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Review

COVID-19 mRNA vaccines

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ABSTRACT

The ongoing COVID-19 pandemic and its unprecedented global societal and economic disruptive impact highlight the urgent need for safe and effective vaccines. Taking substantial advantages of versatility and rapid development, two mRNA vaccines against COVID-19 have completed late-stage clinical assessment at an unprecedented speed and reported positive results. In this review, we outline keynotes in mRNA vaccine development, discuss recently published data on COVID-19 mRNA vaccine candidates, focusing on those in clinical trials and analyze future potential challenges.

Introduction

SARS-CoV-2 was initially identified as the pathogen that caused the outbreak of pneumonia cases in late December 2019 (Juno et al., 2020; Shan et al., 2020; Sun et al., 2020; Xu et al., 2020; Zhou et al., 2020; Zhu et al., 2020c). On March 11th, 2020, SARS-CoV-2 was declared a global pandemic by the World Health Organization (WHO), and the disease was named the coronavirus disease 2019 (COVID-19) (Juno et al., 2020). SARS-CoV-2 is a betacoronavirus closely related to SARS-CoV (with 80% sequence identity) that caused the SARS outbreak in 2003 (Hu et al., 2020; Su et al., 2021). However, the rate of the spread of SARS-CoV-2 is 40-fold higher than that of SARS-CoV (Tang et al., 2020). SARS-CoV-2 has proven to be transmitted from not only symptomatic but also asymptomatic individuals (Hu et al., 2020). As of January 2021, the virus has caused more than 100 million laboratory-confirmed infections and 2.2 million deaths in 223 countries or regions, and still counting. The ongoing COVID-19 pandemic has posed an unprecedented threat to human health and caused widespread social and economic disruption, thus highlighting a desperate need for safe and effective vaccines (Chandrashekar et al., 2020; Haynes et al., 2020; Liu et al., 2020b; Poland et al., 2020).

Over the last decade, mRNA has emerged as a promising platform for developing vaccines against infectious disease and cancer (Pardi et al., 2018b). The overwhelming advantages of mRNA vaccine over traditional vaccines such as live attenuated and inactivated virus, and protein subunit vaccines include versatility and rapid development (Corbett et al., 2020a). In addition, the prior clinical experiences of mRNA vaccine candidates against HIV virus, rabies virus and cancers have proven good safety profiles and potent immunogenicity of this platform (Alberer et al., 2017; Gay et al., 2018; de Jong et al., 2019; Feldman et al., 2019). Therefore, multiple researchers and enterprises chose this platform to develop vaccines against COVID-19. Several mRNA vaccine candidates have been among the most advanced ones in clinical trials. Very recently, two mRNA vaccines against COVID-19 developed by Moderna/NIAID and BioNTech/Pfizer have been shown to exhibit both safety and > 90% protection efficiency in phase III clinical trials and are now authorized for use in some regions (Dai and Gao, 2021; Wouters et al., 2021). In this review, we summarize key points of mRNA vaccine development and discuss COVID-19 mRNA vaccine candidates, focusing on those already advanced into clinical trials and with published data.

mRNA vaccine platform

Traditional vaccine platforms including live attenuated and inactivated virus and subunit vaccines have successfully eradicated many infectious diseases. Today, approximately 30 diseases worldwide can be prevented by vaccination (Maruggi et al., 2019). However, decades of intense efforts toward developing effective vaccines against challenging viruses that can cause chronic or repeated infections, such as respiratory syncytial virus and HIV has failed (Maruggi et al., 2019). In addition, for emerging virus vaccines,

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the main challenge is the desperate need for rapid development that the conventional approaches are powerless, as illustrated by the outbreaks of Middle East Respiratory Syndrome (MERS), Ebola and Zika viruses during 2012–2016 and the current COVID-19 pandemic (Maruggi et al., 2019). Thus, it is crucial to develop a more potent and versatile platform. In 1990, the successful *in vivo* use of *in vitro* transcribed (IVT) mRNA was firstly reported with the detection of protein production from reporter gene mRNA directly administrated into mouse muscle (Wolff et al., 1990). However, concerns about mRNA instability, potent innate immune response and poor efficiency of *in vivo* delivery resulted in no substantial investment in developing mRNA vaccines (Sullenger and Nair, 2016; Pardi et al., 2018b). Building on major technological innovations and research investment in the past decade, mRNA has now become a promising platform in vaccine development. mRNA vaccine usually takes several advantages over conventional approaches including live attenuated and inactivated virus, protein subunit, and DNA vaccines. First, safety: as a non-infectious or non-integrating platform, it takes no potential risk of infection or insertional mutagenesis (Ulmer and Geall, 2016; Maruggi et al., 2019). Second, efficacy: nucleoside modifications can greatly improve mRNA stability and its translational capacity, and lipid nanoparticles (LNPs) have been shown to be an efficient carrier to deliver mRNA *in vivo*, which allows rapid uptake and expression in host cells and finally leads to robust adaptive humoral and cellular immune responses (Ulmer and Geall, 2016; Pardi et al., 2017; Maruggi et al., 2019). Third, rapid preparation and versatility: it takes only several days to develop an mRNA vaccine with gene sequence information obtained, and the approach is highly versatile and applicable to almost all protein targets (Corbett et al., 2020a).

