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Development of leading first-generation vaccines against SARS-CoV-2

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ABSTRACT

SARS-CoV-2 has infected more than 167 million individuals globally. Highly effective and safe vaccines are required to accelerate the development of herd immunity to end the pandemic. This review focuses on vaccines that are being developed at unprecedented speed globally and are completing late phase clinical trials to meet this urgent need.
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1. Background and introduction

A novel Coronavirus disease (Covid-19) emerged in China in late December 2019 [1]. As a result of the rapid spread of Covid-19 throughout the world, the World Health Organization (WHO) declared the disease a pandemic on March 11, 2020 [2]. The novel virus was named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) based on phylogeny and taxonomy and shares high genomic sequence similarity with severe acute respiratory syndrome coronavirus 1 (SARS-CoV-1) from 2003 [3,4].

The first reported US case was seen in Washington state on January 20, 2020 [5]. During an infectious disease outbreak, the accelerated spread of the disease can be curtailed by adopting early surveillance and containment measures that quickly identify infected individuals, trace their contacts, quarantine the asymptomatic contacts, and isolate the symptomatic contacts [6]. Because of the paucity of such rigorous measures in the US, by mid-March 2020 the outbreak had spread to virus-naïve populations in all 50 states and the District of Columbia [7]. It is now known that majority of infected individuals recover from the disease, but to stop the spread of the virus and break the transmission cycle, herd immunity must be reached [8,9]. Because SARS-CoV-2 human-to-human transmission can occur from asymptomatic, pre-symptomatic and mildly symptomatic Covid-19 infected individuals without overt disease, to a highly susceptible population, and the prolonged stability of the virus in different environments, SARS-CoV-2 has the potential to evolve into an endemic virus [10–12]. This review describes the viral pathogenesis and outlines the vaccine development process from vaccine designs, preclinical safety testing in animal models to human clinical trials required for US Food and Drug Administration (FDA) licensure. Additionally, the review highlights the leading first-generation candidate vaccines that have completed late phase clinical trials in the US and globally, with emergency use authorization granted by the FDA to 3 vaccines for use in the US, and emergency use listing granted by the WHO to 6 vaccines for global roll-out during the Covid-19 public health emergency, as of 24 May 2021.

2. Coronaviruses

Coronaviruses are a group of large enveloped viruses with positive-sense, single-stranded RNA genomes [13,14]. These viruses are classified into four genera namely, alpha, beta, gamma and delta coronaviruses [13,14]. Alpha and beta coronaviruses infect mammals including humans, while gamma and delta coronaviruses primarily infect birds [13,14]. Seven coronaviruses are known to cause disease in humans. These include the alpha human coronaviruses (hCoV) hCoV-NL63 and hCoV-229E, beta coronaviruses hCoV-OC43 and hCoV-HKU1, SARS-CoV-1, middle east respiratory syndrome coronavirus (MERS-CoV) and the newly identified SARS-CoV-2 [13,14]. Four human coronaviruses hCoV-NL63, hCoV-229E, hCoV-OC43, and hCoV-HKU1 cause seasonal mild upper respiratory tract infections [13,14]. While the three

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zoonotic coronaviruses SARS-CoV-1, MERS-CoV, and SARS-CoV-2 cause lower respiratory tract infections that can progress to acute lung injury and death [13,14].

3. Viral pathogenesis

The SARS-CoV-2 viral genome encodes four structural proteins specifically, envelope (E), membrane (M), nucleocapsid (N), and spike glycoprotein (S), 16 non-structural proteins and accessory proteins [15]. All these proteins may serve as antigens to stimulate antibodies and induce cellular CD4 and CD8 T-cell immune responses [16]. SARS-CoV-2 uses the angiotensin-converting enzyme 2 (ACE2) receptors on susceptible cells to gain cell entry [17]. ACE2 receptors are expressed on upper respiratory tract epithelial cells, mature lung epithelial cells, epithelial cells lining the oral mucosa, enterocytes, kidney proximal tubular cells, and endothelial cells [18]. Viral cell entry is initiated by binding of the receptor binding domain (RBD) located on the surface N-terminal S1 subunit of the spike protein of the virus, to the ACE2 receptor on target cells [17]. This leads to activation of cellular proteases and cleavage of the spike protein at the S1/S2 and S' site, followed by fusion of the viral membrane with the target cell membrane mediated by the C-terminal S2 subunit and viral entry into cells through endocytosis [17,19]. Because the spike protein induces potent neutralizing antibodies that prevent viral infection of cells, it is the primary target of almost all current candidate vaccines [20].

SARS-CoV-2 initially infects the upper respiratory tract [21]. Following an infection, the ability of the host's innate immune system to arrest viral replication and prevent the virus from subverting the innate immune system, impair dendritic cell activation and delay T cell responses dictates the manifestation of clinical disease [22]. The spectrum of disease can range from an asymptomatic to a pre-symptomatic infection with progression to mild disease with fever, cough, change in taste, smell or to a moderate disease, with clinical and radiographic evidence of lower respiratory tract disease, that can further deteriorate to severe critical disease with a requirement for high-flow oxygen therapy or advanced ventilation, critical care and could be fatal [23]. The failure to curtail viral replication leads to innate immune suppression with impaired interferon type 1 and hyper inflammatory responses, with increased production of chemokines and cytokines including TNF-alpha and IL-6 contributing to a cytokine storm syndrome, shock, respiratory failure, and multi-organ failure [24,25]. The diverse responses to SARS-CoV-2 infection are likely to be determined by the viral load during an infection and host factors such as the age, sex, and other high-risk preexisting comorbidities specially obesity, hypertension, or diabetes [25].

