



Immunological considerations for COVID-19 vaccine strategies

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Abstract | The coronavirus disease 2019 (COVID-19) pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the most formidable challenge to humanity in a century. It is widely believed that prepandemic normalcy will never return until a safe and effective vaccine strategy becomes available and a global vaccination programme is implemented successfully. Here, we discuss the immunological principles that need to be taken into consideration in the development of COVID-19 vaccine strategies. On the basis of these principles, we examine the current COVID-19 vaccine candidates, their strengths and potential shortfalls, and make inferences about their chances of success. Finally, we discuss the scientific and practical challenges that will be faced in the process of developing a successful vaccine and the ways in which COVID-19 vaccine strategies may evolve over the next few years.

The coronavirus disease 2019 (COVID-19) outbreak was first reported in Wuhan, China, in late 2019 and, at the time of writing this article, has since spread to 216 countries and territories¹. It has brought the world to a standstill. The respiratory viral pathogen severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has infected at least 20.1 million individuals and killed more than 737,000 people globally, and counting¹. Although physical-distancing and other transmission-mitigation strategies implemented in most countries during the current pandemic have prevented most citizens from being infected, these strategies will paradoxically leave them without immunity to SARS-CoV-2 and thus susceptible to additional waves of infection. Health-care workers, seniors and those with underlying health conditions are at particularly high risk^{2–4}. It is widely accepted that the world will not return to its prepandemic normalcy until safe and effective vaccines become available and a global vaccination programme is successfully implemented⁵.

As COVID-19 is new to humankind and the nature of protective immune responses is poorly understood, it is unclear which vaccine strategies will be most successful. Therefore, it is imperative to develop various vaccine platforms and strategies in parallel. Indeed, since the outbreak began, researchers around the world have been racing to develop COVID-19 vaccines, with at least 166 vaccine candidates currently in preclinical and clinical development⁶ (FIG. 1). To meet the urgent need for a vaccine, a new pandemic vaccine development paradigm has been proposed that compresses the development timeline from 10–15 years to 1–2 years⁶. However, there remains a lack of clarity as to what may

constitute a safe and immunologically effective COVID-19 vaccine strategy, how to define successful end points in vaccine efficacy testing and what to expect from the global vaccine effort over the next few years. This Review outlines the guiding immunological principles for the design of COVID-19 vaccine strategies and analyses the current COVID-19 vaccine landscape and the challenges ahead.

Natural and vaccine-induced immunity

Although much remains to be understood regarding the immune response to SARS-CoV-2, and vaccine-induced protective immunity may differ from natural immunity owing to the immune-evasion strategies of the virus, improved understanding of the natural immune response will be instrumental in developing effective vaccine and therapeutic strategies. It is particularly relevant to understand the difference in immune responses between asymptomatic, mild and severe cases and at early and late stages of infection, and to understand why seniors are particularly susceptible to COVID-19, whereas the young are better protected. It is estimated that 40–75% of infections may be mild or asymptomatic^{7,8} and asymptomatic individuals may have a significantly longer duration of viral shedding than their symptomatic counterparts⁹. Furthermore, that asymptomatic and mildly ill individuals seem to develop low levels of antibody-mediated immunity has important implications for understanding herd immunity.

The initial site of infection of SARS-CoV-2 is the respiratory tract^{10,11}. On entry, SARS-CoV-2 interacts with the angiotensin-converting enzyme 2 (ACE2) receptor

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<https://doi.org/10.1038/s41577-020-00434-6>

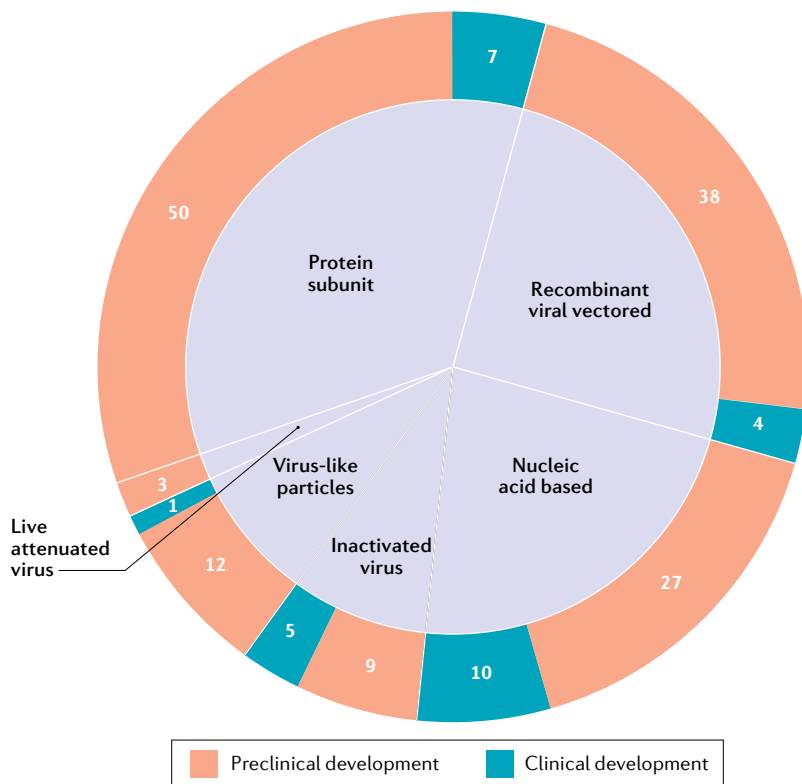


Fig. 1 | The global COVID-19 vaccine landscape. The six major types of candidate vaccine for coronavirus disease 2019 (COVID-19) are illustrated (live attenuated virus, recombinant viral vectored, inactivated virus, protein subunit, virus-like particles and nucleic acid based), showing the number of candidate vaccines that are currently under clinical and preclinical development. The nucleic acid-based platform includes both mRNA vaccines (6 clinical and 16 preclinical) and plasmid DNA vaccines (4 clinical and 11 preclinical). Data obtained from REF⁵.

Acute respiratory distress syndrome

A rapidly developing lung condition characterized by deficient oxygen exchange and shortness of breath, resulting from severe lung injury and inflammation following infection.

Immunosenescence

Age-related changes in the immune system that lead to a progressive reduction in its ability to develop effective antibody and cellular responses to infections and vaccinations.

Trained immunity

A persisting reset state of the innate immune system long after the initial antigen or microbial exposure that leads to enhanced responsiveness to the same or an unrelated antigen or microorganism.

on bronchial and alveolar epithelial cells through its spike (S) protein receptor-binding domain (RBD), which is subsequently primed by a specific cellular serine protease, transmembrane protease serine 2 (TMPRSS2), to gain entry^{12,13}. Analysis of transcripts encoding ACE2 and TMPRSS2 by single-cell RNA sequencing has shown that these transcripts are co-expressed in various cell types^{10,11}, and from autopsy studies SARS-CoV-2 can be detected in multiple organs, including the lungs, pharynx, heart, liver, brain and kidneys¹⁴.

Innate immune responses. Emerging evidence suggests that the immune response to SARS-CoV-2 is similar in several aspects to the response to SARS-CoV or Middle East respiratory syndrome coronavirus (MERS-CoV), the two coronaviruses responsible for the 2002–2004 SARS outbreak and the 2012 MERS outbreak that originated in China and Saudi Arabia, respectively^{15–17}. Like SARS-CoV and MERS-CoV, SARS-CoV-2 suppresses activation of the innate immune system, including dendritic cells^{18,19}, and dampens antiviral type I and type III interferon responses²⁰. This ability of SARS-CoV-2 to subvert the innate immune response may explain the protracted incubation or presymptomatic period of 2–12 days for COVID-19 relative to the 1–4-day incubation period for influenza¹⁶. Thus, uncontrolled SARS-CoV-2 replication in the early phase of infection

resulting from innate immune suppression probably underpins the ensuing dysregulated inflammatory responses^{16,21}, particularly in severe cases of COVID-19. Such cases are characterized by markedly increased numbers of inflammatory monocytes and neutrophils in blood^{20,22,23} and CD14⁺CD16⁺ monocyte-derived macrophages in the airway^{20,24}, and increased systemic levels of inflammatory cytokines and chemokines^{20,22,23}. A failure to accomplish early control of SARS-CoV-2 infection in the respiratory tract likely results in high viral burden and dysregulated, potentially lethal, inflammatory responses and immunopathology, including acute respiratory distress syndrome. For this reason, seniors and those with co-morbidities may be particularly prone to COVID-19 owing to immunosenescence and their propensity to mount exaggerated inflammatory responses^{25–27}. Besides the consideration of vaccine-induced adaptive immunity discussed later, inclusion of the recently emerged concept of trained immunity (BOX 1) in COVID-19 vaccine design might further bolster protection, particularly in the early phases of infection.

Antibody responses. IgM and IgG antibodies to SARS-CoV-2 are detectable within 1–2 weeks after the onset of symptoms in most infected individuals²⁸. Although the relationship between neutralizing antibodies and antigen-specific T cells and disease severity and clinical outcomes remains to be understood, high levels of neutralizing antibodies have been observed in convalescent individuals²⁹, which correlate with T cell responses, particularly those of CD4⁺ T cells³⁰, and seem to offer some benefits in studies of treatment with convalescent plasma³¹. Recent studies indicate that the magnitude of neutralizing antibody responses is positively correlated with disease severity³². Thus, whereas antibody responses wane within weeks after infection in most people infected with SARS-CoV-2 (REF³²), which is a feature of antibody responses to other ‘common cold’ coronaviruses¹⁷, the magnitude of the neutralizing antibody response in asymptomatic individuals is not only smaller but also decreases faster than in symptomatic individuals⁹.

