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Review

Current progress and challenges in the design and development of a successful COVID-19 vaccine

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ABSTRACT

The coronavirus disease 2019 (COVID-19) pandemic, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is still a worldwide concern, with little to no sign of a decreasing trend. There is a general consensus that normal life will be hampered until a safe and effective vaccine strategy is available and globally administered. Numerous countries have accelerated the clinical trials process for the development of a successful COVID-19 treatment, with over 200 candidates presently available for testing against SARS-CoV-2. Here, we provide an overview of the COVID-19 vaccine candidates currently in development, discuss the scientific and practical challenges associated with COVID-19 vaccine design, and share the potential strategies that could be exploited for vaccine design success.

1. Introduction

An unusual viral pneumonia caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was firstly found at the end of 2019 [1,2]. The disease, now termed coronavirus disease 19 (COVID-19), is characterized by high fever, dry cough, difficult breathing, chest discomfort, severe dyspnea, and bilateral lung infiltration [3–5]. Because of the high rate of human-to-human transmission in large-scale, rapid spread manner, the World Health Organization (WHO) officially announced a global pandemic on 11 March 2020, leading to mass isolation and social distancing in an effort to curb the spread [3,4]. Despite many best efforts worldwide, SARS-CoV-2 has spread to 216 countries and regions, and, as of 9 January 2021, has resulted in more than 87 million confirmed cases and at least 1.9 million deaths worldwide [3,5]. This disease poses an extraordinary threat to global health and public safety, and the urgency associated with this pandemic has emphasized the pressing need for effective preventive and therapeutic measures.

SARS-CoV-2 is a single-strand positive RNA virus belonging to the Orthocoronavirinae subfamily [3]. It is closely related to other coronaviruses that have caused similar outbreaks in recent years. Six coronaviruses are known to infect humans, including 229E, NL63, OC43, HKU1, severe acute respiratory syndrome coronavirus (SARS-CoV), and middle east respiratory syndrome coronavirus (MERS-CoV). Zoonotic SARS-CoV, MERS-CoV, and SARS-CoV-2 are betacoronaviruses and crossed into humans in more recent years: the SARS-CoV outbreak in Asia emerged in Guangdong Province, China, with the last reported case in 2003; MERS-CoV was first reported in 2012 and still circulates in the Middle East; and SARS-CoV-2 persists worldwide, only the third type of coronavirus to cause severe pneumonia in humans [6].

Vaccines remain the most cost-effective intervention for the control and prevention of infectious disease. However, there have been no vaccines to date for the treatment of any of the coronaviruses that have made the switch over to humans, including SARS-CoV and MERS-CoV. Whereas these previous coronavirus outbreaks eventually petered out—in part presumably due to good public health containment and the earlier symptomatic response—COVID-19 does not show similar signs of a decreasing trend, with rapid rates of infectivity in clusters. As such, there has been an urgent response within the scientific community to accelerate the development of a vaccine against this particular species.

Phylogenetic analyses suggest that SARS-CoV-2 has a 79% sequence similarity with SARS-CoV, and a lower (50%) similarity with MERS-CoV [7]. SARS-CoV-2 contains four major structural proteins, spike (S), envelope (E), membrane (M), and nucleocapsid (N) proteins [5] and, like SARS, uses the angiotensin-converting enzyme 2 (ACE2) receptor for cell entry [8]. The virions are spherical and decorated with S proteins on the envelope surface. These S proteins play a pivotal role in viral infection and pathogenesis. The S protein comprises two subunits—S1 and S2. The S1 subunit recognizes host receptors, whereas the S2 subunit mediates fusion between the viral envelope and the host cell membrane [6]. The receptor binding domain (RBD) in the S1 subunit is responsible for virus binding to host cell receptors [6].

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Unique to the SARS-CoV-2 S-protein is PRRA amino acid insertion in the furin cleavage site between the S1 and S2 subunits [8]. This long furin cleavage site influences S-protein stability and facilitates subsequent conformational changes that can influence viral entry [8]. The SARS-CoV-2 S-protein is primed by transmembrane serine protease-2 (TMPRSS2) for host cell entry. In addition, neuropilin-1 (NRP1), which is known to bind furin-cleaved substrates, potentiates SARS-CoV-2 infectivity [9]. Earlier work on SARS-CoV and MERS-CoV shows that the S-protein is the principal antigenic constituent that can be harnessed to induce the production of neutralizing antibodies (nAbs) to block virus binding [6,10]. As such, the S-protein has been similarly recognized as a significant target for COVID-19 vaccines. Notably, other studies have reported that the M-protein is capable of inducing the production of nAbs, and the N-protein contains T-cell epitopes in SARS-CoV; these proteins may also offer alternative vaccine targets [11,12]. Collectively, these studies provide potential targets for the development of antivirals against SARS-CoV-2. In this review, we summarize the current advances in the clinical trials associated with the development of COVID-19 vaccines and focus on the challenges and strategies of vaccine development against this unique and difficult species.

2. COVID-19 vaccines in development

There has been an enormous effort to develop an effective and safe vaccine to control the rapid spread of the SARS-CoV-2 virus. Currently, there are 235 candidate vaccines under development, according to a document released by the WHO (https://www.who.int/publications/m/item/draft-landscape-of-covid-19-candidate-vaccines), with 63 candidate vaccines in clinical trials, including 9 inactivated vaccines, 11 non-replicating viral vector vaccines, 6 replicating viral vector vaccines, 19 protein subunit vaccines, 7 RNA vaccines, 8 DNA vaccines, and 2 VLP vaccines. This array of vaccine types provides a chance that at least a few candidates will eventually be approved for further development and marketing.

There are several commonly used platforms for vaccine development: these include 1) the classical and mature approaches using inactivated whole virions, live-attenuated, recombinant protein, or vectored vaccines, which are well-established technologies and have led to the production of numerous licensed vaccines; 2) promising novel vaccine approaches, such as DNA vaccines or mRNA vaccines; there is no precedent for a licensed vaccine based on these platforms [13].

2.1. Classical vaccines

2.1.1. Inactivated vaccines

Inactivated vaccines are the traditional form of vaccine and are formulated using the whole virus that is commonly either physically (heat) or chemically (e.g., β-propiolactone) inactivated. Such viruses are usually produced in Vero cells, and the culture supernatant is purified and formulated with or without adjuvant [14–17]. Inactivated vaccines are easy to produce but require a biosafety level 3 (BSL3) facility. At the time of writing, there were 9 inactivated vaccines [18] in clinical trials, developed by Sinovac Biotech, Ltd. (China); Wuhan Institute of Biological Products; Beijing Institute of Biological Products; Chinese Academy of Medical Sciences; Research Institute for Biological Safety Problems in Kazakhstan; Bharat Biotech (India); Shenzhen Kangtai Biological Products Co., Ltd (China); Valneva, National Institute for Health Research (United Kingdom); and Erciyes University (Turky).

PiCoVacc, developed by Sinovac Biotech (China), is a β-propiolactone inactivated vaccine capable of inducing the production of nAbs in non-human primates (Rhesus macaques) (Table 1). This was achieved after three intramuscular injections of 3 or 6 μg PiCoVacc adjuvanted with aluminum hydroxide per dose at one-week intervals [15]. NAbs titers rose to ~50 after the second boost before virus challenge, which are similar to the titer levels raised by serum from recovered COVID-19 patients. In a SARS-CoV-2 challenge study, the immunized (3 μg/dose) monkeys showed partial protection response as compared with control monkeys following a direct intratracheal inoculation of 10^6 $TCID_{50}$ SARS-CoV-2 into the lungs, with detectable viral loads in the pharynx, anal canal and pulmonary tissues. At higher immunization doses (6 μg/dose), there were no detectable viral loads in any of the aforementioned tissues at 7 days after infection (Table 1). Of note, no antibody-dependent enhancement (ADE) of infection was observed. In another assay, hematological and biochemical analyses showed no notable changes in terms of lymphocyte subset percent and the presence of key cytokines, with no immunopathological exacerbation observed.

Subsequently, Sinovac reported the results from a randomized, double-blind, placebo-controlled phase II trial (NCT04352608) conducted using CoronaVac (previously, PiCovacca) with a total of 600 healthy adults aged 18–59 years [19]. CoronaVac was shown to be well tolerated, favorable, safe, and without any Grade 3 adverse reactions or vaccine-related serious adverse events (SAEs). CoronaVac showed good immunogenicity, with at least 92.4% seroconversion under different vaccination schedules in the lower-dose group (3 μg/dose). The geometric mean titers (GMTs) of the nAbs ranged from 24 to 65 among the different dosage and vaccination schedules (Table 2). The vaccine is now in phase III clinical trials (NCT04456595, NCT04582344, 669/UN6.KEP/EC/2020). In terms of the recent report in news, the CoronaVac vaccine showed 78% effective in Brazil trial.