4. Vaccine designs

According to the WHO, currently 276 vaccines are being developed against SARS-CoV-2 globally with 92 candidate vaccines in clinical testing [26]. Several technologies are used in vaccine designs and these include whole virus vaccines, viral vector vaccines, protein subunit vaccines, virus like particles, nucleic acid vaccines, and antigen presenting cells expressing lentiviral vectored SARS-CoV-2 minigenes and immune modulatory genes. Table 1 lists the number of vaccines of each design that are under clinical evaluation globally [26].

4.1. Whole virus vaccines

In general, whole virus vaccines have the potential to induce broader immune responses by presenting multiple viral antigens and are developed as "live" weakened virus vaccines by passage of the virus through animal or human cell lines, to induce mutations and reduce viral virulence or as "killed" inactivated virus vaccines by physical or chemical inactivation [27,28]. Inactivated virus vaccines don't induce strong immune responses and require multiple booster doses and adjuvants, to enhance the magnitude and alter the quality of specific adaptive immune responses such as antibody, CD4 T helper type 1, and/or CD8 responses to presented antigens [29]. In contrast, live attenuated replication competent viral vaccines are more potent, enabled by their ability to generate both robust humoral and cell-mediated immune responses, with activation of helper CD4 T cells required for both optimal antibody and cytotoxic CD8 T cell production and to initiate immunological memory [27,30]. Although virus neutralizing antibodies prevent infection of target cells, cytotoxic CD8 T cells directly kill virus-infected cells and help to control the spread of an infection once cells are infected [30]. These traditional approaches require the culture of large quantities of infectious virus and take a much longer time to produce, because of the need for extensive safety testing to eliminate the risk of attenuated virus reversion or incomplete inactivation, but with a well-established manufacturing process [27,28].

Currently 12 whole inactivated SARS-CoV-2 virus vaccines are in clinical trials [26]. Despite the past success in developing highly effective attenuated vaccines with superior immune responses against viruses that target multiple organs, such as yellow fever, measles, mumps and rubella viruses, currently this approach is being pursued by only two manufacturers, with one using a proprietary codon deoptimization technology to develop a live attenuated replication competent SARS-CoV-2 virus vaccine [26,27].

4.2. Recombinant viral vectored vaccines

Recombinant viral vectored vaccines use replication-competent viruses such as measles and vesicular stomatitis viruses (VSV) or replication incompetent adenoviruses and modified vaccinia virus Ankara (MVA) as delivery systems to express heterologous viral proteins in infected cells [31–34]. Although these vaccines are highly immunogenic, the presence of pre-existing immunity against viral vectors in the general population caused by past infections or vaccinations, will reduce effective immune responses induced by these vaccines and will require booster doses. Viral vectored vaccines induce antibodies, CD4 and CD8 T cell responses [30]. In 2019, FDA approved the first replication competent VSV viral vectored Ebola virus vaccine, but until recently no replication incompetent adenovirus vectored vaccine has been authorized for human use in the US [32]. In May of 2020, the European Medicines Agency (EMA) human medicines committee (CHMP), recommended granting a two component prophylactic Ebola vaccine composed of Ad26ZEBOV and MVA-BN-Filo marketing authorization under exceptional circumstances for use in a prime-boost vaccine regimen against Ebola [34]. The Ad26ZEBOV is the first non-replicating human adenovirus-26 vectored vaccine to be approved for human use [34].

4.3. Protein vaccines

Protein vaccines are premade purified viral protein subunit vaccines or virus like particles, a type of protein subunit vaccine, and these vaccines can directly stimulate an immune response once inoculated [35,36]. Virus like particles resemble viruses and are made of viral structural proteins that assemble into shells without any viral genetic material and are incapable of replication or infection [36]. Single protein subunit vaccines are poorly immunogenic, require multiple doses and adjuvants to enhance their immune responses and generate T cell responses [29]. Although

Table 1
Number of candidate vaccines under clinical evaluation globally.[a]

Vaccine design	Number of candidates
Whole virus	
Live attenuated	2
Inactivated	12
Viral vector	
Replicating	4 (VSV, measles virus, and influenza virus)
Non-replicating	14 (adenovirus 5, adenovirus 26 and MVA)
Protein subunit	29
Virus like particle	5
Nucleic acid	
RNA	13
DNA	10
Antigen presenting cells with replicating viral vector	1
Antigen presenting cells with non-replicating viral vector	2

[a] WHO candidate vaccines, reference [26]. VSV, vesicular stomatitis virus; MVA, modified vaccinia virus Ankara.

virus like particles stimulate both antibody and T cell-mediated immune responses without adjuvants, adjuvants are added to enhance specific immune responses [36]. The manufacturing process is well established for protein subunit and virus like particle vaccines and several of these vaccines have been commercially produced and licensed for human use, including vaccines against hepatitis B and human papilloma viruses [35].

