

# SARS-CoV-2 vaccines in development

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Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was first reported in late 2019 in China and is the causative agent of the coronavirus disease 2019 (COVID-19) pandemic. To mitigate the effects of the virus on public health, the economy and society, a vaccine is urgently needed. Here I review the development of vaccines against SARS-CoV-2. Development was initiated when the genetic sequence of the virus became available in early January 2020, and has moved at an unprecedented speed: a phase I trial started in March 2020 and there are currently more than 180 vaccines at various stages of development. Data from phase I and phase II trials are already available for several vaccine candidates, and many have moved into phase III trials. The data available so far suggest that effective and safe vaccines might become available within months, rather than years.

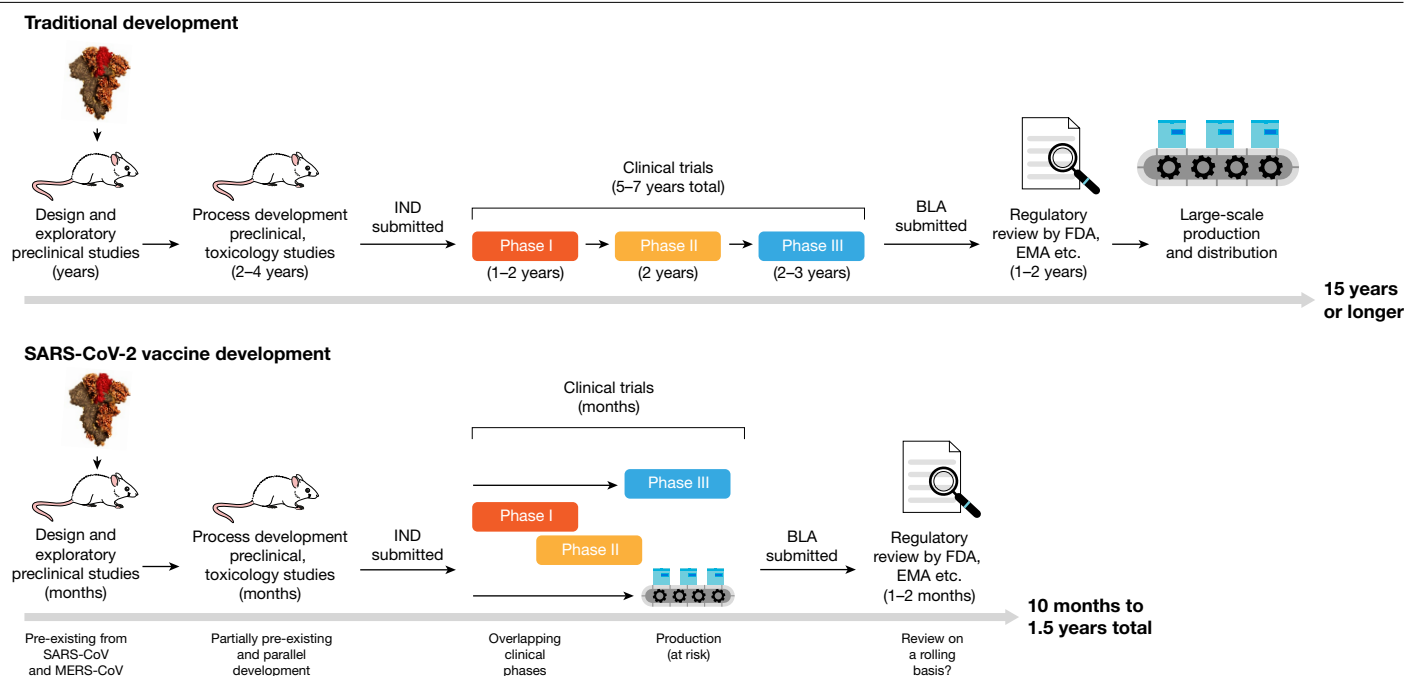
In late December 2019, cases of pneumonia with unknown aetiology were reported in the city of Wuhan, China<sup>1</sup>. The causative agent, identified as the betacoronavirus SARS-CoV-2, is closely related to SARS-CoV, which was responsible<sup>2</sup> for the outbreak of SARS between 2002 and 2004. SARS-CoV-2 caused a sizable epidemic of COVID-19 in China, then spread globally and was declared a pandemic in March 2020. Coronaviruses are enveloped viruses with a large, single-stranded, positive-sense RNA genome. Four such coronaviruses—two alphacoronaviruses (NL63 and 229E) and two betacoronaviruses (HKU1 and OC43)—circulate in humans and cause common colds<sup>3</sup>. All four of these viruses are thought to be zoonotic in origin, and OC43 has been proposed as a potential aetiological agent of the 1889–1890 ‘Russian flu’ pandemic<sup>3,4</sup>; this possibility was suggested by a phylogenetic analysis<sup>4</sup> determining that OC43 and bovine coronavirus (BCoV) split from a common ancestor around 1890. In addition, SARS-CoV and Middle Eastern respiratory syndrome coronavirus (MERS-CoV) have more recently caused zoonotic infections and epidemics with high case fatality rates in humans<sup>3</sup>. No vaccines against coronaviruses have yet been licensed for use in humans. Their development had previously been considered as low priority because the coronaviruses that were circulating in humans caused relatively mild disease; in addition, a vaccine would need to be quadrivalent—effective against four different viruses—and even then would prevent only a minor proportion of colds, because the majority are caused by other viruses. As such, the development of vaccines against human coronaviruses was not pursued. After the 2002–2004 SARS outbreak, vaccines against SARS-CoV were developed preclinically and two were tested in phase I trials<sup>5,6</sup>. However, development was stopped because the virus was eradicated from the human population and has not re-emerged since 2004. Vaccines against MERS-CoV are currently under active development, and have been supported by the Coalition for Epidemic Preparedness Innovations (CEPI). Through preclinical studies of vaccines against SARS-CoV and MERS-CoV, the antigenic target for coronavirus vaccines has become clear<sup>7,8</sup> (Fig. 1b). Most coronaviruses encode only one large surface protein, the spike protein, which is responsible for receptor binding and membrane fusion<sup>9</sup>. In the case of SARS-CoV-2 (and SARS-CoV), the spike protein binds to angiotensin-converting enzyme 2 (ACE2) on host cells and is then endocytosed<sup>10,11</sup>. This step is followed by fusion

of viral and endosomal membranes and release of the viral genome into the cytoplasm<sup>9,12</sup>. Antibodies that bind to the spike protein, especially to its receptor-binding domain (RBD), prevent its attachment to the host cell and neutralize the virus. On the basis of this knowledge, and information gained from preclinical studies with SARS-CoV and MERS-CoV<sup>13</sup>, the spike protein was identified as an antigenic target for the development of a vaccine against SARS-CoV-2 at a very early stage.