The major target of neutralizing antibodies to coronaviruses is the S protein, which is composed of S1 and S2 domains. S1 is membrane distal and contains the RBD that binds to the cellular receptor ACE2. S2 is membrane proximal and has a role in membrane fusion³³. The S proteins of SARS-CoV and SARS-CoV-2 are 88% identical and both bind to ACE2 with high affinity³³. Certain monoclonal and polyclonal antibodies raised to the S protein of SARS-CoV can cross-neutralize SARS-CoV-2 (REFS^{33,34}). Antibodies that bind to the S1 RBD block its interaction with ACE2, whereas those that bind to other regions of S1 and S2 can inhibit conformational change of the S protein and block membrane fusion, respectively^{35–37}.

During natural immune responses to SARS-CoV-2, high titres of antibodies are also generated against nucleoprotein (N) — the most abundant viral protein^{38–40}. Although antibodies to N are unlikely to neutralize the virus, they have been reported to provide protection against mouse hepatitis virus, a coronavirus of mice. Notably, these antibodies were IgG2a, indicating

Box 1 | Trained immunity as a potential COVID-19 vaccine strategy

Innate immune memory (also known as trained immunity) is a recently recognized component of immunological memory that has implications for vaccine strategies^{83,84,168,169}. Several live attenuated human vaccines induce trained immunity that can mediate non-specific protective responses to heterologous infections in addition to pathogen-specific adaptive immune memory^{168–170}. The most well-studied human vaccine that induces trained immunity is the bacillus Calmette–Guérin (BCG) vaccine against tuberculosis¹⁷¹. BCG vaccination endows circulating monocytes with characteristics of trained immunity through epigenetic and metabolic rewiring of myeloid progenitors in the bone marrow^{169,172,173}. These trained monocytes enhance protection against heterologous infections, including respiratory viral infection^{174–176}. BCG may therefore offer a level of protection from coronavirus disease 2019 (COVID-19), which might be supported by the observed inverse correlation between universal BCG vaccination and COVID-19 fatalities¹⁷⁷. Several clinical trials are under way to assess the effects of BCG or measles vaccination on COVID-19 (REF.¹⁷⁸).

A COVID-19 vaccine that can induce trained immunity might enhance early viral control by overcoming virus-imposed innate immune suppression and facilitating adaptive immune activation. The early timing of action by trained immunity is of importance as the overproduction of cytokines by macrophages at later stages of COVID-19 can contribute to immunopathology. Although it remains to be understood how to best harness trained immunity for COVID-19 vaccine strategies, recent evidence suggests that routes of microbial exposure or vaccination determine the tissue distribution of trained immunity^{83,84,169}. As respiratory mucosal immunity is key to early clearance of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), inducing trained immunity in alveolar macrophages and other innate cells^{83,179–181} through respiratory mucosal vaccination could be an effective strategy. Indeed, a human serotype 5 adenovirus-vectored vaccine delivered to the respiratory mucosa induces memory alveolar macrophages capable of trained immunity against heterologous infections⁸⁵. However, it is unclear whether lung memory macrophages may be replaced by inflammatory monocytes in response to SARS-CoV-2.

that they may exert protection through Fc-mediated effector functions rather than direct virus neutralization^{41,42}. Somewhat unusually, several studies have reported that IgA responses to S protein peak earlier than IgM responses and are more pronounced, which makes IgA a potentially attractive target for antibody-based diagnostic assays^{43,44}. The mechanistic basis of this early induction of S-specific IgA is not yet clear.

We do not yet understand the durability of the antibody responses to SARS-CoV-2. However, previous longitudinal studies of patients with SARS-CoV infection reported substantial waning of neutralizing antibody titres between 1 year and 2 years after infection^{45,46}. This is consistent with classical studies showing a relatively rapid waning of antibodies to the seasonal coronavirus 229E⁴⁷. There are currently no immune correlates of protection for SARS-CoV-2 or other human coronaviruses. Thus, it is unclear what titre of neutralizing antibodies is sufficient to confer protection against infection. Establishing such correlates will be essential to guide the development of effective COVID-19 vaccines.

T cell-mediated immunity. Whereas the current successful human antiviral vaccines, such as influenza and measles vaccines, depend largely on the induction of antibody responses, emerging evidence suggests the requirement of both antibody-mediated and T cell-mediated immunity for effective protection against SARS-CoV-2 (REFS^{17,27}). It is well known that CD4⁺ T cell help is important for optimal antibody responses and for CD8⁺ T cell activation in host defence⁴⁸. Furthermore, if neutralizing antibody-mediated protection is

incomplete, cytotoxic CD8⁺ T cells are crucial for viral clearance⁴⁹. One study found that among people who had recovered from COVID-19, 100% had S protein-specific CD4⁺ T cells in the circulation and 70% had S protein-specific CD8⁺ T cells in the circulation³⁰, and preclinical studies show a protective role of T cells in host defence against SARS-CoV⁵⁰.

The 2–12-day incubation or presymptomatic period of SARS-CoV-2 infection is associated not only with virus-mediated innate immune suppression but also with delayed activation of T cells, particularly CD8⁺ T cells^{18,19}, as is the case for SARS and MERS. People who have recovered from COVID-19 seem to have high levels of both neutralizing antibodies and T cells, and, compared with severe cases, milder cases of COVID-19 have greater numbers of memory CD8⁺ T cells in the respiratory tract^{24,29,30}. Evidence suggests that the induction of such lung tissue-resident memory T cells (T_{RM} cells) will depend on the route of vaccination. Respiratory mucosal vaccination induces strong lung T_{RM} cell responses, whereas parenteral vaccination fails to do so^{51–53}. Experimentally, the airway T_{RM} cells elicited by respiratory mucosal vaccination offered robust protection against SARS-CoV infection⁵⁴.

The T helper cell (T_H cell) phenotype of vaccine-induced T cells is also relevant to the protection they mediate. Less severe cases of SARS were associated with accelerated induction of a T_H1 cell response⁵⁵, whereas T_H2 cell responses have been associated with enhancement of lung disease following infection in hosts parenterally vaccinated with inactivated SARS-CoV viral vaccines^{56,57}. Thus, COVID-19 vaccine-induced T_{RM} cells should have a T_H1 cell-like phenotype.

These lines of evidence, together with data suggesting that T cell-mediated immunity generally is a more reliable correlate of vaccine protection than antibody titres in seniors²⁶, strongly support the inclusion of T cell responses in COVID-19 vaccine design^{17,27}.

Pre-existing cross-reactive immunity. Emerging evidence indicates that CD4⁺ T cells in 35% of healthy individuals not exposed to SARS-CoV-2 recognize the SARS-CoV-2 S protein and that CD4⁺ T cells in 40–60% of unexposed individuals are reactive to SARS-CoV-2 proteins other than S protein^{30,58}. This indicates that there is cross-reactivity between CD4⁺ T cells specific for SARS-CoV-2 and CD4⁺ T cells specific for human common cold coronaviruses, SARS-CoV and animal betacoronaviruses^{17,59–61}. There are four human coronaviruses — 229E, NL63, OC43 and HKU1 — that account for ~15% of common colds in humans¹⁷. Adults may be infected with one of these on average every 2–3 years, such that there could be a degree of pre-existing cross-reactive immunity to SARS-CoV-2 antigens in these people, which offers a potential explanation for differing susceptibility to SARS-CoV-2 infection. In addition to understanding the relationship between such pre-existing immunity to human coronaviruses and host defence against SARS-CoV-2, it will also be important to consider the contribution of COVID-19 vaccine-boosted cross-reactive immune responses to vaccine-induced protective immunity.

Fc-mediated effector functions

Immune functions that are mediated through the interaction of the constant Fc region of antibodies with innate immune molecules, complement proteins and specialized Fc receptors expressed by innate immune cells. The resulting functions include complement-dependent cytotoxicity and antibody-dependent cellular phagocytosis or cell-mediated cytotoxicity.

Respiratory mucosal vaccination

Direct administration of a vaccine to the respiratory tract by either intranasal delivery or aerosol inhalation.

Parenteral vaccination

Administration of a vaccine via the skin, muscle or blood vessel.

Antibody-dependent enhancement

(ADE). ADE of disease results when vaccine-induced non-neutralizing or weakly neutralizing antibodies bind to newly infecting virus to promote enhanced virus uptake into host cells via Fcγ receptors. This phenomenon has been observed experimentally or clinically following vaccination against viral pathogens such as dengue virus, respiratory syncytial virus and feline coronavirus.

Macrophage activation syndrome

Also known as cytokine storm or secondary hemophagocytic lymphohistocytosis. A clinical state of systemic hyperinflammation that is characterized by hypercytokinaemia, fever, adenopathy, hepatosplenomegaly, cytopenias and activation of intravascular coagulation.

Importantly, whereas CD4⁺ T cells from patients with COVID-19 equally recognize the S1 and S2 subunits of SARS-CoV-2, cross-reactive CD4⁺ T cells from unexposed individuals recognize the S2 subunit⁵⁸. CD4⁺ T cells from patients with COVID-19 cross-react strongly with S2 subunits of the human coronaviruses OC43 and 229E. More than 90% of tested healthy adults also have IgG antibodies specific for all four human common cold coronaviruses¹⁷. However, similarly to antibody responses to SARS-CoV and SARS-CoV-2, antibody responses to human coronaviruses wane rapidly within months after infection. Therefore, control of reinfection with human coronaviruses seems mainly to be antibody independent but T cell dependent¹⁷.