4.4. Nucleic acid vaccines

Nucleic acid vaccines, both in vitro transcribed messenger RNA (mRNA) and plasmid-based DNA vaccines are much faster to produce than other types of vaccines described once the genetic sequence of the target antigen is known [37]. RNA vaccines rely on the cells that receive the genetic material to produce the target protein. Moreover, RNA replication-based vaccines (RNA replicons) with incorporated viral replication genes can induce self-amplification to increase yields and lower the required vaccine dose [38]. For stability and delivery, RNA is encapsulated in lipid nanoparticles and are safer than DNA vaccines without any possibility of RNA integrating into the host's genome [39]. Compared with RNA vaccines, DNA vaccines must first enter the nucleus to transcribe the gene into mRNA before proteins are produced in the cytoplasm and require a delivery system such as electroporation to optimally deliver DNA into cells [40]. Nucleic acid vaccines also require multiple doses to induce protective immunity and these vaccines induce neutralizing antibodies, CD4, and CD8 T cell responses depending on their formulation [37]. This newer nucleic acid technology has not yet resulted in any FDA licensed vaccine.

5. Animal models

Preclinical animal models provide useful means to test candidate vaccines for safety by identifying potential vaccine risks, guide selection of a safe starting clinical dose, regimen, route of delivery, and provide early evidence on the types of immune responses elicited and effectiveness [41]. An ideal animal model to test SARS-CoV-2 vaccines has not been identified due to the inability of current animal models to fully recapitulate human disease [42]. However, different animals including mice, rats, ferrets, hamsters, cats, and nonhuman primates provide reasonable models of different aspects of the spectrum of human Covid-19 disease [42]. SARS-CoV-2 infected human ACE2 transgenic mice are useful for vaccine immunogenicity and live virus challenge studies [42]. Infected Syrian golden hamsters develop severe disease with rapid weight loss and severe lung pathology and model severe human disease [43]. Ferrets, golden hamsters, and cats are useful to study air-borne transmission of the virus [42,43]. Outbred nonhuman primates with mild clinical disease and transient viral replication in the upper and lower respiratory tracts and mild inflammation in lungs after SARS-CoV-2 infections, provide a suitable model to test effective protection from vaccines at doses that are clinically relevant when challenged with the SARS-CoV-2 virus [44]. Animal models also provide crucial safety information on potential immune enhancement of disease caused by vaccine-induced low concentrations of binding antibodies with poor neutralizing ability, a concern that has received much scrutiny [45]. In support of this concern, when post-vaccinated animals are subsequently challenged with live viruses, these suboptimal antibodies can induce an enhanced uptake of antibody-bound viruses into susceptible cells through Fc receptors or complement receptors with worsening disease [45]. This immune enhancement of disease was seen in mice challenged with SARS-CoV-1 and MERS-CoV viruses, after inoculation with vaccines that induced a T helper cell type 2 biased immune response with eosinophilic pulmonary infiltrates [45]. As a safety measure all current Covid-19 candidate vaccines are required to be tested for vaccine-induced enhanced respiratory disease prior to late phase clinical testing. Animal studies usually precede clinical trials but due to the urgent need for Covid-19 vaccines, some animal studies and human clinical trials are conducted in parallel.

6. Clinical trials, FDA vaccine licensure and emergency use authorization

Several candidate vaccines have completed phase 1 clinical trials for vaccine safety in small numbers of healthy volunteers and have determined a vaccine dose and regimen to advance to phase 2 trials [46–49]. Some have completed phase 2 clinical trials for expanded safety in hundreds of patients and are in phase 3 trials for efficacy, that will involve several thousands of volunteer participants [50–55]. It is anticipated that at least 30,000 volunteer participants will be tested in phase 3 trials before a vaccine is licensed. Table 2 summarizes 6 promising leading candidate vaccines, the Pfizer BNT162b2, Moderna mRNA-1273, AstraZeneca/Oxford ChAdOx1 (AZD1222), Johnson & Johnson/Janssen Ad26CoV2S, Sinovac CoronaVac, and Gamaleya Sputnik V (GamCOVIDVac) vaccines that are in final stages of phase 3 trials and have published their study protocols or phase 3 study results [50–55]. ChAdOx1 and Sputnik V are non-replicating adenovirus vectored vaccines that encode a wild-type SARS-CoV-2 spike protein whereas, BNT162b2 and mRNA-1273, the two mRNA vaccines and Ad26COV2S a non-replicating adenovirus vectored vaccine, encode a modified pre-fusion stabilized spike protein [50–53,55]. The primary efficacy end point of vaccines is prevention of Covid-19 symptomatic disease and the secondary end point is prevention of severe disease following vaccination, with qualifying symptoms for Covid-19 case determination as described in Table 3. It should be noted that the qualifying symptoms used to classify disease as “mild”, “moderate”, “severe” or “very severe/critical/hospitalized” vary between trials. Efficacy will be determined by calculating the illness rate in the vaccine group to the corresponding illness rate in the placebo group as pre-specified in each clinical protocol or as described in the phase 3 trial [50–59]. Symptomatic participants will be confirmed for Covid-19 disease by nucleic acid amplification-based tests to detect SARS-CoV-2 virus in nasopharyngeal swabs, nasal swabs, or saliva [50–59].

The FDA in their June 2020 guidance for Covid-19 vaccine licensure has recommended the primary endpoint for placebo-controlled efficacy trials to be set at $\geq$ 50% efficacy with a >30% lower bound estimate, to protect from SARS-CoV-2 infections and/

Table 2
Leading Covid-19 candidate vaccines in phase 3 clinical trials.