Since the onset of the COVID-19 pandemic we have learned much about the immune response to SARS-CoV-2 after natural infection, and these lessons have corroborated our initial assumptions. Antibodies directed to the spike protein, both those that target the RBD and those that target other regions of the protein, have been shown to neutralize the virus<sup>14–18</sup>. In addition, although the magnitude of the antibody response to the spike protein is very varied, it seems so far to resemble a typical antibody response to a respiratory virus: an initial plasmablast-derived boost of antibodies, followed by some decline and then a potential stabilization at a baseline that is maintained by long-lived plasma cells<sup>17,19,20</sup>. Mucosal antibody responses are also induced by natural infection in humans<sup>19,21</sup>. In addition, it has been demonstrated that the spike protein is a strong target of CD4<sup>+</sup> T cells, whereas fewer CD8<sup>+</sup> T cells are induced by natural infection with SARS-CoV-2 in general<sup>22</sup>. In non-human primates (NHPs), infection with SARS-CoV-2 has been shown to protect against re-infection<sup>23,24</sup>. Vaccination experiments in NHPs showed that neutralizing antibodies, but not T cell responses, correlated with protection<sup>25</sup>. Neutralizing antibodies have now also been implicated as a correlate of protection in humans after studies of an outbreak on a fishing vessel<sup>26</sup>; however, it is important to note that natural infection induces both mucosal antibody responses (secretory immunoglobulin A (IgA)) and systemic antibody responses (IgG). The upper respiratory tract is thought to be mainly protected by secretory IgA, whereas the lower respiratory tract is thought to be mainly protected by IgG<sup>27–29</sup>. Vaccines that are administered intramuscularly or intradermally induce mainly IgG, and no secretory IgA<sup>30</sup>. It is therefore possible that most vaccines currently in development induce disease-preventing or disease-attenuating immunity, but not necessarily sterilizing immunity (Fig. 2).

Traditional vaccine development is a lengthy process, and a development time of 15 years is common (Fig. 1a). The process begins with

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**Fig. 1 | Traditional and accelerated vaccine-development pipelines.** Traditional vaccine development can take 15 years or more, starting with a lengthy discovery phase in which vaccines are designed and exploratory preclinical experiments are conducted. This is usually followed by a phase in which more formal preclinical experiments and toxicology studies are performed and in which production processes are developed. During this process an investigational new drug (IND) application is filed and the vaccine candidate then enters phase I, II and III trials. If, when phase III trials are completed, the predetermined end points have been met, a biologics licence application (BLA) is filed, reviewed by regulatory agencies and finally the

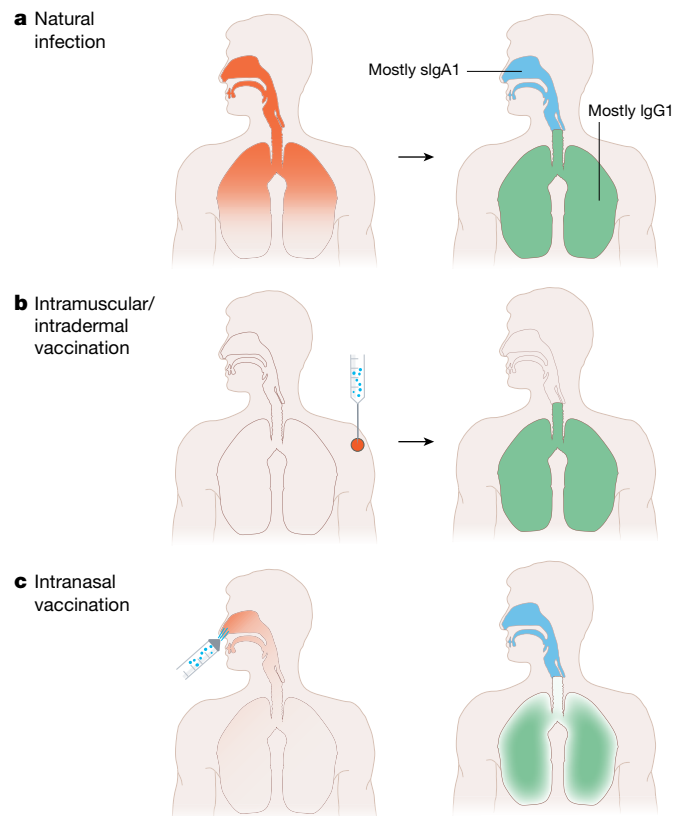
vaccine is licensed. After that point, large-scale production begins. Vaccine development for SARS-CoV-2 is following an accelerated timeline. Because of knowledge gained from the initial development of vaccines for SARS-CoV and MERS-CoV, the discovery phase was omitted. Existing processes were adopted, and phase I/II trials were started. Phase III trials were initiated after the interim analysis of phase I/II results, with several clinical trial stages running in parallel. In the meantime, vaccine producers have started the large-scale production of several vaccine candidates, at risk. The exact pathway by which these vaccine candidates will be licensed—for example, through an initial emergency use authorization—is not yet clear.

exploratory work on vaccine design and evaluation in animal models, which can take years. This is then followed by a stage in which more formal preclinical experiments are conducted, a process for vaccine production is designed and formal toxicology studies are performed; this stage can also last for several years. Next, an application for an investigational new drug is filed and phase I clinical trials (testing in fewer than 100 individuals; approximately 2 years) are performed to generate an initial safety profile of the vaccine candidate and to obtain preliminary immunogenicity data. If the results are promising and funding is available, a vaccine candidate is then moved into phase II clinical trials (testing in a few hundred individuals, also lasting about 2 years) to further investigate immunogenicity and to determine an appropriate dose and optimal vaccine regimens. If the results of phase II trials are encouraging, the decision might be made to move forward with very costly phase III clinical trials (in thousands of individuals; approximately 2 years) in which efficacy and safety are evaluated. If the outcome of phase III trials meets the pre-defined end points, a biologics license application is filed with regulatory agencies (for example, the United States Food and Drug Administration (FDA) or the European Medicines Agency). The licensing process can take another 1–2 years, especially if additional data are requested. Importantly, because it is very expensive, the overall process of vaccine development is slowed by economic risk assessment at every step. Vaccine development progresses through these stages only if the developer is convinced that the data are promising, that the risk of failure is relatively low and that there is (still) a market for the vaccine.

