Molecular Therapy
Review

COVID-19 mRNA vaccines: Platforms and current developments

Gábor Tamás Szabó,[1,2] Azita Josefine Mahiny,[1,2] and Irena Vlatkovic[1]

[1]BioNTech SE, An der Goldgrube 12, Mainz 55131, Germany

Since the first successful application of messenger ribonucleic acid (mRNA) as a vaccine agent in a preclinical study nearly 30 years ago, numerous advances have been made in the field of mRNA therapeutic technologies. This research uncovered the unique favorable characteristics of mRNA vaccines, including their ability to give rise to non-toxic, potent immune responses and the potential to design and upscale them rapidly, making them excellent vaccine candidates during the coronavirus disease 2019 (COVID-19) pandemic. Indeed, the first two vaccines against COVID-19 to receive accelerated regulatory authorization were nucleoside-modified mRNA vaccines, which showed more than 90% protective efficacy against symptomatic severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection alongside tolerable safety profiles in the pivotal phase III clinical trials. Real-world evidence following the deployment of global vaccination campaigns utilizing mRNA vaccines has bolstered clinical trial evidence and further illustrated that this technology can be used safely and effectively to combat COVID-19. This unprecedented success also emphasized the broader potential of this new drug class, not only for other infectious diseases, but also for other indications, such as cancer and inherited diseases. This review presents a brief history and the current status of development of four mRNA vaccine platforms, nucleoside-modified and unmodified mRNA, circular RNA, and self-amplifying RNA, as well as an overview of the recent progress and status of COVID-19 mRNA vaccines. We also discuss the current and anticipated challenges of these technologies, which may be important for future research endeavors and clinical applications.

INTRODUCTION

Vaccines are important tools to prevent, control, and/or eradicate infectious diseases and are fundamental components of public health programs worldwide.[1] The development and approval of effective coronavirus disease 2019 (COVID-19) vaccines represented a significant milestone during the ongoing pandemic. A vast number of parallel vaccine development projects were launched to combat the disease caused by a previously unknown pathogen, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). As of January 14, 2022, there were 333 vaccine candidates in development, of which 139 have entered the clinical phase according to the World Health Organization's vaccine tracker.[2]

Rapid development and highly efficient immune responses brought messenger ribonucleic acid (mRNA) technologies to the forefront of the COVID-19 vaccine race, mainly due to the fact that the technology requires a sequenced viral genome rather than live virus and takes merely a couple of days to design.[3] Furthermore, the upscaling of cell-free vaccine production is relatively straightforward, thereby providing a cutting-edge tool for rapid response in epidemics and pandemics.[4–7] Of the COVID-19 vaccines that have entered clinical trials as of this writing, 23 (17%) are mRNA-based candidates.[2] Moreover, the first two vaccines to receive conditional marketing authorization (CMA) from the European Medicines Agency or emergency use authorization (EUA) from the US Food and Drug Administration (FDA) were nucleoside-modified mRNA vaccines from BioNTech/Pfizer and Moderna, respectively, and showed over 90% protective efficacy against symptomatic SARS-CoV-2 infection in their phase III clinical trials,[8,9] surpassing previous expectations.[10]

In this review, we summarize the preclinical and clinical data of the vaccine candidates, which fall under four mRNA vaccine platforms: non-replicating linear nucleoside-modified and unmodified mRNA, circular RNA (circRNA), and self-amplifying RNA (saRNA). In addition, we discuss potential challenges that may hinder future vaccine development programs.

THE PATH TO COVID-19 mRNA VACCINE DEVELOPMENT

Compared with traditional vaccines, which are relatively slow and laborious to develop,[1,11,12] mRNA-based vaccines have features that allow them to be rapidly designed and upscaled while still being highly potent and low cost.[6] Although mRNA vaccines were already being investigated in clinical trials for other diseases, e.g., cancer, their far-reaching potential was not realized until the COVID-19 pandemic, where mRNA vaccine candidates were some of the first to enter clinical trials and obtain accelerated regulatory approvals.[13,14] Without the extensive research and technological advances over the past three decades, this achievement would not have been possible. This section highlights some of the most important breakthroughs that ultimately

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[2]These authors contributed equally

Correspondence: Gábor Tamás Szabó, BioNTech SE, An der Goldgrube 12, Mainz 55131, Germany.
E-mail: gabor.szabo@biontech.de

carved the way to COVID-19 mRNA vaccine development and approval.

Following the discovery of mRNA,[15,16] research in this area surged and gave rise to multiple scientific breakthroughs ultimately leading to the development of RNA-based vaccines. Examination of the properties of mRNA was previously hampered by the minimal cellular uptake of naked mRNA. However, the development of protective lipid-based formulations, which were first successfully used in 1978 in a study where rabbit reticulocyte 9S mRNA was introduced into mouse lymphocytes, resulting in globin synthesis,[17] made subsequent mRNA research pursuits less complex. In the same year, protein expression was also induced in human cells after liposomal mRNA transport.[18] Later, the efficacy of transfection was further enhanced with the incorporation of a synthetic cationic lipid into liposomes for mRNA delivery.[19]

The identification of deoxyribonucleic acid (DNA)-dependent RNA polymerase enzymes was a crucial step leading to *in vitro* mRNA transcription (IVT) using DNA templates. First published in 1984, IVT made the transcription of a selected functional mRNA from a template in the desired quantity possible.[20,21] It was not until 1993 that mRNA was used as a vaccine for the first time to elicit a specific immune response against the encoded pathogenic antigen in a preclinical setting using lipid-based delivery.[22] Another 20 years later, mRNA vaccines against infectious disease were investigated in phase I, proof-of-concept, clinical trials.[23,24] Based on the potential toxicity of liposomes in clinical application,[25,26] the success of the first approved small interfering RNA-lipid nanoparticle (LNP) therapeutic[27] and mRNA COVID-19 vaccines originates only from their delivery using ionizable lipid-containing LNPs,[28–30] which are known to have significantly higher delivery efficiency in hepatocytes after intravenous (i.v.) injection[31] or in muscle cells after intramuscular (i.m.) injection.[32] Moreover, it was recently found that LNPs possess a potent adjuvant function, which further demonstrates their beneficial effects in vaccine application.[33]

Lack of mRNA stability and innate immune activation were important concerns for many years in the development of mRNA as a drug substance. Uridine-containing mRNA stimulates the innate immune response and has an established adjuvant function when used as a vaccine.[34,35] However, the incorporation of modified nucleosides into mRNA can significantly improve the biological stability and translational capacity of the mRNA while decreasing the innate immune response.[36–38] Further improvement of mRNA quality can be made with purification of IVT mRNA using cellulose,[39] high-pressure liquid chromatography (HPLC),[40] fast protein liquid chromatography (FPLC),[41] oligo(dT) purification,[3] or tangential flow filtration (TFF).[42,43] Details on the existing mRNA vaccines are not disclosed and hence can only be speculated.

In combination with LNPs, modified mRNA is now the basis for the separate mRNA vaccine platform[41,44] that has been proven as the most successful based on its optimal immune response originating from the compromise between LNP adjuvant function[33] and modified mRNA, allowing improved efficacy and safety. Despite the multiple advantages offered by the mRNA-LNP platform, there is still room for improvement, and we will likely see further iterations of this technology. Recently, limitations have been found when predicting clinical outcome based on data generated in murine models regarding downstream effects of systemic inflammation induced by stimulation of toll-like receptor (TLR) 7/8 by mRNA lipoplexes.[45] It has been reported that humans secrete pro-inflammatory interleukin (IL)-1β, whereas mice upregulate the induction of the IL-1 receptor antagonist to control inflammation. Another challenge in this area is the fact that the lipid components themselves may activate immune responses that may differ depending on the type of component and formulation. In an elegant study comparing LNPs and lipoplexes, differences were found in cytokine induction, which indicate that the ionizable lipid of the LNP formulation is likely responsible.[45] With this in mind, future research on mRNA immunological downstream effects after application, which could affect the safety profile of the mRNA, needs to be cautiously investigated, and the type as well as the composition of an mRNA formulation needs to be selected carefully depending on the disease.