Vaccine	BNT162b2	mRNA-1273	AZD1222/ChAdOx1	Ad26COV2S	CoronaVac	GamCOVIDVac/Sputnik V
Manufacturer	Pfizer	Moderna	AstraZeneca	Johnson & Johnson/Janssen	Sinovac	Gamaleya
Partners	BioNTech	NIAID	Oxford University			
Clinical trial	NCT04368728	NCT04470427	NCT04516746	NCT04505722	NCT04456595	NCT04530396
Platform	mRNA	mRNA	adenovirus vector chimpanzee non replicating	adenovirus vector human adenovirus 26 non replicating	Inactivated whole virus aluminum hydroxide adjuvant	adenovirus (Ad) vector human Ad26 (1st dose), Ad5 (2nd dose) non replicating
Virus target	Spike prefusion	Spike prefusion	Spike wild type	Spike prefusion	whole virus	Spike wild type
Doses	2	2	2	1	2	2
Dose intervals	3 weeks	4 weeks	4 weeks	not applicable	not specified	3 weeks
Route	im[a]	im	im	Im	im	Im
Trial size[b]	44,000 (1:1)	30,000 (1:1)	30,000 (2:1)	60,000 (1:1)	13,060 (1:1)[c]	21,977 (3:1)
Participant's age	16–55 years >55 years	18–65 years ≥65 years	18–55 years 56–69 years ≥70 years	18–59 years ≥60 years	18–59 years ≥60 years	18–60 years >60 years
Prespecified Efficacy	60%	60%	50%	60%	60%	not specified
Cases[d]	164	151	150	154	151	78

[a] Intramuscular route of administration.
[b] Total number of volunteer trial participants with randomization ratio in parentheses, randomized to vaccine and placebo groups.
[c] Trial size in Brazil conducted on healthcare professionals by Butantan Institute.
[d] Total target number of virus confirmed Covid-19 cases required for primary endpoint analysis of trial efficacy. NIAID, National Institute of Allergy and Infectious Diseases.

Table 3
Case determination for candidate vaccine efficacy endpoints.

Primary endpoint: Efficacy of the candidate vaccine to prevent Covid-19 disease.
Qualifying Covid-19 symptoms [a]
Fever or chills
Cough, shortness of breath, or difficulty in breathing
Muscle or body aches, fatigue
Headache
New loss of taste or smell
Sore throat
Nasal congestion or runny nose
Nausea, vomiting or diarrhea
SARS-CoV-2 virus confirmed by a nucleic acid amplification-based test (RT-PCR)
Secondary endpoint: Efficacy of the candidate vaccine to prevent severe Covid-19 disease. [b]
Clinical signs of severe systemic illness
Respiratory rate ≥30 breaths per minute
Heart rate ≥125 beats per minute
SpO2 ≤ 93% on room air at sea level or PaO2/FiO2 < 300 mm Hg
Respiratory failure
Requirement for high-flow oxygen, non-invasive ventilation
Mechanical ventilation or extracorporeal membrane oxygenation (ECMO)
Shock
Systolic blood pressure < 90 mm Hg, diastolic blood pressure < 60 mm Hg pressure
Requirement for vasopressors
Acute renal, hepatic, or neurological dysfunction
Intensive care unit admission
Death

For AstraZeneca ChAdOx1 one qualifying symptom: fever, cough, shortness of breath, loss of taste or smell.
[a] Case illness defined by presence of one symptom (Pfizer BNT162b2, Johnson & Johnson Ad26COV2S, Sinovac CoronaVac) or one respiratory symptom, two other symptoms or clinical or radiographical evidence of pneumonia (Moderna) with virus confirmed. For ChAdOx1, based on WHO clinical progression score ≥4 for hospitalization, ≥6 for severe disease.
[b] Case illness defined by presence of at least one severe symptom with virus confirmed. The primary endpoint for the Ad26COV2S vaccine is prevention of moderate to severe/critical Covid-19 disease. For Gamaleya Sputnik V extremely severe course includes multiorgan failure and changes in the lungs on CT (X-ray) or an evidence of adult respiratory distress syndrome. FiO2, fraction of inspired oxygen; PaO2, partial pressure of oxygen, arterial; SpO2, oxygen saturation as measured by pulse oximetry.

or clinical disease in the vaccinated group compared with the placebo group [60]. In addition to full FDA approval through licensure, early access to vaccines can be granted through emergency use authorization (EUA) during a declared public health emergency in response to Covid-19, if no approved alternatives are available, with data to support that the vaccine "may be effective" and the likely benefits outweigh the harms [61]. In the updated October 2020 EUA guidance, the FDA further recommended the inclusion of data from a median follow-up of two months after the last dose of the vaccine for trial participants, as added safety and effectiveness measures to ensure that the vaccine induced protection is due to adaptive and memory immune responses and not early innate immune responses [61]. Additionally, the placebo group should include data from 5 or more severe Covid-19 infected participants to support low risk for vaccine-induced enhanced respiratory disease [61]. Based on first interim analysis of early phase 3 trial data as specified in each vaccine clinical trial protocol, manufacturers may seek EUA for their vaccines [50–53]. Once all trial data and manufacturing information are submitted to the FDA by the manufacturer with a request for EUA, FDA will convene the

Vaccines and Related Biological Products Advisory Committee (VRBPAC) made up of outside scientific and public health experts to discuss the safety and effectiveness of the submitted data [61]. This committee will advise FDA on their evaluations and FDA will then decide whether to authorize a vaccine for emergency use for the prevention of Covid-19 based on the totality of submitted data [61]. Despite the FDA recommendation that trials continue after EUA for as long as it is feasible, an EUA can lead to risks for manufacturers if the study is unblinded to allow participants in the placebo group to cross over and receive the authorized vaccine, before all the safety and efficacy data are collected for subsequent full licensure. Thus, an early unblinding of a clinical trial may result in less reliable safety and efficacy data from the time of unblinding. Due to the urgent need for safe and effective vaccines for Covid-19, clinical development, process development and manufacturing scale up are all being performed in parallel.