Pre-clinical results are also available for another inactivated vaccine candidate, BBIBP-CorV, developed by the Beijing Institute of Biological Products Ltd. BBIBP-CorV adjuvanted with aluminum hydroxide, was evaluated in non-human primates (Cynomolgus macaques) using two-dose immunizations regimens. BBIBP-CorV provided highly efficient protection at both low (2 μg/dose) and high (8 μg/dose) doses. Before intratracheal challenge with 10^6 $TCID_{50}$ of the virus, the GMTs of the nAbs in the low-dose and high-dose groups reached 215 and 256, respectively. At 7 days post-infection (dpi), there was no viral load in the lungs of immunized macaques in either the low- or high-dose groups. In the upper airway, the virus was completely suppressed in the high-dose group, whereas primates in the low-dose group showed a significantly reduced viral load as compared with placebo recipients. The study observed no ADE of infection among any of the vaccinated primates (Table 1).

A randomized, double-blind, placebo-controlled, phase I/II trial with BBIBP-CorV vaccine (ChiCTR2000032459) has since been undertaken, with promising preliminary results [20]. In the phase I trial, 192 healthy participants (18–80 y) received a two-dose schedule at 2, 4, or 8 μg/dose. No SAEs were reported within 28 days post-vaccination. The adverse reactions were mild or moderate in severity (Table 2). Two weeks after the boost, the GMTs of the nAbs measured 87.7 to 228.7 for the younger participants (18–59 y), and 80.7 to 170.87 for the older participants (60 y and over), with a dose-dependent effect observed for the nAb titers. Phase II examined different vaccination schedules. Only one participant in the placebo group experienced and recovered from a grade 3 fever; all other adverse reactions were mild or moderate, and the most common systematic adverse reaction was fever. A two-dose immunization regimen (days 0/21 or days 0/28) with 4 μg of vaccine achieved higher nAb titers (282 and 218, respectively) than a single 8 μg dose (15) or a two-dose regimen of 4 μg on days 0 and 14 (170). This vaccine is currently in phase III human clinical trials (NCT04560881, ChiCTR2000034780). Recently, the interim results in phase III clinical trials showed 79.34% efficacy against COVID-19. This vaccine has been approved by the National Medical Products Administration (NMPA) with conditional marketing authorization on 31 December 2020, which is the first domestic COVID-19 vaccine in China.

An interim analysis from ongoing, randomized phase I, II and III clinical trials from the Wuhan Institute of Biological Product Co. Ltd. has been presented for another β-propiolactone inactivated vaccine adjuvanted with alum (ChiCTR2000031809) [17]. These double-blind, placebo-controlled trials tested the administration of three different doses of the vaccine (2.5, 5, and 10 μg) in 2- vs. 3-injection schedules.

Table 1
Summary of non-human primate results following treatment with COVID-19 vaccine candidates.

Developers	Information	Species	Dose (route)	NAb titer after boost	NAb titer after 2nd boost	Readout	Compared with convalescent serum	T-cell response	Virus challenge dose (route)	Upper airway viral load	Lower airway viral load
Sinovac	PiCoVacc (inactivated aluminum hydroxide)	Rhesus macaques	3 or 6 μg (i.m)	~10	~50	Neutralization assay with CPE	Comparable	NA[a]	10 [6] TCID50(i.t.)	Partially detected	Not detectable
Beijing Institute of Biological Products Ltd	BBIBP-CorV (inactivated aluminum hydroxide)	Cynomolgus macaques	3 or 6 μg (i.m)	215–256 range		Neutralization assay with CPE, 50% inhibition	NA	NA	10 [6] TCID50(i.t.)	Not detected in high-dose; Partially detected in low-dose	Not detectable
AstraZeneca	ChAdOx1nCoV-19 (Non-Replicating Viral Vector)	Rhesus macaques	2.5×10^{10} (i.m)	10–160		Neutralization assay with CPE,	NA	Weak T cell response	2.6×10 [6] $TCID_{50}$ (i.t., i.n.,oral., ocular)	All detected	Significant reduce
Janssen	Ad26.COV2.S (Non-Replicating Viral Vector)	Rhesus macaques	10^{11}(i.m)	113 (53–233)		Neutralization assay, 50% inhibition	4-fold	Th-1	1×10 [5] TCID [50] (i.n., i.t)	Only low in one animal	Not detectable
Novavax	NVX-CoV2373 (Protein Subunit)	Cynomolgus macaques	2.5, 5, 25 μg (i.m, 0, 21)	17,920–23,040 CPE_{100}		Neutralization assay with CPE_{100}	7.9–10.1-fold	NA	1.04×10 [4] pfu (i.n., i.t)	Not detectable	Not detectable
West China Hospital, Sichuan University	RBD monomer (Protein Subunit)	Macaca mulatta	20, 40 μg (i.m, 0, 7)	~100		Neutralization assay with EC_{50}	NA	NA	5×10 [5] pfu (i.n.)	Not detectable	Not detectable
Clover Biopharmaceuticals	S-trimer (Protein Subunit)	Rhesus macaque	30 μg (i.m, 0, 21)	11, 682–20,234		Neutralization assay with CPE, 50% inhibition	16-fold	NA	2.6×10 [6] $TCID_{50}$ (i.n., i.t)	Partially detected	Not detectable
Genexine Consortium	GX-19 (DNA vaccine)	Cynomolgus macaques	3 mg (electroporation, 0, 3, 5.5 week)	285	996	$PRNT_{50}$	NA	Th-1	2.6×10 [7] $TCID_{50}$ (i.t., i.n., oral., conjunctival, intravenous)	Partially detected	Partially detected
Harvard Medical School	DNA vaccine	Rhesus macaques	5 mg (i.m, 0, 3 week)	74		Neutralization assay with CPE,	Comparable	Th-1	1.1×10 [4] pfu (i.n., i.t)	Partially detected	Partially detected
Moderna	mRNA-1273 (mRNA vaccine)	Rhesus macaques	10, 100 μg (i.m, 0, 4 week)	501–3,491		Neutralization assay with CPE	12–84-fold	Th-1 CD4	7.6×10 [5] pfu,	Partially detected (10 μg) Not detectable (100 μg)	Partially detected (10 μg) Not detectable (100 μg)
Pfizer	BNT162b2 (mRNA vaccine)	Rhesus macaques	30, 100 μg (i.m, 0, 21 days)	962–1,689		Neutralization assay, 50% inhibition	10.2–18-fold	Th1 CD4+ and IFNγ+CD8+	1.05×106 pfu,	Not detectable (100 μg)	Not detectable (100 μg)
Walvax Biotech	ARCoV (mRNA vaccine)	Cynomolgus macaques	100, 1000 μg (i.m, 0, 14 days)	699–6,482		Neutralization assay, 50% inhibition	NA	Th-1	NA	NA	NA

a NA denoted as not assessed.
Abbreviations: i.m., intramuscular; i.n., intranasal; i.t., intratracheal; pfu, plaque forming units.

In the 3-injection schedule (days 0, 28, 56), the GMTs in the 2.5, 5, and 10 μg dose groups at day 14 after 3 injections were 316, 206, and 297, respectively, whereas, in the 2-injection schedules, the GMTs were 121 (schedule 1: day 0, 14) and 247 (schedule 2: day 0, 21) at day 14, which was after the boost for the 2-injection schedule (Table 2). The vaccine is in phase III (ChiCTR2000034780, ChiCTR2000039000) to assess the longer-term safety and immunogenicity.

Finally, the Chinese Academy of Medical Sciences recently disclosed their clinical outcomes from trials with another inactivated vaccine (NCT04412538) [14]. In a phase I randomized, double-blinded, placebo-controlled trial involving 192 healthy adults (18–59 y), two injections of three different doses (50 EU, 100 EU and 150 EU) of an inactivated vaccine were administered intramuscularly at a 2- or 4-week interval. The adverse reactions were commonly mild, with no abnormal variations over 28 days. Among the three dose groups (0/28 schedule), the seroconversion rate of nAbs reached 80%, 96%, and 92%, respectively, with GMTs of 10.6, 15.4, and 29.6 at day 28 after immunization. The authors noted that these nAbs could neutralize different pandemic strains with diverse mutations. Furthermore, the specific positive cytotoxic T lymphocyte (CTL) response with IFN-γ was detected, and genes related to T and B cell activation were upregulated by ~40% and ~25%, respectively (Table 2). Genes related to the activation of dendritic cells, mononuclear cells/macrophages, and natural killer cells were also upregulated to different degrees. This vaccine is currently under a phase III assessment (NCT04659239).