The SARS-CoV-2 pandemic has required rapid action and the development of vaccines in an unprecedented timeframe (Fig. 1b). Data from the preclinical development of vaccine candidates for SARS-CoV

and MERS-CoV enabled the initial step of exploratory vaccine design to be essentially omitted, saving a considerable amount of time. In many cases, production processes were simply adapted from those of existing vaccines or vaccine candidates, and in certain cases pre-clinical and toxicology data from related vaccines could be used. As a result, the first clinical trial of a vaccine candidate for SARS-CoV-2 began in March 2020 (NCT04283461). Trials were designed such that clinical phases are overlapping and trial starts are staggered, with initial phase I/II trials followed by rapid progression to phase III trials after interim analysis of the phase I/II data. Currently, several manufacturers have already started the commercial production of vaccines—at risk—without any results from phase III trials. Although the licensure pathways are not yet completely clear, it is possible that reviews could be expedited and that vaccines could even be approved through an emergency use authorization. The FDA has released a guidance document for the development and licensure of SARS-CoV-2 vaccines, which—as well as providing additional details—states that an efficacy of at least 50% will be required<sup>31</sup>. It is very important to point out that moving forward at financial risk is the main factor that has enabled the accelerated development of SARS-CoV-2 vaccine candidates, and no corners have been or should be cut in terms of safety evaluation.

Although vaccine development is moving forward at an unparalleled speed, there are still many open questions. It is likely that two doses of a vaccine will be required, with booster doses potentially necessary at later time points; in this case, at least 16 billion doses will be needed to meet the global demand. Many of the vaccines that are described below are being developed by entities that have never brought a vaccine to market, or use technologies that have never resulted in



**Fig. 2 | Mucosal and systemic immune responses to natural infection with respiratory viruses and to vaccination.** The lower human respiratory tract is thought to be mostly protected by IgG (IgG1 is most prevalent), the main type of antibody in serum, which is transported into the lung. The upper respiratory tract is thought to be mostly protected by secretory IgA (sIgA1). **a**, Natural infection with respiratory viruses induces both a systemic immune response, dominated by IgG1, as well as a mucosal immune response in the upper respiratory tract that is dominated by sIgA1. This process can lead to sterilizing immunity for many respiratory viruses. **b**, Intramuscular or intradermal vaccination leads in many cases to a strong induction of serum IgG but not to an induction of mucosal IgA. Although some IgG can also be found on the mucosal surfaces of the upper respiratory tract, the lack of sIgA often leaves an individual vulnerable to infection of the upper respiratory tract. **c**, Intranasal vaccination can efficiently induce mucosal antibody responses, thereby potentially providing sterilizing immunity in the upper respiratory tract. However, systemic immune responses are often lower after this type of vaccination. Currently, all SARS-CoV-2 vaccine candidates in clinical development are administered intramuscularly, and very few of the more than 180 vaccine candidates in development are designed to induce mucosal immunity. Although mucosal immunity might not be required to protect from severe or even symptomatic disease, it could be required to achieve optimal protection from infection and onward transmission of SARS-CoV-2.

a licensed vaccine. Therefore, unforeseen issues with scaling could cause delays. It is also not yet clear whether bottlenecks will occur in the availability of, for example, syringes or glass vials; how vaccines will be distributed globally; and how rollout will occur within countries. Finally, for certain vaccine candidates against SARS-CoV and MERS-CoV, vaccine-enhanced disease was reported in some animal models (Box 1). For SARS-CoV-2 vaccine candidates, there have so far been no signals of enhanced disease in animal models or in humans; however, such a safety signal would certainly derail the development of a vaccine candidate and would negatively affect vaccine development in general.

Below I review the types of SARS-CoV-2 vaccine in the pipeline, as well as initial data from NHP studies, phase I and phase I/II trials.

## Types of vaccine in development

More than 180 vaccine candidates, based on several different platforms (Fig. 3), are currently in development against SARS-CoV-2<sup>32</sup> (Fig. 4). The World Health Organization (WHO) maintains a working document<sup>32</sup> that includes most of the vaccines in development and is available at <https://www.who.int/publications/m/item/draft-landscape-of-covid-19-candidate-vaccines>. The platforms can be divided into 'traditional' approaches (inactivated or live-virus vaccines), platforms that have recently resulted in licensed vaccines (recombinant protein vaccines and vectored vaccines), and platforms that have yet to result in a licensed vaccine (RNA and DNA vaccines).

### Inactivated vaccines

Inactivated vaccines (Fig. 3c) are produced by growing SARS-CoV-2 in cell culture, usually on Vero cells, followed by chemical inactivation of the virus<sup>33,34</sup>. They can be produced relatively easily; however, their yield could be limited by the productivity of the virus in cell culture and the requirement for production facilities at biosafety level 3. Examples of inactivated vaccine candidates include CoronaVac (initially known as PiCoVacc), which is under development by Sinovac Biotech in China<sup>34,35</sup> and is further discussed below, as well as several other candidates that are being developed in China, by Bharat Biotech in India and by the Research Institute for Biological Safety Problems in Kazakhstan. These vaccines are usually administered intramuscularly and can contain alum (aluminium hydroxide) or other adjuvants. Because the whole virus is presented to the immune system, immune responses are likely to target not only the spike protein of SARS-CoV-2 but also the matrix, envelope and nucleoprotein. Several inactivated vaccine candidates have entered clinical trials, with three candidates from China in phase III trials, and one from India, one from Kazakhstan and two from China in phase I or II clinical trials<sup>32</sup> (Fig. 4).