Results from the efficacy analyses of 6 leading Covid-19 candidate vaccines in ongoing phase 3 randomized, controlled trials are summarized in Table 4. Some important factors need to be considered when assessing efficacy of trials: (a) The dominant SARS-CoV-2 virus that is circulating locally during the time the trial is conducted. Since all first-generation vaccines specifically target the viral spike protein from SARS-CoV-2 isolate Wuhan-Hu-1 (genome reference sequence accession NC_045512) that may not be genetically identical to the locally circulating virus strain. (b) Age of trial participants. (c) The number of participants with comorbidities who are at higher risk of developing disease. (d) Specific populations such as health care professionals who are more likely to recognize and report mild infections than participants from the general population.

Pfizer after an analysis of trial data from over 43,000 participants, reported that the BNT162b2 candidate vaccine efficacy was 95% in preventing symptomatic disease 7 days after the second injection, with 162 confirmed cases in the placebo group and 8 cases in the vaccine group [57]. The vaccine also prevented severe disease with only 1 participant developing severe disease in the vaccine group, while 9 participants developed severe disease in the placebo group [57]. Similarly, Moderna after an analysis of trial data from over 30,000 participants, reported that the mRNA-1273 candidate vaccine was 94.1% effective in preventing symptomatic disease 14 days after the second injection, with 185 Covid-19 cases in the placebo group and 11 cases in the vaccine group [58]. The mRNA-1273 vaccine was also 100% effective in preventing severe disease in the vaccine group, while 30 severe cases and 1 death were reported in the placebo group [58]. Except for transient reactogenicity events (local reactions: pain, redness and swelling; systemic reactions: headache, fatigue, muscle pain) associated with immunization, no serious safety concerns were identified easing the concern over potential vaccine-mediated disease enhancement, during the short duration of these two trials [57,58]. Furthermore, trial participants will be followed for two years for immunogenicity, longer-term safety and efficacy assessments [57,58]. AstraZeneca reported that the chimpanzee adenovirus vectored ChAdOx1 candidate vaccine is overall 66.7% effective after a pooled primary analysis of data from four randomized trials conducted in the UK, Brazil, and South Africa [56]. Overall, there were 248 Covid-19 cases in the control group and 84 cases in the vaccine group [56]. In the placebo group, 127 participants had serious adverse events and an additional 15 hospitalized cases were reported, while 108 serious adverse cases and no hospitalized cases were reported in the vaccine group, with cases determined by WHO clinical progression scores of $\geq$4 for hospitalization and $\geq$6 for severe disease [56,62]. Although 3 cases of neurological disease with transverse myelitis were reported triggering study pauses of the ChAdOx1 vaccine, upon review only 1 case was identified as possibly study related [62]. The Johnson & Johnson human adenovirus 26 vectored Ad26COV2S vaccine has an overall efficacy of 66.9% with 348 cases reported in the placebo group and 116 cases in the vaccine group, with a higher vaccine efficacy of 76.7% against severe-critical Covid-19 [59]. There were 29 hospitalized cases in the placebo group with only 2 in the vaccine group, 16 deaths were also reported in the placebo group with only 5 deaths related to Covid-19 [59]. The 3 deaths in the Ad26COV2S vaccine group were unrelated to Covid-19 [59]. While all the data are currently unavailable for the Sinovac's CoronaVac inactivated SARS-CoV-2 whole virus vaccine with an aluminum hydroxide adjuvant, the study was conducted in Brazil on 12,607 healthcare professionals and a vaccine efficacy of 50.4% was reported after an interim analysis [54]. The Gamaleya Institute human adenovirus 26 and human adenovirus 5 vector-based heterologous prime-boost Sputnik V vaccine study reported an efficacy of 91.6%, with 62 Covid-19 cases in the placebo group and 16 cases in the vaccine group following an interim analysis [55]. The vaccine was also 100% effective against the development of severe Covid-19. The 3 deaths in the vaccine group and 1 death in the placebo group were all unrelated to Covid-19 [55].

Covid-19 infections occur at much higher rates in the elderly, racial and ethnic minorities and these groups develop more severe

Table 4
Phase 3 clinical trial results of Covid-19 candidate vaccines.