2.1.2. Live-attenuated vaccines

Live-attenuated vaccines contain live, whole bacterial cells or viral particles and are treated to have reduced virulence but still retain some antigenicity after attenuating the pathogen [21]. The virulence is reduced through artificial mutations, gene deletions, or by screening from nature. These types of vaccines can simulate naturally occurring recessive infections and induce comprehensive, stable, and persistent responses, with immunization able to be achieved via oral, nasal, and/or aerosol routes. Live-attenuated vaccines can induce antibody, cell and mucosal immune responses [13,21]. Of note, there are some potential safety issues with these types of vaccines that need to be addressed [21]. At present, there is only one live-attenuated vaccine entering to clinical trial (NCT04619628), which is co-developed by Codagenix Inc. (Farmingdale, NY) in collaboration with the Serum Institute of India (India). And another four ones are at the pre-clinical development stage [18], developed by the institutions including 1) Mehmet Ali Aydinlar University in collaboration with Acibadem Labmed Health Services (Turkey); 2) Indian Immunological Ltd. (India) in collaboration with Griffith

Table 2
Overview of clinical results for treatment with COVID-19 vaccine candidates.

Company	Vaccine	Dose (route)	NAb titers after prime	NAb titers after boost	Readout	Convalescent serum titer (Fold)	T-cell response	Registration Number	Efficacy from interim report
Sinovac	PiCoVacc	3 or 6 μg (i.m, 0,14 or 0, 28)		24–65 range	Neutralization assay with CPE	NA	NA[a]	NCT04352608	78%
Beijing Institute of Biological Products Ltd	BBIBP-CorV	2, 4 or 8 μg (i.m, 0, 14 or 0, 21 or 0, 28)		88–282	Neutralization assay, 50% inhibition	128–384 (Comparable)	NA	ChiCTR2000032459	79.34%
Wuhan Institute of Biological Products Ltd	Inactivated virion	2.5, 5, 10 μg (i.m, 0, 28, 56 or 0, 14 or 0, 21)		121–247	Plaque reduction neutralization test ($PRNT_{50}$)	(Comparable)	NA	ChiCTR2000031809	
Chinese Academy of Medical Sciences	Inactivated virion	50, 100, or 150 EU (i.m. 0, 14 or 0, 28)		11–20	Neutralization assay with CPE	NA	CTL response with IFN-γ	NCT04412538	
Cansino	Ad5	5×10^{10} or 1×10^{11} (i.m., 0)	18.3–19.5		Neutralization assay with CPE	NA	CTL response with IFN-γ	NCT04341389	
AstraZeneca	ChAdOx1 nCoV-19	5×10^{10} VP 1× or 2× (i.m.)	218; 51; 4–16	36; 29	$PRNT_{50;;}$ MNA_{80}; Marburg VN IC_{100}	(1×, lower; 2×, Comparable)	IFN-γ response	NCT04324606	70.4%
Gamaleya Research Institute	rAd26-S and rAd5-S	10^{11} VP (i.m., 0, 21)		49.25–49.95	Neutralization assay	(Comparable)	$CD8^+$, $CD4^+$	NCT04437875	91.4%
Novavax	NVX-CoV2373	5, 25 μg (i.m., 0, 21)		3906 and 3305	MN IC>99%	(4–6 fold)	Th1-biased	NCT04368988	
Moderna	mRNA-1273	25, 100 μg (i.m., 0, 28)		317–645	$PRNT_{80}$	(Comparable or above)	Th1-biased	NCT04283461	94.1%
Pfizer	BNT162b1	10, 30, 100 μg (i.m., 0, 21)	168–267	180–578	Neutralization assay with 50% inhibition	(1.9–4.6-fold)	Th1-biased	NCT04368728	
	BNT162b1; BNT162b2	10, 30, 100 μg (i.m., 0, 21)		BNT162b1:37–267; BNT162b2: 84–363	Neutralization assay with 50% inhibition	(1.1–3.8-fold)	NA	NCT04368728	95%
Zhifei	ZF2001	25, 50 μg (i.m., 0, 30)		102.5–69.1	Neutralization assay with 50% inhibition	51 (1–2-fold)	Balanced Th1 and Th2 response	NCT04466085	
Medicago	CoVLP	3.75, 7.5, 15 μg (i.m., 0, 21)	~5 (CpG); 23.6–41.6 (AS03)	71.3–118.1 (CpG); 1200.9–2118.3 (AS03)	Pseudovirion Neutralization Assay ($PsVNA_{50}$)	(1- fold, CpG; 10–20-fold, AS03)	IFN-γ and IL4 response	NCT04450004	

a NA denoted as not assessed.
Abbreviations: i.m., intramuscular;.

University (Brisbane, Australia); 3) Institute Pasteur Lille (French); 4) ALtraBio, TheRex.

2.1.3. Non-replicating viral vectors

Viral vectors are commonly designed using genetic engineering technology. The vector is designed to carry a foreign gene encoding a polypeptide, antigen, or other molecule that can be delivered to the host cell [13,21,22]. Viral vectors can be broadly divided into non-replicating viral vectors and replicating viral vectors. Non-replicating viral vector vaccines are specifically deficient in functions that are essential for viral replication. There are several types of non-replicating viral vectors: Poxvirus, Adenovirus, Alphavirus, Herpes simplex virus, Measles virus, and other viral vectors. Adeno-associated viruses (AAVs) are perhaps the most widely studied in vaccine development because of their safety, ease of production, and capacity to be delivered to numerous host cells through various routes [22]. At present, there are eleven COVID-19 vaccines based on non-replicating viral vectors in clinical evaluation, eight of which are based on AAV, and one based on a modified vaccinia virus Ankara (MVA) [18].

The first-in-human trial against coronavirus was conducted by CanSino (China) using a non-replicating Ad5-based vaccine expressing the wild-type S-protein. The single-center trial was conducted as a dose-escalation, open-label, non-randomized, phase I trial and was carried out in Wuhan, China (NCT04313127) [23]. The vaccine was administrated to 108 participants as a single shot at one of three doses (high, middle, low), and was found to be tolerated and immunogenic at 28 days post-vaccination. Most adverse reactions were mild or moderate, with no SAEs within 28 days. Of note, participants in the high-dose group tended to have higher reactogenicity. The GMTs of the nAbs in the high-, middle-, and low-dose groups post-vaccination were 34.0, 16.2, and 14.5, respectively. Rapid specific T-cell responses for IFN-γ secretion peaked at day 14 (Table 2).

Following this, CanSino then went on to conduct a randomized phase II trial (double-blind, placebo-controlled) of the Ad5-vectored vaccine in 508 participants (NCT04341389) [24], again using three doses. At day 28 post-vaccination, the seroconversion rates in the medium- and low-dose groups were 96% and 97%, respectively, with GMTs of 19.5 to 18.3 (Table 2). Cellular responses, as detected using IFN-γ enzyme-linked immunospot assay were observed in 90% and 88% of the participants in the medium- and low-dose groups, respectively. No SAE was observed. Of note, an advanced age and higher pre-existing anti-Ad5 titers reduced the immune response. The authors suggested that a single-dose immunization schedule of Ad5-vectored COVID-19 vaccine at 5×10^{10} viral particles is an appropriate regimen for healthy adults, and that an additional dose might be needed to induce a better response in older populations. This vaccine has been licensed for use in the Chinese military and is now being evaluated in phase III clinical trials (NCT04526990, NCT0450419, etc.).

Another vaccine based on the AAV vector is the replication-deficient chimpanzee viral vector. This incorporates a weakened version of the adenovirus that contains the gene encoding wild-type S-protein (ChAdOx1nCoV-19) [25]. It was developed by the University of Oxford (UK) with AstraZeneca (Cambridge, UK). Rhesus macaques were administered with an intramuscular injection of the vaccine. NAbs were induced upon prime-only or prime-boosted immunization in all vaccinated animals, with titers of 5~40 or 10~160, respectively (Table 1). Upon challenge with 2.6×10^6 $TCID_{50}$ live SARS-CoV-2 (combined intratracheal, intranasal, oral, and ocular), the viral load in the lung was

significantly reduced in the vaccinated monkeys, but there was no difference in nasal swabs between the vaccinated and control groups. There was no pulmonary pathology in the vaccinated group, and weak T cell responses were detected.