### Live attenuated vaccines

Live attenuated vaccines (Fig. 3d) are produced by generating a genetically weakened version of the virus that replicates to a limited extent, causing no disease but inducing immune responses that are similar to that induced by natural infection (Fig. 2). Attenuation can be achieved by adapting the virus to unfavourable conditions (for example, growth at lower temperature, growth in non-human cells) or by rational modification of the virus (for example, by codon de-optimization or by deleting genes that are responsible for counteracting innate immune recognition<sup>36,37</sup>). An important advantage of these vaccines is that they can be given intranasally, after which they induce mucosal immune responses that can protect the upper respiratory tract (Fig. 2)—the major entry portal of the virus. In addition, because the virus is replicating in the vaccinated individual, the immune response is likely to target both structural and non-structural viral proteins by way of antibodies and cellular immune responses. However, disadvantages to these vaccines include safety concerns and the need to modify the virus, which is time-consuming if carried out by traditional methods and technically challenging when reverse genetics is used. Only three live attenuated vaccines are currently in preclinical development (Fig. 3), all of which attenuated by codon de-optimization and one that is being developed in collaboration between Codagenix and the Serum Institute of India<sup>32</sup>.

### Recombinant protein vaccines

Recombinant protein vaccines can be divided into recombinant spike-protein-based vaccines (Fig. 3e), recombinant RBD-based vaccines (Fig. 3f) and virus-like particle (VLP)-based vaccines (Fig. 3g). These recombinant proteins can be expressed in different expression systems including insect cells, mammalian cells, yeast and plants<sup>15,32,38</sup>; it is likely that RBD-based vaccines could also be expressed in *Escherichia coli*<sup>39</sup>. Yields, and the type and extent of post-translational modifications, vary depending on the expression system. For recombinant



## Box 1

# Vaccine-enhanced disease

Although enhanced disease is usually associated with flaviviruses, pre-existing immunity induced by natural infection with or by vaccination against feline coronavirus can lead to antibody-dependent enhancement of disease. This occurs mostly under experimental conditions and seems to be rare in the field<sup>88</sup>. In several different animal models, the administration of formalin-inactivated, DNA-based, RNA-based, VLP-based and MVA-vectored vaccine candidates against SARS-CoV has resulted in complications—such as liver damage or increased infiltration of eosinophils into the lung (suggesting a T<sub>H</sub>2-type immunopathology)—after challenge with the virus<sup>89–92</sup>. It has been speculated that enhanced disease is driven by non-neutralizing antibodies to the spike protein, but it has also been shown to be triggered by vaccines based on the nucleoprotein<sup>90,93,94</sup>. Bona fide antibody-dependent enhancement of SARS-CoV—even by neutralizing antibodies—has been shown in vitro, although the same antibodies were then found to be protective in vivo<sup>95</sup>. In addition, several vaccine candidates against SARS-CoV induced protective immunity in animal models without signs of enhanced

disease. Enhanced disease has also been reported in rabbits after natural infection and re-challenge with MERS-CoV in the absence of neutralizing antibodies<sup>96</sup>. Mice that had been administered an inactivated MERS-CoV vaccine and were then challenged with infectious virus showed enhanced infiltration of eosinophils into the lung despite the presence of neutralizing antibodies. Notably, as with many SARS-CoV vaccines, the virus was better controlled in these mice than in those from the control group<sup>97</sup>. The mechanism behind this phenomenon is still unclear and the data are inconclusive. In this context it is also important to note that enhanced disease is not necessarily a result of antibody-dependent enhancement and could also be induced by other mechanisms. It seems that, under the appropriate conditions, enhanced disease can be induced in animal models as a result of natural infection or vaccination. However, even in animal models of SARS-CoV-2 infection there is currently no evidence for enhanced disease. Nevertheless, monitoring for the occurrence of this phenomenon both during the development of and after the licencing of vaccines is paramount, especially after antibody titres start to decrease.

spike-protein-based vaccines in particular, modifications such as deletion of the polybasic cleavage site<sup>40–42</sup>, inclusion of two (or more) stabilizing mutations<sup>13,40,43,44</sup>, and inclusion of trimerization domains—as well as the mode of purification (soluble protein versus membrane extraction)—might influence the elicited immune response. The advantage of these vaccines is that they can be produced without handling live virus. In addition, some recombinant protein vaccines—such as the FluBlok vaccine for influenza—have been licensed, and there is considerable experience in producing them. However, such vaccines also have disadvantages. The spike protein is relatively hard to express, and this is likely to have an effect on production yields and on how many doses can be produced<sup>15</sup>. The RBD is easier to express; however, it is a relatively small protein when expressed alone and, although potent neutralizing antibodies bind to the RBD, it lacks other neutralizing epitopes that are present on the full-length spike. This might render RBD-based vaccines more prone to impact from antigenic drift than vaccines that include the full-length spike protein. Many recombinant protein vaccine candidates against SARS-CoV-2 are currently in preclinical development, and several spike-protein-based and RBD-based vaccines have entered clinical trials<sup>32</sup>. Of those, data from NHPs and from phase I trials have been reported for Novavax<sup>42</sup> (Tables 1, 2), which are described in more detail below. VLP-based vaccine candidates, including one produced by Medicago, have also entered clinical trials<sup>32</sup>. Similar to inactivated vaccines, these candidates are typically injected and are not expected to result in robust mucosal immunity.