Vaccine	BNT162b2	mRNA-1273	AZD1222/ChAdOx1	Ad26COV2S	CoronaVac	GamCOVIDVac/Sputnik V
Analysis	Final	Primary	Primary	Primary	Interim	Interim
Total participants	43,548	30,420	17,178	39,321	12,607	21,977
Vaccine group	21,720	15,210	8597	19,630	NA	16,501
Placebo group	21,728	15,210	8581	19,691	NA	5476
Efficacy assessment	7 days after 2nd dose	14 days after 2nd dose	14 days after 2nd dose	14 days after single dose	$\geq$15 days after 2nd dose	21 days after 1st dose
Total confirmed cases	170	196	332	464	NA	78
Vaccine group	8	11	84	116	NA	16
Placebo group	162	185	248	348	NA	62
Severe cases (additional hospitalized cases)						
Vaccine group	1	0	108 (0)	83 (2)	NA	0
Placebo group	9	30	127 (15)	96 (29)	NA	20
Deaths						
Vaccine group	0	0	2[a]	3[a]	NA	3[a]
Placebo group	0	1	5[a]	11[a], 5[b]	NA	1[a]
Efficacy overall	95%	94.1%	66.7%	66.9%	50.4%	91.6%

[a] Death was not Covid-19 related.
[b] Death was Covid-19 related. NA, not available.

disease than younger, healthier individuals of European ancestry [63,64]. Consequently, participants from these groups were included in clinical trials to assess their responses to the candidate vaccines with varied numbers across trials. Importantly, the efficacy of the candidate vaccines was unaffected by race and ethnicity. While the efficacy results for most candidate vaccines greatly exceeded the prespecified 50% efficacy required for vaccine licensure, and are encouraging for the overwhelming numbers of current vaccines that target only the spike protein, the duration of immunity and correlates of protection, two key metrics were not established for all leading candidate vaccines during the short periods of the trials [54–59]. In December 2020, the Pfizer BNT162b2 and the Moderna mRNA-1273 vaccines received FDA emergency use authorization, to prevent Covid-19 disease caused by SARS-CoV-2 during the public health emergency in the US, in individuals 16 years or 18 years of age and older respectively. This approval was closely followed by FDA emergency use authorization of the single-dose Johnson & Johnson Ad26COV2S vaccine in February 2021, for individuals 18 years of age and older.

7. Challenges in the development of safe and effective Covid-19 vaccines

7.1. SARS-CoV-2 viral variants of public health significance

Majority of candidate vaccines target only the viral spike protein with the intent to develop potent virus neutralizing antibodies [20]. Despite having a higher fidelity RNA-dependent RNA polymerase with 3′ to 5′ exonuclease activity and a moderate error rate compared with other RNA viruses such as the influenza virus, sporadic mutations in the spike protein could lead to positive selection of dominant variants, with the potential for immune escape, vaccine resistance, and breakthroughs [65]. To identify variants of public health significance, WHO published definitions for “SARS-CoV-2 Variant of Interest (VOI)” to include variants with mutations that are suspected or known to cause significant phenotypic changes and are circulating widely [66]. A variant of interest becomes a “SARS-CoV-2 Variant of Concern (VOC)” if the variant increases transmissibility or causes a detrimental change in the epidemiology, clinical presentation, or decreases the effectiveness of current public health measures or available diagnostics, treatments or vaccines [66]. Recent analysis has shown that the amino acid substitution mutation D614G, in the S1 domain of the spike protein leads to increased stability of the protein with increased infectivity and has contributed to a greater prevalence of this strain in the US and in the world [65]. More recently newer more transmissible variants of SARS-CoV-2 have emerged independently in the UK (known as 20I/501Y.V1, VOC 202012/01, or B.1.1.7), South Africa (known as 20H/501Y.V2 or B.1.351) and Brazil (known as P1, 20J/501Y.V3, VOC202101/02, or B.1.1.28), with multiple mutations in the spike glycoprotein compared to previous variants, but all share the N501Y amino acid substitution in the receptor binding domain of the spike protein, and has resulted in the rapid spread of these variants in these countries and particularly, the UK B.1.1.7 and South African B.1.351 variants globally [67]. It is reassuring that neutralizing antibodies elicited by the authorized Pfizer BNT162b2 vaccine were effective against engineered USA–WA1/2020 viruses with the full set of spike mutations seen in B.1.1.7 and P1 variants, but were less robust against the engineered B.1.351 variant, based on laboratory virus neuralization assays [68]. Moreover, amino acid substitutions K417N, E484K and N501Y in the receptor binding domain had a greater effect on virus neutralization by BNT162b2 vaccine elicited antibodies, than the deletion of amino acids 241–244 affecting the N-terminal domain of the spike protein [68]. The AstraZeneca ChAdOx1 vaccine did not protect against mild to moderate Covid-19 disease caused by the South African B.1.351 variant, raising concerns about the effectiveness of current vaccines developed against the original Wuhan-Hu-1 virus spike protein, as more variants of concern develop and may require additional booster doses targeting these newer variants [69]. There is an added concern that the reported rapid evolution of SARS-CoV-2 within immunocompromised individuals with new mutations arising in the receptor binding domain and prolonged shedding of infectious virus for months, could give rise to newer more transmissible or pathogenic variants with vaccine resistance leading to new outbreaks [70]. Therefore, a continued global genomic surveillance program for viral evolution will be required as the virus continues to circulate, to detect new virus variants of concern for immune escape from the currently authorized therapeutic antibodies or vaccines, and candidate vaccines that are still being developed against the original Wuhan-Hu-1 spike protein, especially under selection pressure caused by current world-wide vaccination roll-out programs.