IVT mRNA is usually synthesized from a linearized plasmid or PCR product template by a T7, T3, or Sp6 phage RNA polymerase (Pardi et al., 2018b). It optimally contains an open reading frame, which encodes a targeted immunogen and is flanked by 5′ and 3′ untranslated regions (UTRs), and a 5′ cap and a 3′ poly(A) tail at the extremities (Schlake et al., 2012; Sahin et al., 2014; Richner et al., 2017; Pardi et al., 2018a). When mRNA is transmitted to the cytosol via an optimized delivery system, a correctly folded and functional protein with post-translational modifications is yielded by the cellular translation machinery (Kowalski et al., 2019). In addition, mRNA is finally subjected to degradation through normal physiological processes in host cells, thus lowering the toxicity risk caused by drug metabolite (Kowalski et al., 2019).

Exogenous mRNA has inherent immunostimulatory properties due to its recognition by a variety of innate immune receptors localized at the cell surface, endosome, and cytoplasm. This is potentially beneficial for vaccination, since in some cases it may contribute to arouse robust humoral and cellular immune responses by serving as adjuvant itself (Pollard et al., 2013). However, the innate recognition of mRNA has been linked to suppression of target protein expression as well, therefore adversely affecting the immune response. Some advances in clarifying the complex effects of innate immune recognition of mRNA on vaccine immunogenicity have been achieved in recent years. It has been well established that mRNA purification and modification are critical to tailor the elicited immunostimulatory profile. Double-stranded RNA (dsRNA) is produced as aberrant products in mRNA IVT reaction as a result of unwanted activities of phage RNA polymerase (Pollard et al., 2013). Specialized human pattern recognition receptors (PRRs) can recognize dsRNA as pathogen-associated molecular patterns (PAMP) and lead to a robust induction of type I interferon and finally to the shutdown of translation and the degradation of mRNA (Pollard et al., 2013; Sahin et al., 2014). Kariko and colleagues have shown that high-performance liquid chromatography (HPLC) is an efficient method to remove dsRNA from mRNA preparations, and purified mRNA was translated at 10- to 1,000-fold greater levels in human dendritic cells (DCs) (Pardi et al., 2018b). Appropriate purification of mRNA seems to be overall beneficial for maximizing immunogen protein expression and eliminating undesired innate immune activation. Another method to efficiently increase mRNA vaccine potency is introducing modified nucleosides in mRNA during the IVT reaction (Kaczmarek et al., 2017; Pardi et al., 2018a). Modified nucleosides can decrease the production of dsRNA in the IVT reaction. Moreover, recent studies have suggested that post-transcriptional epigenomic modification of RNA could serve as a powerful tool for the evasion of innate immunity (Linares-Fernández et al., 2020). For instance, the natural acetylation of cytidines in epigenomic modifications improves the translation of mRNA by weakening PRR recognition and/or activation (Linares-Fernández et al., 2020). Similarly, nucleoside modifications in mRNA vaccine can increase antigen production *in vivo* and finally improve the adaptive immune response (Linares-Fernández et al., 2020).

An efficient *in vivo* delivery is critical for mRNA vaccines to achieve prophylactic relevance. In order to be translated to immunogen proteins, exogenous mRNA must penetrate the barrier of the host cell membrane into the cytoplasm (Linares-Fernández et al., 2020). LNPs are the most widespread platform and have been shown to present the best clinical outcomes in mRNA delivery. LNPs mainly consisted of ionizable lipids, cholesterol, phospholipids, and polyethylene glycol (PEG)-lipid (Linares-Fernández et al., 2020) (Fig. 1). The ionizable lipids contain amine groups, which become cationic at a low pH and therefore can efficiently complex negatively-charged mRNA. When injected into the host, the amine groups of ionizable lipids in LNPs become neutral or slightly charged at the physiological pH 7.4 and thus have a good safety profile (Linares-Fernández et al., 2020). Once delivered into the endosome of host cells, they are thought to be ionized negatively again upon acidification, which help to induce hexagonal phase structures and finally facilitate the endosomal escape of mRNA into the cytoplasm (Linares-Fernández et al., 2020). In general, phospholipids play a structural role in LNPs, which assist the formulation of LNPs and disruption of the lipid bilayer to promote the endosomal escape of mRNA (Linares-Fernández et al., 2020). Cholesterol serves as a stabilizing element in LNPs. Lipid-anchored PEGs dominantly deposit on the LNP surface as a barrier to sterically stabilize the LNP and reduce nonspecific binding to proteins (Linares-Fernández et al., 2020).