In addition to linear mRNA, other mRNA vaccine platforms have received attention. circRNA was first identified in 1976[46] and was later detected in human cells.[47] Although it was initially thought not to serve as a translational template, later reports contradicted this hypothesis, triggering further research.[48] Given its enhanced biostability compared with linear RNA due to the lack of terminals preventing degradation,[49] circRNA could be a promising research avenue. Another approach to reducing dose levels is the use of an mRNA platform that is able to self-amplify.[50] Subsequent viral biology research led to the insertion of an RNA-dependent RNA polymerase (RdRp) sequence next to the antigen-encoding sequence, leading to the amplification of the antigen of interest in the cytoplasm.[51] Along with the intracellular RNA replication of the antigen of interest, saRNA can lead to high antigen production at a low dose.[52]

THE MECHANISM OF IMMUNIZATION WITH mRNA VACCINES AND SELECTION OF THE SARS-CoV-2 ANTIGEN

Immunization with mRNA vaccines requires an antigen-encoding mRNA transcript formulated into LNPs that is delivered to antigen-presenting cells (APCs) (Figure 1). LNP-mRNA is endocytosed and released through the process of endosomal escape to the cytoplasm, where the antigen of interest is produced and presented as a membrane-bound antigen by transfected cells, including muscle cells and APCs, resulting in activation of B cells, $CD4^+$ helper T cells, and $CD8^+$ cytotoxic T cell responses (Figure 1). The germinal center B cell response and its regulation by $CD4^+$ T follicular helper (Tfh) cells are of key importance for high-affinity neutralization antibody titers and long-lasting B cell responses.[53,54] Tfh cells recognize antigens on the APC surface and help to activate B cells, which in turn produce high-affinity virus-neutralizing antibodies.[6,55–57] Recently, it was found that the LNP component of LNP-mRNA vaccines has adjuvant activity, which is dependent on its ionizable lipid component and IL-6

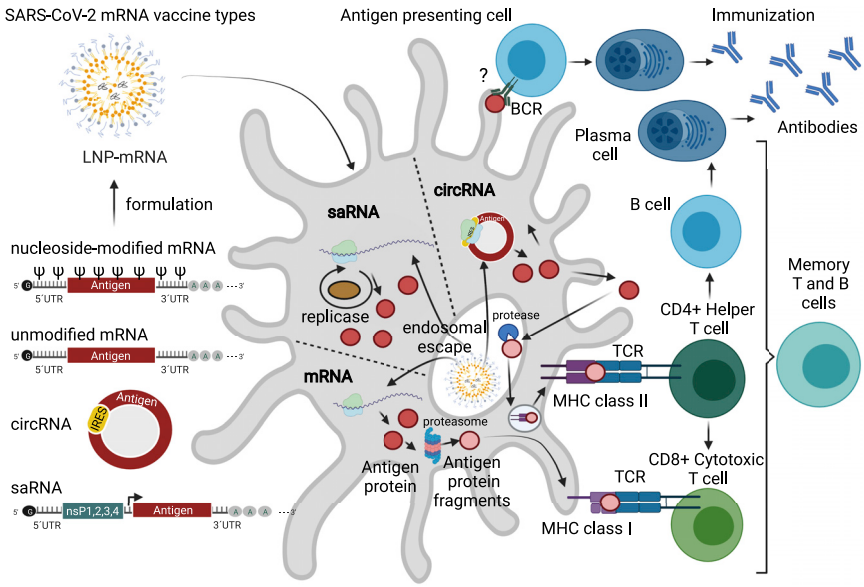

Figure 1. Immunization against COVID-19 with mRNA vaccines

Immunization with mRNA vaccines requires an antigen-encoding mRNA transcript. The linear non-replicating mRNAs consist of a sequence encoding an antigen (e.g., the S protein for SARS-CoV-2) flanked by 5′ and 3′ UTRs, with a cap structure at the 5′ end and a poly(A) tail at the 3′ end.[57] Depending on the use of native or modified nucleosides during IVT, unmodified or modified mRNAs are produced. saRNA consists of the same sequence organization, but in addition contains: (1) a sequence encoding four non-structural proteins (nsP1–4), which form a replicase responsible for amplification of the saRNA, and (2) a subgenomic promoter (black arrow) of viral origin that initiates transcription of antigens.[50] circRNA for vaccine application consists of a covalently closed single-stranded RNA that contains antigen sequence and an IRES that allows initiation of antigen translation.[49,59,60] Antigen-encoding mRNAs are formulated into LNPs, endocytosed, and released through the process of endosomal escape to the cytoplasm. The S protein is produced by the translational machinery of the APCs (red circles), degraded by proteasomes (pink circles), and presented on MHC class I (pink circles), leading to a specific CD8$^+$ cytotoxic T cell response against SARS-CoV-2. Antigens can also be anchored to the membrane of the APC and directly recognized by BCRs leading to B cell responses; however, such a path and its contribution to antibody production is currently under debate. Finally, the antigen protein can be exported from the cell and endocytosed back to the same or another APC, degraded by endosomal proteases, and presented on MHC II structures resulting in a CD4$^+$ helper T cell response. Immunization progresses with CD4$^+$ helper T cells further helping in (1) activation of B cells that produce SARS-CoV-2 neutralizing antibodies and (2) activation of CD8$^+$ cytotoxic T cells that may specifically recognize and eliminate virus-infected cells. APC, antigen-presenting cell; BCR, B cell receptor; circRNA, circular ribonucleic acid; IRES, internal ribosome entry site; IVT, *in vitro* translation; LNP, lipid nanoparticle; MHC, major histocompatibility complex; mRNA, messenger ribonucleic acid; saRNA, self-amplifying ribonucleic acid; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; S protein, spike protein; TCR, T cell receptor; UTR, untranslated region. Figure was created with BioRender.com.

cytokine induction.[33] This LNP-driven adjuvant activity leads to induction of strong Tfh cell responses and humoral immunity, thus enhancing the efficacy of mRNA-based vaccines.[33] Tfh cells further help activate CD8$^+$ cytotoxic T cells that may specifically recognize and eliminate virus-infected cells (Figure 1).[6,55–57] Indeed, a persistent antigen-specific germinal center B cell response and plasmablast response in blood and draining lymph nodes are elicited after vaccination with SARS-CoV-2 LNP-mRNA in humans, leading to the development of a robust and prolonged humoral immunity.[53,58]

The entire surface spike (S) glycoprotein of SARS-CoV-2 or the receptor binding domain (RBD) of the S protein, which is critical for viral entry into the host cell,[61] represent the most widely selected and suitable antigen target sites in COVID-19 vaccine development. Both the full-length S protein and the RBD itself are immunogenic and induce a strong protective neutralizing antibody response after recognition by the immune system.[62,63] Immune responses are improved by introducing two consecutive proline residues (2P), which are known to retain the full-length S protein in the prefusion conformation.[64,65] The S protein is cleaved by the host cell protease furin into subunits (S1 and S2), which support cellular entry.[66] Interestingly, the C-terminal motif of the cleaved S1 subunit can bind to neuropillin-1 (NRP-1),[67,68] which negatively influences T cell memory.[69] Consequently, a loss-of-function modification or deletion of the furin cleavage site of the S protein in vaccine development can be beneficial.[70,71]

The efficacy of a viral antigen-encoding mRNA vaccine is influenced by codon optimization of the coding sequence,[72] but not just in relation to stability and translatability. A recent study revealed that cryptic epitopes, originating from out-of-frame open reading frame translation during SARS-CoV-2 infection,[73] can be modified or lost during codon optimization, leading to either enhanced or diminished immunogenic responses.