As coronavirus cross-reactive T cells can be specific for both structural and non-structural viral proteins^{58,61}, the extent of vaccine-boosted cross-reactive T cell responses induced by most protein subunit and recombinant viral-vectored COVID-19 vaccines, which are currently based only on the S protein, will be different from those boosted by multivalent COVID-19 vaccines such as those based on inactivated SARS-CoV-2 virus. One exception could be the use of live attenuated SARS-CoV-2 vaccines as the pre-existing cross-reactive immunity may limit the potency of such vaccines. Finally, it is noteworthy that the significant presence of cross-reactive immunity in some individuals calls for consideration of stratifying clinical trial participants receiving candidate COVID-19 vaccines according to their status of pre-existing coronavirus immunity.

Antibody-dependent enhancement of disease. A potential barrier to the development of safe and efficacious COVID-19 vaccines (BOX 2) is the risk that insufficient titres of neutralizing antibodies might trigger

antibody-dependent enhancement (ADE) of disease. ADE is most classically associated with dengue virus, whereby cross-reactive but subneutralizing concentrations of antibodies to one virus serotype enhance infection with another serotype in Fcγ receptor (FcγR)-bearing cells, including macrophages⁶². A common property among viruses that cause ADE is an ability to replicate in macrophages and/or cause them to respond abnormally. Although macrophages do not seem to be a major target of SARS-CoV-2 infection, and the expression of ACE2 on different monocyte and macrophage populations is highly variable, previous data regarding SARS-CoV suggest that FcγRs can facilitate uptake of the virus into macrophages and B cells^{21,63}. Cytokine profiles from patients infected with SARS-CoV-2 resemble those in macrophage activation syndrome and are characterized by high levels of inflammatory cytokines and chemokines^{21,64–66}. Furthermore, patients with symptomatic COVID-19 are reported to produce IgG antibodies with reduced fucosylation levels, which in turn promotes their interaction with activating FcγRIIIa⁶⁷.

The evidence for ADE in the context of SARS-CoV infection is circumstantial. Correlations between antibody titres and infection severity have been reported, but it is unclear whether high antibody titres contribute to disease or whether severe infections elicit higher antibody titres⁶⁸. Also, macrophages treated in vitro with serum from patients with SARS had exaggerated inflammatory cytokine profiles^{69,70}.

ADE has been reported in some preclinical animal models vaccinated with experimental SARS-CoV vaccines. Ferrets vaccinated with a modified vaccinia virus Ankara (MVA) vaccine expressing full-length S protein had increased infection and hepatitis following challenge^{71,72}. Antibodies to S protein were reported to induce acute lung injury in experimentally infected macaques on the basis of histological examination⁶⁹. By contrast, hamsters vaccinated with recombinant, full-length SARS-CoV S protein were protected against infection despite the ability of antibodies to mediate entry of SARS-CoV into B cells through FcγRII (REF.⁷³).

Whether ADE occurs in the context of SARS-CoV-2 infection remains unclear but warrants further investigation, focusing directly on whether antibodies increase disease severity and, if so, characterizing the specific properties of these antibodies. What seems clear is that high levels of neutralizing antibodies can mediate protection. Defining the titres of neutralizing antibodies that are protective, ensuring that COVID-19 vaccines can achieve these titres and avoiding waning of antibodies to subneutralizing levels through frequent boosting will be important to minimize the possibility of ADE. Rationally designed COVID-19 vaccines that omit ADE-inducing, non-neutralizing or weakly neutralizing epitopes in favour of those known to mediate protective responses may also minimize the likelihood of disease enhancement. Finally, there is also evidence from mouse models of dengue virus infection that antiviral T cells help to dampen ADE of disease⁷⁴. Therefore, a vaccine strategy designed to induce both neutralizing antibodies and robust T cell-mediated immunity may help to mitigate the risk of ADE.

Box 2 | Safety considerations for COVID-19 vaccines

As most individuals infected with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) are asymptomatic or develop only mild symptoms and coronavirus disease 2019 (COVID-19) vaccines are being developed towards an ultimate goal of global mass immunization, vaccine safety is of paramount importance. Any indication of a lack of safety consideration could also fuel the antivaccination movement and vaccine hesitancy, which would jeopardize the desired effect of achieving herd immunity. In this regard, most of the current COVID-19 vaccine clinical trials were initially conducted in healthy adults aged 55 years or younger, with only some later stage trials including seniors^{98,99,115,146–148}. The highly susceptible elderly populations and those with underlying medical conditions are in particular need of highly safe and effective vaccines. It remains largely unclear whether any of the initially trialled COVID-19 vaccines will be safe for both young children and seniors in both the short term and the long term. It remains likely that a different vaccine strategy with proven safety and efficacy profiles might be required for protection in seniors.

The safety of a vaccine is generally determined by the nature of the vaccine platform, the choice of adjuvant, the mode and route of vaccine administration, the age of vaccinees and the status of pre-existing vaccine immunity⁷⁸. For example, replicating live attenuated virus or viral-vectored vaccines may not be safe for a respiratory mucosal route of vaccination. Neither are certain immune adjuvants such as alum and bacterial-derived proteins. When a prime–boost immunization strategy is required, adverse events are generally more frequent and intense following the booster vaccination¹¹⁵. Vaccine strategies for COVID-19, as for some other respiratory viral infections, require additional safety considerations related to the possibility of antibody-dependent enhancement of disease and the role of overproduction of proinflammatory cytokines in lung immunopathology. The latter pertains particularly to the application of respiratory mucosal vaccine strategies.

Vaccine design

Vaccine design concerns the selection of antigens, vaccine platforms, and vaccination routes and regimen. The choice of vaccine platform determines the relative immunogenic strength of vaccine-derived viral antigens, whether an immune adjuvant is required and the nature of protective immunity. These attributes also determine the suitability of a vaccine for a particular route of vaccination, and whether a prime–boost vaccination regimen is required to increase vaccine-mediated protective immunity and its durability. Furthermore, the selection of live attenuated viral vaccines or a respiratory mucosal route of vaccination will require more stringent safety testing (BOX 2).

Selection of SARS-CoV-2 antigens. The structural proteins present in the infectious virion include S protein, N protein, matrix (M) protein and envelope (E) protein. The N protein coats the large positive-stranded RNA genome, which is encased in a lipid envelope derived from the host cell membrane, into which the other three proteins (S, M and E) are inserted. In the case of SARS-CoV, it has been shown that only antibodies directed to S protein can neutralize the virus and prevent infection⁷⁵. As a result, all SARS-CoV-2 vaccines in development include at least a portion of the S protein. These may be restricted to only the S1 domain or the RBD.

Non-neutralizing antibodies to both S protein and the other exposed proteins (E and M) are generated. As there is a suspected role of these non-neutralizing antibodies, as well as weakly neutralizing antibodies, in ADE of disease, the inclusion of other structural (N) and/or non-structural proteins as vaccine antigens may help to create a more balanced response involving both humoral and T cell-mediated immunity. These could be highly expressed proteins such as N protein or highly conserved functional proteins that have a crucial role in the viral life cycle. For example, inclusion of viral enzymes such as the RNA-dependent RNA polymerase in a vaccine design may ensure that it targets all emerging variant strains, as these proteins are highly conserved^{59,76,77}, even across other bat-derived coronaviruses that could emerge as a threat to humans in the future.

Vaccine platforms. In general, vaccine platforms are divided into six categories: live attenuated virus, recombinant viral-vectored vaccines that are bioengineered to express target pathogen antigens in vivo, inactivated or killed virus, protein subunit vaccines, virus-like particles (VLPs) and nucleic acid-based (DNA or mRNA) vaccines. In broad terms, vaccines require two components: antigens from the target pathogen that are provided to or generated by the vaccine recipient; and an infection signal (such as a pathogen-associated molecular pattern or damage-associated molecular pattern) that alerts and activates the host immune system. Live attenuated vaccines can naturally provide both of these components, whereas non-viral vaccine platforms can provide the antigens but often require the artificial provision of signals to alert the immune system known as adjuvants.

Typically, these non-viral vaccine platforms require multiple vaccinations to induce protective immunity, whereas live virus-based vaccines have the ability to provide ‘one-shot’ immunity. Similarly to non-viral platforms, killed virus vaccines sometimes require the inclusion of an adjuvant and repeated administration for full efficacy⁷⁸. There are immunological pros and cons to each of these technologies as discussed later (TABLE 1).