Although tremendous progress in mRNA stability and delivery has been made in the past years, especially the use of LNPs, many challenges still ahead in the mRNA vaccine field, such as safety concerns. A potential safety risk comes from the potent type I interferon response elicited by mRNA vaccine, which has been shown to be involved not only in inflammation but also possibly in autoimmunity (Pardi et al., 2018b), as evidenced by an increased incidence of allergic reactions induced by COVID-19 mRNA vaccine in clinical trials compared with those by conventional vaccines. Thus,

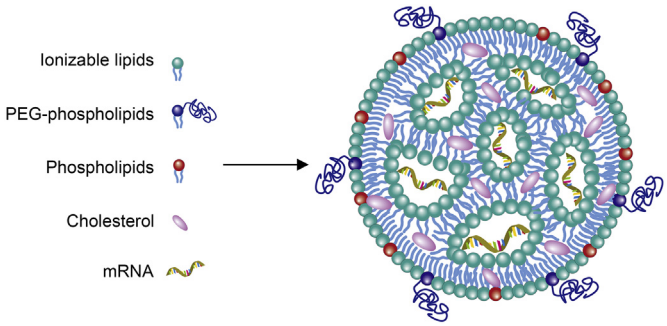

Fig. 1. Schematic representation of mRNA-lipid nanoparticle complex.

the individuals at a higher risk of autoimmune reactions may be excluded from mRNA vaccinations. In addition, although mRNA vaccine showed overall good tolerability in clinical trials in a short term, its long-term safety remains to be further assessed. The stability is another challenge that mRNA vaccine is facing. LNPs-encapsulated mRNA vaccine is vulnerable to degradation at room temperature. It must be stored and shipped at −70°C or −20°C, which poses a huge challenge in some developing countries, where electricity for freezers can be unreliable and dry ice is scarce. Thus, there is huge scope for improvement for mRNA vaccine stability at room temperature.

Antigens of COVID-19 mRNA vaccines

Like other human coronaviruses including SARS and MERS, SARS-CoV-2 is an enveloped, positive-sense, and single-stranded RNA virus (Baum et al., 2020; Santos et al., 2020; Wrapp et al., 2020a; Wu et al., 2020b). Its genome RNA encodes a non-structural polyprotein and structural proteins including the spike (S), envelope (E), membrane (M), and nucleocapsid (N) proteins (Santos et al., 2020). As surface proteins, the S, E, and M proteins are inserted into the viral envelope, and the S glycoprotein gives the viral particle a 'crown' and its name (Santos et al., 2020). The N protein surrounds the positive-stranded genomic RNA (Santos et al., 2020).

