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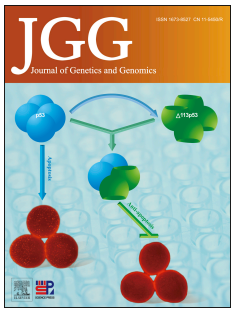

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COVID-19 mRNA vaccines

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Abstract

The ongoing COVID-19 pandemic and its unprecedented global societal and economic disruptive impact highlights the urgent need for safe and effective vaccines. Taking substantial advantages of versality 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 key notes 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.

1. Introduction

SARS-CoV-2 was initially identified as the etiologic agent responsible for an 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, the World Health Organization (WHO) declared SARS-CoV-2 a global pandemic, 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), which caused the SARS outbreak in 2003(Hu et al., 2020; Su et al., 2020). 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 accounted for more than 100 million laboratory-confirmed infections and 2.2 million deaths in 223 countries and territories, and still counting. The ongoing COVID-19 pandemic has posed an unprecedent 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 virus, 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; de Jong et al., 2019; Feldman et al., 2019; Gay et al., 2018). 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. In this article, 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.

2. mRNA vaccine platform

Conventional vaccine approaches, such as live attenuated and inactivated virus and subunit vaccines have resulted in the eradication of many infectious diseases.

Today, approximately 30 diseases worldwide can be prevented by vaccination(Maruggi et al., 2019). Despite this success, there remain hurdles to the production of effective vaccines against challenging viruses that cause chronic or repeated infections, such as HIV-1, herpes simplex virus and respiratory syncytial virus (RSV)(Maruggi et al., 2019). Moreover, for most emerging virus vaccines, the main obstacle is the desperate need for rapid development that the conventional approaches are powerless, as illustrated by the 2014-2016 outbreaks of the Ebola and Zika viruses and the current COVID-19 pandemic(Maruggi et al., 2019). Therefore, the development of more potent and versatile platforms is crucial. The first report of the successful use of *in vitro* transcribed (IVT) mRNA in animals was published in 1990, when reporter gene mRNAs were injected into mice and protein production was detected(Pardi et al., 2018b). However, the concerns associated with mRNA instability, high innate immunogenicity and inefficient *in vivo* delivery resulted in no substantial investment in developing mRNA vaccines(Pardi et al., 2018b; Sullenger and Nair, 2016). Over the past decade, major technological innovations and research investments have enabled mRNA to become a promising therapeutic tool in vaccine development. mRNA vaccines have several beneficial features over subunit, inactivated and live attenuated virus, as well as DNA vaccines. First, safety: as mRNA is a non-infectious, non-integrating platform, there is no potential risk of infection or insertional mutagenesis(Maruggi et al., 2019; Ulmer and Geall, 2016). Second, efficacy: various modifications make mRNA more stable and highly translatable, and efficient *in vivo* delivery can be achieved by formulating mRNA into lipid

nanoparticles, allowing rapid uptake and expression in the cytoplasm and finally robust adaptive humoral and cellular immune responses(Maruggi et al., 2019; Pardi et al., 2017; Ulmer and Geall, 2016). Third, rapid preparation and versatility: mRNA vaccines can be produced rapidly, within days of obtaining gene sequence information, and the platform is versatile and amenable to nearly all protein targets(Corbett et al., 2020a).

IVT mRNA is produced from a linear DNA template or PCR products using a T7, T3, or Sp6 phage RNA polymerase(Pardi et al., 2018b). The resulting product should optimally contain an open reading frame that encodes the antigen of interest, 5' and 3' untranslated regions (UTRs), a 5' cap and a poly(A) tail(Pardi et al., 2018a; Richner et al., 2017; Sahin et al., 2014; Schlake et al., 2012). When the mRNA is transmitted to the cytosol via an optimized delivery system, the cellular translation machinery produces an antigen protein that undergoes post-translational modifications, resulting in a properly folded, fully functional protein(Kowalski et al., 2019). IVT mRNA is finally degraded by normal physiological process, thus reducing the risk of metabolite toxicity(Kowalski et al., 2019).

Exogenous mRNA is inherently immunostimulatory, as it is recognized by a variety of cell surface, endosomal, and cytosolic innate immune receptors. It is potentially advantageous for vaccination because in some cases it may provide adjuvant activity and elicit robust B and T cell immune responses(Pollard et al., 2013). However, the innate sensing of mRNA has also been associated with the inhibition of antigen expression, thus negatively affecting the immune response. Some progress has

been made in recent years in elucidating the paradoxical effects of innate immune sensing on mRNA vaccines. Studies over the past decade have shown that the immunostimulatory profile of mRNA can be shaped by the purification of IVT mRNA and the introduction of modified nucleosides. Enzymatically synthesized mRNA preparations contain double-stranded RNA (dsRNA) contaminants as aberrant products of the IVT reaction(Pollard et al., 2013). As a mimic of viral genomes and replication intermediates, dsRNA is a potent pathogen-associated molecular pattern (PAMP) that is sensed by pattern recognition receptors (PRRs), resulting in robust type I interferon production and finally leading to the inhibition of translation and the degradation of cellular mRNA(Pollard et al., 2013; Sahin et al., 2014). Kariko and colleagues have demonstrated that dsRNA can be efficiently removed from IVT mRNA by chromatographic methods such as reverse-phase fast protein liquid chromatography (FPLC) or high-performance liquid chromatography (HPLC)(Pardi et al., 2018b) (Table 1). Purification by FPLC has been shown to increase protein production from IVT mRNA by up to 1,000-fold in primary human dendritic cells (DCs)(Karikó et al., 2011). It has been shown that appropriate purification of IVT mRNA seems to be beneficial by maximizing antigen production and avoiding unwanted innate immune activation. Another method of efficiently increasing mRNA vaccine potency is the incorporation of modified nucleosides in mRNA during the IVT reaction(Kaczmarek et al., 2017; Pardi et al., 2018a) (Table 1). First, modified nucleosides can decrease the quantity of dsRNA in the reaction. Recent studies have suggested that post-transcriptional epigenomic RNA modifications could be a

powerful tool to evade innate immune responses(Linares-Fernández et al., 2020). For instance, epigenomic modification by natural acetylation of cytidines increases the translational efficiency of mRNA by impairing PRR recognition and/or activation(Linares-Fernández et al., 2020). Similarly, nucleoside modifications in an mRNA vaccine can increase antigen production *in vivo* and finally improve the adaptive immune response(Linares-Fernández et al., 2020).