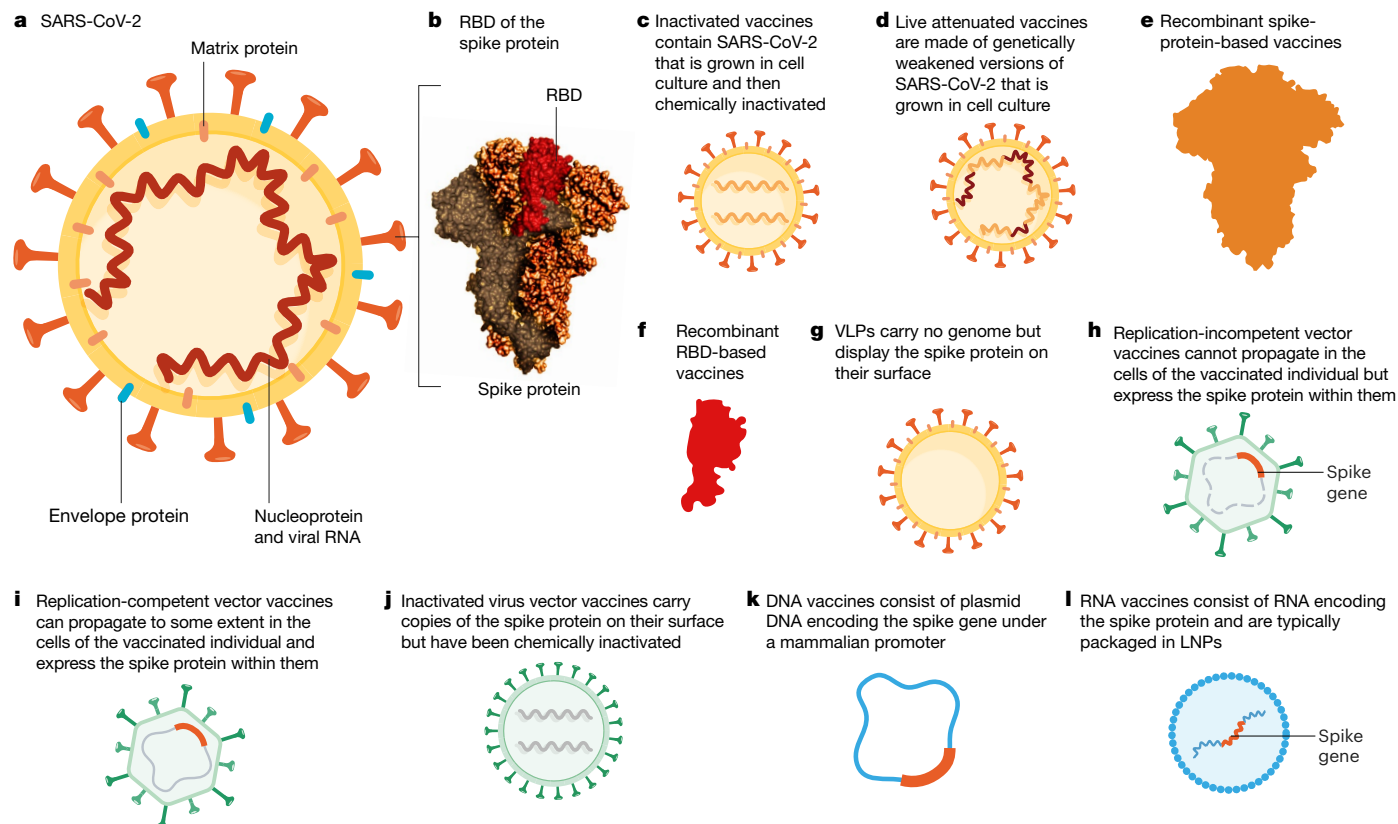
### Replication-incompetent vectors

Replication-incompetent vectors (Fig. 3h) represent a large group of vaccines in development. Such vaccines are typically based on another virus that has been engineered to express the spike protein and has been disabled from replication in vivo by the deletion of parts of its genome. The majority of these approaches are based on adenovirus (AdV) vectors, although modified vaccinia Ankara (MVA), human parainfluenza virus vectors, influenza virus, adeno-associated virus and Sendai virus are used as well<sup>32,41,45–49</sup> (Fig. 3). The majority of these vectors are delivered intramuscularly, enter the cells of the vaccinated individual and then express the spike protein, to which the host immune system responds. These approaches have many advantages. It is not

necessary to handle live SARS-CoV-2 during production, there is considerable experience with producing larger quantities of some of these vectors (an Ad26–MVA-based prime–boost vaccine against the Ebola virus was recently licensed in the European Union), and the vectors show good stimulation of both B cell and T cell responses. A disadvantage is that some of these vectors are affected and are partially neutralized by pre-existing vector immunity<sup>46</sup>. This is circumvented by using vector types that are either rare in humans<sup>41</sup> or are derived from animal viruses<sup>47</sup>, or by using viruses that do not induce much immunity by themselves (for example, adeno-associated viruses). In addition, vector immunity can be problematic when prime–boost regimens are used, although this can be circumvented by priming with one vector and boosting with a different vector. Several replication-incompetent vector vaccine candidates against SARS-CoV-2 have progressed far in clinical development: results from NHP trials and/or clinical trials in humans have been reported for ChAdOx1 nCoV-19 (based on a chimpanzee AdV)<sup>47</sup>, by Janssen (using an AdV26-based vector)<sup>41</sup> and by CanSino (AdV5)<sup>45,46</sup>; in addition, a candidate from the Gamaleya Research Institute (Ad5/Ad26)<sup>50</sup> is in phase III clinical trials and another from ReiThera (gorilla AdV) is in phase I trials<sup>32</sup> (Fig. 4, Tables 1, 2).

### Replication-competent vectors

Replication-competent vectors (Fig. 3i) are typically derived from attenuated or vaccine strains of viruses that have been engineered to express a transgene, in this case the spike protein. In some cases, animal viruses that do not replicate efficiently and cause no disease in humans are used as well. This approach can result in a more robust induction of immunity, because the vector is propagating to some extent in the vaccinated individual and often also triggers a strong innate immune response. Some of these vectors can also be administered through mucosal surfaces, which might trigger mucosal immune responses (Fig. 2). Currently, only two replication-competent vectors are in phase I clinical trials: an engineered measles vaccine strain developed by Institute Pasteur and Themis (now acquired by Merck), and a vector based on the influenza virus that is under development by Beijing Wantai Biological Pharmacy<sup>32</sup> (Fig. 4). However, several others—including vectors based on vesicular stomatitis virus (VSV)<sup>51</sup>, horsepox and Newcastle disease virus (NDV)<sup>52,53</sup>—are currently in development<sup>32</sup>. Vectors based



**Fig. 3 | Vaccine platforms used for SARS-CoV-2 vaccine development.**

**a**, A schematic of the structural proteins of the SARS-CoV-2 virion, including the lipid membrane, the genomic RNA covered by the nucleoprotein on the inside, the envelope and matrix proteins within the membrane, and the spike protein on the surface of the virus. **b**, The structure of the spike protein; one monomer is highlighted in dark brown and the RBD is shown in red. **c–l**, Current

SARS-CoV-2 vaccine candidates include inactivated virus vaccines (**c**), live attenuated vaccines (**d**), recombinant protein vaccines based on the spike protein (**e**), the RBD (**f**) or on virus-like particles (**g**), replication-incompetent vector vaccines (**h**), replication-competent vector vaccines (**i**), inactivated virus vector vaccines that display the spike protein on their surface (**j**), DNA vaccines (**k**) and RNA vaccines (**l**).

on NDV are of interest because this virus grows to high titres in eggs, and the vectors could be produced using the global influenza virus vaccine pipeline. In contrast to measles and the VSV vectors, they are likely to be safe enough to administer intranasally, which could result in mucosal immunity.