This vaccine was then evaluated in single-blind, randomized controlled phase I/II trials (NCT04324606). Participants (18 to 55 y) were allocated to one of two prime groups to receive ChAdOx1 nCoV-19 (n = 543) or a meningococcal conjugate vaccine, MenACWY (n = 534), with a third small group (n = 10) of participants enrolled in the non-randomized ChAdOx1nCoV-19 prime-boost group [26]. Vaccines were administered as a single intramuscular injection. There were no SAEs related to ChAdOx1 nCoV-19 (Table 2). However, the safety profile of ChAdOx1nCoV-19 was poorer than the licensed meningitis vaccine. NAbs responses were measured using three live SARS-CoV-2 neutralization assays, with responses detected in 100% of participants (median titers of 218) using the 50% plaque reduction neutralization assay ($PRNT_{50}$), in 91% participants after prime (median titers of 51) using a microneutralization assay (MNA_{80}), and in 62% participants (titers range, 4–16) using the Marburg virus neutralization assay (Marburg VN IC_{100}). After a booster dose, the titers in the latter two assays increased to 136 (median) and 29 (range) with 100% positivity. These nAbs titers achieved with a two-dose immunization regime were comparable with those measured using convalescent sera. The IFN-γ response peaked at day 14 and then declined, and could not be boosted with additional doses. A low level of anti-vector immunity was observed in subjects (4%). This vaccine is currently in phase III trials (NCT04516746, NCT04540393, etc.). In the recent interim analysis report of phase III, the Oxford-AstraZeneca vaccine was 70.4% effective at preventing SARS-CoV-2 infection overall when combining data from two dosing regimens., The vaccine efficacy was 90% and 62% respectively in the two groups with different dose regimens. The higher efficacy regimen adopted a half dosage for the first dose and a standard one for the second dose. It was claimed that this vaccine is cost effective in production and easier to transport and store (2–8 °C), and has been approved by the U.K. government for emergency use.

Another non-replicating adenovirus vector vaccine candidate based on Ad26 was developed by Janssen Pharmaceutical Companies in collaboration with Harvard and MIT [27]. Fifty-two rhesus macaques were intramuscularly immunized with a single dose of the d26-vectored vaccine, which encoded 7 versions of the S-variant, or the sham control, and were then challenged with SARS-CoV-2. The optimal Ad26 vaccine, Ad26.COV2.S or S.PP, induced robust nAb responses in rhesus macaques, with median titers of 113 (range 53–233); these titers were 4-fold higher than those from human convalescent sera (Table 1). The vaccine also induced detectable S-specific IgG and IgA responses in bronchoalveolar lavage (BAL) fluid samples, and Th-1–biased responses. Challenge with 1×10^5 $TCID_{50}$ SARS-CoV-2—either via intranasal and intratracheal routes—led to no viral load in the lung and only 1 of 6 participants had a low subgenomic mRNA (sgRNA) signal in the nasal swabs. The vaccine-elicited nAb titers correlated with protective efficacy, and thus may be a potential biomarker for vaccine protection. In addition, compared with other versions, the nAbs induced by S.PP did not increase post-challenge, suggestive of sterilizing immunity. The S.PP vaccine contains the wild-type leader sequence, mutations at the furin cleavage site, and two proline mutations to stabilize the pre-fusion conformation. This vaccine is currently being evaluated in phase III clinical trials (NCT04505722).

The Gamaleya Research Institute of Russia has also developed heterologous COVID-19 adeno-based vaccines using the Ad26 and Ad5 vectors carrying the gene for the full-length S protein (rAd26-S and rAd5-S) [28]. In two open, non-randomized phase I/II trials, two formulations (frozen and lyophilized) of the vaccine (NCT04436471, NCT04437875) were tested for safety and immunogenicity, with 38 participants in each study. The safety of the two individual vaccine components (rAd26-S and rAd5-S) was confirmed in phase I. In phase II, both components were then administered as a prime-boost vaccination (rAd26-S+rAd5-S). Most of the adverse events (AEs) were mild, with no SAEs detected. After a two-dose vaccination, the GMTs of the nAbs were 49.25 and 45.95 for the frozen and lyophilized formulations, respectively, both with a seroconversion rate of 100% (Table 2). Notably, the nAb titers after the boost were not significantly different to those titers after COVID-19 infection. Specific $CD4^+$ and $CD8^+$ T-cell responses peaked at day 28 after vaccination. The study also showed that a pre-existing immune response to the components of vaccine vectors (rAd26 and rAd5) does not affect the specific antibody in the serum of participants. These two formulations of the vaccine might be beneficial for vaccine production and distribution in the current pandemic. The vaccine is entering phase III clinical trials (NCT04530396, NCT04564716). In a recent report in news, the efficacy of this vaccine is 91.4% in terms of the results of the third and final control point analysis that was performed 21 days after administering the first dose to volunteers (n = 22,714) in a Russian Phase III clinical trials. This vaccine has been registered via an authorization procedure for emergency use.

There are another seven vaccines based on non-replicating viral vectors in clinical phase I: these are being tested and developed by (1) ReiThera (Italy) in collaboration with Leukocare (Germany) and Univercells (Belgium); (2) CanSino Biotechnology, Inc., in collaboration with Academy of Military Medical Sciences (Beijing, China); (3) The People's Liberation Army of China; (4) Vaxar Inc. (California, USA); (5) Ludwig-Maximilians University of Munich (Germany); (6) City of Hope Medical Center and National Cancer Institute (USA); and (7) Shenzhen Geno-Immune Medical Institute (China).

2.1.4. Replicating vector vaccines

Replicating vector vaccines use engineered viruses or bacteria for the vaccine vector to express a target gene in the host cell. In some cases, viruses that do not replicate efficiently or those that cause no disease in humans are used [13,22]. Measles virus, influenza virus, vesicular stomatitis virus (VSV) and horse pox virus are typical replicating vectors. Replicating vector-based approaches can trigger strong immune responses because the vector will propagate in vivo to some extent. Another advantage is that some of these vectors can also be administered via mucosal surfaces, which can trigger mucosal immune responses [21]. Currently, only six replication active vectors against SARS-CoV-2 are in phase I clinical trials: (1) an engineered measles vaccine strain, developed by Institut Pasteur (France) in collocation with Themis (now acquired by Merck); (2) an influenza virus-based vector by Beijing Wantai Biological Pharmacy in collaboration with Xiamen University, which contains an RBD subunit and can be administrated intranasally; (3) a VSV-based vaccine developed by Israel Institute for Biological Research at the Weizmann Institute of Science (Israel); (4) a replication-competent VSV that delivers the SARS-CoV-2 S-protein vaccine candidate, developed by Merck Sharp & Dohme in collaboration with the International AIDS Vaccine Initiative (IAVI; New York); (5) a lentivirus-modified vector with immune modulatory genes and the viral minigenes to the artificial antigen presenting cells (aAPCs) by Shenzhen Geno-Immune Medical Institute (China); (6) a vaccine consisting of autologous dendritic cells that were loaded with antigens from SARS-CoV-2 by Aivita Biomedical, Inc. and the National Institute of Health Research and Development, Ministry of Health Republic of Indonesia.

2.1.5. Recombinant subunit proteins

Recombinant protein vaccines are based on recombinant subunit proteins, peptides or virus-like particles (VLPs), which can be expressed in various systems, such as E. coli, yeasts, plants, insect cells, and mammalian cells [21,29]. To this end, the RBD, S1, S-protein or N-protein are generally chosen as the principal target antigens. Recombinant protein vaccines need to be combined with potent adjuvants for improved immunogenicity and efficacy, particularly for protein antigens in the non-particulate form. Unlike other vaccine approaches, this vaccine form is safe and easily manufactured by recombinant molecular techniques

[29]. At the time of writing, there were 19 vaccine candidates in clinical trials.

Novavax (Maryland, USA) has developed a recombinant protein vaccine, NVX-CoV2373, which is produced in insect cells and is based on the full-length S-protein stabilized in its prefusion form. NVX-CoV2373 forms into S-nanoparticles with the Matrix-M1 adjuvant [30,31]. In one experiment, Cynomolgus macaques were administered with two doses of 2.5, 5, or 25 μg NXV-CoV2373 at a 21-day interval [30]. After priming, the GMTs of the elicited nAbs in the cytopathic effect assay (CPE) ranged from 17,920 to 23,040, which were 7.9–10.1-fold higher than the measurements taken using convalescent sera. The macaques were then challenged with 1.04×10^4 plaque forming units (pfu) of SARS-CoV-2 via intranasal and intratracheal routes (Table 1). Macaques treated with the higher dose (25 μg) showed no detectable sgRNA in the upper and lower airways; only one monkey (1/4) in the middle-dose (5 μg) group showed detectable sgRNA levels in BAL fluid. Furthermore, there was little to no inflammation observed in the lungs of monkeys at 7 days post-virus challenge.