Vaccination routes and regimens. In addition to the careful selection of vaccine antigens and platform, the route of vaccination is an integral consideration of vaccine strategies^{52,79}. This is particularly important for mucosal pathogens such as SARS-CoV-2 and those pathogens against which optimal protection requires not only neutralizing antibodies but also innate and adaptive cellular immunity^{17,80}. The best window of opportunity for SARS-CoV-2 control and clearance is the asymptomatic or presymptomatic period of COVID-19 (2–12 days), which is likely to require all of the immune protective elements to be present within the respiratory mucosa before viral entry^{16,17,27}. The route of vaccination has a crucial role in determining this^{52,81}. Protective IgG antibodies induced by parenteral vaccination readily appear at the respiratory mucosa, this being the primary mechanism by which intramuscular injection of measles or influenza vaccine offers protection in humans. However, this route of vaccination is unable to effectively induce mucosal IgA antibodies or T_{RM} cells in the lungs^{52,81}. By comparison, the respiratory mucosal route of vaccination is adept at inducing antibodies and T_{RM} cells in the respiratory mucosa, as well as macrophage-mediated trained immunity^{52,54,80–85} (BOX 1). Inactivated virus, protein subunit and nucleic acid vaccines cannot be administered by the respiratory mucosal route owing to their requirement for potentially unsafe immune adjuvants and repeated delivery (TABLE 1). By contrast, recombinant viral-vectored vaccines, particularly those using human serotype 5 adenovirus (Ad5) or chimpanzee-derived adenovirus (ChAd), are safe and highly effective for respiratory mucosal vaccination⁷⁹.

Often, weakly immunogenic vaccines based on inactivated virus, protein subunits, nucleic acids or viral vectors such as Ad26 require a repeated homologous vaccination regimen to be effective. Indeed, most current human vaccines require repeated doses. As it is not yet known which COVID-19 vaccine strategy will be used or for how long the vaccine-induced protection may last in humans, it remains possible that a homologous or heterologous prime–boost vaccination regimen will be required to sustain protection, even with robust stand-alone platforms such as ChAd. The same or a different route may be used for the repeated vaccine delivery.

Major COVID-19 vaccine candidates

As of 31 July 2020, there were 27 vaccine candidates for COVID-19 in clinical evaluation and 139 vaccines in preclinical development⁵ (FIG. 1). Of the 27 vaccines undergoing clinical evaluation (TABLE 2), the three lead candidates are viral-vectored and mRNA-based

Virus-like particles

(VLPs). A type of subunit vaccine based on multiple virus-derived proteins that are assembled to mimic the organization and conformation of authentic native viruses but that lack the viral genome.

Adjuvants

Biochemical components additional to vaccine antigens that are included in a vaccine formulation to help stimulate an adaptive immune response to vaccine antigens by activating innate immune cells. Often, non-live vaccines such as inactivated virus, protein subunit and nucleic acid vaccines require immune adjuvants.

Homologous or heterologous prime–boost vaccination

A repeated immunization regimen designed to increase and sustain vaccine-induced immune responses. It may involve repeated delivery of the same vaccine (homologous) or sequential delivery of different vaccine platforms (heterologous).

Table 1 | Immunological properties of major COVID-19 candidate vaccine platforms

Vaccine platform	SARS-CoV-2 antigens	Neutralizing antibody response	T cell response			Pre-existing antivector immunity	Route of vaccination	Overall immunogenicity	Other attributes
			CD4 ⁺ T _H cells	CD8 ⁺ T cells	Lung T _{RM} cells				
Viral-vectored vaccines									
Ad5 (non-replicating)	S protein	Quality and durability affected by pre-existing antivector immunity	T _H 1 cell	Potent response; negative effects from pre-existing antivector immunity	Induced by RM but not IM route	High, age-dependent, prevalence in blood; low prevalence in respiratory tract	Parenteral (IM) in clinical trials	Strong with single delivery but hindered by pre-existing antivector immunity	Ample human safety data; RM delivery helps bypass antivector immunity; can be delivered by inhaled aerosol
Ad26 (non-replicating)	S protein	Quality and durability affected by pre-existing antivector immunity	T _H 1 cell	Moderate response; negative effects from pre-existing antivector immunity	Induced by RM but not IM route	Medium prevalence	Parenteral (IM) in planned clinical trials	Weak; requires repeated or heterologous boost vaccination	Established human safety from HIV and Ebola vaccine trials; RM delivery helps bypass antivector immunity
ChAd (non-replicating)	S protein	Unimpeded owing to lack of pre-existing antivector immunity	T _H 1 cell	Potent response	Induced by RM but not IM route	Very low prevalence	Parenteral (IM) in clinical trials	Strong with single delivery	Well-established human safety data; amenable to RM delivery; can be used as a stand-alone vaccine or in prime–boost regimens
VSV (replicating)	S protein	Unimpeded owing to lack of pre-existing antivector immunity	T _H 1 cell	Response not as strong as for Ad5 or ChAd when used as a stand-alone vaccine; strong T cell booster	Not induced by IM route	None	Parenteral (IM) in previous successful Ebola vaccine trials	Good with single delivery	Successfully licensed platform for Ebola; not known whether it protects against RM viral pathogens
Measles and influenza viruses (replicating)	S protein?	Quality and durability depend on whether there is pre-existing antivector immunity and vaccination route	T _H 1 cell	Good response when delivered via RM route	Not induced by parenteral route	High prevalence owing to vaccination and natural infection	Parenteral or RM	Weak relative to adenovirus vectors	Not extensively tested in humans; potential recombination of live attenuated influenza vectors in the lung delivered via RM route
Other vaccines									
mRNA-based vaccine	S protein or RBD encapsulated in lipid nanoparticle	Unimpeded owing to lack of pre-existing antivector immunity	T _H 1 cell or T _H 2 cell depending on adjuvant	Depends on choice of adjuvant and formulation	Not induced by parenteral route	None	Parenteral (IM) in clinical trials	Requires repeated delivery	Adjuvant required; unclear whether it is amenable to RM vaccination
DNA-based vaccine	S protein	Unimpeded owing to lack of pre-existing antivector immunity	T _H 1 cell	Response not as strong as for some of the viral vectors	Not induced	None	Parenteral (IM) in clinical trials	Weaker than mRNA-based vaccine; requires repeated delivery	Adjuvant required; not amenable to RM vaccination
Live attenuated virus	Multiple viral antigens	Strong induction	T _H 1 cell	Strong response	Induced by RM but not IM route	No cross-reactive antibodies; cross-reactive T cells from seasonal coronavirus infections	Parenteral (SC)	Requires only a single delivery	Extensive safety testing required for potential recombination with wild-type virus

Table 1 (cont.) | Immunological properties of major COVID-19 candidate vaccine platforms

Vaccine platform	SARS-CoV-2 antigens	Neutralizing antibody response	T cell response			Pre-existing antivector immunity	Route of vaccination	Overall immunogenicity	Other attributes
			CD4 ⁺ T _H cells	CD8 ⁺ T cells	Lung T _{RM} cells				
<i>Other vaccines (cont.)</i>									
Inactivated virus	Multiple viral antigens	Strong induction	T _H 1 cell or T _H 2 cell depending on adjuvant	Weak response	Not induced	None	Parenteral (IM)	Weak; requires repeated vaccination	Adjuvant required; alum often used, which enhances T _H 2 cell responses possibly involved in ADE
Protein subunit vaccine	S protein or RBD	Strong induction	T _H 1 cell or T _H 2 cell depending on adjuvant	Weak response	Not induced	None	Parenteral (IM) in clinical trials	Weak; requires repeated vaccination	Adjuvant required; mostly unsuitable for RM vaccination
Virus-like particle	Multiple viral antigens	Strong induction	T _H 1 cell or T _H 2 cell depending on adjuvant	Weak response	Not induced	None	Parenteral (IM) or RM	Weak, but greater than for protein subunits; requires repeated vaccination	Well-established platform for several commercial human vaccines (hepatitis B and HPV vaccines); adjuvant required

Ad5, human serotype 5 adenovirus; Ad26, human serotype 26 adenovirus; ADE, antibody-dependent enhancement; ChAd, chimpanzee adenovirus; COVID-19, coronavirus disease 2019; HPV, human papillomavirus; IM, intramuscular; RBD, receptor-binding domain; RM, respiratory mucosal; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; S protein, spike protein; SC, subcutaneous; T_H cell, T helper cell; T_{RM} cell, resident memory T cell; VSV, vesicular stomatitis virus.

vaccines that entered clinical trials in China, the UK and the USA in mid-March 2020. Clinical trials for the remaining 24 candidates are currently recruiting volunteers, and a couple of other candidates are also about to enter clinical trials (TABLE 2). Preclinical evaluation of candidate vaccines requires the use of relevant animal models of COVID-19 (BOX 3). Conventionally, the safety, immunogenicity and protective efficacy of experimental vaccines are rigorously evaluated and established in animal models first before clinical trials are begun. In the case of pandemic vaccine development, however, the preclinical and clinical stages of vaccine development are compressed and move forwards in parallel.

Live attenuated viral vaccines. Historically, several successful human vaccines, such as measles vaccine and the bacillus Calmette–Guérin (BCG) vaccine for tuberculosis (TB), have been based on attenuated strains of the actual pathogen⁸⁶, with loss or mutation of virulence genes through in vitro passage. It is now possible to rationally design attenuated virus strains by mutating or deleting virulence genes. These deletion mutants can often replicate to a limited extent in host cells but lose the ability to cause disease in vivo. Coronaviruses have several genes that are not required for replication and that can be deleted, leading to attenuation in vivo. Deletion of various non-structural proteins, as well as of the structural E protein, has been used as a strategy to engineer vaccine strains of several zoonotic and veterinary coronaviruses^{87–89}. Deletion of the E protein leads to attenuation and generation of an efficacious vaccine strain^{87,88}, but reversion of the attenuated phenotype

has been reported⁹⁰. Deletion of virulence factors may therefore provide a preferred mechanism of attenuation. For example, deletion of the 2'-O-methylase gene from the SARS-CoV genome removes the ability of the virus to hide its RNA from the host cell proteins MDA5 (also known as IFIH1) and IFIT1, thereby inducing a robust antiviral response in vivo⁹¹. Another approach to viral attenuation is known as codon deoptimization, whereby the nucleic acid sequence is modified to use suboptimal codons to encode the wild-type amino acid sequence, which considerably slows the translation of the viral protein during infection. This approach can yield a virus that is highly attenuated in vivo but still able to replicate in vitro if the correct viral protein is selected for deoptimization^{92,93}.