NUCLEOSIDE-MODIFIED mRNA VACCINES

Numerous vaccine candidates were being investigated in the early stages of the COVID-19 pandemic, including nucleoside-modified LNP-mRNA vaccines, which were the first to enter clinical trials[74] and receive EUAs, CMAs, and later full regulatory approvals.[75–79] At present, there are a total of eight nucleoside-modified LNP-mRNA vaccine candidates in clinical trials, all of which share the key feature of enabling the replacement of all uridines in the mRNA with 1-methylpseudouridine (m1Ψ) (Table 1).

mRNA and LNP features of nucleoside-modified vaccines have been extensively summarized elsewhere.[55,56,87–90] Here, we highlight the main disclosed differences in the characteristics of the mRNA constructs (Figure 2). BioNTech/Pfizer's modRNA platform RNA sequence (used for BNT162b2) consists of human α-globin 5′ untranslated region (UTR), amino-terminal enhancer of split (AES), and mtRNR1 3′ UTR motifs[91] and a poly(A) tail consisting of A30LA70 (linker [L]: GCAUAUGACU),[81] while the mRNA

Table 1. Nucleoside-modified COVID-19 LNP-mRNA vaccines in clinical trials

Name	Developer	Clinical phase, trial identifier	Dose, regimen	Antigen coding sequence	Formulation	Clinical outcome	Regulatory authority approval date
BNT162b2	BioNTech/Pfizer	phase I/II/III (IV), and phase III booster dose NCT04368728	30 μg (10, 20, 30 μg tested in phase I); p-b in 3 weeks	full-length S prefusion-stabilized; optimized, GC-rich	Acuitas LNP	95% protection against symptomatic COVID-19 in participants 16 years or older[8] and 100% efficacy in participants 12 to 15 years of age[80]	EUA by FDA, December 11, 2020 CMA by EMA, December 21, 2020 Full approval by FDA, August 23, 2021 EUA by FDA for those ≥12 years of age, May 10, 2021; under CMA, by EMA, May 28, 2021
		phase II booster dose, NCT05004181, NCT04955626	30 μg (booster dose); p-b in 3 weeks				single booster dose under EUA, by FDA, September 22, 2021; under CMA, by EMA, October 4, 2021
		phase II/III, NCT04816643	10 μg dose for 5- to 11-year-olds; p-b in 3 weeks			90.7% efficacy in participants 5 to 11 years of age[96]	EUA by FDA for 5- to 11-year-olds. October 29, 2021; under CMA, by EMA, November 25, 2021
BNT162b1	BioNTech/Pfizer	phase I/II/III, NCT04368728	10, 20, 30, 100 μg tested in phase I; p-b in 3 weeks	RBD, secreted	Acuitas LNP	positive clinical data, BNT162 was selected for a pivotal efficacy study based on favorable safety data[81,82]	
	BioNTech/Fosun	phase I, NCT04523571				acceptable safety profile and high levels of humoral and T cell responses in younger (ages 18–55 years) and older adults (ages 65–85 years) in an Asian population[83]	
BNT162b3	BioNTech/Pfizer	phase I/II NCT04537949, EUCTR2020-003267-26-DE	undisclosed, dose escalation study, p-b	RBD trans-membrane	Acuitas LNP	not published	
mRNA-1273	Moderna/ NIAID/BARDA	phase III (IV) NCT04811664, phase III, NCT04470427	100 μg (25, 100, 250 μg tested in phase I); p-b in 4 weeks	full-length S prefusion-stabilized	Moderna LNP	94.1% efficacy at preventing COVID-19 illness, including severe disease in participants 18 years or older[9] 95% efficacy in 12- to 17-year olds[84]	EUA by FDA, December 18, 2020 CMA by EMA, January 6, 2021 full approval by FDA, January 31, 2022[85] single booster dose under EUA, by FDA, October 20, 2021; under CMA, by EMA, October 25, 2021

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Table 1. ***Continued***

Name	Developer	Clinical phase, trial identifier	Dose, regimen	Antigen coding sequence	Formulation	Clinical outcome	Regulatory authority approval date
mRNA-1273.211	Moderna	phase II/III, NCT04927065 booster dose against variants study	50, 100 μg; booster	full-length S prefusion-stabilized	Moderna LNP	boosters increased neutralization titers against key variants[86]	
mRNA-1273.351	Moderna	phase II, NCT04405076 booster dose against variants study (B.1.351)	20, 50 μg; booster	full-length S prefusion-stabilized against B.1.351	Moderna LNP	not published	
TAK-919	Takeda/Moderna	phase I/II, NCT04677660	N/A μg; p-b in 4 weeks	full-length S prefusion-stabilized	Moderna LNP	not published	PMDA, Japan, May 21, 2021
ChulaCov19	Chulalongkorn University	phase II, NCT04566276	10, 25, 50 μg; p-b in 3 weeks	full-length S	Genevant LNP	not published	

EMA, European Medicines Agency; EUA, emergency use authorization; CMA, conditional marketing authorization; FDA, US Food and Drug Administration; GC, guanine-cytosine; LNP, lipid nanoparticle; p-b, prime-boost regimen; PMDA, Pharmaceuticals and Medical Devices Agency; S, spike protein; RBD, receptor binding domain.