Following this, a randomized, placebo-controlled, phase I/II trial was undertaken, with 83 participants assigned to receive the NVX-CoV2373 vaccine in 5-μg or 25-μg doses with Matrix-M1 adjuvant, or as a 25-μg dose without adjuvant [32]. Reactogenicity was absent or mild, and more common in the adjuvant group after a short duration. No SAEs were observed. The two-dose 5-μg and 25-μg adjuvanted regimens induced GMTs (3906 and 3305 MN $IC_{>99\%}$) of neutralization responses that were 4- to 6-times greater than the responses measured with convalescent serum from mostly symptomatic COVID-19 patients (983). The study also showed that NVX-CoV2373 induced a strong Th1 response, and a minimal Th2 response, and that the addition of the adjuvant enhanced this immune response (Table 2).

Pre-clinical data for another three vaccines have been published. One is an RBD-dimer vaccine from Zhifei Longcom Biopharmaceutials and the Institute of Microbiology, Chinese Academy of Sciences. The SARS-CoV-2 RBD-dimer design was guided by structural data, and yielded a stable version of the dimer that retained vaccine potency [33]. This vaccine candidate achieved 10- to 100-fold enhanced nAb titers in mice as compared with delivery of the RBD monomer. In the clinical trial [34], 50 participants (mean age 32.6 y) were enrolled in phase I study and 900 participants were enrolled in phase II study (mean age 43.5 y), and received vaccine or placebo with a two-dose or three-dose schedule. For both trials, there was no serious adverse event that could be related to the vaccine. The SARS-CoV-2 neutralizing antibody of GMTs was 94.5 for the 25 μg group and 117.8 for the 50 μg group in phase I, and 102.5 for the 25 μg group and 69.1 for the 50 μg group in phase II, higher than the average level of a panel of COVID-19 convalescent samples (51). The vaccine could induce balanced Th1 and Th2 responses. This vaccine candidate is currently in phase III clinical trials.

The second vaccine from the West China Hospital at Sichuan University is instead based on the RBD as a monomer and is adjuvanted with aluminum hydroxide. Non-human primates (Macaca mulatta) were immunized with two intramuscular injections of 20 μg or 40 μg vaccine per dose and then challenged with 5×10^5 pfu SARS-CoV-2 intranasally [35]. NAb titers were ~100 in the high-dose group. No detectable viral genomic mRNA (gRNA) and sgRNA in the lung or throat were detected for either of the vaccinated groups at 7 dpi. There was no significant histopathological change in the lung tissues for any of the vaccinated animals (Table 1). In a mouse model deficient in $CD4^{-/-}$, $Sting1^{-/-}$, $Casp1^{-/-}$,$Nlrp3^{-/-}$, IL-1$\beta^{-/-}$,$Tlr2^{-/-}$, and $Tlr4^{-/-}$, several immune pathways and CD4 T-lymphocytes were implicated in the induction of the vaccine antibody response. Currently, the vaccine is in phase II clinical trials (ChiCTR2000039994).

The third vaccine is a native-like S-trimer (wild-type) vaccine based on Trimer-Tag technology from Clover Biopharmaceuticals (Chengdu, Sichuan, China) in collaboration with GSK (Brentford, the United Kingdom) and Dynavax (California, the USA). Rhesus macaques were vaccinated twice with 30 μg S-trimer intramuscularly in combination with one of two adjuvants—AS03 or CpG-1018 plus alum. The two doses were administered 21 days apart [36]. NAb titers in the AS03-adjuvanted group (20,234 MN_{50}) were significantly higher than the levels in human convalescent sera (1232 MN_{50}) after two immunizations. For animals in the CpG-1018 plus alum group, the nAb titers (11,682) were lower than those in the AS03-adjuvanted group. After intranasal and intratracheal challenge with 2.6×10^6 $TCID_{50}$ SARS-CoV-2, macaques immunized with the adjuvanted S-Trimer were protected, with viral loads in the lung tissue reducing from 5 to 7 dpi (Table 1). Viral loads were detected in the nasal swabs. The vaccine is in phase II/III clinical trials (NCT04672395).

2.1.6. VLP vaccines

Virus-like particle (VLP) vaccines are an important form of exogenous expression of the viral capsid protein. The morphological structure is highly similar to that of natural viruses, with considerable immunogenicity and better safety [37]. Compared with traditional attenuated or inactivated vaccines, highly purified VLP vaccines have significant advantages: they comprise a single component, have no viral nucleic acid, have a good safety profile, and offer high immunogenicity. However, their development might be hindered by the need for a suitable scale-up preparation, assembly, and formulation process. At present, marketed genetically engineered vaccines, such as hepatitis B virus (HBV) vaccine, human papillomavirus (HPV) vaccine and hepatitis E virus (HEV) vaccine, are all in the form of VLPs. There are two VLP-based COVID-19 vaccines in clinical trials: RBD-HBsAg VLPs developed by SpyBiotech Ltd. (Oxford, UK) and the Serum Institute of India (Pune, India) in phase I/II (ACTRN12620000817943), and a plant-derived VLP adjuvanted with GSK or Dynavax adjuvant, developed by Medicago Inc. (Quebec, Canada) in phase II/III (NCT04636697).

Medicago Inc. recently reported their candidate VLP vaccine (CoVLP) which is produced in Nicotiana benthamiana plants. These VLPs could spontaneously assemble on the plant cell membrane and carry SARS-CoV-2 stable pre-fusion S trimers on their surface. In a randomized, partially-blinded, prime-boost 21 days apart, dose-escalation Phase I study [38], the vaccine is efficacious at three dose levels (3.75, 7, 5, or 15 μg), either alone or with CPG1018 or AS03. Although both adjuvants increased reactogenicity, all formulations were well tolerated. Antibodies and cellular responses were highest in subjects receiving the AS03 adjuvant. NAb titers on day 42 (21 days after the second dose) achieved were either similar to (CoVLP+CpG1018) or at least 10-times higher (CoVLP+AS03) than those seen in convalescent plasma. Cellular responses (IFNγ and IL4 elispot) were detectable in all subjects who received the adjuvant formulation of all CoVLP doses on day 42, the CoVLP+AS03 groups were higher than those seen in the CoVLP+CpG1018 groups.

2.2. Novel vaccines

2.2.1. DNA vaccines

DNA vaccines comprise a plasmid containing various regulatory elements to ensure efficient production of the plasmid in bacterial systems, including an origin of replication, a selectable marker, and an expression cassette containing the gene of interest under a eukaryotic promoter [21,29]. DNA vaccines are simple and easy to produce using well-established fermentation technologies in E.coli. DNA vaccines can induce humoral and cellular immune responses in systemic and mucosal compartments [13]. The major disadvantage is the poor efficiency of transfection and the need for delivery devices, which may limit the future use of such vaccines [13]. At present, there are eight DNA vaccines in clinical phase.

Preclinical data from Genexine Consortium (Korea) has been recently reported for the GX-19 DNA vaccine. This vaccine contains the entire ectodomain of the S gene and the N-terminal tissue plasminogen activator (tPA) signal sequence [39]. Three cynomolgus macaques were vaccinated three times with GX-19 using electroporation (EP)-enhanced

delivery at approximately 3-week intervals. The nAb titers were 285 ($PRNT_{50}$ assay; week 5.5, after boost) and 996 ($PRNT_{50}$ assay; week 8, after second boost). GX-19 vaccination induced an S-specific, Th1-biased immune response. At ~10 weeks after vaccination, macaques were challenged with 2.6×10^7 $TCID_{50}$ SARS-CoV-2 via combined routes (intratracheal, oral, conjunctival, intranasal and intravenous). Viral loads in the nasal and throat swabs showed 1.58 and 1.57 log_{10} reductions in the GX-19 vaccinated macaques as compared with the unvaccinated macaques (Table 1). Furthermore, virus challenge caused histopathological changes, with moderate to severe inflammation in the control monkeys as compared with the vaccinated monkeys. This vaccine is currently in phase I/II clinical trials (NCT04445389).

Harvard Medical School [40] reports on six different DNA vaccine candidates expressing different forms of the SARS-CoV-2 S-protein. These vaccine candidates have been evaluated in 35 rhesus macaques using a regimen of three intramuscular injections. Vaccinated animals developed humoral and cellular immune responses, including nAb titers (median 74) that were comparable with those found in the serum of convalescent humans and macaques infected with SARS-CoV-2. After virus challenge, the vaccine encoding the full-length S-protein resulted in >3.1 and >3.7 log10 reductions in viral loads in bronchoalveolar lavage and nasal mucosa, respectively (Table 1). These vaccine-elicited nAb titers correlated with protective efficacy.

The first DNA vaccine to enter into clinical trials was INO-4800, developed by Inovio Pharmaceuticals (Pennsylvania, USA). Pre-clinical data of INO-4800 showed nAbs and T cell responses in mice and Guinea pigs [41]; the non-human primate and clinical data have not been reported.

