the two very effective mRNA vaccines during the recent pandemic of COVID-19. mRNA vaccine has been promoted to the core stage of pharmaceutical industry, and the rapid development of mRNA technology has exceeded expectations. Beyond COVID-19, the mRNA vaccine has been tested for various infectious diseases and undergoing clinical trials. Due to the ability of constant mutation, the viral infections demand abrupt responses and immediate production, and therefore mRNA-based technology offers best answers to sudden outbreaks. The need for mRNA-based vaccine became more obvious due to the recent emergence of new Omicron variant. In this review, we summarized the unique properties of mRNA-based vaccines for infectious diseases, delivery technologies, discussed current challenges, and highlighted the prospects of this promising technology in the future. We also discussed various clinical studies as well preclinical studies conducted on mRNA therapeutics for diverse infectious diseases.

KEYWORDS

messenger RNA (mRNA) vaccine, lipid-nanoparticle, infectious disease, drug delivery, coronavirus disease 2019 (COVID-19), virus

1 Introduction

Developing preventive or therapeutic vaccines against infectious pathogens is the most effective means to prevent and curb the epidemics [1]. Traditional vaccines play a decisive role in diseases prevention. However, they show limited efficiency against various viruses such as respiratory syncytial virus (RSV) and human immunodeficiency virus (HIV), which caused great harm to human health. In addition, in the face of sudden and fast spreading coronavirus disease 2019 (COVID-19), the shortcomings of traditional vaccines became highlighted due to their time-consuming and complex developmental processes. Therefore, it is urgent to focus on the development of novel techniques, optimize the designing process of the vaccine as well as enhancing its effectiveness. Many scientists had been working on the mRNA technology for decades before the COVID-19 pandemic led to its breakthrough. In 1990, scientists found that *in vitro* transcribed mRNA showed significant activity and expressed proteins in a dose-dependent manner after injected into the mice [2]. This direct injection of mRNA expresses specific proteins triggering an immune response, which is the prototype of mRNA therapy. RNA based vaccines have several advantages, including high potency, potential for safe administration, low-cost manufacturing, and capacity of rapid development, which paves the way of a capable alternative to traditional-based vaccine approaches [3]. In subsequent studies, although animal experiments have shown that the mRNA can play a role similar with vaccine to achieve therapeutic purposes. However, due to the technical limitations, there were bottlenecks in mRNA stability, drug delivery, and safety. Due to these bottlenecks, more researchers turned to the field of DNA and alternative proteins. However, during the past three decades, the synthesis of mRNA development as well as the modification and delivery platforms made the mRNA therapy back to the prospect of bio-pharmaceutical companies. Without genetic mutation, mRNA became preferable and safer option compared to DNA. It is possible to upgrade the half-life of mRNA by regulating sequence modification and delivery vector. Some of the most key advantages of mRNA vaccine are its great flexibility, easy designing, and large-scale production [4]. Reports suggested that the mRNA-based vaccine causes robust CD4+ or CD8+ T cell response compared to protein immunization [5]. It has been

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reported elsewhere that the mRNA vaccine produces antibodies in animals after administering one or two low-doses [6]. Moreover, mRNA vaccine showed good safety profile in animal studies. The fast and ease features in designing and large-scale production are appropriate for rapid and flexible response to sudden outbreaks, and the relatively simple manufacturing process is also suitable for quality control procedures [7–10]. Numerous preclinical animal studies and the response of the COVID-19 mRNA vaccine in humans have proved the efficacy of the mRNA vaccine against infectious viruses. The landscape of mRNA discovery and its administration in humans at large are shown in Fig. 1.

2 mRNA characteristics and delivery systems

2.1 Overview of mRNA vaccine

mRNA-based therapy is using chemically modified mRNA molecules to enter the cytoplasm, transcribe and express their own nucleotides in the cytoplasm, and generate proteins needed by the body [11]. Compared with inactivated vaccine and recombinant protein vaccine, mRNA vaccine can activate strong cellular immunity [12] (Fig. 2).

On the other hand, the mRNA vaccines express proteins directly in cytoplasm, compared to DNA vaccines. The expression of proteins in cytoplasm does not need to cross cell nuclear, therefore the potential risk of insertion mutation is eliminated [13]. For these reasons, the mRNA vaccine is considered a relatively safe form of vaccine in comparison to others. We can use linear plasmid DNA containing antigen coding sequence as a template to transcribe mRNA *in vitro*. In short, an open reading frames (ORF) with protein coding function is prepared by *in vitro* transcribed (IVT) RNA using RNA polymerase and DNA template [14]. At present, there are two types of RNA used to manufacture mRNA vaccines such as the self-amplifying and non-replicating mRNA [3, 15, 16]. Both the self-amplifying and non-replicating mRNA vaccine types have been effectively used against infectious diseases, particularly for COVID-19, while various mRNA vaccine candidates are under clinical trials and preclinical stages. The antigen encoded by conventional mRNA vaccine contains 5′ untranslated and 3′ untranslated regions (UTRs). Furthermore, the self-amplified RNA encodes both the antigens and the sequences, which are related to the virus-replication process. Therefore, it can replicate inside the cells and expresses protein required for immune response [17]. Once mRNA moves into the cytoplasm, the cell translation-mechanism assembles the sequence of the amino-acid guided by the mRNA, carries out post-translational variation, and folds to form functional proteins properly. These pharmacological features endow mRNA with unmatched benefits in vaccine-based therapy. RNA-based sequence engineering technology is making the synthetic mRNA as an improved translation function than the earlier ones. Efficient and low toxicity vectors improve the *in vivo* expression of antigen significantly. During the last several years, various mRNA vaccine candidates have made huge progress in immunogenicity and efficacy [18–21].

2.2 mRNA structure and biological functions

The coding region is the main component of mature mRNA, and there are non-coding regions on the 5′ upstream and 3′ downstream as well. Eukaryotic mRNA molecules are composed of an ORF flanked by UTR, 5′ 7-methylguanosine triphosphate (m7G) cap, and 3′ poly(A) tail structures (Fig. 3).

Although naked mRNA has been used for *in vivo* immunizations successfully [22–25], however, there are still many challenges in mRNA-based therapy. Exogenous mRNA will stimulate defense mechanism of the body, thereby quickly degrades, and does not maintain stability inside the cells [26]. Moreover, naked mRNA is also easily degraded by extracellular RNases; therefore, many chemical modifications and vectors are used to improve the stability and uptake of mRNA [27–31]. Researchers have explored many chemical modifications to improve the stability of mRNA structures as well as reduce their immune response. The half-life of mRNA, the pharmacokinetics of protein expression, and immunogenicity can be purified by modifying 5' and 3' UTR, optimizing poly(A) tail length, incorporating modified nucleosides, and using various capping strategies [32]. The 5'-cap participates in RNA translation are crucial for recruitment of the translation machinery and initiation of protein synthesis via directly binding to eukaryotic initiation factor 4E (eIF4E), a fundamental effector and rate limiting element of protein synthesis [33]. For the modification of 5'-cap, cap analogues and capping enzymes [34, 35] were synthetized to

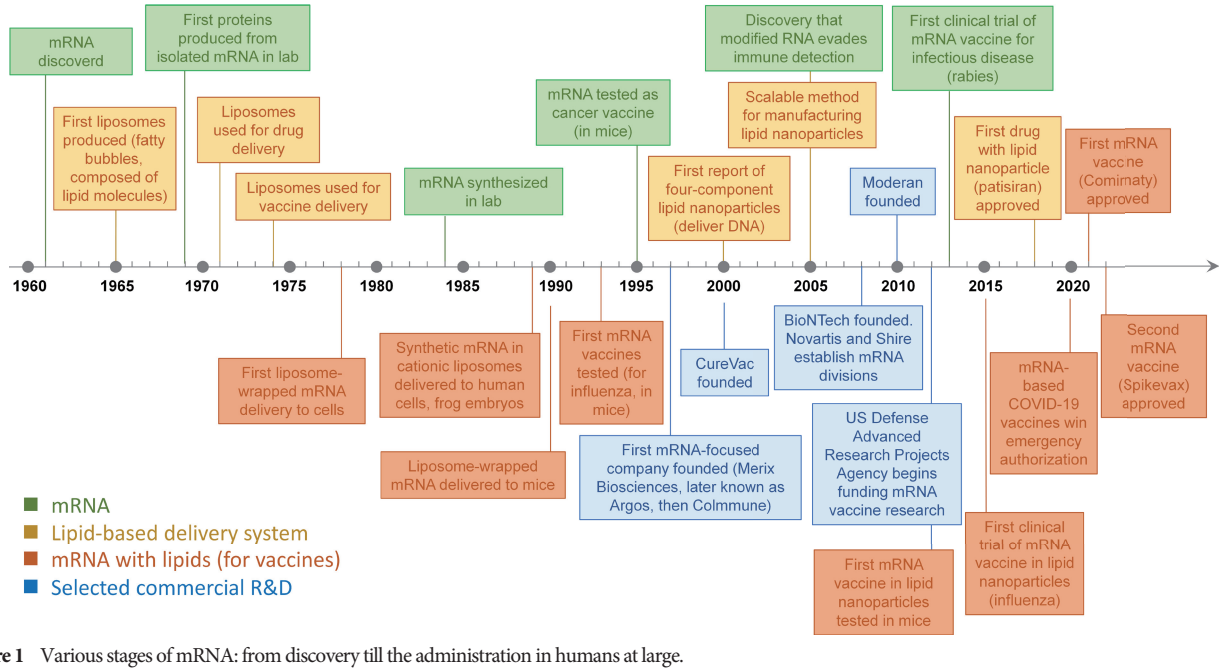

Figure 1 Various stages of mRNA: from discovery till the administration in humans at large.