### Inactivated virus vectors

Some SARS-CoV-2 vaccine candidates that are currently under development rely on viral vectors that display the spike protein on their surface but are then inactivated before use<sup>32</sup> (Fig. 3j). The advantage of this approach is that the inactivation process renders the vectors safer because they cannot replicate, even in an immunocompromised host. Using standard viral vectors, the amount of antigen that is presented to the immune system cannot easily be controlled; however, in inactivated vectored vaccines it can be readily standardized—as is the case for inactivated or recombinant protein vaccines. Examples of inactivated virus vectors include NDV-based vaccines that display the spike protein on their surface—which can be produced in a similar manner to influenza virus vaccines<sup>54</sup>—as well as rabies vectors<sup>32</sup>. These technologies are currently in the preclinical stage.

### DNA vaccines

DNA vaccines (Fig. 3k) are based on plasmid DNA that can be produced at large scale in bacteria. Typically, these plasmids contain mammalian expression promoters and the gene that encodes the spike protein, which is expressed in the vaccinated individual upon delivery. The great advantage of these technologies is the possibility of large-scale production in *E. coli*, as well as the high stability of plasmid DNA. However, DNA

vaccines often show low immunogenicity, and have to be administered via delivery devices to make them efficient. This requirement for delivery devices, such as electroporators, limits their use. Four different DNA vaccine candidates against SARS-CoV-2 are currently in phase I/II clinical trials<sup>32</sup> (Fig. 4).

### RNA vaccines

Finally, RNA vaccines (Fig. 3l) are a relatively recent development. Similar to DNA vaccines, the genetic information for the antigen is delivered instead of the antigen itself, and the antigen is then expressed in the cells of the vaccinated individual. Either mRNA (with modifications) or a self-replicating RNA can be used. Higher doses are required for mRNA than for self-replicating RNA, which amplifies itself<sup>55</sup>, and the RNA is usually delivered via lipid nanoparticles (LNPs). RNA vaccines have shown great promise in recent years and many of them are in development, for example for Zika virus or cytomegalovirus. As potential vaccines against SARS-CoV-2, promising preclinical results have been published for a number of RNA vaccine candidates<sup>43,56–58</sup>: Pfizer and Moderna currently have candidates in phase III trials (Fig. 4, Tables 1, 2), CureVac and Arcturus have candidates in phase I/II trials, and a vaccine candidate from Imperial College London and the Chinese Liberation Army is in phase I trials<sup>32,59,60</sup>. Advantages of this technology are that the vaccine can be produced completely *in vitro*. However, the technology is new, and it is unclear what issues will be encountered in terms of large-scale production and long-term storage stability, because frozen storage is required. In addition, these vaccines are administered by injection and are therefore unlikely to induce strong mucosal immunity (Fig. 2).



**Fig. 4 | Overview of the SARS-CoV-2 vaccine development landscape.** The chart shows the distribution of candidates from different vaccine platforms over the different development phases. \*The two vaccines that are currently licensed include one produced by CanSino, which is currently in use in the Chinese military, and the vaccine from Gamaleya Research Institute in Russia, which was licensed without a phase III trial.

## Results from NHPs

Several animal models of SARS-CoV-2 have been developed, including mice that express human ACE2 (either via adenovirus transduction or by genetic engineering<sup>61,62</sup>) and mouse models with mouse-adapted SARS-CoV-2 strains<sup>63–67</sup>, as well as ferret<sup>68–70</sup>, hamster<sup>71–73</sup> and NHP models (particularly rhesus macaques)<sup>23–25,33,49,74–78</sup>. The hamster model can mimic severe disease, as is seen in a proportion of infected humans, whereas the NHP model reflects mild to moderate infection. For vaccines that have progressed far in clinical trials there are limited data in hamster models, but many of the vaccine candidates have been tested in NHPs, which enables more direct comparisons between them (Table 1). However, these comparisons must be interpreted with caution, because the challenge doses and administration routes vary, as do the vaccine regimens and schedules. Importantly, although all studies report neutralization data, differences in assays can introduce very large biases. Furthermore, most studies did not determine the level of infectious virus in the upper and lower respiratory tracts, and instead measured viral RNA or subgenomic RNA using polymerase chain reaction (PCR) assays.

Sinovac was the first company to test a vaccine candidate—containing SARS-CoV-2 inactivated by  $\beta$ -propiolactone—in the rhesus macaque model; it is now in phase III trials in humans<sup>32,34</sup> (Fig. 3c). The vaccine was formulated on the basis of total protein content and adjuvanted with aluminium hydroxide, then administered to the macaques three times at 1-week intervals at doses of either 3  $\mu$ g (low-dose group) or 6  $\mu$ g (high-dose group). A challenge was performed 1 week post-boost with  $10^6$  times the 50% tissue culture infectious dose (TCID<sub>50</sub>) of virus, via the intratracheal route. This vaccination regimen induced low to moderate neutralizing antibody titres, but protected the lower

respiratory tract from challenge without evidence of vaccine-enhanced respiratory disease (Table 1). Notably, viral RNA was found at very low copy numbers in the lower respiratory tract in the low-dose group, and was present in the throat swabs of both groups but at much lower copy numbers than in the controls. In the same paper<sup>34</sup>, the authors also demonstrated that antiserum from vaccinated mice and rats showed cross-neutralization against diverse SARS-CoV-2 isolates.

Another  $\beta$ -propiolactone-inactivated vaccine candidate (Fig. 3c), developed by the Beijing Institute of Biological Products and currently in phase III trials<sup>32</sup>, was evaluated in cynomolgus macaques. It was administered in two doses of either 2  $\mu$ g or 8  $\mu$ g, with a 2-week interval between doses, and contained aluminium hydroxide as adjuvant<sup>33</sup>. The macaques developed relatively high antibody titres (in the 1:200 range) post-boost, and were challenged 10 days post-boost with  $10^6$  TCID<sub>50</sub> of SARS-CoV-2 administered intratracheally. The results were similar to those with the Sinovac candidate, demonstrating complete protection of the lung but detectable titres in throat swabs (Table 1).