2.2.2. mRNA vaccines

RNA vaccines are prepared with an advanced vaccine technology, with features of both subunit vaccines and live-attenuated vaccines. Production is fast and flexible, and these vaccines can induce both humoral and cellular immune responses. RNA vaccines include messenger RNA (mRNA) and self-amplifying RNA (Replicon) vaccines [21,42]. Unlike with DNA vaccines, RNA vaccines do not integrate into the genome of the immunized host and directly express the antigen in the cytoplasm. As such, there is speculation that RNA vaccines might be more conducive in stimulating antigen-specific immunity [21]. The use of lipid nanoparticles (LNPs) can enhance the delivery of mRNA vaccines via intramuscular or intradermal routes [13]. However, despite the attention that has been given to the development of mRNA vaccines, none have been licensed. At present, there are six COVID-19 vaccines based on RNA technology in clinical evaluation.

The mRNA vaccine, mRNA-1273, developed by Moderna (Massachusetts, USA), consists of the prefusion-stabilized S-protein with 2 proline substitutions, a native furin cleavage site, and a transmembrane-anchored protein [43]. The vaccine was synthesized in vitro and encapsulated into an LNP. Rhesus macaques were vaccinated with an intramuscular injection of 10 or 100 μg mRNA-1273 in a prime-boost regimen with a 4-week interval [44]. The GMTs of the nAbs reached 501 and 3481 in the low- and high-dose groups, respectively, after the boost; these values are 12- and 84-times higher, respectively, than those achieved with human convalescent serum. mRNA-1273 also induced Th1-biased CD4 T-cell responses, and low to undetectable Th2 and CD8 T-cell responses. Two days after challenge with 7.6×10^5 pfu of SARS-CoV-2, viral replication was not detectable in the lower airways of animals in the high-dose group or in 7 of the 8 animals in the low-dose group. No viral replication was detectable in nasal swabs for the high-dose group; limited inflammation cytokine induction was noted in the lungs of animals in both vaccine groups (Table 1). mRNA-1273 thus offers rapid protection, with no pathological changes in the lungs. mRNA-1273 was then further evaluated in clinical studies. First, mRNA-1273 was tested in a dose-escalation, open-label phase I trial. Forty-five volunteers (18 to 55 y) received two vaccinations at a 28-day interval with doses of 25, 100, or 250 μg [45]. Solicited AEs occurred in more than half of the volunteers. One participant in the low-dose group was withdrawn from the study because of unsolicited urticaria, which was judged to be related to the first vaccination. Systemic AEs were more common after the second vaccination, particularly among those in the high-dose group, with three participants (21%) reporting one or more SAEs. NAb titers against SARS-CoV-2 showed GMTs of 339.7 and 645.3 ($PRNT_{80}$) in the low- and medium-dose groups, which were generally at or above the values of convalescent serum. In another similar phase I trial in 40 older adults (56 to 70 years or >71 years) [46], two doses of either 25 μg or 100 μg vaccine were administered via the same regime. SAEs were predominantly mild or moderate, were dose-dependent, and were more common after the second immunization. NAb responses (GMT, 402 in 56–70 group; GMT, 317 in >71 group) appeared to be similar to those reported among the younger cohort (18 and 55 y) and were above the median values of convalescent serum (Table 2). The vaccine elicited a strong CD4 cytokine response involving Th1 cells in both age subgroups. Currently, the vaccine candidate is in a large phase III trial to assess its level of protection against COVID-19.

A randomized, observer-blinded, placebo-controlled phase III trial was conducted in the United States [47]. The trial enrolled 30,420 volunteers who were randomly assigned in a 1:1 ratio to receive either vaccine (100 μg) or placebo (15,210 participants in each group) 28 days apart. The primary end point was prevention of COVID-19 illness with onset at least 14 days after the second injection in participants who had not previously been infected with SARS-CoV-2. Vaccine mRNA-1273 was stored at 2° to 8 °C before preparation and vaccination. Doses could be held in syringes for up to 8 h at room temperature before administration. Moderate and transient reactivity after vaccination was more common in the mRNA-1273 group. The frequency of grade 3 adverse events in the placebo group (1.3%) was similar to that in the vaccine group (1.5%). Overall, the mRNA-1273 vaccine was 94.1% effective at preventing laboratory-confirmed COVID-19 onset, including severe disease. Recently, the U.S. Food and Drug Administration (FDA) authorized the emergency use of mRNA-1273 to prevent COVID-19 in individuals 18 years of age and older on 18 December 2020.

Another mRNA vaccine was developed by BioNTech (Germany) in collaboration with Fosun Pharma (Shanghai, China) and Pfizer (New York, the USA). An mRNA-based vaccine candidate program, BNT162, uses previous knowledge of mRNA-based therapeutics previously developed by BioNTech for cancer. BNT162 comprises 4 vaccine candidates, each of which represents a different formulation in the target antigen (RBD or S-protein), and is formulated using LNPs.

BNT162b1 comprises mRNA encoding RBD in an LNP system. In a placebo-controlled, observer-blinded trial (NCT04368728), 45 participants (range 19–54 years of ages) were randomized and vaccinated with 10 μg or 30 μg BNT162b1 as a prime and boost regime, or 100 μg BNT162b1 prime only. No SAEs were reported. Fourteen days after the boost, GMTs of nAbs reached 180 (10 μg) and 437 (100 μg), respectively, which is 1.9- to 4.6-fold higher than the values achieved with a panel of COVID-19 convalescent human sera [48]. In another non-randomized open-label phase I/II trial in healthy adults, aged 18–55 y (NCT04368728), two doses of 1–50 μg of BNT162b1 were administered [49]. No SAEs or withdrawals due to related AEs were observed for any dose. Seven days after the boost, GMTs of nAbs reached 36 (1 μg), 158 (10 μg), 308 (30 μg), and 578 (50 μg), which were measured as 0.7-fold (1 μg) to 3.5-fold (50 μg) higher than those of the convalescent human sera panel (Table 2). BNT162b1 also induced functional and proinflammatory specific $CD8^+/CD4^+$ T-cell responses with Th1-biased responses [49].

Another pre-clinical study developed by BioNTech (Germany) in collaboration with Fosun Pharma (Shanghai, China) and Pfizer (New York, the USA) focused on the vaccine candidate, BNT162b2, which contains an LNP-formulated 41 nucleoside-modified mRNA [50]. Prime-boost vaccinations (30 or 100 μg) of rhesus macaques elicited GMTs of 962 (30 μg) and 1689 (100 μg), reaching 10.2- to 18.0-times that of the convalescent human serum panel. BNT162b2 induced strong Th1 $CD4^+$

and IFNγ^+CD8$^+$ T-cell responses in the monkeys. The BNT162b2 vaccine candidate at the high-dose protected the lungs of vaccinated animals from infectious SARS-CoV-2 challenge (Table 1). No viral RNA was detected in the BAL fluid from the vaccine group, and viral RNA was detected only in nasal swabs obtained on day 1 after challenge but not in those obtained on day 3 or thereafter. This vaccine candidate is currently being evaluated in a global, pivotal phase II/III trial (NCT04368728).

These two vaccine candidates, BNT162b1 and BNT162b2, were directly compared in a randomized, placebo-controlled, observer-blinded dose-escalation trial (NCT04368728) [51] to select the final vaccine candidate. In both younger (18–55 y) and older adults (65–85 y), the two vaccine candidates elicited similar dose-dependent GMTs of the nAbs: at 7 days after dose two of 30 μg BNT162b1 or BNT162b2, GMTs reached 168, which was approximately 1.1- to 1.6-times the convalescent serum panel GMT in older adults, and from 2.8- to 3.8-times that in younger adults. Although the antibody titers between the two candidates were comparable, BNT162b2 was associated with less systemic reactogenicity, particularly in older adults, and showed a more favorable safety profile (Table 2).

In an ongoing multinational, placebo-controlled, observer-blinded, pivotal efficacy trial [52], persons 16 years of age or older (n = 43,548) were randomly assigned in a 1:1 ratio to receive two doses, 21 days apart, of either placebo or the BNT162b2 vaccine candidate (30 μg per dose) (NCT04368728). The incidence of serious adverse events was low and was similar in the vaccine and placebo groups. Safety over a median of 2 months was similar to that of other viral vaccines. Four related serious adverse events were reported among BNT162b2 recipients (shoulder injury related to vaccine administration, right axillary lymphadenopathy, paroxysmal ventricular arrhythmia, and right leg paresthesia). The BNT162b2 vaccine conferred 95% protection against COVID-19. This vaccine has now been granted a conditional marketing authorization by European Commission.