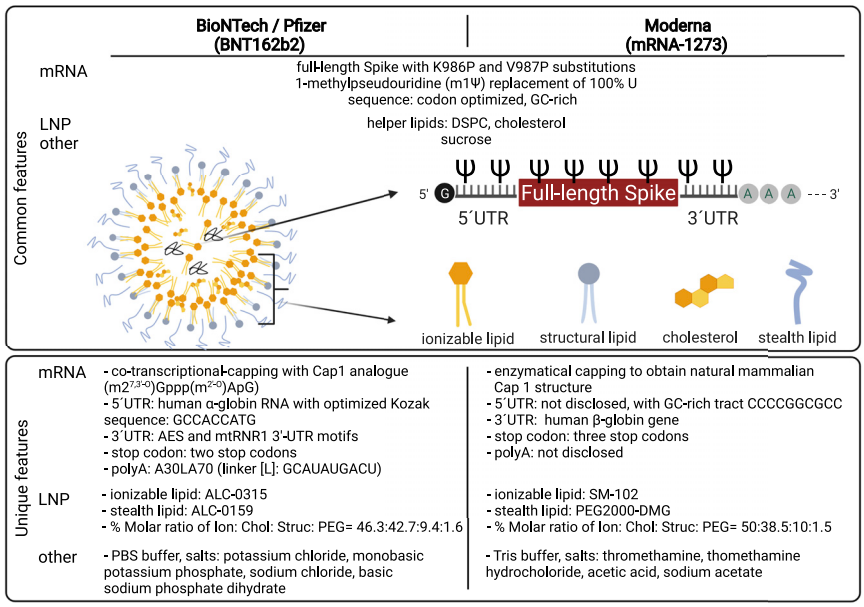

Figure 2. Widely used mRNA-based COVID-19 vaccines: Comparison of ingredients

BNT162b2 (BioNTech/Pfizer) and mRNA-1273 (Moderna) are composed of 1-methylpseudouridine-modified full-length spike mRNA, with proline substitutions, that is GC rich, codon optimized, and composed of standard mRNA components: cap, 5′ UTR, coding sequence, 3′ UTR, and a poly(A) tail. BNT162b2 is co-transcriptionally capped with ((m2$^{7,3'-O}$)Gppp(m$^{2'-O}$)ApG) cap1 and has human α-globin 5′ UTR, AES, and mtRNR1 3′ UTR motifs; two stop codons; and a poly(A) tail consisting of A30LA70.[91,92] mRNA-1273 is enzymatically capped and has an undisclosed 5′ UTR and a human β-globin gene-based 3′ UTR, three stop codons, and a poly(A) tail of undisclosed length.[93] In both cases, the mRNA is formulated using LNPs consisting of ionizable, structural, and stealth lipids and cholesterol. The LNPs of both mRNA vaccines contain DSPC and cholesterol. Unique features of BNT162b2 and mRNA-1273 LNP formulations are the use of ALC-0315 and SM-102 ionizable lipids and ALC-0159 and PEG2000-DMG, PEG-based stealth lipids, respectively.[88,90,94,95] Lipids are integrated into the LNPs under specific molar ratios.[88,90,94,95] In addition to the mRNA and LNP components, the only ingredients are salts (PBS and Tris buffers for BNT162b2 and mRNA-1273, respectively) and 10% sucrose that is used as a cryoprotectant for both mRNA vaccines.[88,90]. ALC-0159, 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide; DSPC, 1,2-distearoyl-sn-glycero-3-phosphocholine; K, lysine; mRNA, messenger ribonucleic acid; LNP, lipid nanoparticle; P, proline; PEG2000-DMG, 1,2-dimyristoyl-sn-glycero-3-methoxypolyethylene glycol; UTR, untranslated region; V, valine; AES, amino-terminal enhancer of split; mtRNR1, mitochondrially encoded 12S rRNA; ALC-3015, ((4-hydroxybutyl)azanediyl)bis(hexane-6,1-diyl)bis; SM-102, 9-heptadecanyl 8-{(2-hydroxyethyl)[6-oxo-6-(undecyloxy)hexyl]amino}octanoate. Figure was created with BioRender.com.

sequences developed by Moderna (mRNA-1273, mRNA-1273.211, mRNA-1273.351), Takeda (TAK-919), and Chulalongkorn University (ChulaCov19) are not fully disclosed. Capping of BNT162b2 was performed co-transcriptionally using a trinucleotide cap1 analog ((m2$^{7,3'-O}$)Gppp(m$^{2'-O}$)ApG) (TriLink),[81,92] while Moderna used enzymatic capping to obtain cap1 for their preclinical mRNA-1273.[3] All eight nucleoside-modified mRNAs are formulated using one of three types of LNP originating from Acuitas, Moderna, and Genevant, which consist of four components: ionizable lipids, structural lipids, stealth lipids, and cholesterol.

The design and preclinical testing of nucleoside-modified mRNA vaccines is based on years of research; thus, at the start of the COVID-19 pandemic they were well placed for rapid adjustments and application to SARS-CoV-2. However, one of the bottlenecks that required significant innovation was manufacturing, process development, and scale-up methods to meet supply demands for large-scale clinical trials and later worldwide marketing. The details of the manufacturing processes are proprietary to the companies producing nucleoside-modified mRNA vaccines and not disclosed in literature.

Figure 3 outlines the basic steps of this manufacturing and scale-up: IVT, mRNA purification, formulation process, and downstream steps up to fill and finish. One of the key steps in this process is mRNA purification, which allows depletion of double-stranded RNA (dsRNA) contaminants. Through recognition of TLR3 in endosomes or retinoic acid-inducible gene I, melanoma differentiation-associated protein 5, and inflammasomes in the cytoplasm,[96–99] depending on the amount, dsRNAs might overactivate innate immunity and lead to adverse events. The exact purification processes performed for BNT162b2 and mRNA-1273 used on a large manufacturing scale are undisclosed. HPLC is a gold standard for mRNA purification in a lab setting;[100] however, it presents challenges when scaling up, and thus it can only be speculated that certain adjustments had to be made between the mRNA vaccine purity and the manufacturing process that allows the scale necessary to produce enough vaccine to meet global requirements during a pandemic. Another possibility is innovation on the IVT level (e.g., by using engineered RNA polymerases and specific reaction conditions)[101,102] that could lead to a significant decrease in dsRNA formation during the IVT reaction, allowing achievement of excellent purity with standard industry purification methods. A thermostable RNA polymerase has been recently used for mRNA IVT and to prevent formation of dsRNA, allowing a lower immune response to such mRNA without the purification step.[102] In 2021, Moderna provided details on their T7 RNA polymerase, which is able to minimize dsRNA formation;[103] however, its potential use in mRNA-1273 vaccine production is currently not publicly disclosed and can only be speculated.

The dosing of nucleoside-modified LNP-mRNAs against COVID-19 applied in clinical trials ranged between 10 and 250 μg RNA (Table 1). BNT162b2 was granted EUA by the FDA on December 11, 2020, for a 30 μg dose for individuals 16 years or older for the prevention of COVID-19, and the EUA for mRNA-1273 was granted shortly after on December 18, 2020, for individuals 18 years of age and older at a dose of 100 μg. Full approval by the FDA was granted for BNT162b2 for individuals 16 years and older and mRNA-1273 for

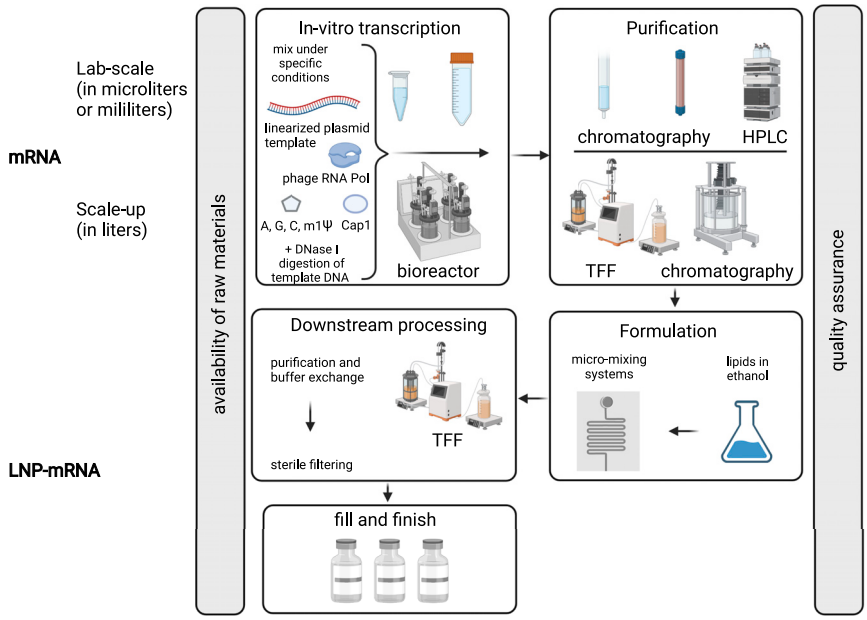

Figure 3. Manufacturing and scale-up of nucleoside-modified mRNA vaccines

The first step in nucleoside-modified mRNA vaccine production consists of an IVT reaction. This reaction, which is conducted under specific conditions, is based on mixing linearized plasmid template, phage RNA polymerase, nucleoside-triphosphates (including m1Ψ), and the Cap1 structure when a co-transcriptional capping process is used.[57] The IVT reaction can be performed at different scales and is typically followed by DNase I digestion, which allows DNA template depletion. Purification of mRNA is a process that allows depletion of unwanted IVT reaction by-products and other impurities. Depletion of dsRNA formed during IVT reactions by diverse types of chromatography, such as HPLC or TFF techniques, means that mRNA vaccine-triggered adverse events caused by systemic innate immune system responses are kept to a minimum. Purified mRNA is diluted in an appropriate buffer and then formulated with lipid components, which are dissolved in ethanol by a micro-mixing technology.[104–106] Downstream processes include further purification, buffer exchange, and sterile filtering prior to fill and finish.[106] Availability of raw materials is of key importance for continual large-scale production when demands are high, such as during a pandemic. The process is tightly controlled by numerous quality assessments at the LNP, mRNA, and LNP-mRNA levels. HPLC, high-performance liquid chromatography; LNP, lipid nanoparticle; m1Ψ, 1-methylpseudouridine; mRNA, messenger ribonucleic acid; RNA Pol, RNA polymerase; TFF, tangential flow filtration. Figure was created with BioRender.com.

individuals 18 years and older on August 23, 2021, and on January 31, 2022, respectively. All nucleoside-modified LNP-mRNAs referred to in Table 1 are administered i.m. in a prime-boost (p-b) regimen with a 3 week interval for BNT162b2 and ChulaCov19, or a 4 week interval for mRNA-1273 and TAK-919.