Efficient *in vivo* mRNA delivery is critical for mRNA vaccines to achieve prophylactic relevance. Exogenous mRNA must penetrate the barrier of the lipid membrane in order to reach the cytoplasm and be translated to antigens(Linares-Fernández et al., 2020). Lipid nanoparticles (LNPs) are the most widespread platform used and have been shown to present the best clinical outcomes in mRNA delivery. LNPs are mainly composed 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 can efficiently complex negatively-charged mRNA. When injected into the host, the amine groups of ionizable lipids in LNPs change to become neutral or slightly charged at physical 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. They help to induce hexagonal phase structures, and finally facilitate endosomal escape of mRNA into the cytoplasm(Linares-Fernández et al., 2020). Generally, phospholipids play a structural role in LNPs. They help with 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 preferentially deposit on the LNP surface, where they act as a barrier that sterically stabilizes the LNP and reduces nonspecific binding to proteins(Linares-Fernández et al., 2020).

Although many advances in mRNA stability and delivery have been made in the past years, especially using LNPs, there are still many challenges in mRNA vaccine field. A possible safety concern could be that mRNA vaccine usually induces potent type I interferon responses, which have been associated not only with inflammation but also potentially with 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, screening of individuals at a low risk of autoimmune reactions before mRNA vaccination may be necessary. In addition, although mRNA vaccine showed an overall good tolerability in clinical trials in a short term, its long-term safety remains to be further assessed. LNPs-encapsulated mRNA vaccine is vulnerable to degradation at room temperature. It has to be stored and shipped at -70°C or -20°C, which poses great challenges in developing countries, where electricity for freezers can be unreliable and dry ice scarce. Thus, there is huge scope for improvement for mRNA vaccine stability at room temperature.

3. Antigens of COVID-19 mRNA vaccines

Like other human coronaviruses including SARS and Middle East respiratory

syndrome (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 S, envelope (E), membrane (M) and nucleocapsid (N) proteins(Santos et al., 2020). As surface proteins, the S, E, and M proteins are embedded in the viral envelope, and the S glycoprotein gives the viral particle a “crown” and therefore 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; V'Kovski et al., 2020; Wu et al., 2020a; Xia et al., 2020). The S glycoprotein is 1,273 amino acids in length and consists of a signal peptide (amino acids 1-14) located at the N terminus, an extracellular domain (amino acids 14-1,211), a transmembrane domain (amino acids 1,2114-1,234) and an intracellular domain (amino acids 1,234-1,273) (Fig. 1A)(Cai et al., 2020). It can be functionally categorized into S1 and S2 subunits, where the receptor-binding domain (RBD), located at the C-terminal of S1 subunit, engages human angiotensin-converting enzyme 2 (hACE2) as the receptor, 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 when two membranes approach each other during viral membrane

fusion(Cai et al., 2020). The prefusion S structure revealed that the four domains of the S1 fragments, 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 (Fig. 2B)(Cai et al., 2020; Wang et al., 2020). Structure of the SARS-CoV-2 S trimer in postfusion conformation showed that after a substantial structural rearrangement, HR1 and central helix (CH) form an unusually long, central, three-stranded coilded coil (Fig. 2C)(Cai et al., 2020). 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 >50-fold improvement in yield of MERS-CoV-2 S in prefusion state resulting from proline substitutions at residues V1060 and L1061(Pallesen et al., 2017). The restricted backbone torsion angles from 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, cryo-EM structure of SARS-CoV-2 S-2P mutant indicated that the 2P substitutions do not alter the

conformation of the S protein (Fig. 2C)(Wrapp et al., 2020b). Meanwhile, a variety of structures including RBD-hACE2, RBD-monoclonal antibodies have also been reported and showed that RBD contains two structural domains, and one is the conserved core subdomain with five antiparallel β strands, and the other is the external subdomain, which is dominated by a disulfide bond-stabilized flexible loop and responsible for the recognition of hACE2 (Fig. 2D)(Wang et al., 2020). In addition, both RBD and the S-2P proteins are capable of inducing highly potent neutralizing antibodies and cellular immunity(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; Zhu et al., 2020b). Therefore, they have been widely selected as antigens in COVID-19 mRNA vaccine development.

4. COVID-19 mRNA vaccines

Taking advantages of versality and rapid development, two COVID-19 mRNA vaccines (mRNA-1273 and BNT162b2) have been approved for market, one candidate 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 summarize their safety profiles, neutralizing antibody responses, and protection efficacy, where available, according to published data from preclinical experiments or clinical trials.

4.1 mRNA-1273

The mRNA-1273 vaccine candidate which was developed by Moderna, is an LNP-encapsulated mRNA with complete replacement of uridine by

N1-methyl-pseudourine, that encodes a SARS-CoV-2 full-length S-2P immunogen (Table 1). The structure-based antigen design builds upon previous studies of the MERS-CoV-2 mRNA vaccine which indicated that full-length S-2P mRNA was more immunogenic than wild-type full-length S or secreted S-2P mRNA(Corbett et al., 2020a; Pallesen et al., 2017). mRNA-1273 was the first SARS-CoV-2 vaccine candidate entering a phase I clinical trial on 16 March 2020, 66 days after the viral sequence was released. The 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. Preclinical study demonstrated that mRNA-1273 induces potent neutralizing antibody responses to both wild-type and D614G mutant SARS-CoV-2 as well as CD8+ T cell responses in several mouse strains, and protects against SARS-CoV-2 infection in the lungs of mice(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 3481, respectively, values that are 12 times and 84 times as high, respectively, as in human convalescent serum, and the 100-μg dose protected against SARS-CoV-2 viral replication in both the upper (nose) and lower (lung) airways(Corbett et al., 2020b). A phase I clinical trial with mRNA-1273 was conducted in 45 healthy adults, 18-55 years of age, to assess its safety and the immunogenicity of three doses (25 μg, 100 μg and 250 μg) in a prime-boost