However, the generation of an attenuated strain of a pathogen for use as a vaccine requires demonstration of its inability to revert genetically to become pathogenic (TABLE 1; BOX 2). This is particularly challenging in the case of coronaviruses as they are known to recombine in nature⁹⁴, and an attenuated vaccine strain could, in theory, recombine with wild coronaviruses to recreate a pathogenic strain. So far, there are only three attenuated SARS-CoV-2 vaccines generated by codon deoptimization under preclinical development, by Mehmet Ali Aydinlar University in Turkey, Codagenix and Serum Institute of India, and Indian Immunologicals Ltd and Griffith University⁵.

Recombinant viral-vectored vaccines. Recombinant viral-vectored vaccines are built on either a replication-deficient viral backbone or an attenuated replication-competent viral backbone that is bioengineered to

Table 2 | COVID-19 vaccine candidates in or entering clinical trials

Vaccine	Platform	Developer	Clinical trial phase	Immunization attributes	Preclinical data	Clinical data	Clinical Trial registrations	Refs
ChAdOx1 nCov-19 (AZD-1222) ^a	ChAd-vectored, non-replicating	University of Oxford, AstraZeneca	Phases I-III in UK, South Africa, USA and Brazil	Expressing S protein; single dose or two repeated doses of IM injection	Published data showing prevention of pneumonia but not transmission in NHPs	Published data showing safety and good induction of neutralizing antibodies and T cell activation in >90% of vaccinees	ISRCTN89951424, EudraCT 2020-001228-32, PACTR202006922165132, EudraCT 2020-001072-15, NCT04324606	114,115
Ad5-nCoV	Ad5-vectored, non-replicating	CanSino Biologics Inc., Beijing Institute of Biotechnology	Phases I and II; phase II studies in China and Canada	Expressing S protein; single dose of IM injection	NA	Published data showing high dose unsafe, low and medium doses elicit neutralizing antibodies in ~50–60% of vaccinees; antibody levels negatively associated with pre-existing antivector immunity and age (>55 years)	ChiCTR2000031781, ChiCTR2000030906, NCT04341389	98,99
mRNA-1273 ^a	Lipid nanoparticle–mRNA	Moderna, NIAID	Phases I-III in USA	Expressing S protein; two repeated doses of IM injection	Published report showing induction of neutralizing antibodies and CD8 ⁺ T cells, as well as protection, in mouse models	Published data showing safety, but highest dose causes severe AEs in 20% of vaccinees; induction of neutralizing antibodies in 100% of vaccinees and CD4 ⁺ T cell responses in some	NCT04405076, NCT04283461, NCT04470427	145,146
PiCoVacc	Inactivated SARS-CoV-2	Sinovac Biotech	Phases I-III; phase III in China and Brazil	Multiple viral antigens; two repeated doses of IM injection	Published data from NHP model showing protection	Interim phase I/II information released to indicate safety and immunogenicity	NCT04456595, NCT04383574, NCT04352608	126
NVX-CoV2373 ^a	Protein subunit	Novavax	Phases I and II in Australia	Recombinant S protein; two repeated doses of IM injection	Unpublished information indicates high levels of S-specific neutralizing antibodies	NA	NCT04368988	–
BNT162b1 ^a	Lipid nanoparticle–mRNA	BioNTech, Pfizer, Fosun Pharma	Phases I-III; dose- and candidate-finding in Germany, USA and China	RBD of S protein; two repeated doses of IM injection	Published data from mouse model showing strong antibody and T cell responses	Submitted report indicating safety, high neutralizing antibody titres and T _H 1 cell-type CD4 ⁺ and CD8 ⁺ T cell responses	NCT04368728, Eudra CT 020-001038-36, ChiCTR2000034825	147,148, 166
BBIBP-CoV	Inactivated SARS-CoV-2	Sinopharm, Beijing Institute of Biological Products Co. Ltd	Phases I-III in China and United Arab Emirates	Multiple viral antigens; two repeated doses of IM injection	Published data from rodents, rabbits and NHP models showing neutralizing antibodies and protection	Interim information released to indicate safety and high antibody conversion rates in vaccinees	ChiCTR2000034780, ChiCTR2000032459	127

Table 2 (cont.) | COVID-19 vaccine candidates in or entering clinical trials

Vaccine	Platform	Developer	Clinical trial phase	Immunization attributes	Preclinical data	Clinical data	Clinical Trial registrations	Refs
COVID-19 vaccine	Inactivated SARS-CoV-2	Sinopharm, Wuhan Institute of Biological Products Co. Ltd	Phases I–III in China	Multiple viral antigens; two repeated doses of IM injection	NA	Interim information released to indicate safety	ChiCTR2000034780, ChiCTR2000031809	–
INO-4800 ^a	Plasmid DNA	Inovio Pharmaceuticals, International Vaccine Institute	Phases I–III in USA	Expressing S protein; two repeated doses of intradermal injection plus electroporation	Published data showing immunogenicity in mice and guinea pigs	Interim information released to indicate safety and overall immune responses	NCT04447781, NCT04336410	157
LNP-nCoVsaRNA	Lipid nanoparticle–saRNA	Imperial College London, Morningside Ventures	Phases I and II in UK	Expressing S protein; two repeated doses of IM injection	Published report showing induction of neutralizing antibodies and T _H 1 cell responses in mouse models	NA	ISRCTN17072692	167
COVID-19 vaccine	Inactivated SARS-CoV-2	Chinese Academy of Medical Sciences	Phases I and II in China	Multiple viral antigens; two repeated doses of IM injection	NA	NA	NCT04470609, NCT04412538	–
CVnCoV	Lipid nanoparticle–mRNA	CureVac	Phase I in Germany and Belgium	Expressing S protein; two repeated doses of IM injection	Information released suggesting protection in animal models	NA	NCT04449276	–
Gam-COVID-Vac Lyo	Ad5- or Ad26- vectored, non-replicating	Gameleya Research Institute	Phases I and II in Russia	Single dose and heterologous Ad26 prime–Ad5 boost doses of IM injection	NA	NA	NCT04436471, NCT04437875	–
GX-19	Plasmid DNA	Genexine Consortium	Phases I and II in South Korea	Expressing S protein; two repeated doses of IM injection	NA	NA	NCT04445389	–
SCB-2019	Protein subunit	Clover Pharmaceuticals, GlaxoSmithKline, Dynavax	Phase I in Australia	Trimeric S protein; two repeated doses of IM injection	Information released suggesting induction of neutralizing antibodies in multiple animal species	NA	NCT04405908	–
COVID-19 vaccine	Protein subunit	Anhui Zhifei Longcom Biologic Pharmacy, Chinese Academy of Medical Sciences	Phases I and II in China	Dimeric RBD; two or three repeated doses of IM injection	NA	NA	NCT04445194, NCT04466085	–
ARCoV	mRNA	Academy of Military Medical Sciences, Walvax Biotechnology, Suzhou Abogen Biosciences	Phase I in China	Expressing S protein; two repeated doses of IM injection?	Information released suggesting induction of neutralizing antibodies in mice and NHPs	NA	ChiCTR2000034112	–
COVID-19 vaccine	Plasmid DNA	AnGes Inc., Osaka University, Takara Bio	Phases I and II in Japan	Expressing S protein; two repeated doses of IM injection	NA	NA	JapicCTI-205328, NCT04463472	–

Table 2 (cont.) | COVID-19 vaccine candidates in or entering clinical trials

Vaccine	Platform	Developer	Clinical trial phase	Immunization attributes	Preclinical data	Clinical data	Clinical Trial registrations	Refs
COVID-19 vaccine	Virus-like particle	Medicago, Laval University	Phase I in Canada	Multiple viral antigens; two repeated doses of IM injection	Information released to indicate antibody responses in mice	NA	NCT04450004	–
Lunar-COV19	Self-replicating mRNA	Arcturus Therapeutics, Duke-National University of Singapore	Phases I and II to be launched in Singapore	Expressing S protein; one dose of IM injection	Information released to indicate high levels of neutralizing antibodies after single injection	NA	NCT04480957	–
Covaxin	Inactivated SARS-CoV-2	Bharat Biotech, Indian Council of Medical Research, National Institute of Virology	Phases I and II to be launched in India	Multiple viral antigens; two repeated doses of IM injection	NA	NA	CTRI/2020/07/026300, NCT04471519	–
ZyCov-D	Plasmid DNA	Zydus Cadila	Phases I and II to be launched in India	Expressing S protein; three repeated doses of intradermal injection	Information released to indicate immune responses in several animal species	NA	CTRI/2020/07/026352	–
COVID-19 vaccine	Protein subunit	University of Queensland	Phase I in Australia	Molecular clamp-stabilized S protein; two repeated doses of IM injection	Information released to indicate neutralizing antibodies in animal models	Information released to indicate safety	ACTRN12620000674932p	–
Ad26.COVS2-S ^a	Ad26-vectored, non-replicating	Johnson & Johnson	Phases I and II in USA and Belgium	Expressing S protein; two repeated doses of IM injection	Published data from NHPs showing induction of robust neutralizing antibodies and protection by single dose	NA	NCT04436276	¹¹⁰
KBP-COVID-19	Protein subunit	Kentucky Bioprocessing Inc.	Phases I and II in USA	Recombinant RBD-based protein; two repeated doses of IM injection	NA	NA	NCT04473690	–
COVID-19 vaccine ^a	VSV-vectored, replicating	Merck, IAVI	Phases I and II to be launched in USA?	Expressing S protein; IM injection	NA	NA	–	–
COVAX19	Protein subunit	Vaxine Pty Ltd, Medytox, Central Adelaide Local Health Network	Phase I in Australia	Recombinant S protein with Advax-SM adjuvant; single escalating dose of IM injection	NA	NA	NCT04453852	–
MVC-COV1901	Protein subunit	Medigen Vaccine Biologics, Dynavax	Phase I to be launched in Taiwan	Recombinant S protein; two repeated doses of IM injection	Information released indicating induction of neutralizing antibodies and T cells	NA	NCT04487210	–