In preclinical studies, one i.m. injection of BNT162b1 or BNT162b2 was sufficient to elicit an inhibitory antibody response with high titers as well as $CD4^+$ helper and $CD8^+$ cytotoxic T cell responses.[107] Two injections of BNT162b2 administered to rhesus macaques elicited SARS-CoV-2 neutralizing antibody titers 8.2- to 18.2-fold higher than in convalescent human sera.[107] The safety and immunogenicity data of the phase I/II trials of BNT162b1 and BNT162b2 supported the selection of BNT162b2 for a phase II/III trial based on milder systemic reactions[82] and strong adaptive humoral and poly-specific cellular immune responses against epitopes conserved in diverse SARS-CoV-2 virus variants.[81] In the BNT162b2 phase II/III trial, with a total of 43,548 participants 16 years of age or older, two doses of BNT162b2 resulted in confirmation of the favorable safety profile and 95% vaccine efficacy (prevention of COVID-19).[8] In 12- to 15-year-olds, two 30 μg doses of BNT162b2 showed a favorable safety profile and resulted in 100% vaccine efficacy.[80] Recently, as a part of the clinical trial examining BNT162b2 in 5- to 11-year-olds (NCT04816643), two 10 μg doses of BNT162b2 were found to give rise to 90.7% vaccine efficacy (prevention of COVID-19).[108] Owing to the reduced efficacy of LNP-mRNA vaccines over time and the emergence of highly transmissible SARS-CoV-2 variants such as delta (B.1.617.2) and omicron (B.1.1.529),[109,110] the effects of booster vaccination are being examined. An Israeli study looking at the population ≥60 years of age receiving a single booster of BNT162b2 given at least 5 months after completion of the p-b primary regimen demonstrated 95% fewer reports of severe illness and significantly lower rate of confirmed COVID-19 cases.[111] Recently, multiple trials have investigated vaccine-induced immunity against the delta (B.1.617.2) and omicron (B.1.1.529) variants.[110,112,113] In all cases, neutralizing antibody titers after the second dose of mRNA-based vaccines were highly reduced for omicron (B.1.1.529) compared with delta (B.1.617.2) and especially compared with the wild-type variant, while the third dose significantly improved antibody neutralization titers.[110,112,113] This suggests that application of a booster dose of mRNA vaccines may protect against these highly transmissible variants and supports booster vaccine doses, which are now being rolled out globally. A booster dose of BNT162b2 obtained EUA by the FDA on September 22, 2021, for individuals ≥65 years of age or ≥18 years at high risk of severe COVID-19.[114] Currently, clinical trials with authorized vaccines in specific population groups, including 6-month- to 4-year-old children, pregnant women, immunocompromised individuals, and cancer patients, are ongoing (Table 2).

mRNA-1273 went through a clinical development process similar to that of BNT162b2,[3] and a favorable safety profile and 94.1% vaccine efficacy (protection in preventing COVID-19 illness, including severe disease) for those ≥18 years of age was demonstrated in a pivotal clinical phase II/III trial.[9] In an interim analysis

Table 2. Clinical development of BioNTech/Pfizer and Moderna COVID-19 vaccines in specific population groups

Name	Sponsor of clinical trial	Clinical phase, identifier	Population type	Population	Age	Estimated primary completion date
BNT162b2	BioNTech SE	phase II/III, NCT04816643	healthy	healthy individuals	6 months to 18 years	June 18, 2024
	BioNTech SE	phase III, NCT04754594	healthy	pregnant women	≥18 years	October 15, 2022
	BioNTech SE	phase II, NCT04895982	immunocompromised	immunocompromised	≥12 years	February 11, 2023
	Centre Hospitalier Régional d'Orléans	phase IV, NCT04952766	immunocompromised	kidney transplant, myeloma, cancer, hematologic malignancy, multiple sclerosis, hypergammaglobulinemia, malignant tumors, HIV, diabetes mellitus type 2	≥18 years	March 2022
	University of Liege	phase III, NCT04951323	immunocompromised	allogeneic stem cell recipients	18 to 100 years	December 1, 2021
	Humanity & Health Medical Group Ltd.	phase IV, NCT04775069	immunocompromised	chronic liver disease	≥18 years	December 31, 2021
	National Institute of Allergy and Infectious Diseases (NIAID)	phase II, NCT04761822	immunocompromised	high-allergy/mast cell disorder	≥12 years	October 2021
	Assistance Publique-Hôpitaux de Paris	Phase I/II, NCT04969601	cancer	acute leukemia	≥12 years	April 2022
	Moderna	phase III, NCT04470427	healthy	healthy individuals	≥18 years	October 27, 2022
mRNA-1273	Moderna	phase III, NCT04649151	healthy	healthy individuals	12 to 17 years	June 30, 2022
	Moderna	phase III, NCT04796896	healthy	healthy individuals	6 months to 11 years	June 12, 2023
	Moderna	phase III, NCT04860297	immunocompromised	solid organ transplant recipients	≥18 years	March 31, 2023
	McGill University Health Centre/Research Institute of the McGill University Health Centre	phase III, NCT04806113	immunocompromised	rheumatic diseases, rheumatoid arthritis, systemic lupus erythematosus	≥18 years	June 13, 2021
	NIAID	phase II, NCT04761822	immunocompromised	high-allergy/mast cell disorder	≥12 years	October 2021
	National Cancer Institute (NCI)	phase II, NCT04847050	cancer	solid tumor malignancies, hematologic malignancies, lymphoma, multiple myeloma	≥18 years	January 1, 2022

HIV, human immunodeficiency virus.

of a single booster dose following p-b vaccination, Moderna tested mRNA-1273 and multiple variant-modified versions and found high safety and tolerability, as well as increased neutralization titers against key variants of concern and variants of interest of SARS-CoV-2, including beta (B.1.351), gamma (P.1), delta (B.1.617.2), and omicron (B.1.1.529).[86] In 12- to 17-year-olds, mRNA-1273 showed 95% vaccine efficacy and had an acceptable safety profile.[84] A single booster dose of mRNA-1273 obtained EUA by the FDA on October 20, 2021, while numerous clinical trials for specific population groups are currently recruiting (Table 2).