SARS-CoV-2 makes use of the S protein to enter host cells (Amanat et al., 2020; Cao et al., 2020; Du et al., 2020; Hoffmann et al., 2020; Lv et al., 2020; Monteil et al., 2020; Wu et al., 2020a; Xia et al., 2020; V'Kovski et al., 2021). The S glycoprotein is 1,273 amino acids in length and consists of an N-terminal signal peptide (amino acids 1−14), an extracellular domain (amino acids 14−1,211), a transmembrane domain (amino acids 1,211−1,234), and an intracellular domain (amino acids 1,234−1,273) (Cai et al., 2020) (Fig. 1A), which is functionally divided into S1 and S2 subunits. The receptor-binding domain (RBD), localized at the C-terminal of S1 subunit, is responsible for engaging human angiotensin-converting enzyme 2 (hACE2), and S2 mediates membrane fusion (Cai et al., 2020; Hsieh et al., 2020; Walls et al., 2020; Watanabe et al., 2020; Yuan et al., 2020). Refolding of the S protein from an initial, metastable prefusion conformational state on the viral surface to a stable, postfusion state affords free energy to overcome kinetic barriers with two membranes approaching each other during viral membrane fusion (Cai et al., 2020). The prefusion S structure reveals that the four domains of the S1 subunits, N-terminal domain (NTD), RBD, C-terminal domain (CTD)1, and CTD2, wrap around the threefold axis of S trimer and cover the S2 fragment underneath; S2 subunit appears to be a symmetric trimer, in which the first heptad repeat (HR1) bends back toward the viral membrane (Cai et al., 2020; Wang et al., 2020) (Fig. 2B). The structure of the SARS-CoV-2 S trimer in postfusion conformation shows that after a substantial structural rearrangement, HR1 and central helix (CH) form an unusually long, central, three-stranded coiled coil (Cai et al., 2020) (Fig. 2C). In addition, the spontaneous transition of SARS-CoV-2 S protein to the postfusion state has been reported to be independent of target cells, and when the full-length S-encoding plasmids are transfected into cells, both prefusion and postfusion S proteins are produced (Cai et al., 2020; Liu et al., 2020a). The high instability of the S protein in the prefusion conformational state is undoubtedly a large hurdle in S-based vaccine development. Fortunately, an introduction of two consecutive proline residues (2P) at the beginning of CH has been demonstrated to be a general strategy for retaining betacoronavirus S protein in the prefusion conformation, as evidenced by a greater than 50-fold improvement in yield of MERS-CoV-2 S in the prefusion state resulting from proline substitutions at residues V1060 and L1061 (Pallesen et al., 2017). The restricted backbone torsion angles of prolines presumably disfavor the refolding of the linker between the CH and HR1 and thus prevent the transition of S protein to postfusion conformation (Pallesen et al., 2017). Moreover, the cryo-EM structure of SARS-CoV-2 S-2P mutant indicates that the 2P substitutions do not alter the conformation of the S protein (Wrapp et al., 2020b) (Fig. 2D). Meanwhile, a variety of structures including RBD-hACE2 and RBD-monoclonal antibodies have also been reported (Shi et al., 2020; Wang et al., 2020; Wu et al., 2020b). RBD contains two structural subdomains, in which five antiparallel β strands comprise one conserved core subdomain, and the other external subdomain is dominated by a disulfide bond-stabilized flexible loop that recognizes hACE2 (Wang et al., 2020) (Fig. 2E). In addition, both RBD and the S-2P proteins can induce potent SARS-CoV-2 neutralizing antibody and T cell responses (Chen et al., 2020; Dai et al., 2020; Laczkó et al., 2020; Lu et al., 2020; Mercado et al., 2020; Smith et al., 2020; Yang et al., 2020; Yu et al., 2020; Zhu et al., 2020a, 2020b). Therefore, they are widely used as immunogens in COVID-19 mRNA vaccine development.

COVID-19 mRNA vaccines

Taking advantage of versatility and rapid development, two COVID-19 mRNA vaccines (mRNA-1273 and BNT162b2) have been approved for market, one candidate is in phase III clinical trials, and three other candidates are currently in phase I or II clinical assessment. Table 1 lists all those COVID-19 mRNA vaccines or vaccine candidates in clinical trials and summarizes their safety profiles, neutralizing antibody responses, and protection efficacy, according to published data from preclinical experiments or clinical trials.

mRNA-1273

mRNA-1273 developed by Moderna is an LNP-encapsulated mRNA with complete substitution of uridine by N1-methyl-pseudourine and encodes SARS-CoV-2 full-length S-2P protein (Table 1). The structure-based antigen design builds upon previous studies of the MERS-CoV-2 mRNA vaccine that full-length S-2P mRNA was more immunogenic than wild-type full-length S or secreted S-2P mRNA (Pallesen et al., 2017; Corbett et al., 2020a). mRNA-1273 is the first COVID-19 vaccine candidate entering phase I clinical trial on 16th March 2020, only 66 days after SARS-CoV-2 genome sequence was published. Its phase II clinical trial was initiated 74 days later on 29 May 2020 and the phase III clinical trial was initiated in July 2020, demonstrating the substantial advantage of mRNA vaccines with regard to development and manufacturing speed.