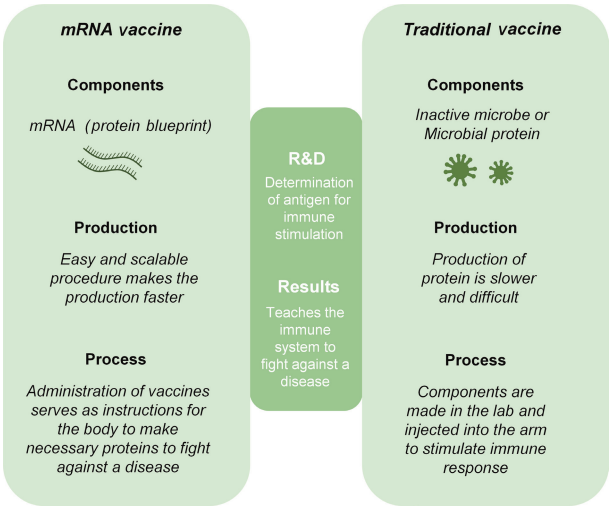

Figure 2 Comparison of mRNA vaccine and traditional vaccine.

stabilize mRNA and increase protein translation. In addition, UTRs can improve the stability of the complex during transcription [36], so that the artificially manufactured mRNA can play the role of transcriptional assembly like mature mRNA molecules. Introduction of stabilizing elements into UTRs also improves the half-life of mRNA and the α- and β-globin are the most important factors [37]. It was demonstrated that the longer tails increase the protein expression in various cell types [38]. However, some scientists also suggested that the longer poly(A) tail is not the better strategy [39, 40]. It is worth noting that the different codon optimization strategies should be practice to improve the translation rate of mRNA and ensure the quality of expressed antigens. In order to obtain the high therapeutic efficiency, optimization of the combination of mRNA regions is necessary.

2.3 Delivery strategies for mRNA

Since the naked mRNA is easily degraded in the body, therefore the efficient mRNA delivery is the guarantee of vaccine with high efficacy. Generally, vaccines effectively target antigen-presenting cells via subcutaneous injection, intradermal injection, or intramuscular injection, while naked mRNA has been studied after direct intravascular administration [25, 41, 42]. Some physical methods were also investigated to deliver mRNA into cells, such as gene gun [43] and electroporation [44]. In addition, vast majority of carriers are used for mRNA delivery [26], which can protect mRNA from degradation. The mRNA vaccine encapsulated by delivery carrier: After injection into the body, the mRNA encapsulated liposomes are endocytosis into the cell, and the mRNA is released in the cell, using the organelles of body to translate and express antigen proteins, and stimulate the body to produce an immune response. In this section, we summarized various mRNA delivery systems and made future directions and challenges in advancing this promising vaccine platform to widespread therapeutic use.

2.3.1 Lipid and lipidoid based delivery system

Liposomes are spherical vesicles composed of a single or multilayer phospholipid bilayer containing a water-based core containing target genes. Positively charged cationic lipids can interact with negatively charged mRNA for delivery purposes. BioNTech developed lipoplexes (LPX) delivery system, which embeds mRNA between lipid bilayers, to delivery mRNA to dendritic cells.

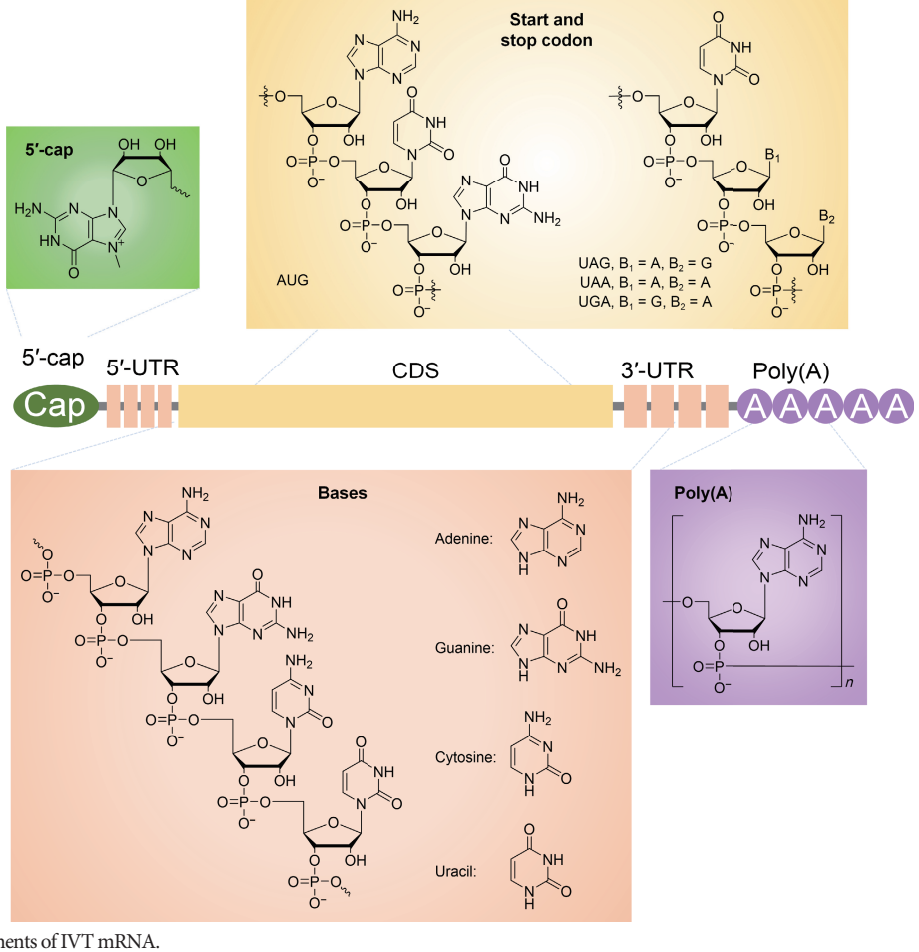

Figure 3 Structural elements of IVT mRNA.

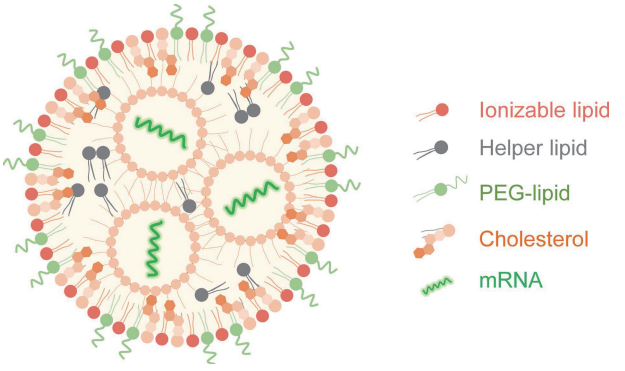

Figure 4 Schematic illustration of mRNA-LNP.

Lipid nanoparticle (LNP) is one of the most studied mRNA delivery systems (Fig. 4). Arbutus Biopharma Corporation is the pioneer of LNPs delivery technology, which was originally designed to deliver hepatitis-B RNAi drugs. LNPs were initially shown to be safe and effective in the delivery of siRNA [45–47]. Inspired by siRNA delivery, LNP delivery technology was favored by many mRNA development companies, such as Moderna, CureVac, and BioNTech. For example, LNP delivery system is also used for the three COVID-19 mRNA vaccine delivery systems. BioNTech has replaced its original LPX delivery system with LNP delivery system, and Moderna has also used LNP delivery system for COVID-19 vaccine research. In addition to protecting mRNA, LNPs also promote cellular uptake, enhance endosomal escape, protect mRNA molecules from being recognized by Toll-like receptors (TLRs), and avoid the role of innate immune system over-activation. LNP formulations are typically composed by four parts: amine-containing lipid or lipid-like material, helper phospholipid, cholesterol, and lipid-anchored polyethylene glycol [46, 48–51].

Amine-containing lipid or lipidoid materials play crucial roles in LNP delivery systems [52]. Among them, ionizable amino lipid with the pKa is usually between 6 and 7, which is positively charged at low pH and can promote carrier self-assembly and endosomal release of mRNA. During the preparation of LNP, the positively charged lipids interact with negatively charged mRNA, promoting the self-assembly of LNP. Once entering the endosomes with the low pH, the positively charged lipid interacts with the ionic endosomal membrane, facilitating membrane destabilization and facilitating mRNA release from both LNPs and endosome. A series of lipid or lipidoid molecules have been developed for mRNA delivery (Fig. 5). Common ionizable lipids include KC2, Dlin-MC3-DMA [53], Lipid 319 [54], C12-200 [55], 5A2-SC8 [56], etc. The Dlin-MC3-DMA was used as ionizable lipid to deliver mRNA, and many pre-clinical and clinical studies have been conducted [57]. Jayaraman et al. [58] reported Dlin-MC3-DMA with highest nucleic acid delivery potency and supported pKa (6.44) value as the dominant factor for siRNA activity. Arteta et al. indicated that Dlin-MC3-DMA-formulated mRNA-LNPs can be administered to rats and monkeys intravenously, and produced EPO protein [59]. Robinson and colleagues realized the treatment of cystic fibrosis with mRNA-LNPs [60]. Kauffman et al. optimized erythropoietin-mRNA-loaded C12-200 lipid nanoparticles which increased the potency of mRNA with 7-fold change. A formulation containing the cationic lipidoid C12-200 was developed for mRNA delivery in human disease mouse model [61]. Cas9 mRNA was encapsulated to C12-200 based LNPs to induce repair of a disease gene in adult animals [62]. Zwitterionic lipids are commonly used to improve ion pairing with membrane phospholipids, and modulate fluidity of particle [63]. While, cholesterol (chol) primarily acts as a stabilizer, and polyethylene glycol (PEG) lipids are commonly used to enhance systemic circulation half-life of vaccines and improve surface hydration. Moderna Inc. developed another ionizable lipid SM-102, the tail of which has larger branches to increase its effectiveness [64]. BioNTech uses ALC-0315 [65] as the core ionizable lipid, which has analogical functional groups in their structure. It was found that the diolefin tail in MC3 was slowly degraded, and repeated administration had potential cumulative toxicity. The improvement of core lipids on Moderna and BioNTech is the basis for successful approval of COVID-19 vaccine and promotes the research progress of mRNA vaccine in other infectious diseases. The principle of RNA delivery by LNP is not completely clear, but it is believed that the LNP is absorbed through non-covalent affinity and endocytosis of cell membrane. It is worth noting that the LNP can also be excreted from cells through opposite exocytosis.