immunization regimen administrated 4 weeks apart(Jackson et al., 2020). As for safety, the results showed that solicited adverse events including fatigue, chills, headache, myalgia and pain at the injection site were reported in more than half of the participants(Jackson et al., 2020). Systemic adverse events were more common after the second vaccination, particularly with the highest dose (Table 1). This was illustrated by fever events, which showed that none of the participants had fever after the first vaccination, whereas 40% in the 100-μg group and 57% in the 250-μg group reported fever (38-38.9℃), and one adult in the 250-μg group reported one severe fever event (maximum temperature, 39.6℃)(Jackson et al., 2020). Vaccine-induced neutralizing activity was determined by a plague-reduction neutralization testing (PRNT) assay as $PRNT_{80}$ (the highest serum dilution that is capable of reducing SARS-CoV-2 infectivity by 80%)(Jackson et al., 2020). The 25-μg and 100-μg doses elicited geometric mean $PRNT_{80}$ responses of 339.7 and 654.3, respectively, generally at or above values of convalescent serum specimens(Jackson et al., 2020). Since increased incidences of illness and death from SARS-CoV-2 have been associated with an older age, the phase I clinical trial was later expanded to include 40 older adults, who were stratified according to age (56 to 70 years and ≥71 years) and assigned to receive two doses of either 25 μg or 100 μg of mRNA-1273 with four weeks apart(Anderson et al., 2020). In this small study involving older adults, adverse events were mainly mild or moderate and 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 4 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., 2020). The results demonstrated that the mean $PRNT_{80}$ value 4 months after the first vaccination remained at 430 in participants aged 18 to 55 years, 269 in those between 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., 2020). Very recently, Moderna announced the primary efficacy analysis in phase III clinical trials, indicating mRNA-1273 vaccine efficacy of 94.1% (Table 1).

4.2 BNT162b1 and BNT162b2

BioNTech and Pfizer developed two COVID-19 lipid nanoparticle-formulated, nucleoside-modified RNA vaccine candidates: BNT162b1, which encodes a secreted RBD antigen, trimerized by the addition of a T4 fibritin foldon domain to increase its immunogenicity throμgh multivalent display; and BNT162b2, which encodes the full-length S-2P protein (Table 1). Two placebo-controlled, observer-blinded dose-escalation phase I/II clinical trials (one in the USA; the other in Germany) 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 μg, 30 μg or 100 μg) in a two-dose immunization regimen(Mulligan et al., 2020). Since the prime vaccination with 100 μg showed an increased reactogenicity and a lack of meaningfully increased

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 fatigue, headache, chills, muscle pain and joint pain(Mulligan et al., 2020)(Table 1). Systemic events increased with dose level and after the second dose. As for immunogenicity, geometric mean NT50 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 that of a panel of COVID-19 convalescent human sera, respectively(Mulligan et al., 2020). The clinical trial in Germany investigated five dose levels: 1 μg, 10 μg, 30 μg, 50 μg, 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. Two doses of 1-50 μg of BNT162b1 elicited robust Th1-biased T cell immune response with RBD-specific $CD4^+$ and $CD8^+$ T cell expansion(Sahin et al., 2020). Geometric mean NT50 titers were 0.7-fold (1 μg dose) to 3.5-fold (50 μg dose) those of the recovered individuals(Sahin et al., 2020). These results demonstrated that BNT162b1 has the potential to protect against COVID-19 throμgh multiple beneficial mechanisms(Sahin et al., 2020). Meanwhile, another placebo-controlled, observer-blinded, dose-escalation phase I trial was conducted in the USA and aimed to compare the safety and immunogenicity profiles of BNT162b1 and BNT162b2 vaccine candidates among adults 18-55 years of age and those 65-85 years of age(Walsh et al., 2020). The trial results indicated that BNT162b2 was associated with

a lower incidence and severity of systemic reactions than BNT162b1, particularly in older adults, for example, only 8% of the older participants receiving BNT162b2 reported mild fever (38.0-38.4 ℃), whereas 17%, 8% and 8% of those receiving the same dose of BNT162b1 reported mild(38.0-38.4 ℃), moderate (>38.4-38.9 ℃) and severe (>38.9-40.0 ℃) fever, respectively(Walsh et al., 2020). Moreover, in both younger and older adults, the two vaccine candidates elicited similar dose-dependent SARS-CoV-2-neutralizing geometric mean titers, which were similar to or higher than those of convalescent human serum samples(Walsh et al., 2020). Taking all of the above data into consideration, the developers finally selected BNT162b2 at 30 μg in a two-dose immunization regimen for advancement to a pivotal phase III safety and efficacy evaluation. In the phase III clinical trial, 43,448 participants were randomly assigned to receive injections: 21,720 with BNT162b2 and 21,728 with placebo(Polack et al., 2020). There were 8 cases of COVID-19 with onset at least 7 days after boost vaccination in the BNT162b2 group and 162 cases in the placebo group. Therefore, BNT162b2 showed a 95% efficacy in preventing COVID-19(Polack et al., 2020) (Table 1). In addition, among 10 cases of severe COVID-19, 9 occurred in placebo recipients and 1 in a BNT162b2 recipient(Polack et al., 2020). The safety profile of BNT162b2 was characterized by short-term, mild-to-moderate pain at the injection site, fatigue, and headache(Polack et al., 2020). The incidence of serious adverse events was low and similar in the vaccine and placebo groups(Polack et al., 2020). BNT162b2 has currently been approved for emergency use in USA and Germany.

4.3 Other COVID-19 mRNA vaccines

In addition to the above three mRNA vaccines, there are four other COVID-19 mRNA vaccines 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. Preclinical study showed that two doses of ARCoV immunization conferred complete protection against the challenge of a SARS-CoV-2 mouse-adapted strain in mice and also elicited robust neutralizing antibodies against SARS-CoV-2 as well as Th1-biased cellular response 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 observed in convalescent sera from COVID-19 patients(Kremsner et al., 2020) (Table 1). Unlike these above mRNA vaccine candidates which encode only the SARS-CoV-2 antigen of interest, the ARCT-021 and LNP-nCoVsaRNA vaccine candidates are self-amplifying mRNA derived from the genome of positive-stranded RNA viruses, which encodes not only the antigen of interest but also the viral replication machinery required for intracellular RNA

amplification. 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, encapsulated in LNPs, was derived from an alphavirus genome and encodes the alphaviral replicase and SARS-CoV-2 prefusion stabilized S(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).