Another LNP-encapsulated mRNA vaccine candidate, ARCoV, which encodes the RBD, was developed by Walvax Biotechnology (China) in collaboration with the Academy of Military Medical Sciences (China) and Suzhou Abogen Biosciences Co., Ltd. (China). ARCoV is manufactured in a liquid formulation and can be stored for at least 1 week at room temperature. Cynomolgus monkeys were immunized with 100 or 1000 μg ARCoV via an intramuscular injection and booster with the same dose over a 14-day interval [53]. Pre-clinical results (Table 1) showed that 50% of animals in the high-dose group developed low-level nAbs on day 14 after the prime. However, there was a notable increase in the 50% neutralization titer (NT_{50}) after the booster (699 and 6,482 in the low- and high-dose ARCoV groups, respectively). IFN-γ assays showed that ARCoV elicited a Th1-biased cellular response. In mice, ARCoV vaccination confers complete protection against SARSCoV-2 challenge. ARCoV is currently being evaluated in phase I clinical trials.

3. Challenges in COVID-19 vaccine development

Over the past few decades, there have been numerous efforts to develop a vaccine against human coronaviruses. However, despite intense research, there is still no vaccine available for any of the diseases. Furthermore, even though there are numerous COVID-19 vaccines candidates in the pipeline, with 10 vaccine candidates already entering phase III trials, we are still faced with many challenges associated with vaccine safety, efficacy, and production, with some of these important considerations highlighted below.

An important concern for COVID-19 vaccine development is the ADE of infection or disease that ensues after vaccination. Although ADE is not yet well-defined, it is thought to increase the rate of viral infection and/or trigger immunopathology because of the presence of low-affinity nAbs or suboptimal titers of nAbs, which not only have a limited neutralizing activity but also have enhanced uptake and can cause the virus to spread by entering Fc receptor-expressing cells [54–56]. ADE has been documented clinically with the use of respiratory syncytial virus (RSV) or measles vaccines and in patients with dengue hemorrhagic fever [54,55]. An earlier report regarding the SARS-CoV and MERS-CoV vaccines raised concerns of the immunopathology associated with the Th2 response. Th2 is a subgroup of T cells that can secrete Th2-type cytokines, such as interleukin 4 (IL-4), IL-5, IL-10, and IL-13. Aberrant levels of Th2 cytokines can cause immune reactions that lead to eosinophil infiltrations. In mice models, the SARS-CoV vaccine caused Th2 immunopathology with high eosinophil infiltration, which is an indicator of Th2 hypersensitivity [57,58]. Eosinophilic infiltration was also reported in mice vaccinated with the inactivated MERS-CoV vaccine along with elevated levels of IL-5 and IL-13 compared with before vaccination. Recent studies on cytokine changes in patients with SARS-CoV-2 infection also found elevated Th2 cytokine secretion, which may be related to lung immune pathology [59,60]. Although many of the vaccine candidates in clinical trials have observed no evidence of ADE, this issue still remains unclear.

One recent North American study reported the first case of re-infection with SARS-CoV-2. A 25-year-old man, who had laboratory-confirmed SARS-CoV-2 infection, was found to have a secondary infection within a period of around 6 weeks [61]. Genomic analysis showed that the two viral agents were genetically distinct. The second infection was symptomatically more severe than the first. There are several similar cases of reinfection with distinct viruses in pre-print publications. This potential for reinfection could impact on our understanding of acquired immunity after natural infection and the mechanism of ADE, which needs to be well-validated. While the immunological response to and mechanism of SARS-CoV-2 infection has not been well elucidated and still cannot confirm the presence of SARS-CoV-2–associated ADE, it will remain important to control the balance of T-cell response (Th1/Th2) when designing vaccines against SARS-CoV-2.

Another issue is the need for a suitable animal model for testing vaccine safety and efficacy. Not all animal models perfectly mimic the human COVID-19 infection and immune response [62]. It is necessary to determine whether small animal and non-human primate virus infection models of SARS-CoV-2 can predict the benefits or risks associated with these various vaccines in humans. When optimizing these models, it is necessary to understand the mechanism of infection of SARS-CoV-2 in humans and how to protect against such infection, and find ways to evaluate the mode of action of vaccines and antibodies in humans [54]. In vivo studies of these processes are essential if we are to better respond to future pandemics. It is also necessary to directly test safety in human clinical trials and determine the relevance of vaccines and antibodies in protecting against SARS-CoV-2.

Reactogenicity after immunization is another major concern in clinical trials. Most of the current COVID-19 vaccine clinical trials are being conducted in healthy adults aged 18–55 years, with some later-staged trials enrolling older participants over the age of 55 years. Older people and children fall into the high-risk population, and it remains largely unclear whether COVID-19 vaccines will be safe for both young children and older patients over the short and long term. Several studies have reported that the immunogenicity and reactogenicity levels are stronger with higher doses, which tend to be needed for older people to achieve protective immunity. Children are more likely to require a lower dose, since they commonly display more reactogenicity. Currently, most vaccines are performing better with a two-dose regimen, but such a regime will increase the reactogenicity of the treatment. Therefore, adequate assessment and monitoring of the safety of the COVID-19 vaccine in clinical trials are needed, especially for trials exploring the use of a novel vaccine technology, such as DNA or mRNA vaccines.

Immune sensing studies suggest that SARS-CoV-2 suppresses the activation of the innate immune system in a manner similar to that of SARS and MERS, with responses from dendritic cells and impaired antiviral type I interferon (IFN-I) and type III interferon (IFN-III) responses [63–65]. Studies indicate that dysregulation of the IFN-I response plays a pivotal role in the pathogenicity of COVID-19[66]. Animal models of SARS-CoV and MERS-CoV infections indicate that failure to induce an

early IFN-I response is associated with the severity of the disease [67]. Importantly, these results show that the timing is critical: IFN has a protective effect in the early stages of the disease, but its delayed expression is pathological; a recent study showed that IFN-induced upregulation of ACE2 in nasal epithelial cells may be involved [68]. These pro-inflammatory processes may lead to the "cytokine storm" observed in COVID-19 patients. Therefore, clarifying the delicate balance between antiviral and inflammatory innate immune programs is crucial for the development of effective COVID-19 vaccines and antiviral drugs [66]. In addition to considering the effects of the vaccine-induced adaptive immunity, innate immune memory might also play a role, perhaps by enhancing viral control, particularly in the early phases of infection [65].

An important characteristic of the SARS-CoV-2 virus mode of infection is the high affinity binding between the S-protein and the ACE2 receptor, with a K_D value of ~15 nM; this is approximately 10- to 20-fold higher affinity than the binding between SARS-CoV and ACE2 [69], 10-fold higher than that between insulin-like growth factor-1 receptor (IGF1R) and RSV (118 nM), and 100,000-fold higher than sialolactos binding to influenza virus (mM range) [70]. Currently, most nAbs isolated from cohorts of SARS-CoV-2 recovered participants exhibited affinities in the range of 1 to 100 nM [71]. Thus, the affinity of the S-protein:ACE2 receptor binding is comparable to that of nAbs to the S-protein. This underscores the high affinity binding requirement of the nAbs induced by the COVID-19 vaccine.

Another issue is whether the nAbs can protect against SARS-CoV-2 infection. In results from non-human primate studies, most of the vaccine candidates showed complete or partial protection after virus challenge in the upper and lower respiratory tract, and a few vaccines (Novavax, Jassen, West China Hospital, Moderna) administered at higher doses led to complete protection in the upper respiratory tract. This may prevent symptomatic disease while still allowing the virus to spread. Thus, sterilizing immunity might be crucial in the upper respiratory tract. Vaccine candidates based on live-attenuated vaccines or viral vectors could be used intranasally and may also cause a strong mucosal immune response (predominately induced secretory IgA and cellular response). There are two vaccines in clinical trials that aim to evaluate the mucosal immune response (Academy of Military Medical Sciences, Beijing Wantai Biological Pharmacy); the data for these studies have yet to be disclosed. One vaccine, Ad5-S-nb2, which is based on the Ad5 vector and is administered intranasally, confers effective protection against SARS-CoV-2. Indeed, the work suggests that intranasal vaccination provides mucosal immunity in the respiratory system in a manner that can effectively eliminate the spread of the virus into the lower respiratory tract [72]. Although the systemic antibody and cellular response is lower than that achieved via intramuscular vaccination, intranasal vaccination plays an important role in protection efficacy. In another similar study, mucosal vaccination of the Ad5-vectored vaccine led to better protective efficacy than intramuscular delivery in the upper respiratory tracts of mice and ferrets after SARS-CoV-2 challenge [73].