2.3.2 Polymer based delivery system

Polymer have shown considerable potential in efficient mRNA delivery (Fig. 6) [66, 67]. In the design of polymer as carrier, the molecular weight and charge need to be considered first. Cationic polymers combine with mRNA to form nanoparticles through electrostatic interaction. Too large carrier molecules and charge both usually led to strong stable binding between carrier and mRNA molecules, resulting in lower efficiency of mRNA expression. Polyethylenimine (PEI) is the earliest polymer that widely used in RNA delivery [68, 69]. It was reported that PEI was applied to package self-amplifying mRNA encoding influenza virus hemagglutinin and nucleocapsid to protect people from virus infection [70]. In addition, many different polymers [71–73] have been synthesized and used alone or together with other lipids to deliver mRNA. In addition, it was reported that oligomers can non-covalently deliver mRNA, achieving highly efficient protein translation. Compared with lipids, polymers are relatively unpopular in the development of nucleic acid therapeutics, due to the complexity and uncontrollability of molecular preparation [74]. In addition, many polymers displayed high toxicity *in vivo*, which further limits the wide use of polymers.

Stemirna therapeutic is developing the lipopolyplex (LPP) nano-delivery platform for mRNA-based therapeutic. LPP is a double-layer structure with polymer-coated mRNA as the core and phospholipid as the shell, which exhibits better results at mRNA protection compared with LNPs. CureVac has a polymer-based delivery system that uses a proprietary PEG-based polymer system Curevac carrier molecule (CVCM) to deliver therapeutic drug candidates to the eye and lung. McKinlay et al. reported a tunable and effective material: charge-altering releasable transporters (CARTs) for mRNA delivery [102]. Cationic CARTs bind to RNA through electrostatic interactions initially and deliver mRNA into cellular and then change physical properties, leading to mRNA release and protein translation. Patel et al. synthesized DD90-188, hyperbranched poly(beta amino esters) (hPBAEs), to deliver mRNA to lung. Luciferase mRNA targets to the lung and produces protein after 24 h inhalation [103]. Jeught et al. explored an advanced hybrid lipid polymer shell mRNA nanoparticle (lipopolyplex) as a delivery vehicle for systemic mRNA vaccination. For the synthesis, mRNA complex to a cationic polypeptide PEG-HpK, and then mix with TriMan-liposome to form a hybrid lipid-shell polymer core mRNA nanoparticle (LPR). LPR exhibited excellent hemocompatibility and largely restricted mRNA expression to splenic antigen presenting cells upon systemic administration [104].

2.3.3 Other delivery system

Protamine is a natural cationic protein, which can load negatively

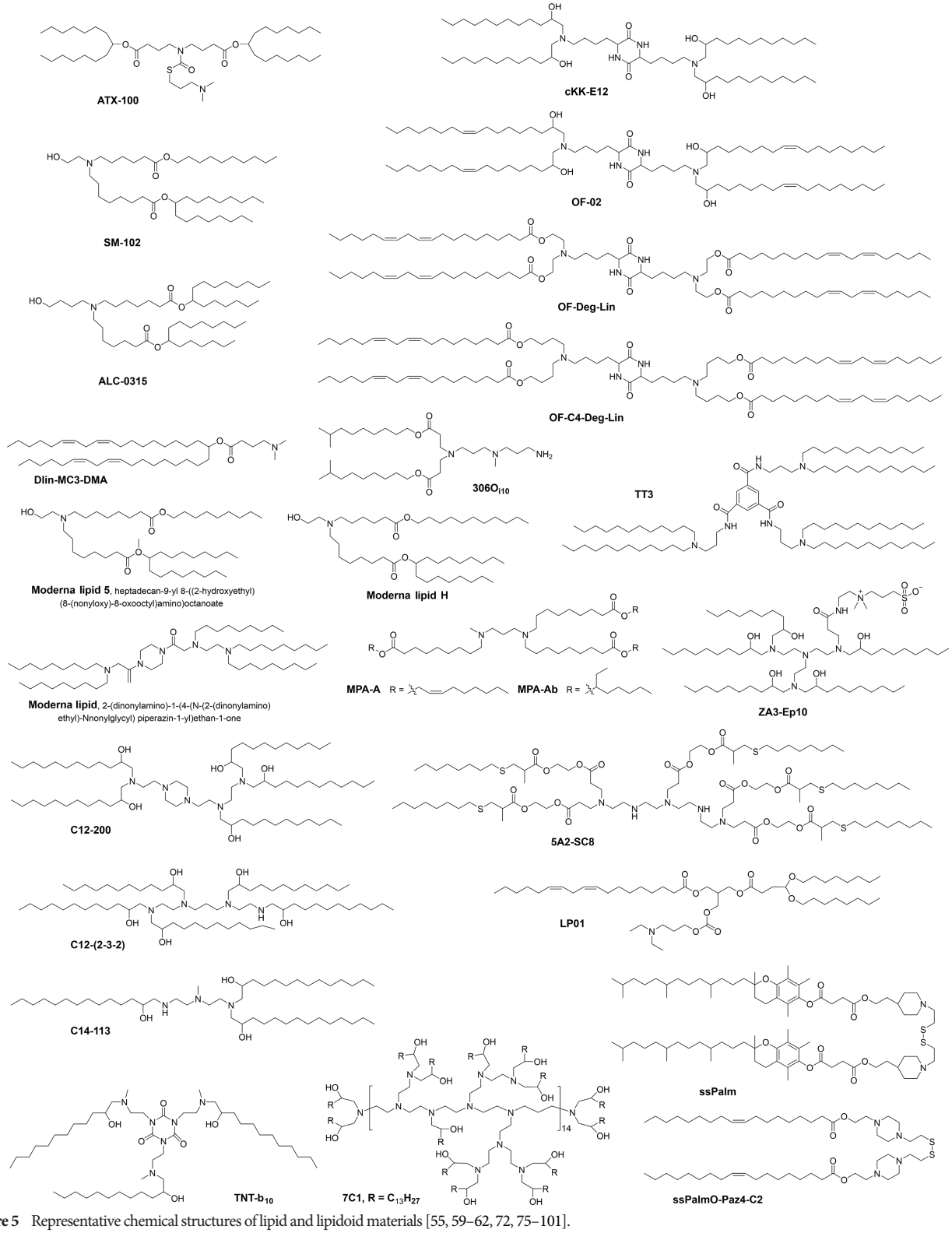

Figure 5 Representative chemical structures of lipid and lipidoid materials [55, 59–62, 72, 75–101].

charged mRNA molecules into nano nucleic acid particles [105]. In a study, the results taken via dynamic light scattering (DLS) showed that the size of free mRNA was close to 50 nm, whereas after complexation, the size ranges between 250 and 350 nm [106]. It has been proved that the protamine protects mRNA from RNase degradation as well as can cause strong immune response from the immune cells (such as dendritic cell, monocytes, B-cells, natural killer cells, and neutrophils). Protamine-mRNA complex is obtained through RNA activity platform, in which protamine can induce Th1 cell response as TLR7/8 antagonist, and the mRNA can express target protein and induce specific immune response. However, research also showed that protamine reduces the expression of protein, which may be caused by its tight assembly with mRNA. In addition, the ratio of protamine to mRNA also affects the expression of antigen [107]. For this reason, protamine is now commonly used only as an adjuvant in vaccines. CureVac adopted this method for their vaccine candidate against rabies termed as CV7201, which is a freeze-dried and temperature stable non-modified mRNA composed of free and protamine complex mRNA encoding rabies virus glycoprotein (RABV-g) [106]. But this vaccine has been suspended due to the inadequate immunogenicity in clinical trials.

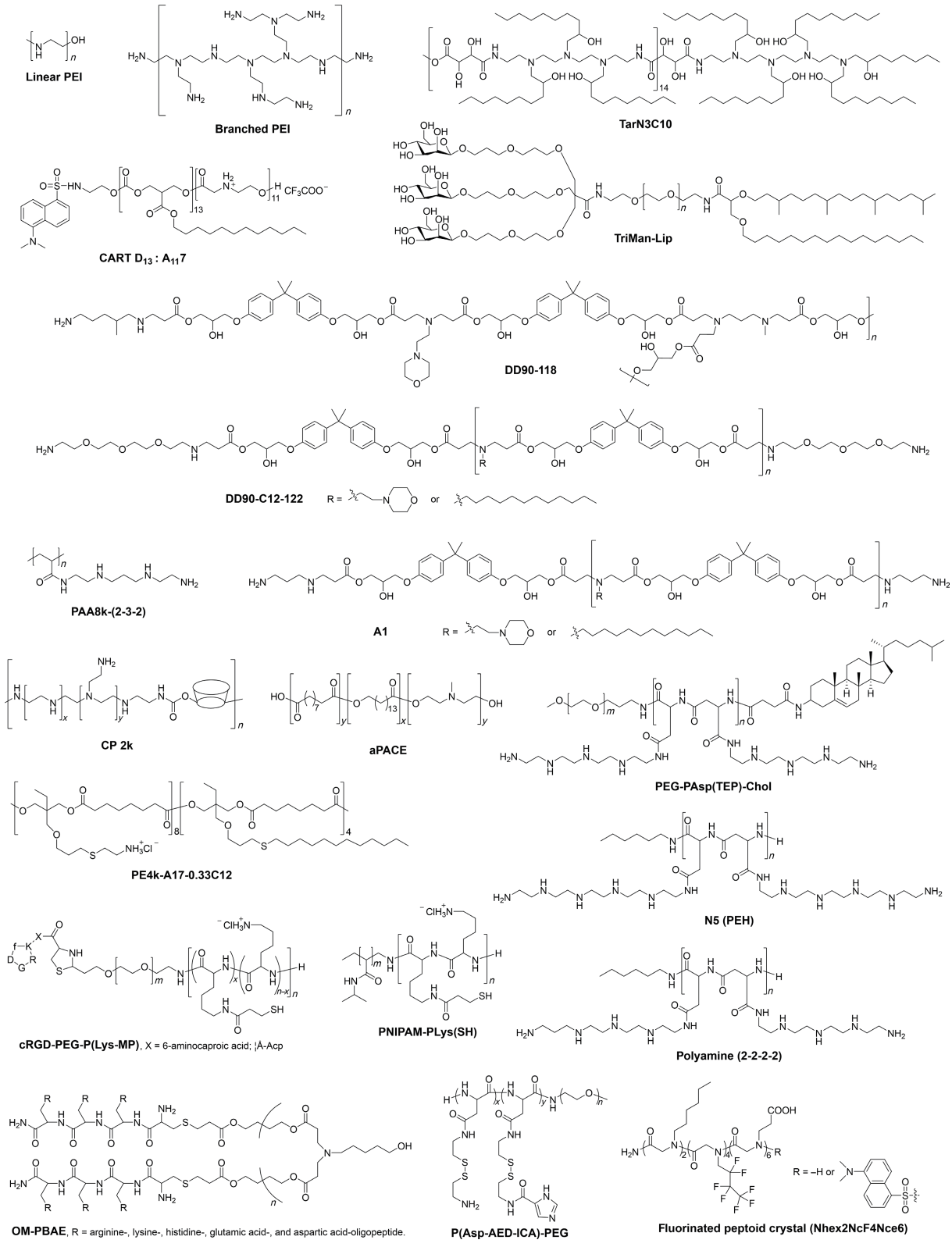

Figure 6 Representative chemical structures of polymer and hybrid materials [27, 66, 71, 102–104, 112–127].