5. 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 versality, 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, which can protect the upper respiratory tract, may be necessary for sterilizing immunity, and IgA levels induced by mRNA vaccines have not been determined in current clinical trials. In addition, although mRNA-1273 has shown that high neutralizing antibody titers persist for at least 4 months following prime vaccination in humans(Widge et al., 2020), 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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Author contributions

Jinghua Yan and Qingrui Huang carried out the concepts, design and definition of intellectual content. Qingrui Huang and Jiawei Zeng conducted literature search and manuscript preparation. Jinghua Yan carried out manuscript editing. All authors have read and approved the content of the manuscript.

References

Alberer, M., Gnad-Vogt, U., Hong, H.S., Mehr, K.T., Backert, L., Finak, G., Gottardo, R., Bica, M.A., Garofano, A., Koch, S.D., *et al.* (2017). Safety and immunogenicity of a mRNA rabies vaccine in healthy adults: an open-label, non-randomised, prospective, first-in-human phase 1 clinical trial. Lancet (London, England) *390*, 1511-1520.

Amanat, F., Stadlbauer, D., Strohmeier, S., Nguyen, T.H.O., Chromikova, V., McMahon, M., Jiang, K., Arunkumar, G.A., Jurczyszak, D., Polanco, J., *et al.* (2020). A serological assay to detect SARS-CoV-2 seroconversion in humans. Nature medicine *26*, 1033-1036.

Anderson, E.J., Rouphael, N.G., Widge, A.T., Jackson, L.A., Roberts, P.C., Makhene, M., Chappell, J.D., Denison, M.R., Stevens, L.J., Pruijssers, A.J., *et al.* (2020). Safety and Immunogenicity of SARS-CoV-2 mRNA-1273 Vaccine in Older Adults. The New England journal of medicine *383*, 2427-2438.

Baum, A., Fulton, B.O., Wloga, E., Copin, R., Pascal, K.E., Russo, V., Giordano, S., Lanza, K., Negron, N., Ni, M., *et al.* (2020). Antibody cocktail to SARS-CoV-2 spike protein prevents rapid mutational escape seen with individual antibodies. Science *369*, 1014-1018.

Cai, Y., Zhang, J., Xiao, T., Peng, H., Sterling, S.M., Walsh, R.M., Jr., Rawson, S., Rits-Volloch, S., and Chen, B. (2020). Distinct conformational states of SARS-CoV-2 spike protein. Science *369*, 1586-1592.

Cao, Y., Su, B., Guo, X., Sun, W., Deng, Y., Bao, L., Zhu, Q., Zhang, X., Zheng, Y., Geng, C., *et al.* (2020). Potent Neutralizing Antibodies against SARS-CoV-2 Identified by High-Throughput Single-Cell Sequencing of Convalescent Patients' B

Cells. Cell *182*, 73-84.e16.

Chandrashekar, A., Liu, J., Martinot, A.J., McMahan, K., Mercado, N.B., Peter, L., Tostanoski, L.H., Yu, J., Maliga, Z., Nekorchuk, M., *et al.* (2020). SARS-CoV-2 infection protects against rechallenge in rhesus macaques. Science.

Chen, W.H., Tao, X., Agrawal, A.S., Algaissi, A., Peng, B.H., Pollet, J., Strych, U., Bottazzi, M.E., Hotez, P.J., Lustigman, S., *et al.* (2020). Yeast-expressed SARS-CoV recombinant receptor-binding domain (RBD219-N1) formulated with aluminum hydroxide induces protective immunity and reduces immune enhancement. Vaccine *38*, 7533-7541.

Corbett, K.S., Edwards, D.K., Leist, S.R., Abiona, O.M., Boyoglu-Barnum, S., Gillespie, R.A., Himansu, S., Schäfer, A., Ziwawo, C.T., DiPiazza, A.T., *et al.* (2020a). SARS-CoV-2 mRNA vaccine design enabled by prototype pathogen preparedness. Nature *586*, 567-571.

Corbett, K.S., Flynn, B., Foulds, K.E., Francica, J.R., Boyoglu-Barnum, S., Werner, A.P., Flach, B., O'Connell, S., Bock, K.W., Minai, M., *et al.* (2020b). Evaluation of the mRNA-1273 Vaccine against SARS-CoV-2 in Nonhuman Primates. The New England journal of medicine *383*, 1544-1555.

Dai, L., Zheng, T., Xu, K., Han, Y., Xu, L., Huang, E., An, Y., Cheng, Y., Li, S., Liu, M., *et al.* (2020). A Universal Design of Betacoronavirus Vaccines against COVID-19, MERS, and SARS. Cell *182*, 722-733.e711.

de Jong, W., Aerts, J., Allard, S., Brander, C., Buyze, J., Florence, E., van Gorp, E., Vanham, G., Leal, L., Mothe, B., *et al.* (2019). iHIVARNA phase IIa, a randomized,

placebo-controlled, double-blinded trial to evaluate the safety and immunogenicity of iHIVARNA-01 in chronically HIV-infected patients under stable combined antiretroviral therapy. Trials *20*, 361.

Du, S., Cao, Y., Zhu, Q., Yu, P., Qi, F., Wang, G., Du, X., Bao, L., Deng, W., Zhu, H., *et al.* (2020). Structurally Resolved SARS-CoV-2 Antibody Shows High Efficacy in Severely Infected Hamsters and Provides a Potent Cocktail Pairing Strategy. Cell.

Feldman, R.A., Fuhr, R., Smolenov, I., Mick Ribeiro, A., Panther, L., Watson, M., Senn, J.J., Smith, M., Almarsson, Pujar, H.S., *et al.* (2019). mRNA vaccines against H10N8 and H7N9 influenza viruses of pandemic potential are immunogenic and well tolerated in healthy adults in phase 1 randomized clinical trials. Vaccine *37*, 3326-3334.

Gay, C.L., DeBenedette, M.A., Tcherepanova, I.Y., Gamble, A., Lewis, W.E., Cope, A.B., Kuruc, J.D., McGee, K.S., Kearney, M.F., Coffin, J.M., *et al.* (2018). Immunogenicity of AGS-004 Dendritic Cell Therapy in Patients Treated During Acute HIV Infection. AIDS research and human retroviruses *34*, 111-122.