SARS-CoV-2 viruses that adapt to a new animal host species could also result in establishing mutant SARS-CoV-2 animal reservoirs and provide a source for zoonotic transmissions with increased pathogenesis and transmissibility [71]. In Denmark transmission of a Covid-19 mink variant with mutations in the SARS-CoV-2 spike protein was reported in 12 individuals resulting in the culling of millions of animals in mink pelt farms in the country, to eliminate the potential threat of this variant establishing in the country [71].

7.2. Vulnerable populations

Initial vaccine efficacy trials excluded pregnant, lactating women, very young children, and severely immunocompromised individuals [54–59]. If these groups are not included in clinical trials, then safety and effectiveness of vaccines in these groups cannot be simply assumed. A recent study examining the ability of authorized mRNA vaccines to induce humoral immunity in pregnant and lactating women, found that the vaccines induced comparable antibody titers in pregnant and lactating women as in non-pregnant women [72]. Moreover, the vaccine-induced immune responses were greater in vaccinated women than those seen in naturally infected pregnant women [72]. Additionally, vaccine generated antibodies were also present in the umbilical cord blood and breast milk after maternal vaccination, leading to immune transfer to neonates through the placenta and breast milk [72]. Similar studies will need to be performed for all other currently authorized vaccines. Trials are also set to begin in children younger than 12 years with the mRNA vaccines and these trials plan to include children as young as 6 months [73]. Because children have encountered fewer pathogens, they are likely to produce stronger immune responses than adults. In the pediatric trials Pfizer and Moderna plan to assess immune markers after vaccination as measures of efficacy [73].

7.3. Protection from severe disease and virus transmission

Importantly, clinical trials for the vaccines that were evaluated were not powered to directly test whether these vaccines will prevent severe disease, instead these trials were designed to test whether the vaccine will protect against development of milder disease with RT-PCR-confirmed infection, with the assumption that the vaccine will most likely protect from severe disease, if the vaccines are proven to protect against milder disease [74]. The efficacy trials in the US were conducted after public health safety measures such as wearing masks, washing hands and social distancing were recommended that could have resulted in trial

participants being exposed to a lower dose of infectious virus potentially leading to milder infections. It is currently unknown if the vaccines will protect when exposed to higher doses of the infectious virus in the absence of these mitigating safety measures. It remains unknown whether first-generation Covid-19 vaccines will provide sterilizing immunity against infections and the BNT162b2 and mRNA-1273 trials did not assess asymptomatic infection with virus shedding and rates of transmission [57,58]. Hence, vaccinated individuals who are infected with newer variants may still be capable of transmitting an infection, and it remains to be seen whether vaccinated individuals will continue to adhere to public health measures to protect the vulnerable, in a country that has shown an aversion to universal mask mandates.

7.4. Candidate vaccine-induced rare adverse events

Additionally, the trials were also not powered to detect rare adverse events with an incidence less than 0.01% during the short follow up time of two months [57]. The mass roll-out of the Pfizer and Moderna mRNA vaccines has resulted in rare acute allergic reactions in vaccinated individuals that were not detected during the trials [57,58,75]. The FDA EUA guidance for both vaccines is to not administer the vaccine to individuals with a known history of a severe allergic reaction (e.g., anaphylaxis) to any component of the Covid-19 vaccine [75]. The CDC additionally advises individuals with a history of an immediate allergic reaction to a vaccine or injectable or any history of anaphylaxis be observed for 30 minutes after Covid-19 vaccination [75]. All other individuals should be observed for 15 minutes after Covid-19 vaccination [75].

Recent reports of several rare cases of a thrombosis-thrombocytopenia syndrome with blood clots that unusually develop in the brain and abdomen, with low levels of blood platelets after vaccination, with two recombinant adenovirus vectored Covid-19 vaccines, the AstraZeneca ChAdOx1 and Johnson & Johnson Ad26COV2S vaccines resulted in temporary vaccination pauses for safety analysis [76]. These rare clinical and laboratory features were also seen in another rare condition known as heparin-induced thrombocytopenia (HIT), in patients following the administration of the anticoagulant heparin [76]. HIT is caused by platelet factor 4 and heparin complexes that induce production of antibodies against platelet factor 4, that then result in platelet destruction and promote clot formation, although none of the Covid-19 vaccinated individuals received heparin before their symptom onset [76]. While the adenovirus vaccine components that cause blood clots are unknown, clinicians are now made aware that blood clots can develop at unusual sites such as in the brain or abdomen, with reduced blood platelets between 5 and 20 days after vaccination as rare adverse events [76]. Because these blood clots are rare events, where 6 cases were identified out of 7 million vaccinated resulting in 1 death as of 13 April 2021, the CDC and FDA after thorough safety reviews of the Johnsons & Johnson vaccine data concluded that the vaccine's known and potential benefits outweigh risks and the pause should be lifted [76,77]. FDA recommended a warning label to alert clinicians and providers to the potentially rare but serious blood clotting disorder, with women younger than 50 years also alerted to these rare risks and informed of the availability of other vaccines where this risk was not observed [77]. A similar response was made by the EMA on the AstraZeneca vaccine.

Even with the availability of effective and safe vaccines, due to “vaccine hesitancy” significant numbers of individuals will likely delay acceptance or refuse to get vaccinated making it a challenge to achieve herd immunity and end the pandemic [78]. It will be necessary to continue to gain public trust by effectively communicating the risks associated with the current Covid-19 candidate vaccines.