2.4 Progress in pre-clinical and clinical research of mRNA vaccine for infectious disease

Clinical reports suggested that the mRNA vaccines against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) have shown significant results compared to traditional inactivated counterparts as well as adjuvant protein-subunit vaccines [108]. These results further strengthened the promise of mRNA vaccine. The available vaccines against COVID-19 in clinical practice include replicating and non-replicating viral vectors, subunit vaccines, virus-like particles (VLPs), live-attenuated vaccines, DNA and RNA vaccines, and inactivated vaccines. In this section, we have explained the mRNA-based vaccines against COVID-19 in pre-clinical stage (Table 1) and clinical stage (Table 2). In addition to the prevention of COVID-19, the mRNA vaccines developed for other infectious diseases are mainly targeted at influenza, RSV, rabies virus, and HIV, among which the fastest progress is Moderna's prevention of cytomegalovirus (CMV) infection via mRNA-1647 candidate. The parainfluenza virus vaccines, RSV vaccines, Chikungunya virus vaccines, and Zika

Table 1 Progress in mRNA vaccine undergoing clinical trials for various infection except COVID-19

Name	Developers	Phase trials	Trial number	mRNA type	Source
			RSV		
		Phase 1	NCT04528719		
mRNA-1345	ModernaTX, Inc.	Phase 2	NCT05127434	Modified	https://www.modernatx.com
		Phase 3	Approved and will start soon		
			Flu vaccine		
mRNA-1010	ModernaTX, Inc.	Phase 2	NCT05333289	Modified	
mRNA-1020	ModernaTX, Inc.	Phase 2	NCT05333289	Modified	https://www.modernatx.com
mRNA-1030	ModernaTX, Inc.	Phase 2	NCT05333289	Modified	
BNT161	BioNTech/Pfizer	Phase 1	NCT05052697	Modified	https://biontech.de/
			COVID + flu + RSV vaccine		
mRNA-1230	ModernaTX, Inc.	Early stages	ND[a]	Modified	https://www.modernatx.com
			COVID + flu vaccine		
mRNA-1073	ModernaTX, Inc.	Phase 1	Expected to begin in 2022	Modified	https://www.modernatx.com
			CMV vaccine		
mRNA-1647	ModernaTX, Inc.	Phase 3	NCT05085366	Modified	https://www.modernatx.com
			Rabies virus		
CV7202	CureVac	Phase 1	NCT03713086	Non-modified	https://www.curevac.com
GSK3903133A	GSK	Phase 1	NCT04062669	Self-amplifying	NCT04062669
			Herpes simplex virus (HSV) vaccine		
mRNA-1608	ModernaTX, Inc.	Phase 1	NCT04762511	Modified	https://www.modernatx.com
			HIV vaccine		
mRNA-1644	ModernaTX, Inc.	Phase 1	NCT05001373	Modified	https://www.modernatx.com
mRNA-1574	ModernaTX, Inc.	Phase 1	NCT05217641	Modified	
			Zika vaccine		
mRNA-1893	ModernaTX, Inc.	Phase 1	NCT04064905	Modified	https://www.modernatx.com
		Phase 2	NCT04917861		
			Nipah vaccine		
mRNA-1215	ModernaTX, Inc.	ND[a]	ND[a]	Modified	https://www.modernatx.com
			EVB vaccine		
mRNA-1189	ModernaTX, Inc.	Phase 1	NCT05164094	Modified	https://www.modernatx.com
			Chikungunya virus		
mRNA-1944-P101	ModernaTX, Inc.	Phase 1	NCT03829384	Modified	https://www.modernatx.com

[a]ND, no data.

virus vaccines are also in clinical stages [109]. Various mRNA vaccine candidates for diverse infectious diseases are listed in Table 3 and Table 4.

2.4.1 mRNA vaccines for SARS-CoV-2

Until 13 May 2022, there are 25 mRNA-based vaccine candidates are in pre-clinical evaluation, and 30 were in clinical evaluation. The mRNA-based vaccines currently in clinical trials mostly encode full-length spike protein (S protein) of SARS-CoV-2. One of the vaccines, BNT-162b2, developed by Germany and USA based organization named BioNTech and Pfizer, respectively. The BNT-162b2 became the first mRNA vaccine against COVID-19, which got approved by the United States Food and Drug Administration (U.S. FDA), marking a milestone. The mRNA-1273, developed by Moderna Inc., became the other leading COVOD-19 vaccine got huge acceptance around the world to fight against COVID-19. Regardless of global acceptability, the two mRNA vaccines for COVID-19 were also evaluated for possible side effects. Therefore, two rare side effects myocarditis and pericarditis were found especially in the USA and Canada. These side effects were commonly reported in males under the age of 30 within one week of their second vaccination dose. Moreover, these effects were observed with Moderna Spikevax®, while less pragmatic with vaccine manufactured by PfzerBioNTech Comirnaty®. The common symptoms include shortness of breath, pain in the chest, and palpitations. However, the American Heart Association reported a study which revealed that these side effects in young people are mild and improved quickly with minimum care [110].

In addition to BNT-162b2 and mRNA-1273, we also discussed several other mRNA-based vaccines which are currently in relatively rapid development against COVID-19 and various infectious diseases.

(1) BNT-162b2

On 24 August 2021, the U.S. FDA has approved a biologics license

Table 2 mRNA vaccines in preclinical stage for COVID-19 and other infectious disease

Organization	Target pathogen	Generic name	Global stage	Latest update	Source
Shenzhen NeoCura Biotechnology	Anti-viral (unspecified)	NeoCura PV002	Pre-clinical	1/26/2022	http://www.neocura.net/bk_24848574.html
Sanofi	Chlamydia	Chlamydia vaccine	Pre-clinical	3/30/2022	https://www.sanofi.com
BioNet-Asia	SARS-CoV-2 (multiple variants)	COVID-19 mRNA vaccine	Pre-clinical	2/2/2022	https://bionet-asia.com
Afrigen Biologics	SARS-CoV-2	COVID-19 vaccine	Pre-clinical	3/7/2022	https://medicinespatentpool.org
Argorna Pharmaceuticals	SARS-CoV-2	COVID-19 vaccine	Pre-clinical	3/28/2022	http://www.chictr.org.cn/showproj.aspx?proj=162513
CanSino Biologics	SARS-CoV-2	COVID-19 vaccine	Pre-clinical	4/5/2022	https://www.prnewswire.com
CSPC Pharmaceutical	SARS-CoV-2	SYS 6006	Pre-clinical	4/14/2022	https://doc.irasia.com/listco/hk/cspc/announcement/a220403.pdf
CureVac(Collaboration with UK government)	SARS-CoV-2	CureVac-1	Pre-clinical	2/14/2022	https://www.curevac.com
Eyam Vaccines	SARS-CoV-2	COVID-19 vaccine	Pre-clinical	9/27/2021	https://eyamhealth.com
Globe Biotech	SARS-CoV-2 (D614G-specific)	BANCOVID	Pre-clinical	12/23/2021	https://www.biorxiv.org/content/10.1101/2020.09.29.319061v1.full.pdf+html
HelixNano	SARS-CoV-2	COVID-19 vaccine	Pre-clinical	10/27/2021	https://www.helixnano.com
Longuide	SARS-CoV-2	COVID-19 vaccine	Pre-clinical	8/17/2021	http://www.longuide.com
mCureX Therapeutics	SARS-CoV-2	MCX 101	Pre-clinical	3/17/2022	Press release, mCureX Therapeutics, 8 Feb 2021
Providence Therapeutics	SARS-CoV-2	PTX COVID19-BT	Pre-clinical	9/16/2021	https://www.providencetherapeutics.com
RNACure Biopharma (collaboration with Fudan University and Shanghai Jiao Tong University)	SARS-CoV-2	RNA Cure	Pre-clinical	4/19/2022	https://www.raps.org
Stemirna Therapeutics	EBV	EBV mRNA vaccine	Pre-clinical	7/22/2021	http://www.stemirna.com
Bdgene Technology	HSV	BD-102	Pre-clinical	8/4/2021	https://www.bdgenetherapeutics.com
Eyam Vaccines	Influenza	ND[a]	Pre-clinical	9/28/2021	https://eyamhealth.com
mCureX Therapeutics	Influenza	MCX 102	Pre-clinical	3/21/2022	https://www.olixpharma.com
Sanofi	Influenza	QIV influenza mRNA vaccine	Pre-clinical	3/30/2022	https://www.sanofi.com

[a]ND, no data.

Table 3 mRNA vaccines candidates in pre-clinical stage against COVD-19

S. No.	Type	Organization/developers
1	Self-amplifying RNA formulated in a nanostructured lipid carrier	Infectious Disease Research Institute/Amyris, Inc.
2	Lipid nanoparticle encapsulated mRNA encoding spike protein	Max-Planck-Institute of Colloids and Interfaces
3	Self-amplifying RNA (saRNA)	Gennova
4	mRNA	Selcuk University
5	Lipid nanoparticle encapsulating messenger-RNA	Translate Bio/Sanofi Pasteur
6	Lipid nanoparticle encapsulating messenger-RNA	CanSino Biologics/Precision NanoSystems
7	Lipid nanoparticle encapsulated messenger-RNA combination encoding virus like particle	Fudan University/Shanghai JiaoTong University/RNACure Biopharma
8	Lipid nanoparticle encapsulated messenger-RNA encoding receptor-binding domain	Fudan University/Shanghai JiaoTong University/RNACure Biopharma
9	Replicating defective SARS-CoV-2 derived RNAs	Centro Nacional Biotecnología (CNB-CSIC), Spain
10	Lipid nanoparticle encapsulated messenger-RNA	University of Tokyo/Daiichi-Sankyo
11	Liposome encapsulated messenger-RNA	BIOCAD
12	various messenger-RNA candidates	RNAimmune, Inc.
13	Messenger-RNA	FBRI SRC VB VECTOR, Rospotrebnadzor, Koltsovo
14	Messenger-RNA	China CDC/Tongji University/Stermina
15	Messenger-RNA in an intranasal delivery system	eTheRNA
16	Messenger-RNA	Greenlight Biosciences
17	Messenger-RNA	IDIBAPS-Hospital Clinic, Spain
18	Messenger-RNA	Providence Therapeutics
19	Messenger-RNA	Cell Tech Pharmed
20	Messenger-RNA	ReNAP Co.