Table 2 (cont.) | COVID-19 vaccine candidates in or entering clinical trials

Vaccine	Platform	Developer	Clinical trial phase	Immunization attributes	Preclinical data	Clinical data	Clinical Trial registrations	Refs
COVID-19 vaccine	Plasmid DNA	Entos Pharmaceuticals	Phases I and II to be launched in Canada and USA	Expressing S protein, IM injection	Information released indicating induction of neutralizing antibodies and T cells	NA	–	–

Ad5, human serotype 5 adenovirus; Ad26, human serotype 26 adenovirus; AEs, adverse events; ChAd, chimpanzee adenovirus; COVID-19, coronavirus disease 2019; IM, intramuscular; NA, not available; NHP, non-human primate; RBD, receptor-binding domain; saRNA, self-amplifying RNA; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; S protein, spike protein; T_H1 cell, T helper 1 cell; VSV, vesicular stomatitis virus. *Selected for US Operation Warp Speed.

express antigens derived from the target pathogen. Although only a couple of viral-vectored vaccines have been approved for human use for the control of infections such as Ebola, this platform has been widely investigated and has a well-established track record for infectious diseases and cancer, given its genetic malleability, safety and ability to induce strong T cell responses without the need for an adjuvant^{95,96}. Some viral vectors, such as Ad5 and ChAd, usually need to be administered only once for protection and have natural tropism for the respiratory mucosa, which means they are amenable to respiratory mucosal vaccination⁷⁹. The technology already exists for their large-scale clinical grade production and storage.

Thus, recombinant viral vectors are the second most common platform for COVID-19 vaccine development, with 4 candidates currently in clinical trials (TABLE 2), 38 under preclinical development⁵ and 3 (ChAdOx1 nCoV-19, Ad26-S and VSV-S) selected for US Operation Warp Speed⁹⁷ (TABLE 2). The non-replicating viral platforms are mostly based on Ad5 or MVA, and most of these vaccine candidates express the S protein or RBD of SARS-CoV-2. Replication-competent viral vectors are mainly based on the vaccine strains of other human pathogens (such as measles or influenza viruses) or veterinary pathogens (such as vesicular stomatitis virus (VSV)). However, it will be important to consider whether humans have pre-existing immunity against the viral backbone (TABLE 1). Pre-existing antibodies can impair the ability of such vaccines to engage the immune system. Use of viral backbones such as ChAd, for which humans have little to no pre-existing immunity, can help to circumvent this issue⁷⁹.

Ad5-nCoV, which is being developed by the Chinese vaccine company CanSino Biologics, is designed to induce neutralizing antibodies to SARS-CoV-2 S protein following intramuscular injection (TABLE 2). Without published preclinical data, it entered phase I/II clinical trials with three doses of vaccine tested^{98,99}. Of note, these doses are 10–30 times higher than those used in previous trials of intramuscular vaccines^{100–102}. Whereas the highest dose generated unacceptable toxicity and was dropped from the phase II study⁹⁹, the smaller doses induced S protein-specific neutralizing antibodies in only 50% of the vaccine recipients⁹⁸. The phase II study largely reaffirms the phase I observations that, although the vaccine induces both antibody and T cell responses, its potency is

reduced by pre-existing immunity to Ad5, particularly in elderly participants⁹⁹. Depending on geographical region, 35–95% of humans have significant circulating levels of neutralizing antibodies to Ad5 (REF.¹⁰³). This is consistent with the rapidly declining antibody titres observed in a phase II Ad5-Ebola vaccine study¹⁰⁴. The vaccine is entering further advanced trials in China and Canada, but the efficacy of this strategy is now in question¹⁰⁵. Another human adenovirus-based COVID-19 vaccine, known as Ad26-S, is being developed by Johnson & Johnson, although there is still 40% seroprevalence for Ad26 in humans¹⁰⁶. As Ad26 is inherently less immunogenic than Ad5 (REF.¹⁰⁷), effective immunity requires repeated homologous or heterologous vaccination, as has been shown in Ad26-HIV and Ad26-Ebola vaccine studies in humans^{108,109}. Nevertheless, a single parenteral administration of an Ad26-vectored COVID-19 vaccine (Ad26.COVS.2) offered robust protection in a non-human primate model of SARS-CoV-2 (REF.¹¹⁰).

ChAdOx1 nCoV-19 (also known as AZD-1222), which is being developed by Oxford University, UK, and AstraZeneca, is the most clinically advanced COVID-19 vaccine (TABLE 2). Humans have low seroprevalence for ChAd, hence its strong immunogenicity and utility for heterologous prime-boost COVID-19 vaccination^{79,107,111}. The development of ChAdOx1 nCoV-19 is based on promising human studies with ChAdOx1-MERS vaccine¹¹² and ChAdOx1-TB vaccine¹¹³. However, although intramuscular delivery of ChAdOx1 nCoV-19 reduced SARS-CoV-2 viral load in the lungs and prevented pneumonia in rhesus macaques, it did not reduce viral loads in the upper respiratory tract¹¹⁴. A recently reported phase I/II study shows its safety and the induction of potent neutralizing antibody and T cell responses following a single parenteral injection, which are boosted further by a second homologous vaccination¹¹⁵. It remains unclear from this trial to what extent both CD4⁺ and CD8⁺ T cell subsets were activated.

VSV-S is a replication-competent COVID-19 vaccine under development by Merck¹¹⁶ and other groups. Merck's vaccine is built upon the licensure of its highly efficacious VSV-Ebola vaccine, which induces neutralizing antibodies and cellular immunity against Ebola virus surface glycoprotein¹¹⁷. VSV is a veterinary virus to which humans have no pre-existing immunity. However, the cloning capacity of the VSV vector is

US Operation Warp Speed

A public-private partnership initiated and funded by the US government to accelerate and coordinate the development, manufacture and distribution of coronavirus disease 2019 (COVID-19) vaccines, therapeutics and diagnostics. It was introduced by the Trump administration in early April 2020.

Box 3 | Animal models of COVID-19 for vaccine testing

There is an urgent need to identify suitable animal models for the preclinical evaluation of coronavirus disease 2019 (COVID-19) vaccines¹⁸². A large number of animal species have differing degrees of susceptibility to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, depending on the relative binding affinity of the virus to the host angiotensin-converting enzyme 2 (ACE2) receptor or on host protease activities on the S protein¹⁸³.

Among the animal species tested, ACE2 of rhesus macaques has the greatest binding activity for SARS-CoV-2 (REF. 183). Infected macaques shed SARS-CoV-2 from the upper and the lower respiratory tract but they do not develop the same clinical signs and age-dependent disease severity as humans^{184,185}. Cats, ferrets and hamsters are also susceptible to SARS-CoV-2 infection. Notably, natural airborne and contact transmissions of SARS-CoV-2 have been reported in cats and hamsters, respectively, but not in ferrets¹⁸⁶. Hamsters, but not cats and ferrets, manifest severe clinical symptoms. Thus, these animal models are differentially capable of recapitulating relevant aspects of COVID-19.

Mouse models are widely used for vaccine testing owing to their affordability and the availability of immunoreagents and transgenic mouse strains. However, the ACE2 of conventional mice does not bind well to SARS-CoV S protein¹⁸⁷. Transgenic mice expressing human ACE2 were initially developed and thoroughly characterized for the study of SARS-CoV and have now been shown to support SARS-CoV-2 replication in the lung, and these mice develop interstitial pneumonia similar to humans¹⁸⁸. Human ACE2-expressing mice that are further humanized to express human HLA genes and/or to have human immune cells will be useful for studying human immune responses and immunodominant epitopes following vaccination and viral infection with SARS-CoV-2. Beyond animal models, of further relevance to human applications is the ongoing ethical debate regarding intentional challenge of vaccinated young people with SARS-CoV-2.

limited to 4 kb, and its suitability for respiratory mucosal vaccination is unclear. A single parenteral vaccination with a VSV vector expressing S protein provides protection against SARS-CoV-2 in both mouse and hamster models^{118,119}. Among other viral-vectored candidates is non-replicating MVA. MVA has widely been explored as a vaccine carrier and has a cloning capacity of up to 30 kb. However, as it is not robustly immunogenic, MVA is often used as a booster vaccine or repeated injection is required to be effective, as was the case in clinical testing of an MVA-MERS-S vaccine¹²⁰.