While initial clinical trials included healthy volunteers, an important aspect is the safety and efficacy in pregnant women, who were not included. A recent observational study in a cohort of 10,861 pregnant women showed that BNT162b2 had 96% effectiveness in the prevention of COVID-19.[115] In a smaller patient cohort with 131 individuals, BNT162b2 and mRNA-1273 were studied in pregnant or lactating women, and both vaccines led to robust humoral immunity with no significant differences in postvaccination reactogenicity between pregnant and non-pregnant women.[116] Importantly, functional neutralizing antibodies against SARS-CoV-2 were found in infant cord blood and in the breast milk of vaccinated pregnant women, suggesting that there is a protective effect for newborns.[116–118] In January 2022, the European Medicine Agency's COVID-19 task force finalized a detailed review of multiple studies in pregnant woman, including about 65,000 pregnancies. Based on the results, the use of mRNA-based COVID-19 vaccines during pregnancy is encouraged, since no increased risk of pregnancy complications for expectant mothers and their unborn babies was found, and effectiveness was not compromised compared with non-pregnant women.[119]

Table 3. Unmodified mRNA COVID-19 vaccine candidates being tested in clinical trials

Name	Developer	Clinical phase, trial identifier	Dose, regimen	Antigen coding sequence	Formulation	Clinical outcome
CVnCoV	CureVac	phase IIb/III, NCT04652102 phase I, II, NCT04449276, NCT04515147	12 μg, p-b, 4 weeks	full-length S, 2P	Acuitas LNP	48% efficacy for the prevention of COVID-19 in age group 18–60 years[123]
MRT5500 (VAW00001)	Translate Bio/Sanofi	phase I/II, NCT04798027	7.5 μg, p-b, 3 weeks	full-length S 2P, modified furin cleavage site	LNP	not published
ARCoV	AMS/Walvax/Suzhou	phase II, ChiCTR2100041855 phase III, NCT04847102	15 μg, p-b, 2 to 4 weeks	RBD	LNP	not published
PTX-COVID19B	Providence Therapeutics	phase I (II), NCT04765436	16, 40, 100 μg, p-b, 4 weeks; 40 μg was selected for phase II	full-length S	LNP	well tolerated in seronegative 18- to 64-year-old individuals, strong IgG antibody response[124]
		phase II, NCT05175742 comparison with BNT162b2	60, 80 μg, p-b, 4 weeks			not published
DS5670	Daiichi Sankyo	phase I/II, NCT04821674	dose not disclosed, p-b	not disclosed	LNP	neutralizing activity without any safety concerns in both age groups (20–64 and 65–74 years)[125]
SW-0123	Stemirna Therapeutics/ Shanghai East Hospital	phase I, ChiCTR2100045984	10, 30, 60, 100 μg, p-b	full-length S	LPP	not published
EG-COVID	eyeGENE	phase I/IIa	50, 100, 200 μg, p-b, 3 weeks	full-length S	cationic liposome	not published

2P, two consecutive proline residues; AMS, Academy of Military Science of the Chinese People's Liberation Army; IgG, immunoglobulin G; LNP, lipid nanoparticle; LPP, core-shell structured lipopolyplex; p-b, prime-boost regimen; RBD, receptor binding domain; S, spike protein.

UNMODIFIED mRNA VACCINES

The optimization of mRNA constructs, including the coding sequence, can regulate immunogenicity without incorporation of modified nucleosides. Uridine depletion to reduce counterproductive immune reactions can be achieved by the replacement of uridine by codon optimization, but this must be balanced with regard to transfer RNA abundance.[120–122] Currently, there are no marketed unmodified mRNA vaccines, but candidates are being investigated in clinical trials (Table 3).

The favorable safety profile of the CVnCoV vaccine candidate[126,127] using the RNActive mRNA vaccine platform developed by CureVac[128] was confirmed at up to a 12 μg dose,[129] but later in the phase IIb/III trial, using a p-b regimen with 12 μg 4 weeks apart, only 48% efficacy was disclosed for the prevention of COVID-19 at any severity in all age groups among 39,680 participants,[130] which finally led to its withdrawal from the regulatory approval process. The inherent limitations of unmodified mRNA vaccines against COVID-19 are yet to be overcome. These limitations are based on the intrinsic immunogenicity of unmodified mRNA due to intracellular detection[131,132] and type I interferon (IFN) production,[34] which in turn is responsible for the inhibition of translation, the reduction of $CD8^+$ T cell activation, and the inhibition of T helper cell generation. These alterations possibly lead to a blunted specific immune response.[37,133–135] However, CureVac further engineered the UTRs of the mRNA and showed a superior immunological profile in preclinical tests,[136,137] and subsequently, in collaboration with GlaxoSmithKline, CureVac plans to investigate this second-generation candidate in a clinical program.[138] MRT5500, developed by Translate Bio and Sanofi, was examined in a phase I/II trial where different doses (15, 45, or 135 μg) were administered with a 3 week interval without safety or tolerability concerns. Interestingly, the MRT5500 vaccine candidate was designed using the full-length S protein with a loss-of-function modified furin cleavage site (RRAR682-685GSAS)-encoding sequence. However, the developers decided not to proceed with a phase III clinical trial for the vaccine candidate.[139] BioNTech/Pfizer started COVID-19 vaccine development in three different mRNA vaccine platforms, including one utilizing unmodified mRNA. BNT162a2, a full-length S protein encoding unmodified linear mRNA, was not selected for later-stage clinical trials. The ArCoV vaccine candidate, developed through the cooperation of Walvax Biotechnology, Suzhou Abogen Biosciences, and the Academy of Military Science of the Chinese People's Liberation Army, is registered for entering late-stage clinical assessment. The investigational product is an RBD-encoding, LNP-mRNA, which is administered as a 15 μg dose in a p-b regimen. The developers emphasize thermostability, as their vaccine can be stored at room temperature for at least 1 week, which was confirmed in preclinical studies.[140,141] PTX-COVID19-B, developed by Providence

Therapeutics, entered a phase II clinical trial[142] following preclinical testing[143] and a successful phase I trial, which showed that it had a tolerable safety profile at a dose of up to 100 μg among seronegative individuals, and induced high neutralizing antibody levels. In January 2022, a clinical trial was started to compare PTX-COVID19-B with the authorized BNT162b2 COVID-19 vaccine.[144] DS5670, developed by Daiichi Sankyo, is being tested in four different doses up to 100 μg in a Japanese population focusing on age-specific immunogenic responses in the phase I/II clinical trial. Recently, the company disclosed that no relevant safety concerns have been observed in different age groups, with appropriate immunogenic response.[125] In a phase I clinical trial, Stemirna Therapeutics in cooperation with Shanghai East Hospital are testing their SW-0123 vaccine candidate. The mRNA encodes the natural full-length S protein rather than a prefusion-stabilized form, and is formulated into a core-shell structured lipopolyplex (LPP), making it highly selective to dendritic cells, according to preclinical study results.[145] EyeGene developed a proprietary formulation using cationic liposome to avoid the use of polyethylene glycol and to facilitate freeze-drying during production. Their candidate is scheduled to enter testing in a phase I/IIa trial in Korea in late 2021.[146,147]

Furthermore, numerous development projects in preclinical phases aim to create an effective COVID-19 vaccine using an unmodified linear mRNA platform, including the Max Planck Institute of Colloids and Interfaces (Germany), Selçuk University (Turkey), CanSino (China) in collaboration with Precision NanoSystems, BIOCAD (Russia), RNAimmune (USA), Greenlight Biosciences (USA), IDIBAPS (Spain), Cell Tech Pharmed (Iran), ReNAP (Iran), and Globe Biotech Ltd. (Bangladesh).[148] In particular, a unique formulation developed by eTheRNA makes their candidate suitable for intranasal administration, which could prove advantageous in vaccination against SARS-CoV-2 by activation of mucosal immunity.[149] A consortium of RNACure BioPharma, Fudan University, and Shanghai Jiao Tong University is developing three candidates, including one that encodes membrane (M) and envelope (N) proteins in addition to S protein, resulting in the production of virus-like particles,[150] marking a different approach with regard to the chosen antigen and in contrast to other mRNA vaccine candidates.