(Continued)

S. No.	Type	Organization/developers
21	D614G variant lipid nanoparticle encapsulated messenger-RNA	Globe Biotech Ltd
22	Messenger-RNA	CEA
23	Recombinant, prefusion stabilized SARS-CoV-2 S antigen	Medigen Vaccines Biologics Corp (MVC)/Vaxess Technologies (MIMIX)
24	ZIP1642, a saRNA vaccine encapsulated in a Lipid Nanoparticle encoding multiple antigens, including the S-protein.	Ziphius Vaccines and Ghent University

Table 4 mRNA vaccines in clinical stage against COVID-19

Number	Type of candidate vaccine	Number of doses	Schedule	Route of administration	Developers	Description	Phase
1	mRNA-1273	2	Day 0 + 28	IM[b]	Moderna + National Institute of Allergy and Infectious Diseases (NIAID)	ND[d]	Phase IV
2	BNT162b2 (LNP-mRNAs)	2	Day 0 + 21	IM[b]	Pfizer/BioNTech + Fosun Pharma	Also known as "Comirnaty"	Phase IV
3	CvnCoV vaccine	2	Day 0 + 28	IM[b]	CureVac AG	ND[d]	Phase III
4	ARCT-021	ND	ND	IM[b]	Arcturus Therapeutics	ND[d]	Phase II
5	LNP-nCoVsaRNA	2	ND	IM[b]	Imperial College London	ND[d]	Phase I
6	SARS-CoV-2 (ARCoV)	2	Day 0 + 14 or Day 0 + 28	IM[b]	Academy of Military Science (AMS), Walvax Biotechnology and Suzhou Abogen Biosciences	ND[d]	Phase III
7	ChulaCov19	2	Day 0 + 21	IM[b]	Chulalongkorn University	ND[d]	Phase I
8	PTX-COVID19-B	2	Day 0 + 28	IM[b]	Providence Therapeutics	ND[d]	Phase I
9	CoV2 SAM (LNP)	2	Day 0 + 30	IM[b]	GlaxoSmithKline	A self-amplifying mRNA (SAM) LNP platform + spike antigen	Phase I
10	mRNA-1273.351	3	Day 0 or Day 0 + 28 or Day 56	IM[b]	Moderna + National Institute of Allergy and Infectious Diseases (NIAID)	A LNP-encapsulated mRNA-based vaccine that encodes for a full-length, prefusion stabilized S protein of the SARS-CoV-2 B.1.351	Phase IV
11	MRT5500	2	Day 0 + 21	IM[b]	Sanofi Pasteur and Translate Bio	An mRNA vaccine candidate	Phase II
12	DS-5670a	2		IM[b]	Daiichi Sankyo Co., Ltd.	ND[d]	Phase I/II
13	HDT-301	2	Day 0 + 28	IM[b]	SENAI CIMATEC	Self-replicating mRNA vaccine formulated as a lipid nanoparticle.	Phase I
14	mRNA-1283	2	Day 0 + 28	IM[b]	ModernaTX, Inc.	ND[d]	Phase I
15	EXG-5003	1	Day 0	ID[c]	Elixirgen Therapeutics, Inc	A temperature-sensitive self-replicating RNA vaccine expressing the receptor binding domain of the SARS-CoV-2 spike protein.	Phase I/II
16	mRNA COVID-19 vaccine	2	TBD[a]	IM[b]	Shanghai East Hospital and Stemirna Therapeutics	ND[d]	Phase I
17	LNP-nCOV saRNA-02 vaccine	2	Day 0 + 28	IM[b]	MRC/UVRI and LSHTM Uganda Research Unit	Self-amplifying RNA (saRNA) encapsulated in LNP	Phase I
18	mRNA-1273.211	1	Day 0	IM[b]	ModernaTX, Inc.	A multivalent booster candidate combining mRNA-1273 plus mRNA-1273.351.	Phase II/III

[a]TBD, to be determined; [b]IM, intra muscular; [c]ID, intra dermal; [d]ND, no data; .

application (BLA) for BNT-162b2 vaccine (COMIRNATY), co-developed by Pfizer and BioNTech. BNT-162b2 marked the first officially approved COVID-19 vaccine and the first mRNA based therapeutic for human in the history. BNT-162b2 is a LNP encapsulated nucleotides modified mRNA, which is transcribed and synthesized in an *in vitro* cell-free system. Encoding optimized COVID-19 spike glycoprotein is the main candidate vaccine for BioNTech/Pfizer BNT-162 (PF-07302048) mRNA vaccine project. According to a study published in middle of July 2021, the mRNA vaccine showed 88% effectiveness in preventing symptomatic infections with the Delta variant. The Ragon Institute of MGH, MIT, and Harvard demonstrated the effectiveness of two widely used mRNA based COVID-19 vaccines. For this purpose, they have constructed pseudo virus, a harmless version of Omicron. This pseudo virus was used by these scientists to evaluate mRNA COVID-19 vaccines effectiveness. Blood samples were collected from 239 participants, who got vaccine shots of the two prominent mRNA vaccine (BNT162b2 or mRNA-1273). The 70 individuals among 239 participants also got additional dose (booster shot) of mRNA vaccine. The results showed the low neutralization against pseudo virus. On the other hand, against Omicron variant, a significant neutralization was

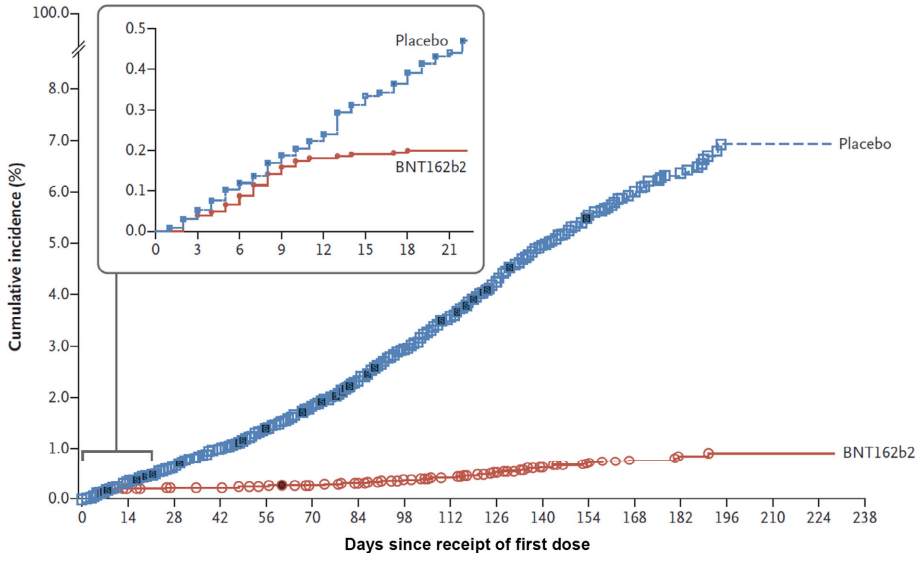

Figure 7 Efficacy of BNT162b2 against COVID-19. The above figure is the cumulative incidence curves rate COVID-19 occurrence after the initial dose in population above 12 years of age. While the figure below demonstrated the rate of COVID-19 occurrence in the efficacy analysis population and surveillance time. Reproduced with permission from Ref. [128], © Massachusetts Medical Society 2021.

observed in participants who got the booster shot of the mRNA vaccine. These results showed that the additional dose or booster shot has the ability to bind firmly to the spike protein [111].

Regarding safety and efficacy studies (Fig. 7), BNT162b2 demonstrated constant safety and efficacy profile in a trial. According to the study reported, the BNT162b2 showed 91.3% (confidence interval of 95%) efficacy against COVID-19 in a six-month trail in a population having no past COVID-19 infection. However, in a diverse population across various regions in the world, a gradual efficacy decline was observed with the course of time. In severe cases of B.1.351 in South Africa, an efficacy of 100% (confidence interval of 95%) was observed, concluding that the BNT162b2 had a promising safety profile and found exceedingly efficacious to prevent COVID-19 infection [128].

(2) mRNA-1273

The mRNA-1273 is developed by Moderna Inc., comprising a novel LNP-encapsulated mRNA vaccine, which encodes a full-length and perfusion stabilized spike protein of SARS-CoV-2. It is included in the 18 vaccine candidates among total 270 therapeutic agents against COVID-19. The National Institute of Health (NIH) and Moderna's infectious disease research team finalized the sequence for mRNA-1273 in January 2020, soon after the outbreak of COVID-19. Moderna mobilized towards clinical manufacturing. The mRNA vaccine (mRNA-1273) developed by Moderna company has confirmed its safety and effectiveness in large-scale human clinical trials. A phase II randomized controlled clinical trial study showed that the mRNA-1273 vaccine was safe and effective for adolescents aged 12 to 17 years. The phase III clinical trial of mRNA-1273 started in July 2020. In October 2020, the United Kingdom (UK) Medicines and Health Products Regulatory Authority (MHRA) launched a rolling review process for mRNA-1273. The data published in November 2020 showed that the trial met the preset statistical standard for vaccine efficacy in the research protocol, and the vaccine efficacy evaluation was observed almost 94.5% ($P < 0.0001$). The mRNA-1273 got an Emergency Use Authorization (EUA) from the U.S. FDA's Vaccines and Related Biological Products Advisory Committee (VRBPAC). It has also been urgently approved by FDA for listing and mass vaccination in December 2020. In February 2021, in order to increase its own and partnered manufacturing capabilities, Moderna Inc. announced new capital investments. This new capital investment is expected to increase its worldwide capacity to around 1.4 billion doses (100 μg dose) of COVID-19 in the year 2022. The newest trial results have demonstrated that the mRNA-1273 manufactured by Moderna Inc. has an acceptable safety profile in younger population. This report is supporting the previous safety and efficacy claim of mRNA-1273 in adults.