Haynes, B.F., Corey, L., Fernandes, P., Gilbert, P.B., Hotez, P.J., Rao, S., Santos, M.R., Schuitemaker, H., Watson, M., and Arvin, A. (2020). Prospects for a safe COVID-19 vaccine. Science translational medicine.

Hoffmann, M., Kleine-Weber, H., Schroeder, S., Kruger, N., Herrler, T., Erichsen, S., Schiergens, T.S., Herrler, G., Wu, N.H., Nitsche, A., *et al.* (2020). SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. Cell *181*, 271-280.e278.

Hsieh, C.L., Goldsmith, J.A., Schaub, J.M., DiVenere, A.M., Kuo, H.C., Javanmardi, K., Le, K.C., Wrapp, D., Lee, A.G., Liu, Y., *et al.* (2020). Structure-based Design of Prefusion-stabilized SARS-CoV-2 Spikes. bioRxiv : the preprint server for biology.

Hu, B., Guo, H., Zhou, P., and Shi, Z.L. (2020). Characteristics of SARS-CoV-2 and COVID-19. Nat Rev Microbiol, 1-14.

Jackson, L.A., Anderson, E.J., Rouphael, N.G., Roberts, P.C., Makhene, M., Coler, R.N., McCullough, M.P., Chappell, J.D., Denison, M.R., Stevens, L.J., *et al.* (2020). An mRNA Vaccine against SARS-CoV-2 - Preliminary Report. The New England journal of medicine.

Juno, J.A., Tan, H.X., Lee, W.S., Reynaldi, A., Kelly, H.G., Wragg, K., Esterbauer, R., Kent, H.E., Batten, C.J., Mordant, F.L., *et al.* (2020). Humoral and circulating follicular helper T cell responses in recovered patients with COVID-19. Nature medicine.

Kaczmarek, J.C., Kowalski, P.S., and Anderson, D.G. (2017). Advances in the delivery of RNA therapeutics: from concept to clinical reality. Genome medicine *9*, 60.

Karikó, K., Muramatsu, H., Ludwig, J., and Weissman, D. (2011). Generating the optimal mRNA for therapy: HPLC purification eliminates immune activation and improves translation of nucleoside-modified, protein-encoding mRNA. Nucleic acids research *39*, e142.

Kowalski, P.S., Rudra, A., Miao, L., and Anderson, D.G. (2019). Delivering the Messenger: Advances in Technologies for Therapeutic mRNA Delivery. Molecular

therapy : the journal of the American Society of Gene Therapy *27*, 710-728.

Kremsner, P., Mann, P., Bosch, J., Fendel, R., Gabor, J.J., Kreidenweiss, A., Kroidl, A., Leroux-Roels, I., Leroux-Roels, G., Schindler, C., *et al.* (2020). Phase 1 Assessment of the Safety and Immunogenicity of an mRNA- Lipid Nanoparticle Vaccine Candidate Against SARS-CoV-2 in Human Volunteers. medRxiv, 2020.2011.2009.20228551.

Laczkó, D., Hogan, M.J., Toulmin, S.A., Hicks, P., Lederer, K., Gaudette, B.T., Castaño, D., Amanat, F., Muramatsu, H., Oguin, T.H., 3rd, *et al.* (2020). A Single Immunization with Nucleoside-Modified mRNA Vaccines Elicits Strong Cellular and Humoral Immune Responses against SARS-CoV-2 in Mice. Immunity.

Linares-Fernández, S., Lacroix, C., Exposito, J.Y., and Verrier, B. (2020). Tailoring mRNA Vaccine to Balance Innate/Adaptive Immune Response. Trends in molecular medicine *26*, 311-323.

Liu, C., Yang, Y., Gao, Y., Shen, C., Ju, B., Liu, C., Tang, X., Wei, J., Ma, X., Liu, W., *et al.* (2020a). Viral Architecture of SARS-CoV-2 with Post-Fusion Spike Revealed by Cryo-EM. bioRxiv : the preprint server for biology, 2020.2003.2002.972927.

Liu, Y., Wang, K., Massoud, T.F., and Paulmurugan, R. (2020b). SARS-CoV-2 Vaccine Development: An Overview and Perspectives. ACS pharmacology & translational science *3*, 844-858.

Lu, J., Lu, G., Tan, S., Xia, J., Xiong, H., Yu, X., Qi, Q., Yu, X., Li, L., Yu, H., *et al.* (2020). A COVID-19 mRNA vaccine encoding SARS-CoV-2 virus-like particles induces a strong antiviral-like immune response in mice. Cell research, 1-4.

Lv, Z., Deng, Y.Q., Ye, Q., Cao, L., Sun, C.Y., Fan, C., Huang, W., Sun, S., Sun, Y., Zhu, L., *et al.* (2020). Structural basis for neutralization of SARS-CoV-2 and SARS-CoV by a potent therapeutic antibody. Science.

Maruggi, G., Zhang, C., Li, J., Ulmer, J.B., and Yu, D. (2019). mRNA as a Transformative Technology for Vaccine Development to Control Infectious Diseases. Molecular therapy : the journal of the American Society of Gene Therapy *27*, 757-772.

McKay, P.F., Hu, K., Blakney, A.K., Samnuan, K., Brown, J.C., Penn, R., Zhou, J., Bouton, C.R., Rogers, P., Polra, K., *et al.* (2020). Self-amplifying RNA SARS-CoV-2 lipid nanoparticle vaccine candidate induces high neutralizing antibody titers in mice. Nature communications *11*, 3523.

Mercado, N.B., Zahn, R., Wegmann, F., Loos, C., Chandrashekar, A., Yu, J., Liu, J., Peter, L., McMahan, K., Tostanoski, L.H., *et al.* (2020). Single-shot Ad26 vaccine protects against SARS-CoV-2 in rhesus macaques. Nature.

Monteil, V., Kwon, H., Prado, P., Hagelkrüys, A., Wimmer, R.A., Stahl, M., Leopoldi, A., Garreta, E., Hurtado Del Pozo, C., Prosper, F., *et al.* (2020). Inhibition of SARS-CoV-2 Infections in Engineered Human Tissues Using Clinical-Grade Soluble Human ACE2. Cell *181*, 905-913.e907.