Inactivated viral vaccines. Physically or chemically inactivated viruses have been used successfully in human vaccines against polio, hepatitis A and influenza^{121,122}. Inactivated viruses can be rapidly generated and scaled up in a pandemic situation using well-established infrastructure and methods¹²³. Inactivated viral vaccines have few safety concerns, unlike their live attenuated counterparts, and they express a wide range of native viral antigens, including surface antigens with retained epitope conformations that can induce conformation-dependent antibody responses^{124,125}.

Currently, there are five early clinical trials to assess inactivated SARS-CoV-2 vaccines (TABLE 2), with an additional nine candidates in preclinical development⁵. PiCoVacc, an inactivated SARS-CoV-2 and alum-adjuvanted vaccine developed by Sinovac Biotech Ltd in China, is the most advanced candidate with published preclinical results¹²⁶. It protects rhesus macaques against SARS-CoV-2, with reduced viral titres and immunopathology associated with antibodies to S protein and nucleocapsid¹²⁶. BBIBP-CorV, another inactivated virus candidate, which is being developed

by Chinese state-owned Sinopharm, was tested in a range of animal models, with demonstrated efficacy in non-human primates¹²⁷. Although these findings provide optimism, the observations were made in rather short-term studies and should be interpreted with caution.

Inactivated viral vaccines often require an adjuvant and repeated administration to be effective (TABLE 1). The use of alum as an adjuvant^{126,127} makes them unsuitable for respiratory mucosal delivery¹²⁸. Although the protection mediated by intramuscular immunization with PiCoVacc or BBIBP-CorV indicates some level of mucosal immunity, probably through the transport of systemic antibodies to the lungs, the durability of such immunity remains unclear as SARS-CoV-2 challenge was performed 1–4 weeks after vaccination^{126,127}. Furthermore, similarly to protein subunit vaccines, inactivated viral vaccines are poor inducers of cytotoxic CD8⁺ T cells, which are likely to be required for an effective COVID-19 vaccine.

Studies with inactivated SARS-CoV and respiratory syncytial virus vaccines have reported vaccine-related enhancement of disease, likely involving a T_{H2} cell response and lung eosinophilia, which may be worsened in aged hosts^{56,74,129}. Although PiCoVacc or BBIBP-CorV did not worsen lung disease within 7 days after infection, alum is known to drive T_{H2} cell-mediated immune responses, which warrants further safety investigations. The use of T_{H1} cell-skewing modified alum or other adjuvants such as CpG may avert such safety concerns^{130,131}.

Protein subunit vaccines. Currently, there are seven COVID-19 subunit vaccines in clinical trials (TABLE 2), with 50 other candidates under preclinical development, making this the most common platform⁵. Subunit vaccines primarily induce CD4⁺ T_H cell and antibody responses. Therefore, most of these vaccines contain full-length SARS-CoV-2 S protein or portions of it with the goal of inducing neutralizing antibodies, similarly to the majority of SARS and MERS vaccines, which had differing levels of efficacy^{132–134}.

Subunit vaccines can be designed to focus the immune response towards neutralizing epitopes, thereby averting the production of non-neutralizing antibodies that may promote ADE of disease¹³⁵. However, unlike nucleic acid-based or viral-vectored vaccines, recombinant S proteins in subunit vaccines could have an improper epitope conformation unless they are produced in mammalian cells¹³⁶. Proteins or peptides alone are poorly immunogenic and generally require not only an adjuvant but also repeated administration, and they are poor activators of CD8⁺ T cell responses (TABLE 1). Furthermore, this platform is generally unsuitable for respiratory mucosal vaccination. As is the case for inactivated viral vaccines, use of unmodified alum as an adjuvant skews the immune response towards T_{H2} cell-like responses⁵⁶, which is undesirable for host defence against SARS-CoV-2 and may have a role in ADE of disease^{74,130}. In this regard, subunit COVID-19 vaccines being developed by GlaxoSmithKline and Novavax use AS03 and Matrix-M adjuvants, respectively⁵.

Virus-like particles. VLPs are spontaneously forming particles composed of several structural viral proteins that are co-expressed or admixed. Several commercial vaccines, such as hepatitis B and human papillomavirus vaccines, are based on VLPs¹³⁷. In the case of enveloped coronaviruses, VLPs form when the viral proteins S, M and E, with or without N, are co-expressed in eukaryotic producer cells^{138,139}. This results in active budding from the producer cells of VLPs that are structurally identical to the infectious virus but lack the viral genome and thus are non-infectious. The presence of S protein on the surface of VLPs enables them to bind and enter ACE2⁺ cells in the same manner as the parent virus¹⁴⁰. Unlike subunit vaccines, the array of S protein on the VLP surface crosslinks the B cell receptor and directly activates B cells, but, like subunit and inactivated viral vaccines, VLPs also typically require an adjuvant and repeated administration¹³⁷. Notwithstanding this, the VLP technology is well established, the biology and safety of coronavirus VLPs are understood and their large-scale production to Good Manufacturing Practice standards is relatively straightforward.

Currently, there is only 1 VLP-based COVID-19 vaccine in clinical trials (TABLE 2), with 12 more under preclinical development⁵. These are produced either *in vivo* from a viral vector, such as MVA, that expresses the VLP components (a platform being developed by GeoVax) or more often *in vitro* from producer cells. Notably, Medicago, a Canadian company, produces its SARS-CoV-2 VLPs from genetically engineered plants. Its unpublished results seem to suggest efficacy in inducing neutralizing antibodies in mice¹⁴¹.

Nucleic acid-based vaccines. Recombinant plasmid DNA has been explored as a vaccine platform for decades, whereas mRNA has emerged more recently as a promising platform^{142,143}. Currently, there are 6 mRNA-based COVID-19 vaccines and 4 DNA-based COVID-19 vaccines in clinical trials (TABLE 2), with 27 such vaccines (16 mRNA-based and 11 DNA-based vaccines) under preclinical development⁵.

The antigen-encoding mRNA complexed with a carrier such as lipid nanoparticles can be efficiently delivered *in vivo* into the cytoplasm of host cells for protein translation and post-translational modifications^{142,144}, which is an advantage over recombinant protein subunit vaccines. mRNA vaccines are non-infectious and are synthesized by *in vitro* transcription, free of microbial molecules. These beneficial features differentiate mRNA vaccines from live attenuated viral vaccines, inactivated viral vaccines, subunit vaccines and recombinant viral-vectored vaccines in terms of safety, efficacy and issues of antivector immunity, enabling their rapid and inexpensive production and repeated vaccination¹⁴² (TABLE 1).

mRNA-1273, which is produced by Moderna, an American biotech company that has experience with mRNA-based MERS vaccines, encodes a prefusion-stabilized SARS-CoV-2 S protein encapsulated in lipid nanoparticles. It entered clinical testing even before the release of preclinical data¹⁴⁵. Recently published phase I clinical trial data indicate that low and medium

doses of two repeated parenteral injections are generally safe and induce strong S protein-specific antibody responses and a primarily CD4⁺ T cell response in most trial participants¹⁴⁶. Pfizer and BioNTech are also assessing an mRNA–lipid nanoparticle vaccine encoding the S protein RBD (known as BNT162b1) in humans, who developed robust S protein-specific antibody and CD4⁺ and CD8⁺ T cell responses following two repeated parenteral injections^{147,148}. The Pfizer/BioNTech and Moderna vaccines have both been selected for US Operation Warp Speed⁹⁷ (TABLE 2).

Although no mRNA vaccine has yet been licensed for human use, their potential is supported by previous studies of influenza, rabies and Zika virus infections in animals^{149–153}. For example, an mRNA vaccine for influenza virus induced long-term humoral immunity in young and aged mice¹⁴⁹, and an mRNA vaccine for Zika virus induced both antibodies and cytotoxic CD8⁺ T cells in mice¹⁵⁴. However, two clinical studies show disparities in the magnitude and longevity of immune responses induced by mRNA vaccines^{152,155}. Thus, although mRNA-based COVID-19 vaccines show promise from early clinical testing, questions remain about their protective efficacy in humans. It is also unclear whether mRNA vaccines are amenable to respiratory mucosal delivery.

Plasmid DNA vaccines share several characteristics with mRNA vaccines, including safety, ease of production and scalability¹⁵⁶. However, they are poorly immunogenic, requiring multiple doses and the addition of an adjuvant. Currently, there are four plasmid DNA-based COVID-19 vaccines in clinical testing (TABLE 2), with 11 more under preclinical development. INO-4800, a plasmid DNA vaccine expressing SARS-CoV-2 S protein, is being developed by the US biotech company Inovio Pharmaceuticals. A preclinical study in mice and guinea pigs examined the immunogenicity of this vaccine but did not provide any data pertaining to protection against challenge¹⁵⁷. Two repeated injections of an S protein-expressing plasmid DNA vaccine resulted in robust protective immunity in rhesus macaques¹⁵⁸.

Conclusions and outlook

The world is in dire need of safe, effective COVID-19 vaccine strategies. Many laboratories and companies have scrambled to rapidly develop these vaccines, resulting in more than 160 vaccine candidates, with a handful having entered phase I, II and III clinical trials within a short period of 6 months. Although we are just beginning to understand COVID-19 and its vaccine requirements, most of the advanced vaccine platforms have been extensively explored for other infections and cancer^{79,95,96,159}. While it is important to pursue various vaccine strategies in parallel, it is equally important not to lose sight of this existing scientific knowledge to make well-informed decisions around which strategies to prioritize.