circRNA VACCINES

circRNA (also called endless RNA) can be translated in eukaryotic cells[48] and, due to its closed-ring structure, is significantly more resistant to exonuclease-mediated degradation compared with linear mRNA.[151] Despite these potential benefits, there is only one circRNA COVID-19 vaccine candidate under development, which is produced using a group I ribozyme autocatalysis strategy[49] but, as of January 31, 2022, has not yet entered clinical trials. The RBD antigen-encoding sequence of this circRNA, which is encapsulated in LNPs, was fused with signal peptide sequences to ensure secretion of antigens and to improve binding capacity. To drive translation, an undisclosed internal ribosome entry site (IRES) was positioned in front of the antigen-encoding sequence. In the preclinical study, the candidate was administered to mice i.m. at two different doses (10 and 50 μg) in a p-b regimen 2 weeks apart, which elicited a high titer of neutralizing antibodies in a dose-dependent manner, with Th1-biased T cell responses.[59]

saRNA VACCINES

Similar to other mRNA vaccine platforms, saRNA facilitates rapid candidate design and development, as well as cell-free molecule synthesis.[152] Moreover, owing to its self-replicative nature, in which the encoded replicase enables multiple copies of the antigen-encoding RNA, lower doses seem to be required to reach the same translational level compared with non-replicative mRNAs.[153,154] saRNA vaccines are inherently self-adjuvanting, as their replicase activity leads to dsRNA and replicon intermediates during transcription mimic a viral infection. This triggers a broader immune response, which was also considered to have potential to be effective with a single-dose inoculation.[155,156]

Several clinical trials investigating the safety and efficacy of saRNA vaccines against COVID-19 are currently ongoing (Table 4). The front-running and disclosed candidates are designed with Venezuelan equine encephalitis virus (VEEV)-derived replicase in one RNA molecule with the antigen coding sequence predominantly full-length S protein. Most of the clinical trials are using p-b immunization or comparing a single dose with a p-b regimen. Only one candidate, EXG5003, is undergoing testing with only a single-dose administration. One of the main obstacles concerning the platform is the large size of the saRNA, as it contains the non-structural protein encoding sequence derived from the alphavirus genome necessary for replicative activity. The length of the RNA substantially influences the stability and ease of delivery. However, there is no saRNA COVID-19 vaccine candidate that was developed using the previously successfully applied size reduction method.[157,158]

After a preclinical *in vivo* experiment demonstrating proof of principle for an effective saRNA candidate against COVID-19,[161] Imperial College London entered LNP-nCoVsaRNA into a phase I/II clinical trial.[162] Doses of 0.1–10 μg showed a dose-dependent immune response; however, there was no seroconversion in a significant portion of the trial participants, all of whom were seronegative at inclusion for SARS-CoV-2 infection[159] and, accordingly, the clinical development process was halted. The self-adjuvant effect of saRNA is partly related to the activation of types I and III IFN,[153] which has the potential to induce a negative feedback loop and inhibit translation. Accordingly, despite lower doses, the use of saRNA vaccines lead to higher reactogenicity.[50,157]

To avoid this, a candidate with an undisclosed redesigned saRNA backbone to dampen IFN production is being studied in the phase I COVAC-Uganda trial (LNP-nCOV saRNA-02), which assesses immune response in SARS-CoV-2 antibody seronegative and seropositive individuals, but is not directly testing efficacy against COVID-19.[163] One of the first saRNA vaccine candidates to enter the clinical phase, BNT162c2, was developed and tested by BioNTech; however, this vaccine format was not selected for further clinical investigation. Arcturus

Table 4. Self-amplifying mRNA vaccines in clinical trials

Name	Developer	Clinical phase, trial identifier	Dose, regimen	Antigen coding sequence	LNP features	Clinical outcome
LNP-nCoVsaRNA	Imperial College London	phase I/II, ISRCTN17072692	0.1 to 10 μg, p-b, 4 weeks	full-length S, prefusion-stabilized	LNP	dose-dependent immunological effect up to 5 μg, seroconversion 8%–61% by ELISA, 46%–87% by immunoblot assay[159]
LNP-nCOV saRNA-02	Imperial College London/ MRC/UVRI/LSHTM/ Uganda Research Unit	phase I, NCT04934111	5 μg, p-b, 4 weeks	full-length S, prefusion-stabilized	LNP	not published
EXG5003	Elixirgen Therapeutics	phase I/II, NCT04863131	N/A μg, one dose	RBD	LNP	not published
ARCT 021 ARCT 154 ARCT 165	Arcturus Therapeutics/ Duke-NUS Medical School	phase II/III, NCT05012943 phase I/II, NCT04480957 phase II, NCT04728347 phase I/II NCT05037097	0.2 to 10 μg ARCT 021: 5 μg, one dose or p-b, 4 weeks	full-length S, prefusion-stabilized	LUNAR	dose-dependent binding and neutralizing antibody responses in interim data[160]
CoV2 SAM LNP	GSK	phase I, NCT04758962	1 μg, p-b	full-length S	LNP	not published
HDT301 (repRNA-CoV2S; HGCO19)	SENAI Cimatec/HDT/ Gennova/Quratis	phase I, NCT04844268	1, 5, 25 μg, one dose or p-b in 4 or 8 weeks	full-length S	LION	not published
VLPCOV-01	VLP Therapeutics Japan	phase I, jRCT2071210067	N/A	N/A	N/A	not published
SAM-SARS-CoV-2	Gritstone	phase I	10 μg, one dose as booster	full-length S, perfusion stabilized, mutated furin cleavage site	LNP	not published

GSK, GlaxoSmithKline; LION, lipid inorganic nanoparticles; LNP, lipid nanoparticle; LSHTM, London School of Hygiene and Tropical Medicine; LUNAR, lipid-enabled nucleic acid delivery reagent; MRC, Medical Research Council; N/A, not applicable/not disclosed; p-b, prime-boost regimen; RBD, receptor binding domain; S, spike protein; UVRI, Uganda Virus Research Institute.

Therapeutics, in collaboration with Duke-NUS Medical School, is studying three candidates (ARCT-021, ARCT-154, ARCT-165) using their STARR mRNA saRNA platform. The company's first candidate, ARCT-021, was tested in a phase I/II trial with a single-dose regimen and p-b dosing in the range of 1–10 μg. Currently, a single-dose regimen is being investigated in an ongoing phase II trial. ARCT-154 has started a phase I/II/III trial in Vietnam using a modified antigen-encoding sequence focusing on the recent alpha (B.1.1.7), beta (B.1.351), gamma (P.1), and delta (B.1.617.2) virus variants. Addressing the question of the boost application of saRNA, all three candidates will be directly compared in a phase I/II trial in previously vaccinated and unvaccinated individuals in the United States and in Singapore. First results have been released from 24 participants, in which a 5 μg booster dose of ARCT-154 or ARCT-165 5 months after immunization with BNT162b2 resulted in a robust increase in neutralizing antibody responses against the omicron variant (B.1.1.529) after booster injection.[164]

All saRNA candidates that are being tested in the clinic were designed for i.m. administration, with the exception of EXG-5003 (Elixirgen Therapeutics), which is formulated for intradermal administration, rendering it effective only at the injection site in a temperature-sensitive manner.[165] In an extensive collaboration of SENAI Cimatec, Gennova Biopharmaceuticals, HDT Biotech, and Quratis, another candidate, HDT301 (also called repRNA-CoV2S or HGCO19), is being tested in a phase I/II trial. In the preclinical setting, the p-b regimen has been shown to induce a more potent T cell response in comparison with the administration of a single dose;[166] however, in the ongoing clinical trial, a one-shot regimen is also being tested. Data from a phase I trial in India demonstrated that Gennova's COVID-19 RNA vaccine candidate HGCO19 has an acceptable safety and tolerability profile.[167]

Recently, another important question has emerged regarding the boost potential of different vaccine modalities, specifically, the heterologous administration of two different products. In the preclinical setting, a heterologous vaccine regimen of an adenoviral vectored vaccine (ChAdOx1 nCoV-19/AZD1222) followed by saRNA was tested, which resulted in improved antigen-specific antibody responses and a higher neutralization effect compared with the p-b homologous vaccine regimen.[168] Similarly, Gritstone Bio has an saRNA vaccine candidate (SAM-SARS-CoV-2), which is being investigated in a phase I clinical trial among individuals previously immunized with an adenoviral-vectored vaccine[169]. SAM-SARS-CoV-2 is designed to elicit immune responses against S and the nucleoprotein, as well as the membrane protein and open reading frame 3, which may bolster its efficacy against variants of concern[170].