Mulligan, M.J., Lyke, K.E., Kitchin, N., Absalon, J., Gurtman, A., Lockhart, S., Neuzil, K., Raabe, V., Bailey, R., Swanson, K.A., *et al.* (2020). Phase 1/2 study of COVID-19 RNA vaccine BNT162b1 in adults. Nature.

Pallesen, J., Wang, N., Corbett, K.S., Wrapp, D., Kirchdoerfer, R.N., Turner, H.L.,

Cottrell, C.A., Becker, M.M., Wang, L., Shi, W., *et al.* (2017). Immunogenicity and structures of a rationally designed prefusion MERS-CoV spike antigen. Proceedings of the National Academy of Sciences of the United States of America *114*, E7348-e7357.

Pardi, N., Hogan, M.J., Naradikian, M.S., Parkhouse, K., Cain, D.W., Jones, L., Moody, M.A., Verkerke, H.P., Myles, A., Willis, E., *et al.* (2018a). Nucleoside-modified mRNA vaccines induce potent T follicular helper and germinal center B cell responses. The Journal of experimental medicine *215*, 1571-1588.

Pardi, N., Hogan, M.J., Pelc, R.S., Muramatsu, H., Andersen, H., DeMaso, C.R., Dowd, K.A., Sutherland, L.L., Scearce, R.M., Parks, R., *et al.* (2017). Zika virus protection by a single low-dose nucleoside-modified mRNA vaccination. Nature *543*, 248-251.

Pardi, N., Hogan, M.J., Porter, F.W., and Weissman, D. (2018b). mRNA vaccines - a new era in vaccinology. Nature reviews Drug discovery *17*, 261-279.

Polack, F.P., Thomas, S.J., Kitchin, N., Absalon, J., Gurtman, A., Lockhart, S., Perez, J.L., Pérez Marc, G., Moreira, E.D., Zerbini, C., *et al.* (2020). Safety and Efficacy of the BNT162b2 mRNA Covid-19 Vaccine. The New England journal of medicine.

Poland, G.A., Ovsyannikova, I.G., Crooke, S.N., and Kennedy, R.B. (2020). SARS-CoV-2 Vaccine Development: Current Status. Mayo Clinic proceedings *95*, 2172-2188.

Pollard, C., De Koker, S., Saelens, X., Vanham, G., and Grooten, J. (2013). Challenges and advances towards the rational design of mRNA vaccines. Trends in

molecular medicine *19*, 705-713.

Richner, J.M., Himansu, S., Dowd, K.A., Butler, S.L., Salazar, V., Fox, J.M., Julander, J.G., Tang, W.W., Shresta, S., Pierson, T.C., *et al.* (2017). Modified mRNA Vaccines Protect against Zika Virus Infection. Cell *169*, 176.

Sahin, U., Kariko, K., and Tureci, O. (2014). mRNA-based therapeutics--developing a new class of drugs. Nature reviews Drug discovery *13*, 759-780.

Sahin, U., Muik, A., Derhovanessian, E., Vogler, I., Kranz, L.M., Vormehr, M., Baum, A., Pascal, K., Quandt, J., Maurus, D., *et al.* (2020). COVID-19 vaccine BNT162b1 elicits human antibody and T(H)1 T cell responses. Nature *586*, 594-599.

Santos, I.A., Grosche, V.R., Bergamini, F.R.G., Sabino-Silva, R., and Jardim, A.C.G. (2020). Antivirals Against Coronaviruses: Candidate Drugs for SARS-CoV-2 Treatment? Frontiers in microbiology *11*, 1818.

Schlake, T., Thess, A., Fotin-Mleczek, M., and Kallen, K.J. (2012). Developing mRNA-vaccine technologies. RNA biology *9*, 1319-1330.

Shan, C., Yao, Y.F., Yang, X.L., Zhou, Y.W., Gao, G., Peng, Y., Yang, L., Hu, X., Xiong, J., Jiang, R.D., *et al.* (2020). Infection with novel coronavirus (SARS-CoV-2) causes pneumonia in Rhesus macaques. Cell research *30*, 670-677.

Smith, T.R.F., Patel, A., Ramos, S., Elwood, D., Zhu, X., Yan, J., Gary, E.N., Walker, S.N., Schultheis, K., Purwar, M., *et al.* (2020). Immunogenicity of a DNA vaccine candidate for COVID-19. Nature communications *11*, 2601.

Su, S., Du, L., and Jiang, S. (2020). Learning from the past: development of safe and effective COVID-19 vaccines. Nat Rev Microbiol, 1-9.

Sullenger, B.A., and Nair, S. (2016). From the RNA world to the clinic. Science *352*, 1417-1420.

Sun, S.H., Chen, Q., Gu, H.J., Yang, G., Wang, Y.X., Huang, X.Y., Liu, S.S., Zhang, N.N., Li, X.F., Xiong, R., *et al.* (2020). A Mouse Model of SARS-CoV-2 Infection and Pathogenesis. Cell host & microbe *28*, 124-133.e124.

Tang, Z., Kong, N., Zhang, X., Liu, Y., Hu, P., Mou, S., Liljeström, P., Shi, J., Tan, W., Kim, J.S., *et al.* (2020). A materials-science perspective on tackling COVID-19. Nature reviews Materials, 1-14.

Ulmer, J.B., and Geall, A.J. (2016). Recent innovations in mRNA vaccines. Current opinion in immunology *41*, 18-22.

V'Kovski, P., Kratzel, A., Steiner, S., Stalder, H., and Thiel, V. (2020). Coronavirus biology and replication: implications for SARS-CoV-2. Nat Rev Microbiol, 1-16.

Walls, A.C., Park, Y.J., Tortorici, M.A., Wall, A., McGuire, A.T., and Veesler, D. (2020). Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein. Cell *181*, 281-292.e286.

Walsh, E.E., Frenck, R.W., Jr., Falsey, A.R., Kitchin, N., Absalon, J., Gurtman, A., Lockhart, S., Neuzil, K., Mulligan, M.J., Bailey, R., *et al.* (2020). Safety and Immunogenicity of Two RNA-Based Covid-19 Vaccine Candidates. The New England journal of medicine *383*, 2439-2450.