Today, several animal and clinical evaluation results about mRNA-1273 have been published. The preclinical study demonstrated that mRNA-1273 induced robust neutralizing antibody responses against both wild-type and D614G variant virus and $CD8^+$ T cell responses in several mouse strains, and confers protection for mouse lungs from SARS-CoV-2 infection (Corbett et al., 2020a). Two injections of 10 or 100 μg mRNA-1273 in nonhuman primates had 50% neutralizing antibody titers (NT_{50}) of 501 and 3,481, respectively, which were 12 and 84 folds higher than that in human convalescent serum, respectively, and the 100-μg dose protected against SARS-CoV-2 replication in both the upper (nose) and lower (lung) airways (Corbett et al., 2020b). To evaluate vaccine safety and immunogenicity in humans, Moderna conducted a phase I clinical trial in 45 healthy adults (18−55 years of age), who were randomly assigned to receive 25, 100, or 250 μg of mRNA-1273 with a prime-boost vaccination regimen at a four-week internal (Jackson et al., 2020). As for safety, the results showed that solicited adverse events including pain, headache, fatigue, myalgia, and chills were reported in more than half of the participants (Jackson et al., 2020). Systemic adverse events were more frequent following the second inoculation,

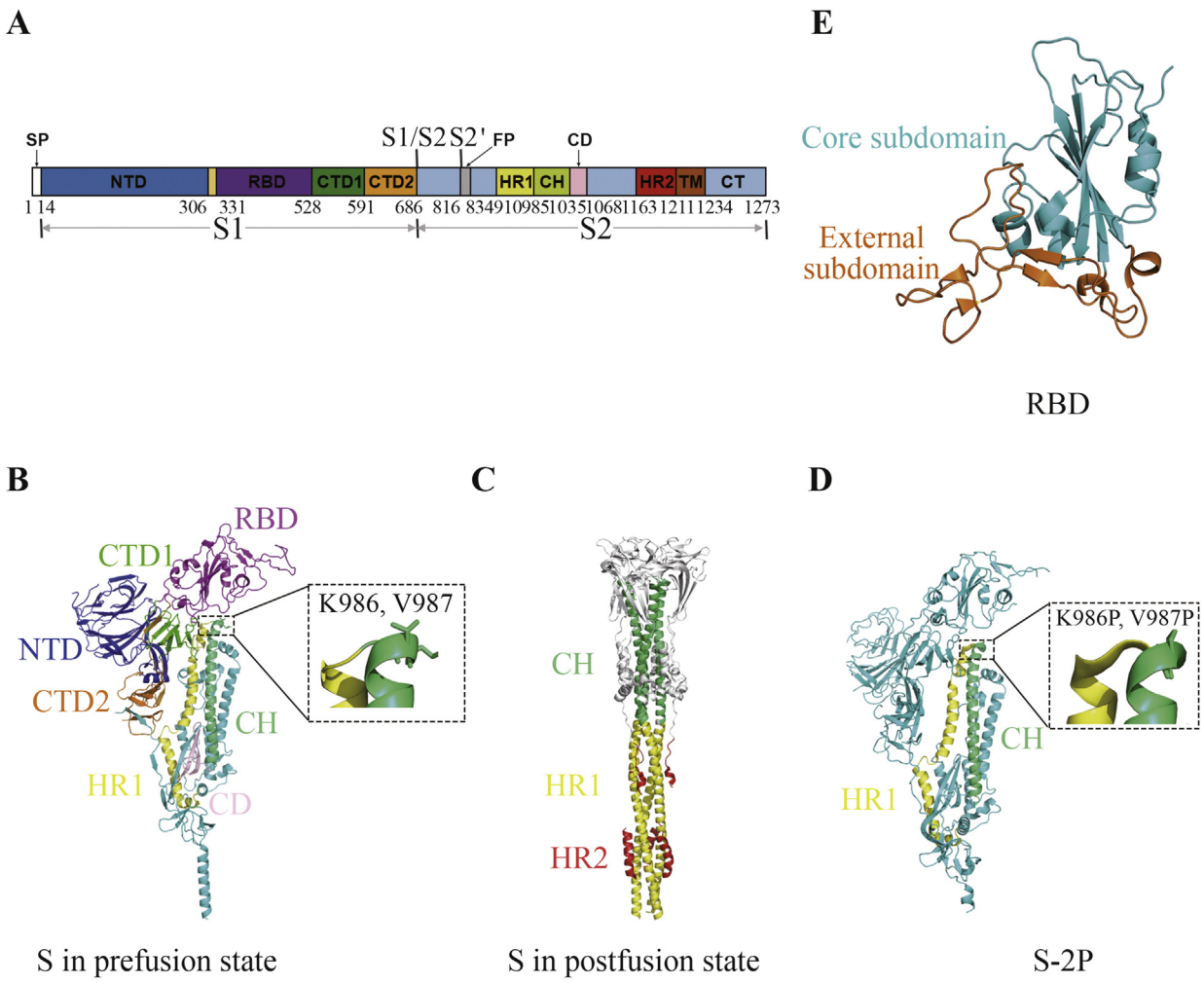

Fig. 2. Antigens of COVID-19 mRNA vaccines. **A**: Schematic representation of full-length SARS-CoV-2 S primary structure colored by domain. SP, signal peptide; NTD, N-terminal domain; RBD, receptor-binding domain; CTD, C-terminal domain; FP, fusion peptide; HR1, heptad repeat 1; CH, central helix; CD, connector domain; HR2, heptad repeat 2; TM, transmembrane domain; CT, cytoplasmic tail. **B**: The structure of SARS-CoV-2 wild-type S protein in prefusion state (PDB:6XR8). **C**: The structure of SARS-CoV-2 wild-type S protein in postfusion state (PDB:6XRA). **D**: The structure of SARS-CoV-2 S-2P (PDB:6VSB). **E**: The structure of SARS-CoV-2 RBD (PDB:6LZG).

particularly with the highest dose (Table 1), which was illustrated by fever events. No participants had fever after the first vaccination, whereas following the second injection, 40% of 100 μg group and 57% of 250 μg group reported fever (38–38.9°C), and one severe fever event (39.0–40.0°C) in the 250 μg group was recorded (Jackson et al., 2020). Vaccine-induced neutralizing activity was determined by a plaque-reduction neutralization testing (PRNT) assay as $PRNT_{80}$ (the highest serum