The latest study reported demonstrated that mRNA-1273 efficacy against COVID-19 and severe COVID-19 among all subgroups including those at risk for severe complications in the trial was sustained for more than five months after the 2nd dose (Fig. 8). Asymptomatic COVID-19 infections were also decreased. There were no safety concerns recorded in the trial [129].

Due to the persistent emergence of new variants having virulence capabilities, transmissibility, and concern to evade immune responses, it is necessary to re-evaluate the efficacy mRNA COVID-19 vaccines, especially the Omicron variant [130]. Accordingly, scientists have evaluated the efficacy of various vaccination regimens in eliciting humoral immune responses against the SARS-CoV-2 Omicron variant. Recently in Singapore, the National Vaccine Programme (NVP) has permitted various vaccines, i.e., two mRNA-based vaccines developed by Pfizer-BioNTech/COMIRNATY (BNT162b2) and Moderna (mRNA-1273) and an inactivated virus vaccine, for mass vaccination. NVP introduced booster vaccination in July 2021, by reviewing the available reports about the waning of vaccine-induced protection over time and its efficacy against the new SARS-CoV-2 variants. The study revealed that the people inoculated with mRNA booster after two doses of any inactivated virus vaccines demonstrated greater neutralization of 77.85% against Omicron receptor binding domain (RBD). This outcome is in line with previous studies which showed people who got an mRNA booster following a two-dose regimen of inactivated virus vaccine showed a higher neutralizing capability to both new prevalent variants [131].

(3) mRNA1273.351/mRNA-1273.211

Viruses have the capability to frequently mutate at a fast pace. Therefore, sometimes the mutation makes the virus weaker or gives an extra advantage to the virus in escaping the therapeutic options. The World Health Organization (WHO) has labelled some variants as variant of concern (VOC). Therefore, for the new mutant virus strain B.1.351, which is believed to escape immune response, Moderna has developed an mRNA-1273.351 vaccine against the spike protein of this virus strain. The Moderna also

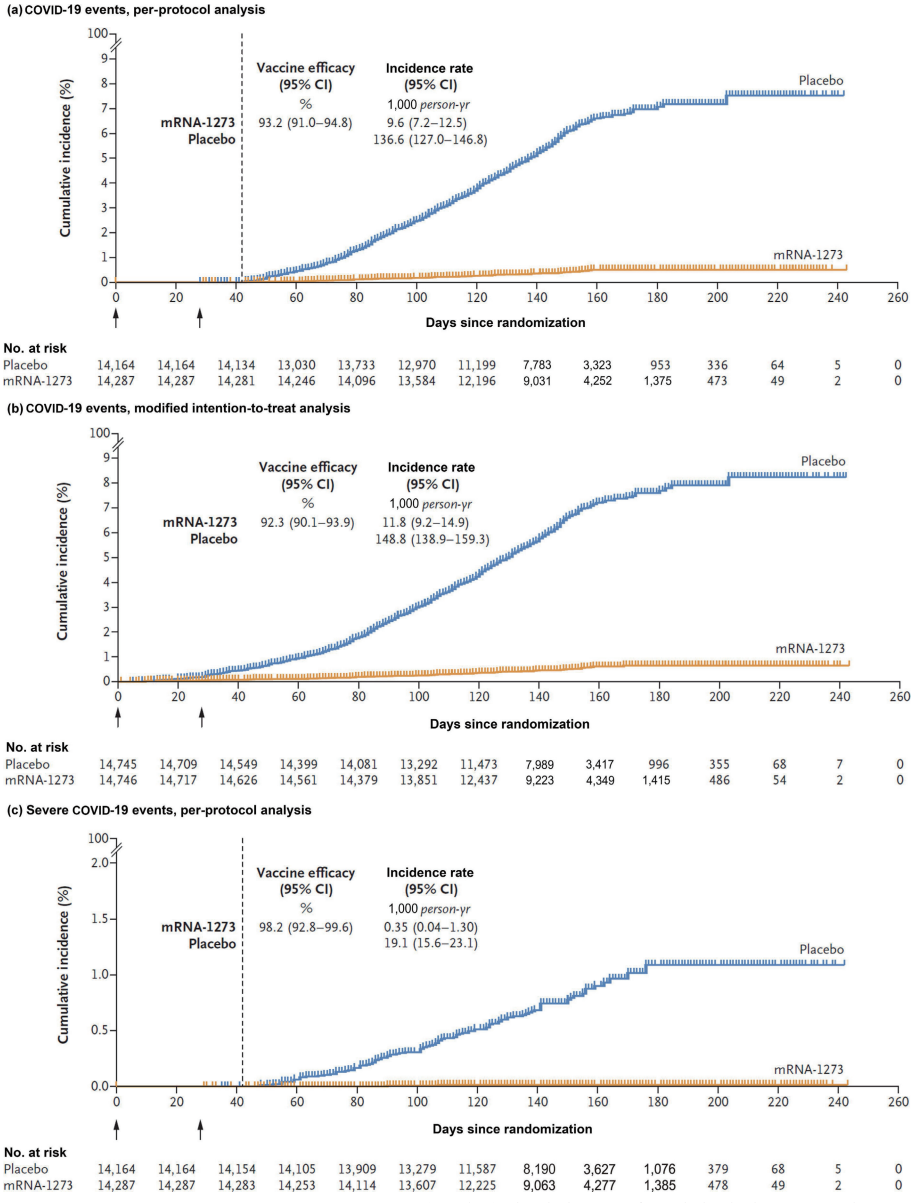

Figure 8 Efficacy of the mRNA-1273 vaccine in preventing COVID-19. In (a) and (c), the dashes signify settled calculation started at 42nd day, 14 days after the 2nd dose of placebo or vaccine. In (a), (b), and (c), the data cut-off date was 26th March, 2021. Reproduced with permission from Ref. [129], © Massachusetts Medical Society 2021.

developed multivalent vaccine mRNA-1273.211, which is a combination of wild-type mRNA-1273 and B.1.351 mRNA-1273.351, aiming to provide a wide range of protection. A study published revealed that the two doses of mRNA-1273.351 or mRNA-1273.211 could improve the neutralizing antibody titer against the B.1.351 mutant virus strain in mouse model. The multivalent candidate vaccine mRNA-1273.211 provides the most extensive neutralizing immunity in the mentioned study [132].

(4) CvnCoV

CureVac, a German biopharmaceutical company, started the development of its own mRNA vaccine against COVID-19 as soon as the initial outbreak of COVID-19 began. Their mRNA vaccine candidate, CvnCoV, comprising a chemically-modified mRNA, an optimized, and non-encoding perfusion, stabilized full-length S-protein of the virus causing COVID-19. The code is encapsulated within lipid to form lipid nanoparticles-encoding S protein. In June and September 2020, the initial phase I and IIa clinical trials of CvnCoV started, respectively. Soon in November 2020, phase I interim data was reported, which demonstrated that the vaccine was commonly well tolerated among all the range of tested doses. Moreover, in addition to the first indication of T cell activation, the CvnCoV also induced strong antibody responses. The results also suggested that the immune response triggered by CvnCoV has approximate quality compared to the recovered COVID-19 patients. In December 2020, a HERALD study, phase IIb/III, was also conducted using a 12-μg dose of CvnCoV. CureVac also began a rolling submission in February 2021 with the European Medicines Agency (EMA) for its mRNA-based vaccine. CureVac published the efficacy study in June 2021 which showed that the CvnCoV proved a total efficacy of 48% against SARS-CoV-2 with any severity, including single non-respiratory mild symptoms. The results also demonstrated that a substantial protection was observed among participants in various age groups, especially from 18 to 60 years. The CvnCoV showed an efficacy of 53% against COVID-19 with severity, and most importantly across 15 reported strains identified. Moreover, CvnCoV demonstrated protection against moderate to severe COVID-19 was almost 77%. In 18 to 60 years old age group, the vaccine provided 100% protection against more severe cases and prevented hospitalization and death [133].

(5) ARCoV

ARCoV is a mRNA COVID-19 vaccine developed by Suzhou

Abogen Biosciences, China, in which the mRNA encoding the RBD of SARS-CoV-2 is encapsulated in a LNP. In May 2020, Watson and Abogen collaborated on the development of RNA vaccine, and worked together to carry out pre-clinical research, clinical research, and commercial production of ARCoV. The ARCoV is comprised of a white to off-white dispersion for injection. The vaccine can be stored at room temperature for at least 7 days. ARCoV is supplied in a single-dose prefilled syringe with 0.5 mL dispersion for intramuscular (IM) injection. According to the details available, each dose (0.5 mL) of the ARCoV contains 0.339 mg of total lipids such as 9001, distearoylphosphatidylcholine (DSPC), cholesterol (Chol), and DMG-PEG2000, and 15 μg of mRNA. The reports about ARCoV suggested that the vaccine also induces protective T cell immune response along with induction of high-level of neutralizing antibodies in cynomolgus monkeys and mice. The attack test of cynomolgus monkeys showed that the vaccine immunized animal could tolerate COVID-19 attack with high titer, effectively preventing virus replication and lung pathological progress, and showed good protective effect. In July 2021, Watson biology registered a clinical trial (phase III) of ARCoV to further test the efficacy, safety as well as immunogenicity in participants over 18 years of age.

In a study conducted from Oct. 30 to Dec. 2, 2020, a total of 230 participants were screened and a total of 120 eligible individuals were randomly allocated to get 5 dose levels of placebo or ARCoV. The results demonstrated that the ARCoV is safe and well tolerated, and immunogenicity profile of the vaccine was also evaluated (Fig. 9). An excellent stability profile was observed, because of the storage condition as well as the temperature required for transportation is highly appreciable. The storage temperature presents great convenience for vaccine acceptability to the general public and to all regions where the temperature is on the higher side. Recently, a phase III clinical trial in multiple regions has been started for the purpose to test the efficacy of ARCoV [134].

(6) mRNA vaccine developed by CanSino Biologics

In early April 2022, CanSino Biologics, a Chinese based company, has publicized that the National Medical Products Administration of China (NMPA), the Chinese drug regulated authority, approved the application for clinical trials of mRNA vaccine against COVID-19. A higher titer neutralizing antibody was observed against multiple VOC causing COVID-19, especially the Omicron variant. The Omicron has been considered the highly transmissible, and therefore the most prevailing strain around the globe and specifically in China as well. The neutralizing antibodies induced by CanSino Bio's mRNA vaccine provide stronger protection against VOCs, and have greater cross-reactivity against these variants compared to the original variant.