Wang, Q., Zhang, Y., Wu, L., Niu, S., Song, C., Zhang, Z., Lu, G., Qiao, C., Hu, Y., Yuen, K.Y., *et al.* (2020). Structural and Functional Basis of SARS-CoV-2 Entry by Using Human ACE2. Cell.

Watanabe, Y., Allen, J.D., Wrapp, D., McLellan, J.S., and Crispin, M. (2020). Site-specific glycan analysis of the SARS-CoV-2 spike. Science *369*, 330-333.

Widge, A.T., Rouphael, N.G., Jackson, L.A., Anderson, E.J., Roberts, P.C., Makhene, M., Chappell, J.D., Denison, M.R., Stevens, L.J., Pruijssers, A.J., *et al.* (2020). Durability of Responses after SARS-CoV-2 mRNA-1273 Vaccination. The New England journal of medicine.

Wrapp, D., De Vlieger, D., Corbett, K.S., Torres, G.M., Wang, N., Van Breedam, W., Roose, K., van Schie, L., Hoffmann, M., Pöhlmann, S., *et al.* (2020a). Structural Basis for Potent Neutralization of Betacoronaviruses by Single-Domain Camelid Antibodies. Cell *181*, 1004-1015.e1015.

Wrapp, D., Wang, N., Corbett, K.S., Goldsmith, J.A., Hsieh, C.L., Abiona, O., Graham, B.S., and McLellan, J.S. (2020b). Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. Science *367*, 1260-1263.

Wu, Y., Li, C., Xia, S., Tian, X., Kong, Y., Wang, Z., Gu, C., Zhang, R., Tu, C., Xie, Y., *et al.* (2020a). Identification of Human Single-Domain Antibodies against SARS-CoV-2. Cell host & microbe *27*, 891-898.e895.

Wu, Y., Wang, F., Shen, C., Peng, W., Li, D., Zhao, C., Li, Z., Li, S., Bi, Y., Yang, Y., *et al.* (2020b). A noncompeting pair of human neutralizing antibodies block COVID-19 virus binding to its receptor ACE2. Science.

Xia, S., Liu, M., Wang, C., Xu, W., Lan, Q., Feng, S., Qi, F., Bao, L., Du, L., Liu, S., *et al.* (2020). Inhibition of SARS-CoV-2 (previously 2019-nCoV) infection by a highly potent pan-coronavirus fusion inhibitor targeting its spike protein that harbors

a high capacity to mediate membrane fusion. Cell research *30*, 343-355.

Xu, Y., Kang, L., Shen, Z., Li, X., Wu, W., Ma, W., Fang, C., Yang, F., Jiang, X., Gong, S., *et al.* (2020). Dynamics of SARS-CoV-2 genome variants in the feces during convalescence. Journal of Genetics and Genomics.

Yang, J., Wang, W., Chen, Z., Lu, S., Yang, F., Bi, Z., Bao, L., Mo, F., Li, X., Huang, Y., *et al.* (2020). A vaccine targeting the RBD of the S protein of SARS-CoV-2 induces protective immunity. Nature.

Yu, J., Tostanoski, L.H., Peter, L., Mercado, N.B., McMahan, K., Mahrokhian, S.H., Nkolola, J.P., Liu, J., Li, Z., Chandrashekar, A., *et al.* (2020). DNA vaccine protection against SARS-CoV-2 in rhesus macaques. Science.

Yuan, M., Wu, N.C., Zhu, X., Lee, C.D., So, R.T.Y., Lv, H., Mok, C.K.P., and Wilson, I.A. (2020). A highly conserved cryptic epitope in the receptor-binding domains of SARS-CoV-2 and SARS-CoV. Science.

Zhang, N.N., Li, X.F., Deng, Y.Q., Zhao, H., Huang, Y.J., Yang, G., Huang, W.J., Gao, P., Zhou, C., Zhang, R.R., *et al.* (2020). A Thermostable mRNA Vaccine against COVID-19. Cell.

Zhou, P., Yang, X.L., Wang, X.G., Hu, B., Zhang, L., Zhang, W., Si, H.R., Zhu, Y., Li, B., Huang, C.L., *et al.* (2020). A pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature *579*, 270-273.

Zhu, F.C., Guan, X.H., Li, Y.H., Huang, J.Y., Jiang, T., Hou, L.H., Li, J.X., Yang, B.F., Wang, L., Wang, W.J., *et al.* (2020a). Immunogenicity and safety of a recombinant adenovirus type-5-vectored COVID-19 vaccine in healthy adults aged 18 years or

older: a randomised, double-blind, placebo-controlled, phase 2 trial. Lancet (London, England) *396*, 479-488.

Zhu, F.C., Li, Y.H., Guan, X.H., Hou, L.H., Wang, W.J., Li, J.X., Wu, S.P., Wang, B.S., Wang, Z., Wang, L., *et al.* (2020b). Safety, tolerability, and immunogenicity of a recombinant adenovirus type-5 vectored COVID-19 vaccine: a dose-escalation, open-label, non-randomised, first-in-human trial. Lancet (London, England) *395*, 1845-1854.

Zhu, N., Zhang, D., Wang, W., Li, X., Yang, B., Song, J., Zhao, X., Huang, B., Shi, W., Lu, R., *et al.* (2020c). A Novel Coronavirus from Patients with Pneumonia in China, 2019. The New England journal of medicine *382*, 727-733.

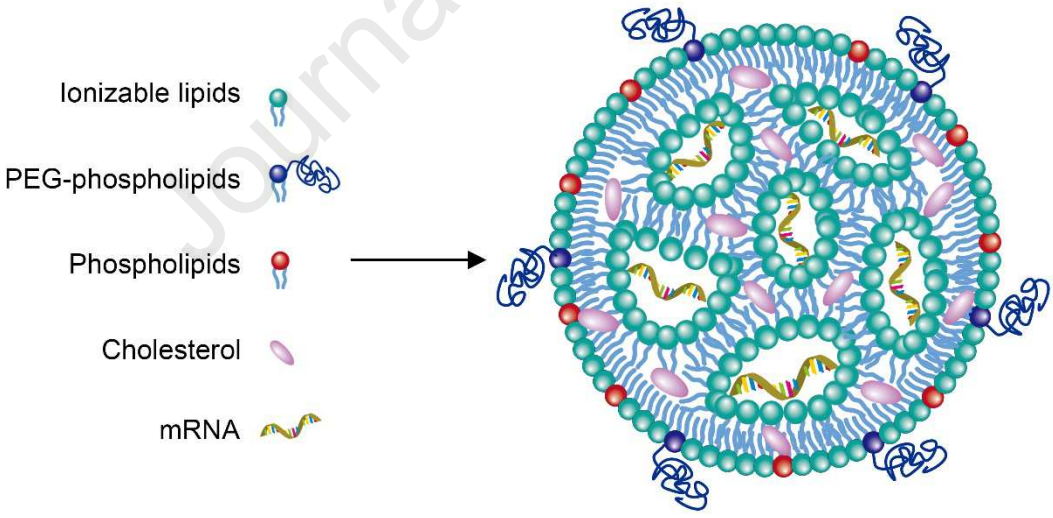

Figure 1. Schematic representation of mRNA-lipid nanoparticle complex.