dilution inhibiting 80% of SARS-CoV-2 infection) (Jackson et al., 2020). The 25-μg and 100-μg doses elicited geometric mean $PRNT_{80}$ titers of 339.7 and 654.3, respectively, generally comparable to or above that in human convalescent sera (Jackson et al., 2020). Since both COVID-19 morbidity and case fatality increased with older age, the phase I clinical trial was later extended to involve 40 elderly adults, who were further stratified into two groups of the age of 56–70 years and the age of ≥ 71 years and were administrated two injections of either 25 or 100 μg of mRNA-1273 with four weeks apart (Anderson et al., 2020). This small elders-involved study demonstrated that adverse events were mostly mild or moderate and were similar to the safety profile of young adults aged 18–55 years; in response to the 100-μg dose, the $PRNT_{80}$ mean value reached 878 among participants who were between 56 and 70 years of age and 317 among those who were 71 years of age or older (Anderson et al., 2020). Recently, Moderna also reported immunogenicity data four months after the first vaccination in 34 adult participants in the phase I clinical trials who received two injections of 100 μg of mRNA-1273 (Widge et al., 2021). The results showed that the mean $PRNT_{80}$ value four months after the first vaccination remained at 430 in participants aged 18–55 years, 269 in those at the age of 56–70 years, and 169 in those 71 years of age or older, indicating that mRNA-1273 has the potential to provide durable humoral immunity (Widge et al., 2021). Very recently, Moderna announced the primary efficacy analysis in phase III clinical trials, indicating mRNA-1273 vaccine efficacy of 94.1% (Baden et al., 2021) (Table 1).

BNT162b1 and BNT162b2

BioNTech and Pfizer developed two COVID-19 nucleoside-modified, LNP-encapsulated mRNA vaccine candidates. One is BNT162b1 that encodes a secreted RBD immunogen, trimerized by a T4 fibritin foldon domain to improve its immunogenicity through multimerization (Table 1). The other is BNT162b2 that encodes the full-length S-2P protein (Table 1). Two placebo-controlled, observer-blinded dose-escalation phase I/II clinical trials in the USA and Germany, respectively, were conducted to assess the safety and immunogenicity profile of BNT162b1 among healthy adults aged 18–55 years (Mulligan et al., 2020; Sahin et al., 2020). The USA trial investigated three doses of the BNT162b1 vaccine (10, 30 or 100 μg)

Table 1
COVID-19 mRNA vaccine candidates in clinical trials.