2.4.2 mRNA vaccines for CMV

CMV is a globally spread virus, generally contracted by children as well as adults in their life span. However, the indicators of the disease range from asymptomatic to severe conditions. The severe cases mostly cause end-organ dysfunction in immunocompromised patients suffering congenital CMV disease. CMV can be transmitted from pregnant mothers to unborn children, so it is urgent to prevent CMV in women of childbearing age.

To address this, the mRNA-1647 candidate has been developed, comprising six mRNAs encoding CMV pentamer (glycoprotein H complex composed of GH, GL, UL128, UL130, and UL131A proteins) and glycoprotein B (GB). Among the above 6 different mRNAs, one type encodes GB protein, while the rest of the five encode the subunits of CMV, however all of them are observed to be highly immunogenic. Pentamer and GB protein are essential for CMV to enter epithelial cells, which is the first step of CMV infection. Phases I and II studies demonstrated functional antigen-specific responses which supported the potential to prevent CMV infection. The neutralizing antibody titers resulted in the CMV-positive group showed that the ratio of neutralizing antibody titers compared to baseline (geometric mean ratios, or GMRs) against epithelial cell infection ranged 13.4–40.8 and against fibroblast infection ranged 4.0–7.1. Based on the phase II interim analysis, neutralizing antibody GMRs against fibroblast infection increased to approximately 2-fold over baseline. At present, mRNA-1647 vaccine for CMV was generally well tolerated, and the phase III clinical trial is expected to be launched to test the 100 μg dose level.

2.4.3 mRNA vaccines for Zika

Zika is an arbovirus and member of the Flaviviridae family. The Zika virus is a single-stranded positive RNA virus with a diameter of 20 nm and transmitted by mosquitoes. The primary source of Zika virus infection in humans is from bites of infected mosquitoes. There have also been cases of sexual, perinatal, and suspected blood-transfusion transmission. The activity of Zika virus is fully understood and further investigation is underway, however, the periodic cases of Zika virus infection have been found rarely in Asia, America, and Pacific region, while most cases appeared in the African region. The first outbreak occurred on Jas spread island in Micronesia in the Western Pacific in 2007, and the larger outbreak occurred in French Polynesia in Oceania from 2013 to 2014, infecting about 32,000 people. The mRNA-1893 developed by Moderna Therapeutics is a gene vaccine, which contains an mRNA sequence encoding the structural protein of Zika virus. It aims to make cells secrete virus like particles and simulate the response of cells after natural infection. Preclinical studies published in the Journal of infectious diseases showed that the administration of mRNA-1893 can prevent the transmission of Zika virus during mouse pregnancy [135]. At present, the mRNA-1893 is in phase I clinical study to evaluate its safety, pharmacokinetics, and pharmacodynamics in healthy volunteers.

2.4.4 mRNA vaccines for influenza

Influenza is an annual global health concern, with the WHO estimating that influenza leads to approximately 290,000 to 650,000 deaths annually. Hekele et al. demonstrated that the mRNA vaccine can be generated quickly in the case of genetic sequence of the target influenza hemagglutinon (HA) antigen [136]. In 2013, during an outbreak of a deadly strain of H7N9 influenza in China, the HA gene was cloned in a self-amplifying mRNA vaccine pDNA template, and the self-amplifying mRNA vaccine was produced within 8 days once the HA gene sequence was obtained.

Influenza viruses are prone to recombination and mutation. In the traditional influenza vaccine, the target pathogen or antigen must be produced in the specific cell culture or fermentation process, and the development and production cycles are long, which cannot achieve the purpose of prevention. mRNA influenza vaccine can quickly produce and detect antigens, which can greatly shorten the vaccine development and production cycle, and is expected to overcome the challenges faced by the influenza vaccine in industry. Phase I clinical studies of the first mRNA vaccines against H10N8 and H7N9 influenza viruses in Moderna elicited robust immune responses, further supporting the potential of mRNA as a vaccine platform [137].

Moderna has three influenza vaccines: mRNA-1010, mRNA-1020, and mRNA-1030. In July 2021, the company launched the

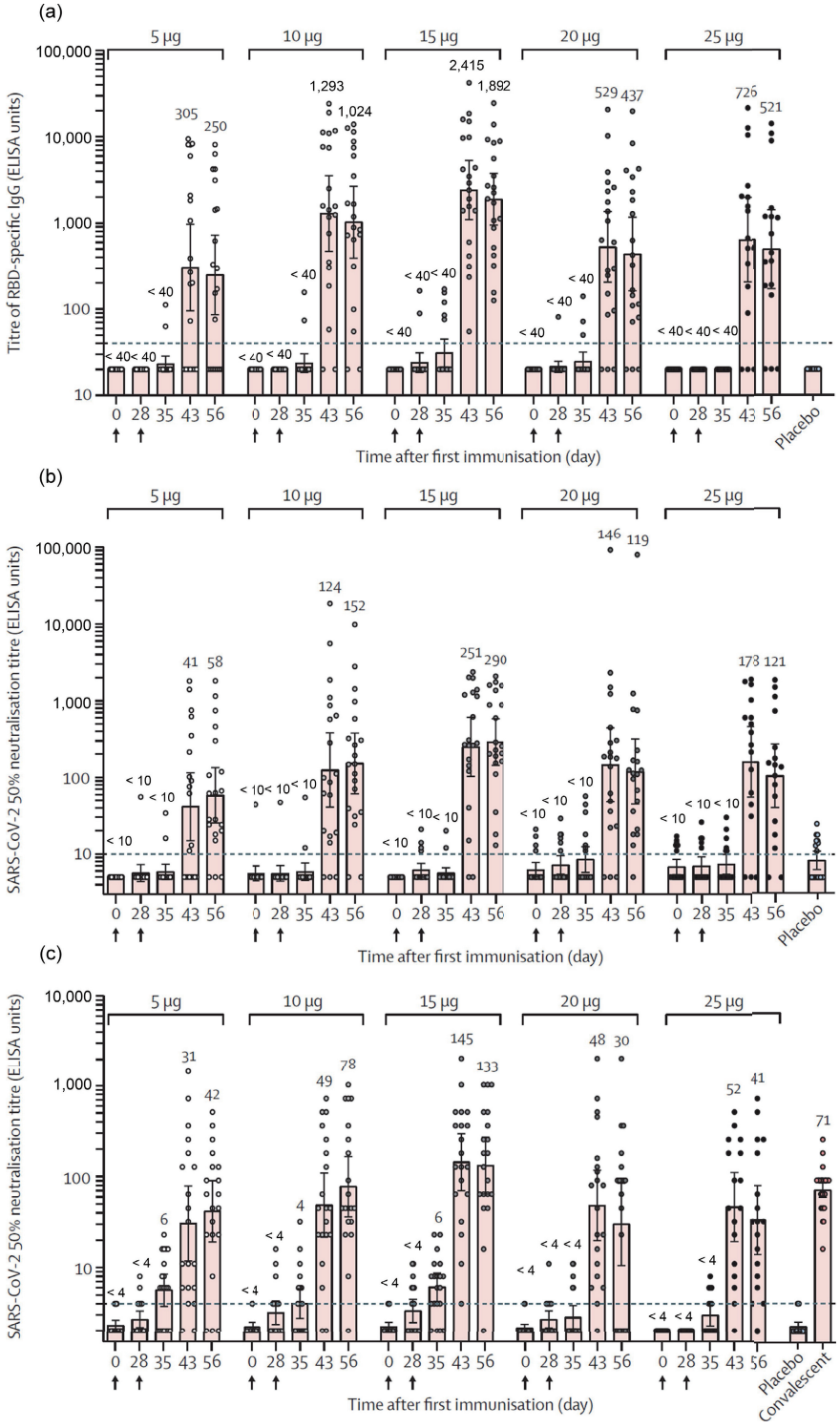

Figure 9 ARCoV antibody and neutralization responses in phase 1 trials. (a) Showing the RBD-specific IgG geometric mean titers, (b) representing neutralizing antibodies against pseudo virus, while (c) showing live SARS-CoV-2. The collected serum samples of the participants at baseline (day 0), before the second dose (day 28), and after the second dose (day 35, 43, and 56). Dots represent a serum sample. Bars represent geometric mean titer (SD). Numbers above dots show the geometric mean titer of the group. Dashed line indicates the lower limit of quantification. Reproduced with permission from Ref. [134], © Chen, G. L. et al. 2021.

phase I/II clinical trial of mRNA-1010, an mRNA candidate vaccine for 4-valent seasonal influenza, targeting four seasonal influenza virus strains, including influenza A virus strains H1N1 and H3N2, and influenza B virus strains Yamagata and Victoria. The company recruited 180 healthy adult participants in the United States to participate in phase I/II clinical randomized, observer blind, dose range clinical trial to evaluate the safety, and immunogenicity of mRNA-1010. The vaccine is based on the same mRNA technology as used for COVID-19 developed by Moderna. The effectiveness of this technology is more than 90%. If the test proves successful, it will bring a new generation of more protective influenza vaccine. CV7301 is a second-generation LNP flu vaccine, which has demonstrated strong and durable immunogenicity in non-human primates.

2.4.5 mRNA vaccines for RSV

RSV is a common respiratory virus, which can cause cold symptoms. Most infected people can recover within one to two weeks, but a few people, especially infants and the elderly, may become seriously ill. RSV is the most common cause of

bronchiolitis and pneumonia in children under one-year-old in the United States. It can also lead to pneumonia and respiratory distress in the elderly. According to the U.S. Centers for Disease Control and Prevention, RSV causes an average of 58,000 hospitalizations of children under the age of 5 and 177,000 and 14,000 hospitalizations and deaths of adults aged 65 and over every year. At present, no RSV vaccine has been approved. mRNA-1345 is an anti-RSV vaccine, encoding a stabilized perfusion F glycoprotein. Compared with post fusion, it can induce better neutralizing antibody response. The LNP used in this vaccine is the same as COVID-19 vaccine and contains optimized protein and codon sequences [138].