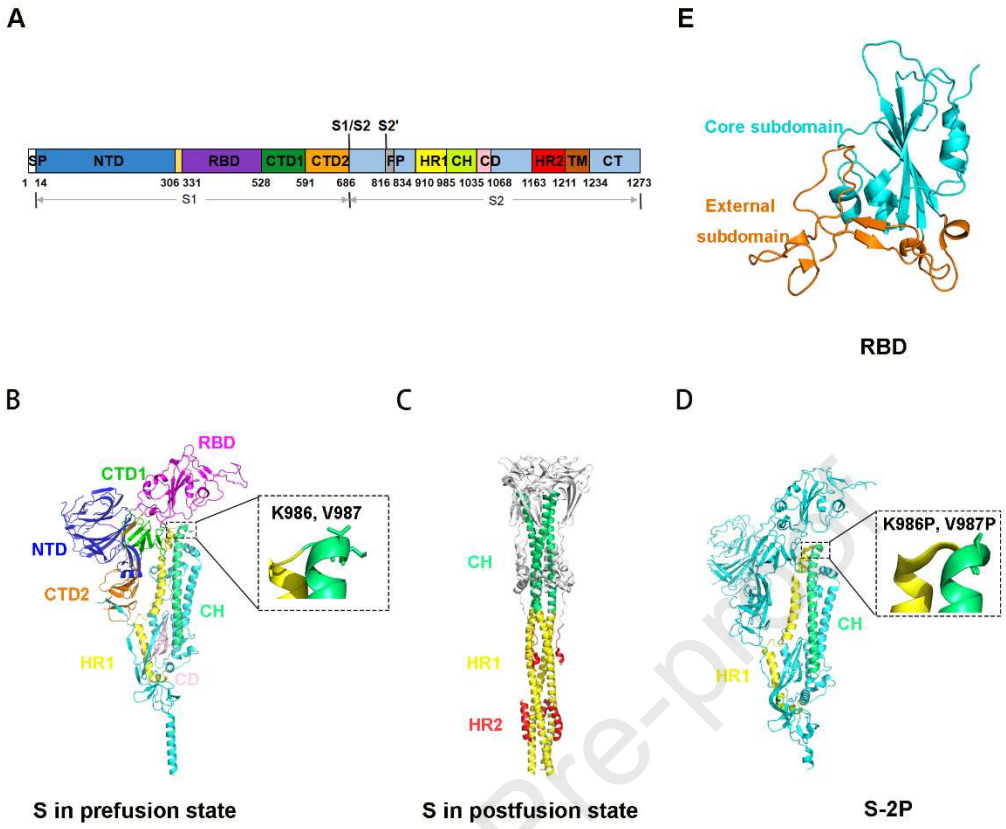

Figure 2. Antigens of COVID-19 mRNA vaccines. (A) Schematic representation of full-length SARS-CoV-2 S primary structure colored by domain. SS, signal peptide; NTD, N-terminal domain; RBD, receptor-binding domain; CTD, C-terminal domain; FP, fusion peptide; HR1, heptad repeat 1; CH, central helix; CD,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

Table 1. COVID-19 mRNA vaccines in clinical trials.

Table 1 COVID-19 mRNA vaccine candidates in clinical trials

Vaccine name	Developers	Location	Route	Targets	Immunogenicity and protection	Safety	Phase
mRNA-1273	Moderna	USA	IM	S-2P	A two-dose regimen of mRNA-1273 was 94.1% effective in preventing COVID-19 (95% CI 89.3%, 96.8%)	Severe adverse reactions occurred in 0.2% to 9.7% of participants, were more frequent after dose 2 than after dose 1, and were generally less frequent in participants ≥65 years of age as compared to younger participants.	Phase III[a] NCT04470427
BNT1626b2	BioNTech	Germany	IM	S-2P	A two-dose regimen of BNT162b2 was 95% effective in preventing Covid-19 (95% CI, 90.3 to 97.6).	The safety profile of BNT162b2 was characterized by short-term, mild-to-moderate pain at the injection site, fatigue, and headache. The incidence of serious adverse events was low and was similar in the vaccine and placebo groups.	Phase III[a] NCT04368728
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 solicited systemic adverse events, but the majority were mild or moderate and transient in duration	Phase III NCT04674189
ARCoV	People's Liberation Army (PLA) Academy of Military Sciences/Walvax Biotech	China	IM	RBD	Two doses of ARCoV immunization elicited robust neutralizing antibodies and cellular immune responese in non-human primates and protected mice from SARS-CoV-2 challenge.	Not available.	Phase II ChiCTR2000039212
ARCT-021	Arcturus Therapeutics, Inc.	USA	IM	Not available.	Favorable immunogenicity results for both single-dose and prime-boost regimens. Binding IgG:100% seroconversion in younger adults; 4 out of 5 older adult participants seroconversion; GMT > 2300 in all cohorts.	Favorable safety and tolerability profile in both younger (ages 21-55) and older (ages 56-80) subjects; no severe adeverse events.	Phase II NCT04668339
LNP-nCoVsaRNA	Imperial College London	England	IM	S-2P	Two doses of LNP-nCoVsaRNA immunization in mice elicited higher neutralizing antibody titers than those of COVID-19 convalescent patients and cellular immune responese	Not available.	Phase I SRCTN17072692
ChulaCov19 mRNA vaccine	Chulalongkorn University	Thailand	IM	Not available.	Not available.	Not available.	Phase I NCT04566276

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