Name	Developers	Location	Route	Targets	Immunogenicity and protection	Safety	Phase	Reference
mRNA-1273	Moderna	USA	IM	S-2P	Two doses of mRNA-1273 afforded an efficacy of 94.1% (95% credible interval, 89.3–96.8%) in preventing COVID-19.	Systemic adverse events were more common after the second vaccination. Serious adverse events were rare.	Phase III[a] NCT04470427	Jackson et al., 2020; Baden et al., 2021
BNT162b2	BioNTech	Germany	IM	S-2P	The BNT162b2 vaccine with two injections showed 95% efficacy (95% credible interval, 90.3–97.6%) at preventing COVID-19.	The adverse events included mild-to-moderate pain at the injection site, fatigue, and headache. The frequency of serious adverse events was low and was similar in the vaccine and placebo groups.	Phase III[a] NCT04368728	Polack et al., 2020; Walsh et al., 2020
CVnCoV	CureVac AG	Germany	IM	S-2P	Neutralizing antibody titers in participants after two injections were comparable to those of convalescent human sera.	There were dose-dependent increases in frequency and severity of systemic adverse events, but the majority were mild or moderate and transient in duration.	Phase III NCT04674189	Kremsner et al., 2020
ARCoV	Abogen	China	IM	RBD	Two doses of ARCoV immunization elicited robust neutralizing antibodies and cellular immune responses in non-human primates and protected mice from SARS-CoV-2 challenge.	Not available.	Phase II ChiCTR2000039212	Zhang et al., 2020
ARCT-021	Arcturus	USA	IM	Not available	Neutralizing antibody levels in both single-dose and prime-boost groups were within those observed in convalescent patient sera.	ARCT-021 was well tolerated, the majority of adverse local and systemic adverse events were mild.	Phase II NCT04668339	https://clinicaltrials.gov/ct2/show/NCT04480957
LNP-nCoVsaRNA	Imperial College London	England	IM	S-2P	Two injections of LNP-nCoVsaRNA elicited higher neutralizing antibody titers than those of COVID-19 convalescent patients and cellular immune responses in mice.	Not available.	Phase I SRCTN17072692	McKay et al., 2020
ChulaCoV19 mRNA vaccine	Chulalongkorn University	Thailand	IM	Not available	Not available.	Not available.	Phase I NCT04566276	https://clinicaltrials.gov/ct2/show/NCT04566276

[a] Represents that the vaccines have been approved for emergency use.

in a two-dose immunization regimen (Mulligan et al., 2020). Since the prime vaccination with 100 μg revealed higher reactogenicity and no superior immunogenicity compared with the 30-μg dose, a second injection was not administered (Mulligan et al., 2020). The most-common systemic events included mild to moderate muscle pain, headache, fatigue, chills, and joint pain (Mulligan et al., 2020) (Table 1). Systemic events increased with the dose level and after the second dose. As for immunogenicity, geometric mean NT_{50} titers of SARS-CoV-2 serum-neutralizing antibodies following the second vaccination in the 10 μg and 30 μg groups approached 180 and 437 and were 1.4- and 4.6-fold those of the convalescent patient sera, respectively (Mulligan et al., 2020). The clinical trial in Germany investigated five doses including 1, 10, 30, 50, and 60 μg. All doses except 60 μg were conducted with a two-dose immunization regimen administrated 3 weeks apart (Sahin et al., 2020). Clinical data showed that there were no serious adverse events and the safety profiles were similar to those of the USA trial. BNT162b1 induced potent Th1-skewed immune response with high expansion of SARS-CoV-2-specific $CD4^+$ and $CD8^+$ T cells (Sahin et al., 2020). Antibody neutralizing NT_{50} titers were 0.7-fold (dose of 1 μg) to 3.5-fold (dose of 50 μg) those of the recovered patient sera (Sahin et al., 2020). These results demonstrated that BNT162b1 potentially provided protection against SARS-CoV-2 through multiple beneficial mechanisms (Sahin et al., 2020). Meanwhile, to investigate the difference of safety and immunogenicity profiles between BNT162b1 and BNT162b2, the developer conducted another observer-blinded and dose-escalation phase I trial that included both young adults (18–55 years of age) and the elderly (65–85 years of age) in the USA (Walsh et al., 2020). The trial results indicated that compared to BNT162b1, BNT162b2 was better tolerated and exhibited a lower frequency and severity of systemic events, particularly in the elderly, for example, only 8% of the older participants receiving BNT162b2 reported mild fever (38.0–38.4°C), whereas 17%, 8% and 8% of those receiving the same dose of BNT162b1 reported mild (38.0–38.4°C), moderate (>38.4–38.9°C) and severe (>38.9–40.0°C) fever, respectively (Walsh et al., 2020). Moreover, in both young and elderly participants, similar dose-dependent neutralizing antibody responses were elicited by BNT162b1 and BNT162b2, and the NT50 titers were comparable to or higher than those of convalescent human sera (Walsh et al., 2020). Taking all the above data into consideration, the

developers finally selected BNT162b2 at 30 μg in a two-dose immunization regimen for advancement into a pivotal phase III clinical trial for safety and efficacy evaluation. In the phase III clinical trial, 43,448 participants were recruited with 21,720 receiving BNT162b2 and 21,728 receiving placebos (Polack et al., 2020). Interim data demonstrated that 170 confirmed COVID-19 cases were occurring at least seven days following boost vaccination with eight in the BNT162b2 group and 162 in the placebo group. Therefore, BNT162b2 showed a 95% efficacy in preventing COVID-19 (Polack et al., 2020) (Table 1). In addition, among ten cases of severe COVID-19, nine occurred in the placebo group and one in the BNT162b2 group (Polack et al., 2020). Adverse events induced by BNT162b2, which involved pain at the injection site, fatigue, and headache, were usually self-limiting and mild to moderate in magnitude (Polack et al., 2020). The frequency of serious adverse events was low and similar in the placebo and vaccine groups (Polack et al., 2020). BNT162b2 has currently been approved for emergency use in the USA and Germany.