The phase I study of mRNA-1345 tolerance and reactivity in people is ongoing. At present, all young subjects in all four age groups (18–49 years old) have been enrolled, and the dose study of the elderly group (65–79 years old) is in progress. mRNA-1345 boosts RSV neutralizing antibodies in younger adults. The age range of infant subjects is 12–59 months. On the annual vaccine day on April 14, 2021, Moderna first published the interim analysis data of the adult cohort (50 or 100 μg dose) of the phase I study. The neutralizing antibody produced in one month after vaccination showed a geometric average increase of at least 11.7 times compared with the baseline. The result indicates that the mRNA-1345 is well tolerated. The most common locally induced adverse reaction was injection site pain, which was reported by at least 73.7% of vaccinators.

2.4.6 mRNA vaccines for HIV

HIV is a retrovirus that causes defects in the human immune system. This virus attacks and gradually destroys the human immune system, so that the host cannot be protected when infected. People who are infected with human immunodeficiency virus often die with secondary infection or cancer. AIDS is the last stage of human immunodeficiency virus infection. The diameter of HIV is about 80–140 nm, which is round or oval in shape. The outer membrane of the virus is a lipid envelope, which comes from the host cell and is embedded with viral proteins Gp120 and Gp41. Gp41 is a transmembrane protein. Gp120 is located on the surface and binds to Gp41 through non-covalent interaction. Inward, there is a spherical matrix formed by protein p17 and a semi conical capsid formed by protein p24. The capsid shows high electron density under electron microscope. The capsid contains the RNA genome of the virus, enzymes (reverse transcriptase, integrase, protease), and other components from the host cell. The NIH has started the clinical trial phase 1 trials to combat HIV using mRNA technology. The trial comprises three vaccines: BG505 MD39.3, BG505 MD39.3 gp151, and BG505 MD39.3 gp151 CD4KO HIV Trimer mRNA vaccines. Moderna has developed mRNA-1574 and mRNA-1644 to against HIV. The recent study, which was carried out by NIAID, Moderna, and other institutions, revealed that mRNA vaccine co-expressed the membrane-affixed simian immunodeficiency virus (SIV) Gag proteins and HIV-1 envelope (Env) [139]. This co-expression induces antibodies after generating virus-like particles (VLPs). These antibodies have the ability of broad neutralization and reduce the risk of infection in rhesus macaques. Moreover, the immunization with env and gag mRNAs co-formulation was found to induce higher neutralizing antibodies compared to env mRNA alone.

2.4.7 mRNA vaccines for Nipah

Nipah virus (NiV) is an enveloped non-segmented negative-strand RNA virus in the Henipavirus genus of the *Paramyxoviridae* family. The members of the *Paramyxoviridae* and *Pneumoviridae* virus family have two membrane-anchored glycoproteins that are targets for neutralizing antibodies, the attachment protein (G, H, or HN) and the fusion (F) protein. Nipah's attachment protein is a type II membrane protein that facilitates binding of the NiV virions to the host cells through the ephrin B2/B3 receptors [140]. Nipah virus is a new zoonotic virus belonging to *Paramyxoviridae*, which can cause extensive vasculitis. Infected people have symptoms such as fever, severe headache, and meningitis, which brings serious harm to people and animals. Nipah virus disease (NVD) is another zoonotic disease which has aroused widespread concern and panic all over the world after mad cow disease in Britain, pig foot-and-mouth disease in Taiwan, China, and avian influenza in Hong Kong, China. From the perspective of public health, this disease should be highly valued by domestic medical and animal husbandry and veterinary circles. In late 2021, Moderna announced that it has started the development of mRNA vaccine to tackle the deadly Nipah virus termed as mRNA-1215. The mRNA-1215 vaccine is believed to target the attachment proteins of the virus to disrupt the attachment of the virus to the host [141].

2.4.8 mRNA vaccines for Epstein-Barr virus (EBV)

EBV is a member of lymphophilic virus of herpesvirus family, and its genome is DNA. EB virus has the biological characteristics of specifically infecting human and some primate B cells *in vitro* and *in vivo*. Human is the host of Epstein–Barr virus infection, which is mainly transmitted through saliva. Asymptomatic infection mostly occurs in children. More than 90% of children aged 3–5 have been infected with EB virus, and more than 90% of adults have virus antibodies. Recently, Moderna has already administered its mRNA vaccine (mRNA 1189) to the first subject in phase I trial. The trial was termed as Eclipse clinical trial against EBV. The randomized, observer-blind, placebo-controlled, and dose-ranging Eclipse phase I clinical trial includes 18–30 years old healthy adults. The mRNA-1189 is designed to target EBV targeting the glycoprotein 350 (gp350) and recently it showed encouraging outcomes in preclinical studies. Moreover, it also induced antibody titers after two doses in Balb/c mice against the viral protein facilitating the epithelial cell entry. These encouraging outcomes brought hope for feasible protection and defense against EBV-related infections and complications [142].

2.4.9 mRNA vaccine for monkeypox

Recently, the monkeypox has been reported in almost 11 countries other than Africa, which shows the early outbreak has been started. The virus was first identified in monkeys in 1958 in laboratory. Earlier, the virus was causing infections in West African population, but the recent spread to other continent forces the scientists on high alert. A leading virologist at the US Army Medical Research Institute of Infectious Diseases in Fort Detrick, Jay Hooper, said that regardless of its painful symptoms, the virus is far more different than the virus causing COVID-19. In case of COVID-19, the virus spread more quickly, such as through a tiny air-borne droplets called aerosols, but the virus causing monkeypox is highly related to smallpox, and does not show direct human-human transmission. However, this virus spreads from close contact with bodily fluids, such as saliva from coughing. Moreover, there are therapies already developed to curb the spread smallpox and therefore, the vaccines development for monkeypox will be a lot faster, if needed [143].

The common symptom of monkeypox mostly includes flu, while it also triggers enlarge lymph nodes. The later symptoms include distinctive lesions on various parts of the body such as face, hands, and feet. However, a patient suffering from monkeypox can be relieved in weeks without taking any kind of treatment [144].

According to Andrea McCollum, a leading epidemiologist at the US Centers for Disease Control and Prevention in Atlanta, Georgia, said that the monkeypox does not usually go unobserved when infecting people around because of the skin lesions. However, the concerning this would be if it spread asymptomatically, then it might cause severe trouble, because this will make the virus harder to detect and track [143].

Moderna Inc. started the pre-clinical testing of its mRNA candidate against smallpox based on the genome reported recently. Till date, the initial pre-testing has been carried out, however, no further details shared.

2.5 Storage issue related to mRNA vaccines

During lyophilization, the removal of water takes place via sublimation at low temperature under vacuum. Generally, lyophilization is comparatively a mild drying method and believed to enhance the stability issues mostly related to colloidal nanoparticles (NPs) and/or macro-biomolecules [145, 146].

As of now, the mRNA vaccines have become global cost-effective preventive measure against COVID-19. Till date, billions of doses of mRNA COVID-19 vaccines have been injected worldwide but it is hard for the low-income developing countries to afford to get at least one dose of vaccine. In principle, it is necessary to develop an mRNA vaccine which can be easily stored and transported at 2–8 °C, or better at room temperature. A Chinese based company Recogen recently published the data that revealed the lyophilization of mRNA encapsulated in LNP. The resultant lyophilization of mRNA technology could significantly expand the convenience of mRNA vaccines or other therapeutics, predominantly in remote areas around the globe. The data presented an optimized lyophilization technique, significantly maintained the physio-chemical characteristics as well as the bioactivity of mRNA. Moreover, the lyophilized form of mRNA-LNP achieved long-term storage in the range of 2–8 °C. The significant improved thermostability was further confirmed with various LNPs encapsulating different mRNA molecules, which determined their widespread applicability [147].

Furthermore, the method was also used to develop the first ever lyophilized and thermostable mRNA-LNP vaccines encoding the antigen of wild-type (WA1, Lyo-mRNA-WT), and two most dominant variants: Delta (Lyo-mRNA-Delta) and Omicron COVID-19 variant (LyomRNA-Omicron). The three vaccines were tested on mice and the results revealed their high level antibody responses and prevention ability against the infection. The lyophilized vaccine also induced strong immune response and generated high level neutralizing antibody titer. A cross-protection capability was also observed by mRNA-Delta and mRNA-Omicron vaccine against other variants [147].

Another recent study was also published by researchers from University of Pennsylvania, USA, describing an effective lyophilization method which can take its application to develop freeze-dried cake of nucleoside-modified mRNA-LNPs. They have generated the lyophilized mRNA-LNPs and evaluated various storage conditions such as −80, −20, 4, 25, and 42 °C for 4, 12, or 24 weeks. They observed that the physico-chemical characteristics of mRNA-LNPs do not significantly alter after storage at 25 °C (room temperature) for almost 12 weeks. They also noticed that the mRNA-LNPs was stable at 4 °C for at least 24 weeks. They further evaluated that the firefly luciferase-encoding mRNA-LNPs do not lose their high translatability in mice measured via bioluminescence imaging. They also tested the same lyophilized system in a comparative mouse immunization study conducted to evaluate the potency of the nucleoside-modified mRNA-LNP influenza virus vaccine, which showed a sustained potency after 12 weeks of storage at room temperature and/or for at least 24 weeks at 4 °C [148].

3 Conclusions and prospective

mRNA vaccine is attracting more attention due to its simple production process, rapid synthesis, fast development, and low cost. Compared to other pharmaceutical modalities, mRNA therapeutics have many advantages. The mRNA has the adjuvant effect of activating immune response, and translation occurs in the cytoplasm without entering the nucleus, and there is no risk of integrating the host genome. In the process of drug development, attention should be paid to the biological distribution of mRNA after administration. There is no systematic and comprehensive comparative study on whether the relationship between mRNA dose protein prognostic responses varies with individual differences and whether different injection methods in the same individual change this relationship. Fortunately, mRNA drug research is being combined with vaccines, nanotechnology, and immune and gene therapy, which provides us with new methods and ideas to solve human diseases. Furthermore, the dose-response titers of the mRNA-based vaccines under development for infectious disease need an optimization. The clinical dose should be designed in such a way to effectively record the immune response. However, the humoral response induction by the mRNA vaccine noticed by par compared to live-attenuated vaccine candidates. Therefore, there is a need of in-depth analysis in order to develop formulations which help to achieve desired immune response. Since there is a lack of proper regulatory guidelines for the evaluation of safety and efficacy of mRNA formulations, proper regulatory guidelines are necessary to speed up the development process to address various treatment option for infectious diseases.

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