Previous studies on humoral and cellular immunity in convalescent patients showed that both B cells and T cells are associated with protection from viral infection, suggesting that the humoral and cellular immune response may be critical for virus clearance [66,74]. Of note, the results from several vaccine candidate studies suggest that the level of neutralization is correlated with the level of protection. However, we cannot ignore the potential roles of cell-mediated and mucosal-associated immunities, which are not well elucidated. Indeed, an effective COVID-19 vaccine may elicit multiple immune responses, including humoral, cellular, systemic, and local mucosal immunity. The potential important roles of these other types of immunity need to be addressed in future studies. To date, there are six vaccine candidates with the efficacy data (Beijing Institute of Biological Products Ltd., Oxford/AstraZeneca, Gamaleya, Pfizer/BioNTch, Moderna, Sinovac), all of them satisfied the lowest requirements (50% efficacy) of the WHO standards. These vaccines are hopeful for humans, but the efficacy of a vaccine may actually change as more and more people receive it.

Another unknown is the duration of vaccine-acquired immunity. The pandemic required quick action and an accelerated rate of vaccine development [13]. Similarly, the vaccine developmental timeline was shortened for SARS-CoV and MERS-CoV in the initial stages. However, an evaluation in humans is critical and such time requirement should not be simply skipped. Earlier studies found that induced antibodies after SARS-CoV-2 infection began to wane to undetectable levels within a few months. But more recently, the results from serological studies have shown that nAbs targeting the RBS and S2 are stable for at least 5–7 months after SARS-CoV-2 infection, as compared with those against the N-protein, for which titers more quickly diminish [75]. In comparison, SARS-CoV nAbs can still be detected 12–17 years after the initial induction [76], with long-lasting memory T cells shown to be reactive 17 years after infection [77]; this T-cell reactivity displayed robust cross-reactivity to the SARS-CoV-2 N-protein and may have offered those with previous SARS-CoV infection some immunity against SARS-CoV-2. However, natural infection-associated immunity does not compare with vaccination-derived immunity, which is much more potent. In terms of the current vaccines against COVID-19, it is still unknown whether the immune response induced by the vaccine will last longer or shorter than the immune response induced by natural infection.

Once the vaccine is licensed, there still could be delays in the production process. The global demand for COVID-19 vaccines will exceed ordinary pharmaceutical industry supplies, with there likely to be a requirement for billions of doses. The production of RNA and DNA vaccines could be simpler than other methods, but biotech-based companies involved in these approaches have never licensed a vaccine or produced a compound in such high demand [13]. Also concerning are the unforeseen challenges associated with world-wide distribution, with there likely to be the need for frozen storage for delivery to some lower-income countries; this will be problematic for some mRNA vaccines.

It is worth noting that China is committed to developing and deploying a vaccine for COVID-19 (if any) as a global public interest, which is part of China's contribution to providing affordable vaccines for developing countries. In addition, the WHO aims to ensure near 2 billion doses of a COVID-19 vaccine by the end of 2021 [78]. Under such circumstances, we are facing unprecedented demand for vaccines and urgently need to improve the global production, procurement, and distribution of safe and effective vaccines. Vaccines based on different technology platforms may play important roles in meeting this global demand.

4. Potential strategies for COVID-19 vaccines

Antigen design is particularly important in terms of vaccine efficacy. NAbs and/or T-cell immune responses can be raised directly against several proteins of SARS-CoV, SARS-CoV-2, or MERS-CoV. However, most of these vaccines target the S protein, which may indicate that S-protein–induced specific immune responses play an important role in the fight against coronavirus infection. Previous studies in RSV, HIV, and MERS have shown the importance of structure-guided construction for precise and fast vaccine design [79–81]. Previous studies have developed a generalizable strategy for retaining the coronavirus S-protein in an antigenically optimal prefusion conformation by substituting two prolines, hereafter referred to as S-2P. These prolines maintain the prefusion conformation [81], and can elicit high nAb titers against MERS-CoV. Recent COVID-19 vaccine candidates (mRNA-1273, NVX-CoV2373, Jassen, Pfizer) based on S-protein immunogens have a similar design and can induce high immune responses. The updated version of the S-protein variant, referred to as HexaPro, contains an additional four proline substitutions (F817P, A892P, A899P, A942P), which further increases the protein yield and stability. This higher yield may promote industrial production and improve immunogenicity of recombinant protein vaccines, nucleic acid-based vaccines or even vector vaccines. In the case of vaccines based on the RBD, this domain has been further engineered by structure-guided design as a tandem repeat single-chain dimer (RBD-sc-dimer), which could enhance nAb titers. This strategy could be

adopted for beta-CoV and COVID-19 vaccine designs. The optimal structural design for immunogens deserves further investigation to enhance the antigen presentation capacity and the induction of efficient immune responses.

Another aspect of effective vaccine design is the recognition of immunodominant B-cell and T-cell epitopes. One study identified that 8 immunodominant CD4+ T cell epitopes are distributed among the S-, E- and M-proteins and are relevant for effective T cell and B cell responses in subunit vaccines [82]. An additional study assessed the utility of 65 vaccine peptides predicted to activate CD4 and CD8 T cells. These peptides were highly dissimilar to the self-proteome, and were conserved across 15 related coronaviruses, including SARS-COV-2 [83]. The epitopes are expected to drive long-term immunity in most of the population. These predictions can facilitate effective vaccine design against this virus and thus warrant further investigation.

Adjuvants are key components in vaccine formulation and are used to enhance or prolong the immune response. Adjuvants can activate the innate immune response and induce robust adaptive immune responses. There are a range of adjuvants certified for use in humans (aluminum adjuvant [Alum], MF59, virosomes, AS04, RC-529, AS03, ISA-51, AS01B, CpG-1018). For SARS-CoV-2 vaccines, other novel adjuvant systems are being tested in clinical trials, including Matrix-M and Advax [84]. Previous work shows that alum tends to induce Th2-biased responses and is unable to elicit cell responses against intracellular pathogens such as those causing malaria, tuberculosis, or AIDS [85]. In the case of SARS-CoV, studies in animal models showed that alum-formulated vaccines induced inadequate Th1-biased responses, which might be associated with the resultant lung eosinophilic immunopathology [86]. More recently, however, alum-adjuvanted inactivated and RBD subunit vaccines showed no abnormal changes to the lungs in non-primates [15,16]. Whether alum is suitable for COVID-19 development needs careful research. Adjuvant systems developed by GSK and AS03 (vitamin E/Surfactant polysorbate 80/Squalene) are presently being evaluated in several combination with COVID-19 vaccines (Sanofi Pasteur, Clover Biopharmaceuticals, Medicago Inc., Innovax). Clover Biopharmaceuticals reported that immunization with an S-trimer adjuvanted with AS03 induced high levels of nAbs and a Th1-biased immune response in an animal model. Another study showed that CpG-1018, a Toll-like receptor 9 adjuvant agonist, in addition with alum appeared to induce a durable cellular immune response (as measured by lymphocyte frequency) in nonhuman primates and was more strongly associated with a Th1-biased response in rodents. However, in nonhuman primates, there was no clear difference in the degree of immune protection against SARS-CoV-2 challenge with the two adjuvant systems. Another saponin-based Matrix-M adjuvant administered with the NVX-CoV2373 vaccine was shown to enhance functional immunity, including nAb response, Tfh cell production, and the generation of antigen-specific germinal center (GC) B cells.

Finally, delivery and administration routes are extremely important considerations for vaccine design. DNA and RNA vaccines depend on the use of a gene gun, electroporation, or delivery with LNPs. Encapsulated LNP technology appears to offer enhanced immunogenicity in COVID-19 mRNA vaccine development. LNPs usually comprise four key components: a charged lipid, a lipid-linked polyethylene glycol (PEG), cholesterol, and a phospholipid; these components support the formation and in-vivo stability of LNPs [29,87]. Despite this, the safety of LNPs for delivery requires further investigation. Other novel vaccine delivery platforms, such as polymeric particles, inorganic particles, plant-like, bacteria and viruses material, immunostimulating complexes (ISCOMS), complex emulsions system, are well studied [87] and could be implemented and tested for COVID-19 vaccines.

Novel technologies, such as transdermal delivery (microneedles), and other routes of administration, such as mucosal (oral or intranasal), may offer alternative ways to induce strong mucosal immune responses [87]. A few COVID-19 vaccines are being explored to target mucosal immunity. Antigen-specific IgA plays a pivotal role in protecting mucosal surfaces from both microbe adhesion and virus activities [88]. Thus, the development of novel vaccine delivery platforms that elicit specific IgA and systemic IgG will be critical to improve vaccine effectiveness.

5. Conclusions and perspectives

The COVID-19 pandemic is an ongoing, global concern. Vaccines could be the only effective and economical means to curtail and manage this outbreak. The rapid development of COVID-19 vaccines could make this a reality. Despite the challenges ahead, we believe that, through different vaccine platforms and strategies, combined with collaborative global research efforts, we can overcome the issues facing vaccine design, formulation, and delivery to ultimately achieve a safe and effective COVID-19 vaccine.

Declaration of Competing Interest

The authors have declared that no competing interests exist.

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