ChAdOx1 nCoV-19—developed by the University of Oxford, AstraZeneca and the Serum Institute of India—is based on a replication-incompetent chimpanzee adenovirus (Fig. 3i) expressing a wild-type version of the spike protein<sup>49</sup> (that is, containing no stabilizing mutations and with the polybasic cleavage site present; Tables 1, 2). This vaccine candidate was tested in rhesus macaques in a prime-only and a prime-boost regimen, at a dose of  $2.5 \times 10^{10}$  viral particles administered intramuscularly. The prime and boost doses were given at a 4-week interval and the macaques were challenged 4 weeks after the last vaccination. Macaques in both groups developed moderate neutralizing antibody titres (1:5–1:40 after the prime, 1:10–1:160 after the boost), and challenge with SARS-CoV-2 delivered through a combined intranasal, intratracheal, ocular and oral route showed that both groups were protected from lung disease; they were also mostly protected from viral replication in the lung, as assessed by the copy numbers of subgenomic RNA. However, viral replication in the upper respiratory tract was not controlled. In addition, T cell responses were detected (Table 1).

Another replication-incompetent adenovirus vector vaccine candidate (Fig. 3i), based on AdV26, is under development by Janssen and has been tested in rhesus macaques<sup>41</sup> (Table 1). Several constructs were tested in parallel in a single-shot regimen of  $10^{11}$  virus particles given intramuscularly; one of the most successful included a full-length version of the spike protein in which the polybasic cleavage site was removed and two stabilizing proline residues were introduced (named S.PP)<sup>10,13</sup>. Macaques were challenged with  $10^5$  TCID<sub>50</sub> of SARS-CoV-2, administered through intranasal and intratracheal routes, 6 weeks after vaccination. The S.PP construct—which ultimately progressed into clinical trials in humans—achieved neutralization titres in the 1:100 range at week 4 post-boost. Challenged S.PP macaques showed no trace of subgenomic viral RNA in the lung, and for only one macaque out of six was a low PCR signal observed in the upper respiratory tract. In addition, antibody titres in these macaques did not increase after infection, which is indicative of sterilizing immunity. Other constructs tested in parallel fared less well, but all induced some degree of protection with no sign of enhanced disease. CD8<sup>+</sup> T cell responses were also assessed but were not particularly high, especially in the S.PP group (Table 1).

An mRNA vaccine candidate (Fig. 3l), termed mRNA-1273, is under development by the Vaccine Research Center (VRC) at the National Institutes of Health and Moderna. It was tested in rhesus macaques, at doses of 10  $\mu$ g or 100  $\mu$ g, in a prime-boost regimen with a 4-week interval<sup>57</sup> (Table 1). The vaccine induced considerable neutralizing antibody levels, which—especially in the high-dose group—reached titres in the range of 1:1,000 even after only the prime dose had been administered. Neutralization titres reached geometric mean titres (GMTs) of 1:501 and 1:3,481 in the low-dose and high-dose groups, respectively, post-boost. CD4<sup>+</sup> T cell and T follicular helper cell responses were also detected. After challenge with  $7.6 \times 10^5$  plaque-forming units of virus via the intranasal and intratracheal routes, the macaques were

**Table 1 | Overview of NHP results**

Company (ref.)	Vaccine candidate (type)	Dose range (route)	Neut. titre after prime	Neut. titre after boost	T cell response	Challenge dose (route)	URT protection	LRT protection	Species
Sinovac <sup>34</sup>	PiCoVacc (inactivated virion + aluminium hydroxide)	3–6 µg (i.m.)	None <sup>a</sup>	1:10 range <sup>a</sup> after first boost; 1:50 range <sup>a</sup> after second boost	ND	10 <sup>6</sup> TCID <sub>50</sub> (i.t.)	Partial <sup>b</sup>	Partial (low dose) <sup>b</sup> Complete (high dose)	Rhesus macaques
Beijing Institute of Biological Products <sup>33</sup>	BBIBP-CorV (inactivated virion + aluminium hydroxide)	4–8 µg (i.m.)	1:100 range <sup>a</sup>	1:200 range <sup>a</sup>	ND	10 <sup>6</sup> TCID <sub>50</sub> (i.t.)	Partial <sup>b</sup>	Complete <sup>b</sup>	Cynomolgus macaques
AstraZeneca <sup>49</sup>	ChAdOxCoV-19 (non-replicating AdV)	2.4 × 10 <sup>10</sup> VP; 1 × or 2 × (i.m.)	1.5–1:40 range <sup>a</sup>	1:10–1:160 range <sup>a</sup>	Yes	2.6 × 10 <sup>6</sup> TCID <sub>50</sub> (i.t., oral, i.n., ocular)	None (1 ×) <sup>c</sup> None (2 ×) <sup>c</sup>	Partial (1 ×) <sup>c</sup> Complete (2 ×) <sup>c</sup>	Rhesus macaques
Janssen <sup>41</sup>	Ad26COVS1 (non-replicating AdV)	1 × 10 <sup>11</sup> VP (i.m.)	1:100 range <sup>d</sup>	NA	Low	10 <sup>5</sup> TCID <sub>50</sub> (i.n, i.t.)	Complete in S.PP group <sup>c</sup>	Complete in S.PP group <sup>c</sup>	Rhesus macaques
Moderna <sup>57</sup>	mRNA-1273 (mRNA via LNPs)	2 × 10–100 µg (i.m.)	ND <sup>e</sup>	1:501–1:3,481 range <sup>d</sup>	Yes, CD4, T <sub>FH</sub>	7.6 × 10 <sup>5</sup> TCID <sub>50</sub> (i.n., i.t.)	None (10 µg) <sup>c</sup> Partial (100 µg) <sup>c</sup>	Partial (10 µg) <sup>c</sup> Complete (100 µg) <sup>c</sup>	Rhesus macaques
Novavax <sup>79</sup>	NVX CoV2373 (spike protein + Matrix-M)	2 × 2.5–25 µg	Not reported	17,920–23,040 range <sup>a</sup>	ND	10 <sup>4</sup> plaque-forming units (i.n., i.t.)	Partial (low dose) <sup>c</sup> Complete (higher doses) <sup>c</sup>	Complete <sup>c</sup>	Cynomolgus macaques

<sup>a</sup>Based on microneutralization assay with CPE as readout.