The various vaccine platforms and strategies have their immunological pros and cons (TABLE 1), but modern immunological principles and data from prior studies of similar platforms lead us to surmise that a parenteral COVID-19 vaccine strategy capable of inducing a robust, durable response involving both neutralizing antibodies

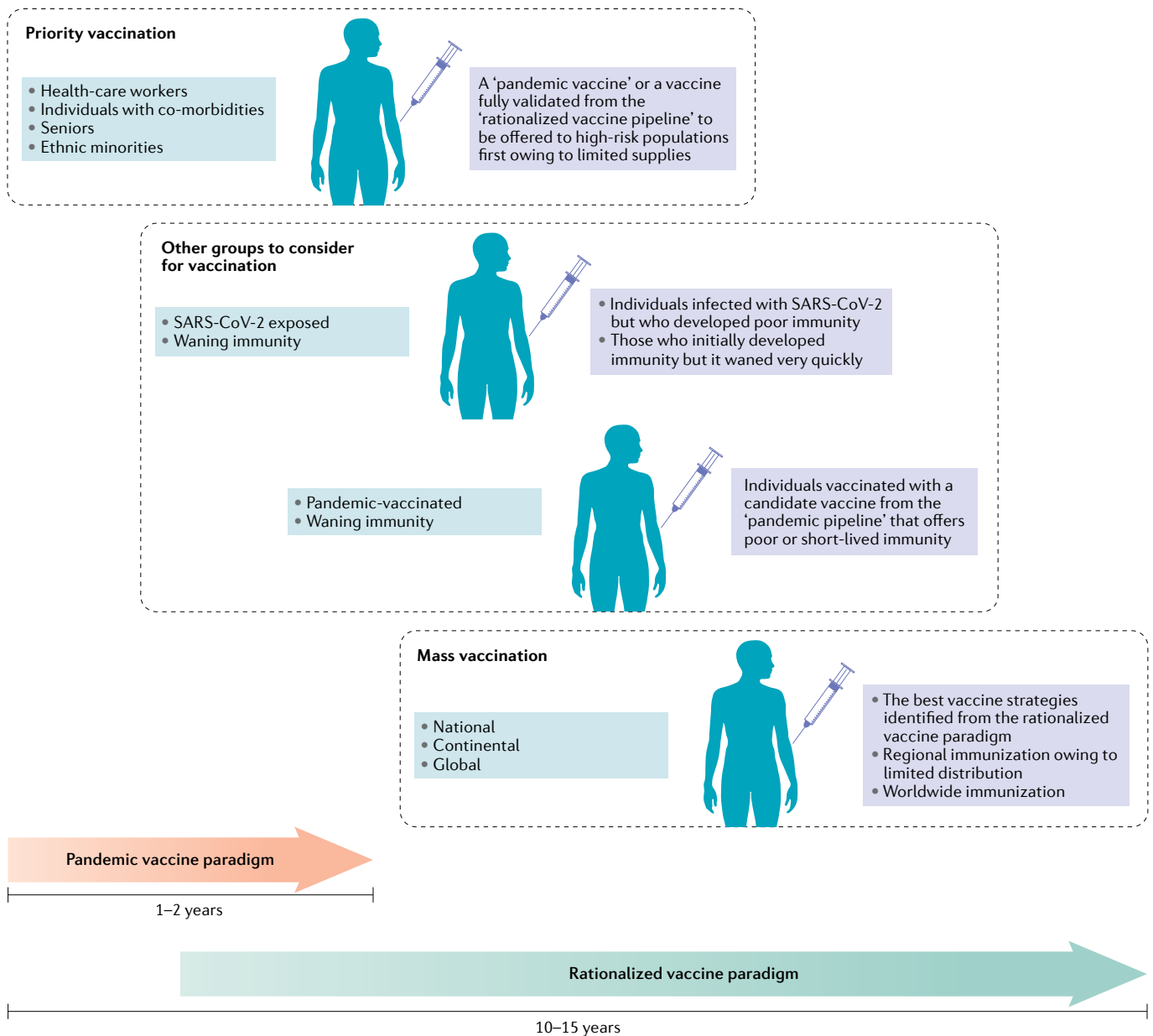


Fig. 2 | Evolving scenarios for global COVID-19 vaccine development and demand. In response to the urgent demand for a vaccine, more than two dozen candidate vaccines are advancing through clinical trials following an expedited pandemic vaccine development paradigm, with many steps of the development process occurring in parallel before a successful outcome of previous steps has been confirmed. Vaccine candidates will continue to be preclinically and clinically evaluated following conventional and/or rationalized vaccine development processes over the next few years. These efforts will evolve to meet the demands for vaccination in several likely scenarios that are predicted on the basis of sociopolitical challenges and the emerging data regarding the trajectory of the coronavirus disease 2019 (COVID-19) pandemic and the host response to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). One scenario is the priority

vaccination of high-risk populations such as health-care workers, seniors, people with co-morbidities and ethnic minorities, who have been disproportionately affected by COVID-19, when vaccine supply is initially limited. Aside from these prioritized groups, it may also be necessary to consider that asymptomatic individuals, patients who have recovered from COVID-19 but generated poor immunity or whose immunity quickly waned, and individuals who received a rapidly developed 'pandemic' vaccine that provided suboptimal protection or rapidly waning immune responses may require a booster vaccination to ensure sufficient levels of population protection for herd immunity. Ultimately, regional, continental and global populations will be subject to mass vaccination programmes based on the extent of national and global vaccine distribution and also likely according to the relative regional severity of outbreaks.

and T cells should provide a significant level of protection. Almost all of the current vaccines in the human immunization programme are delivered via the skin or muscle, and most of the current COVID-19 vaccine strategies also focus on the parenteral route of vaccination (TABLE 2). We further surmise that a respiratory mucosal

vaccine strategy capable of inducing these responses directly in the respiratory mucosa will be most effective in the early control or clearance of SARS-CoV-2. This is particularly relevant to high-risk elderly populations, who will require a particularly robust vaccine strategy. In this regard, a respiratory mucosal vaccine strategy for

COVID-19 may draw on the successful experience in respiratory mucosal delivery of influenza, measles and TB vaccines to humans^{160–162}. Respiratory mucosal vaccination also has the advantages of being needle-free and requiring a much smaller dose than the parenteral route. However, compared with the parenteral route, fewer vaccine platforms are safe and effective for respiratory mucosal vaccination. Furthermore, the use of inhalational devices for respiratory mucosal delivery may potentially be a limiting factor for widespread application in resource-poor settings.

According to the pandemic vaccine development paradigm (FIG. 2), the conventional vaccine development milestones are compressed from a time frame of 10–15 years to 1–2 years, with overlapping preclinical, clinical and scale-up manufacturing processes occurring in parallel⁶. Owing to the accelerated development process, the interim data from ongoing clinical and preclinical vaccine studies are being published almost in real time. As a result, crucial information about the longevity and quality of vaccine-induced protective immunity is unavailable. As transmission rates and the numbers of new cases have reduced in many countries, it is uncertain whether the phase II and phase III studies of the front-runner candidates will reach a reliable conclusion with regard to their protective efficacy. Furthermore, these vaccine candidates have been studied in isolation, which makes it difficult to directly compare the effectiveness of different candidates. Thus, it would be premature to hail the safety and immunogenicity observed in COVID-19 vaccine trials as a real success. To a large extent, such outcomes could be anticipated from past studies testing the same platforms and delivery routes. Nevertheless, rapid development of a vaccine

with preclinical efficacy data but limited clinical data to high-risk populations may be necessary (FIG. 2).

The evolving process of vaccine development will continue over the next few years until more clinical trials are completed, additional vaccine strategies are evaluated and host defence against SARS-CoV-2, including postinfection immunity, is better understood (FIG. 2). Probably not until then will global mass immunization become a reality. It is possible that the populations that receive the first round of vaccines will have waning immunity and require boosting using improved second-generation COVID-19 vaccines. Furthermore, in addition to unexposed individuals, some individuals who have recovered from COVID-19 who develop poor or waning immunity may also require vaccination¹⁶³.

Given the challenges in resources, manufacturing and issues associated with distribution and regional protectionism, the implementation of vaccination programmes will likely be uneven, asynchronous and variable — involving different vaccine platforms and strategies around the globe^{164,165}. In this regard, some resource-rich countries have already secured large numbers of doses of different candidate vaccines without knowing which one may prove effective. The heated debate has begun globally over who should be at the front of the line when vaccine supply is limited. The founding of the COVID-19 Vaccines Global Access (COVAX) Facility by Gavi, the Coalition for Epidemic Preparedness Innovations (CEPI) and the WHO is an attempt to garner resources and unite higher- and lower-income countries for the coordinated, rapid, transparent and equitable access to COVID-19 vaccines worldwide.

Published online 4 September 2020

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Acknowledgements

The work was supported by the Canadian Institutes of Health Research (CIHR) COVID-19 Rapid Research Program, the CIHR Foundation Program, the National Sanitarium Association of Canada Innovative Research Program, the Natural Sciences and Engineering Research Council of Canada, the Canadian Foundation for Innovation, Ontario Government, and McMaster University.

Author contributions

The authors contributed equally to all aspects of the article.

Competing interests

The authors declare no competing interests.

Peer review information

Nature Reviews Immunology thanks P. Klenerman and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

Publisher's note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

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