7.5. Human challenge studies

Phase 3 clinical trials need to be conducted in regions where the virus is actively spreading so that significant numbers of participants in the control group are infected and vaccinated individuals are protected to derive meaningful conclusions from these trials. But with the availability of authorized vaccines, waning of infections in some countries and multiple vaccines that still need to be tested in thousands of participants, it will be difficult to recruit volunteers to participate in randomized placebo-controlled trials, to complete these studies. To speed up future efficacy trials, some have proposed the deliberate infection of vaccinated human participants under tightly controlled conditions to carefully track rates of infection, the immune response and identify biomarkers [79]. There is an ongoing debate on the ethics of infecting humans with a novel virus that can cause severe disease for which only 3 treatment modalities namely, remdesivir, a combination of remdesivir plus baricitinib, two treatments with modest efficacies or dexamethasone are available for hospitalized patients as approved treatments in the US [80–83]. The UK government has announced the intention to launch human challenge studies in 2021, to accelerate the development of future treatments and vaccines for Covid-19. For these trials healthy volunteer participants aged 18–30 years will be vaccinated and then infected with a well characterized medical grade SARS-CoV-2 virus inoculum to cause an infection but not disease [80].

7.6. Global authorization, vaccine access, distribution, and storage

Covid-19 Vaccines Global Access (COVAX) partners, the Gavi vaccine alliance, WHO, and Coalition for Epidemic Preparedness Innovations (CEPI), support the participation of 92 low- and middle-income economies in the COVAX Facility, enabling access to donor-funded doses of safe and effective COVID-19 vaccines [66]. Through COVAX these low- and middle-income countries gain expedited access to unlicensed Covid-19 vaccines that are authorized by the WHO through Emergency Use Listing (EUL), after regulatory review as a global vaccine regulator for vaccine quality, safety, and efficacy during the current public health emergency [66]. As of 24 May 2021, vaccines from Pfizer, the AstraZeneca/Oxford vaccine locally produced by AstraZeneca-SKBio (Republic of Korea) and the Serum Institute of India (Covishield), Johnson & Johnson, Moderna vaccines, and the inactivated vaccine from Sinopharm (BB1BP-CoV) are listed by WHO for emergency use and global roll-out by COVAX [47]. Currently, the inactivated vaccine from Sinovac (CoronaVac) is under WHO review [54].

Many of the vaccines that are currently authorized and are being developed require intramuscular injections with 2 doses of the same vaccine to stimulate protective immunity, and the logistics of vaccine distribution and keeping track of individuals in large populations, to ensure that they receive both doses of a vaccine will be a challenge that needs to be addressed [54–58]. Additionally, for long-term storage, mRNA vaccines require ultralow freezing temperatures to maintain vaccine stability. The Pfizer BNT162b2 mRNA vaccine requires a storage temperature of minus 70 °C (−94 F) or below, while the Moderna mRNA1273 vaccine requires a storage temperature of minus 20 °C (−4 F) [50,51]. This requirement will act as a barrier to deployment of these vaccines to low and middle-income countries. In contrast to the mRNA vaccines, the AstraZeneca ChAdOx1, Johnson & Johnson Ad26COVS, Sinovac CoronaVac, and the freeze-dried Gamaleya Sputnik V vaccines can be stored at refrigerator temperature and can utilize the currently available “cold chain” infrastructure for storage and distribution of these vaccines [52–55].

7.7. Other notable first-generation candidate vaccines in development

Although several other candidate vaccines including inactivated whole-virus vaccines Sinopharm BBIBP-CorV with aluminum hydroxide adjuvant and Bharat Biotech's Covaxin, and CanSino Biologic's adenovirus type 5 vaccine Ad5-nCoV have been developed and authorized for emergency use by some countries, this review has focused only on vaccines where the manufacturer's have published their phase 3 clinical trial data or made the full study protocols publicly available for scrutiny [46–48].

In a recent press release, Novavax announced its protein-based Covid-19 candidate vaccine NVX-CoV2373, containing a full-length, prefusion spike protein made using Novavax' recombinant nanoparticle technology and the company's proprietary saponin-based Matrix-M™ adjuvant met the primary endpoint, with a vaccine efficacy of 89.3%, in a Phase 3 clinical trial conducted in the UK [84]. Similarly, Bharat Biotech announced their whole virus inactivated COVID-19 candidate vaccine BBV152/Covaxin adjuvanted with imidazoquinoline, a toll-like receptor 7/8 agonist chemisorbed on alum to induce cell-mediated immune responses, demonstrated an interim vaccine efficacy of 81% in a Phase 3 clinical trial in India [48,85]. All the above vaccines require only refrigerator temperature for storage.

8. Conclusions

According to the most recent United Nations estimates, the world population is 7.875 billion as of May 2021, populating 195 countries. Several effective and safe vaccines will be required to induce herd immunity in the global population, including vaccines specifically targeted to the very young, elderly, pregnant and lactating women, women of child-bearing age and the immune compromised. To stop a pandemic, a global vaccination campaign will require multilateral cooperation between governments, regulatory authorities, and equitable worldwide distribution of vaccines. How we meet these challenges in the coming years will foretell whether the SARS-CoV-2 virus can be effectively controlled or will remain as an endemic virus constantly evolving and giving rise to periodic and devastating outbreaks.

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Declaration of competing interest

No conflicts to disclose.

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