There are several other vaccine candidates on the horizon that will enter clinical trials in the immediate future, such those developed by Ziphius Vaccines with Ghent University and by Amyris (USA) in collaboration with the Infectious Disease Research Institute, which has promising preclinical data justifying a clinical development program.[171]

CONCLUSION AND OUTLOOK

The COVID-19 pandemic led to the fastest vaccine development in history.[172] Although the therapeutic potential of mRNA had previously been investigated for many decades without being available as a marketed medical treatment, the time was now ripe to harness its unique features compared with conventional vaccine approaches. In this review, we aimed to give an overview of the approved COVID-19 mRNA vaccines and vaccine candidates as well as their different molecular biology approaches in this fast-moving field.

If a suitable antigen sequence is available to target, mRNA vaccine technology facilitates rapid design and production because it does not involve pathogens or growing the vaccine by specific cell culture processes or fermentation. In contrast, these challenges make traditional vaccine research, development, and production processes more complex and lengthy.[173]

The two vaccines against COVID-19 to first gain accelerated approvals and meet the required safety and efficacy standards were mRNA vaccines.[8,81,82] Nucleoside-modified mRNA in LNPs seems to be the superior choice over unmodified candidates, as demonstrated by the clinical efficacy and safety data. While relatively similar dose levels of unmodified mRNA and saRNA vaccine candidates were tested in phase I trials, unmodified mRNA vaccines that proceeded to later stages of clinical testing were used at lower doses compared with nucleoside-modified mRNA vaccines. Since none of the unmodified mRNA and saRNA candidates have made it to an approval stage as of this writing, it may be speculated that this is due to intrinsic adverse effects caused by the unmodified nucleosides or immunostimulatory by-products. circRNA represents a favorable concept, but it seems that further improvement is necessary prior to continuing their development as drug candidates. The circRNA platform is theoretically compatible with the replacement of uridine; however, to our knowledge, the functionality of an IRES element in combination with modified nucleosides is yet to be demonstrated.

The flexibility of LNP-mRNA-based vaccine design and scalability of manufacturing, in contrast to conventional technologies, may allow for a rapid response to other novel SARS-CoV-2 variants in the case where the efficacy of the vaccine designed for the original strain is lower. Nowadays, different approaches are in the pipeline with the use of mRNA platforms that aim to have a variant-specific response, including modified, adjusted coding sequences for new strains or heterologous vaccine administration. In January 2022, BioNTech and Pfizer announced that a new clinical trial has started to test an omicron (B.1.1.529)-specific mRNA vaccine in healthy adults.[174] Similarly, Moderna started a phase II study to test their omicron-specific booster candidate (mRNA-1273.529).[175]

The success of mRNA vaccines in the COVID-19 vaccine race emphasized the promising potential of this technology in a wide variety of future applications,[176,177] including other infectious diseases,[4] cancer therapy,[178,179] and protein replacement therapies,[180,181] which is reflected in the current pipelines of the developers.

However, it must be considered that, in addition to the success of the first mRNA vaccines, the proof of a versatile future usage of this technology must be delivered. Profound immunogenicity data for therapeutic usage in non-inflammatory environments is still missing. It is also expected that the world will have to deal more frequently with new pandemics, and, in terms of COVID-19, novel virus variants of concern may arise and manifest in the population. Here, the mRNA technology could prove its suggested supremacy regarding speed of design and scale-up and cost efficiency when adapting to new sequences or even other diseases. Israel, a country with extensive real-world data on COVID-19 vaccine effectiveness due to its rapid vaccine rollout, reported a drop in neutralizing antibody titer to less than 10% of the initial titer after vaccination.[182] This raises concerns regarding additional booster doses of the highly similar mRNA vaccine and if it may cause host tolerance, which is particularly pertinent if the vaccine needs to be administered several times a year or seasonally. Although immune escape of the omicron (B.1.1.529) variant has been reported,[183] data from Israel have shown that there is a significantly lower rate of SARS-CoV-2 infection after a booster-immunization compared with after the primary two-dose series.[184] Ultimately, the world will need multiple adaptable vaccine technologies in parallel to cover supply demands. In terms of mRNA vaccine use for other diseases, the fundamentals of a therapeutic mRNA vaccine for the induction of antigen-specific tolerization in the treatment of experimental autoimmune encephalomyelitis, a mouse model for multiple sclerosis, have been developed.[185] Some of the future applications of mRNA technology include individualized cancer vaccines,[186,187] co-stimulatory ligand receptors, cytokines, immunomodulators, and mRNA-encoded antibodies, as well as chimeric antigen receptors (CARs), and are extensively reviewed elsewhere.[179] Despite the current hype of mRNA technology, many researchers are already pursuing novel methods to improve mRNA composition and expansion into their fields of application.

The administration routes of mRNA therapies are mainly limited to i.m. and i.v.; however, there are encouraging results addressing different modes of delivery. It seems beneficial to reach the respiratory tract epithelium in airborne diseases in order to prime lung-resident memory T cells.[188] In addition to different indications,[189,190] a COVID-19 vaccine candidate for intranasal delivery is being investigated.[149] For enteral use, an interesting approach is the milli-injector capsule with nanoparticle formulations of nucleic acids, which is able to deliver to the gastric epithelia.[191] Furthermore, norovirus and SARS-CoV-2 sequences have been combined to generate an orally delivered saRNA vaccine.[192] Recombinant murine cytomegalovirus was successfully used in mice as a vector-based vaccine against influenza and SARS-CoV-2.[193] Others pursue DNA-based vaccine approaches to fight drug resistance in cancer.[194] Adenoviral-based drug delivery is an option to deliver nucleic acids (reviewed by Sakurai et al.[195]); however, repeated administration could fail to induce

or greatly reduce the desired immune response due to induction of anti-adenoviral antibodies by the host.[196]

The new mRNA vaccines must still overcome several challenges to meet the world market needs. The further development of an adjustable formulation platform resulting in even lower or no adverse effects is crucial, especially in terms of choice of lipids and their charge and biodegradability. Hurdles for continuous production could be the availability and scalability of essential reagents. Furthermore, low-income countries should have equitable access to these new vaccines, which will be instrumental in tackling the pandemic. BioNTech and Pfizer have designed a three-step price system depending on the income level of a country, of which the lowest price is a non-profit price, financed by the higher price for high-income countries, such as the United States or the European Union.[197] Similarly, Moderna states that they are "aiming to provide effective and affordable vaccines and therapeutics to all populations."[198]

In summary, mRNA-based therapeutics have key advantages in terms of their versatility and adaptability, which could lead to promising therapeutics not only for infectious diseases but for other indications such as cancer, and thus, further research and investment into this technology is warranted. Given the success of the technology thus far, the expectations are high and so could be the reward.

Source of the data

References published as of January 31, 2022, about mRNA vaccine candidates were selected from the PubMed online library and from publications without finalized peer review available on the medRxiv and bioRxiv preprint servers. In addition, announcements, communications, and press releases from pharmaceutical companies and health care agencies were cited to enable a timely review. Relevant information about ongoing clinical trials was identified using ClinicalTrials.gov and the Chinese Clinical Trial Registry websites.

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DECLARATION OF INTERESTS

G.T.S., A.J.M., and I.V. are all full-time employees at BioNTech SE, Mainz, Germany, and may hold shares from BioNTech SE.

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