Other COVID-19 mRNA vaccines

In addition to the above three mRNA vaccines, four other COVID-19 mRNA vaccines are in clinical trials as well: ARCoV (Abogen, China) (Zhang et al., 2020), CVnCoV (CurVac, Germany) (Kremsner et al., 2020), ARCT-021 (Arcturus, USA), LNP-nCoVsaRNA (Imperial College London, England) (McKay et al., 2020), and ChulaCoV19 mRNA vaccine (Chulalongkorn University, Thailand) (Table 1). ARCoV is an LNP-encapsulated nucleoside-modified mRNA encoding SARS-CoV-2 RBD. A preclinical study showed that ARCoV with a prime-boost vaccination regimen afforded complete protection for mice from a mouse-adapted SARS-CoV-2 strain and elicited both potent neutralizing antibody and cellular responses in non-human primates (Zhang et al., 2020) (Table 1). CVnCoV is an LNP-encapsulated sequence-optimized mRNA encoding SARS-CoV-2 S-2P (Kremsner et al., 2020) (Table 1). A phase I clinical trial assessment of this vaccine has been reported. The analysis showed that the majority of systemic adverse events were mild or moderate and transient in duration (Kremsner et al., 2020). Moreover, neutralizing antibody titers following a second 12 μg dose were comparable to those in convalescent patient sera (Kremsner et al., 2020) (Table 1). Unlike the abovementioned mRNA vaccine candidates that encode only the SARS-CoV-2 antigen of interest, the ARCT-021 and LNP-nCoV saRNA vaccine candidates are self-amplifying mRNA derived from the genome of positive-stranded RNA viruses. They encode not only the SARS-CoV-2 target antigen but also the viral replication machinery required for mRNA replication in host cells. ARCT-021 is currently in phase II clinical trials (Table 1). In its phase I trial, the safety and immunogenicity of escalating doses as a single injection were investigated. However, the detailed data of the trial are currently not available. LNP-nCoVsaRNA, which is delivered by LNPs, encodes an RNA replicase derived from an alphavirus and SARS-CoV-2 prefusion stabilized S protein (McKay et al., 2020) (Table 1). Preclinical study of this vaccine demonstrated that two injections induced higher neutralizing antibody titers than those of recovered COVID-19 patients and high cellular responses, which were characterized by IFN-γ production upon restimulation with SARS-CoV-2 peptides (McKay et al., 2020).

Concluding remarks and perspectives

The ongoing COVID-19 pandemic poses an enormous threat to human health, and a safe and effective vaccine is urgently needed. The characteristics of versatility, rapid development, safety, and potent immunogenicity make the mRNA approach very suitable for vaccine development against newly emerging viruses such as SARS-CoV-2. Building upon major technological innovation and progress in the mRNA vaccine platform during the last decade, mRNA vaccines against COVID-19 were successfully developed at an unprecedented speed. However, given that COVID-19 vaccines are the first mRNA vaccines licensed for market, some issues might be encountered with regards to future large-scale production and the long-term storage stability of the vaccine. COVID-19 mRNA vaccines showed a higher rate of systemic adverse events such as fever and fatigue compared with protein subunit and inactivated virus vaccines in clinical trials. Thus, long-term monitoring of the safety of COVID-19 mRNA vaccines is very necessary. Most mRNA vaccines aim to generate neutralizing IgG antibodies, which only efficiently protect the lower respiratory tract by intramuscular immunization. However, IgA antibodies, which are mainly responsible for protecting the upper respiratory tract, may be necessary for sterilizing immunity, and IgA antibody levels induced by mRNA vaccines have not been determined in current clinical trials. Moreover, although mRNA-1273 has shown that high neutralizing antibody titers persist for at least four months following prime vaccination in humans (Widge et al., 2021), how long these mRNA vaccines can protect humans against COVID-19 still needs to be further elucidated.

Conflict of interest

The authors declare that they have no conflict of interest.

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