<sup>b</sup>Based on viral genome RNA copy number.

<sup>c</sup>Based on subgenomic RNA copy number.

<sup>d</sup>Based on microneutralization assay with a SARS-CoV-2 reporter virus; 50% reduction in relative light units as readout.

<sup>e</sup>Not assessed using authentic SARS-CoV-2.

Neut., neutralizing antibody; NA, not applicable; i.m., intramuscular; i.n., intranasal; i.t., intratracheal; T<sub>FH</sub>, T follicular helper cells.

almost completely protected from challenge in the lower respiratory tract—except for a single macaque in each group that showed low copy numbers of viral subgenomic RNA. The upper respiratory tract of the low-dose group contained subgenomic RNA copy numbers similar to those of the control group; however, viral replication in the high-dose group was mostly controlled, except for three out of eight macaques on day 1 post-infection and one out of eight on day 4 post-infection.

A recombinant spike-protein-based vaccine candidate with Matrix-M adjuvant (Fig. 3e) is under development by Novavax. It was tested in cynomolgus macaques at three different doses (2.5 µg, 5 µg and 25 µg) in a prime–boost regimen with a 3-week interval, and administered intramuscularly<sup>79</sup> (Table 1). Macaques were then challenged on day 37 with 1.04 × 10<sup>4</sup> plaque-forming units of virus administered via the intranasal and intratracheal routes. Those in vaccinated groups showed neutralizing titres in the range of 17,920 to 23,040, and the lower and upper respiratory tracts were protected, except for one macaque (out of four) in the low-dose group that had detectable viral subgenomic RNA in the bronchoalveolar lavage on day 2. No subgenomic RNA could be detected in the high-dose group, which is suggestive of sterilizing immunity. This vaccine candidate has been tested in phase I trials and has now advanced into phase II and phase III trials<sup>42</sup>.

## Results from phase I/II clinical trials

More than 42 vaccine candidates have so far entered into clinical trials in humans, and 10 are currently in phase III trials<sup>32</sup> (Fig. 4). As mentioned above, owing to the speed of vaccine development in this area, I refer to the WHO working document that includes most of the vaccines in development<sup>32</sup>. The first phase I trial, of the Moderna/VRC vaccine, began in March 2020—barely 3 months after SARS-CoV-2 was reported for the first time. For several of the candidates described above for which data are available from NHP experiments (Table 1)—as well as candidates for which no data are yet available—data from phase I,

phase I/II or phase II trials have already been released. Here I will discuss these findings in detail with a focus on neutralizing antibody responses, T cell responses, where available, and safety data. Again, although neutralizing antibody titres are compared, it is important to point out that the assays used to measure neutralizing antibodies vary greatly and comparisons must be made with a degree of caution.

### CoronaVac from Sinovac

Very recently, Sinovac reported results from a randomized, double-blind, placebo-controlled phase II trial (NCT04352608) of the inactivated vaccine CoronaVac (Fig. 3c; the name PiCoVacc was used in the paper describing the NHP results, Table 1) in 600 healthy adults (18–59 years of age)<sup>35</sup>. Two different doses—3 µg or 6 µg—adjuvanted with aluminium hydroxide were administered in a 2-week or a 4-week prime–boost regimen. PBS was used as a placebo control (Table 2). Immunogenicity readouts included RBD enzyme-linked immunosorbent assays (ELISAs) and neutralization assays (cytopathogenic effect (CPE)-based) with authentic SARS-CoV-2. The safety profile of the vaccine was excellent, and for both doses was comparable to that of the placebo. No grade 3 adverse reactions were reported. For both doses, the 2-week prime–boost interval resulted in low neutralization titres with GMTs of around 1:30; the 4-week interval fared slightly better, with GMTs in the 1:60 range at 28 days post-boost. Overall, more than 90% of individuals showed seroconversion. Notably, the authors also stratified the titres by age. It was found that individuals between 18 and 39 years old had notably higher antibody responses than older individuals, suggesting that perhaps higher doses or different adjuvants might be needed for the latter group. This vaccine candidate is currently being evaluated in phase III clinical trials in adults, including in older adults (NCT04456595)<sup>32</sup>.

### Inactivated whole virus COVID-19 vaccine from Sinopharm

Another betapropiolactone-inactivated SARS-CoV-2 vaccine candidate (Fig. 3c), developed by Sinopharm and the Wuhan Institute of

**Table 2 | Overview of phase I/II results**

Company (reference)	Vaccine (type)	Dose range (route)	Neut. titre after prime	Neut. titre after boost	T cell response	Trial registration number
Sinovac <sup>35</sup>	CoronaVac (inactivated SARS-CoV-2 + aluminium hydroxide)	3–6 µg (i.m.) 2× (day 0 and 14 (0/14) or 0/28)	ND	1:30–1:60 range <sup>a</sup>	ND	NCT04352608
Sinopharm <sup>80</sup>	Inactivated whole virus COVID-19 vaccine (inactivated SARS-CoV-2 + aluminium hydroxide)	2.5, 5 or 10 µg (i.m.) 3× (0/28/56) 5 µg (i.m.) 2× (0/14 or 0/21)	Not reported in detail	1:316 (2.5 µg, 0/28/58) <sup>b</sup> , 1:206 (5 µg, 0/28/58) <sup>b</sup> , 1:297 (10 µg, 0/28/58) <sup>b</sup> , 1:121 (5 µg, 0/14) <sup>b</sup> , 1:247 (5 µg, 0/21) <sup>b</sup>	ND	ChiCTR2000031809
CanSino <sup>46</sup>	Ad5 nCoV (non-replicating AdV5 expressing spike protein)	5 × 10 <sup>10</sup> , 10 <sup>11</sup> VP (i.m.)	1:18.3–1:19.5 range <sup>c</sup>	NA	Yes	NCT04341389
AstraZeneca <sup>47</sup>	ChAdOx1 nCoV-19 (non-replicating chimpanzee AdV expressing spike protein)	5 × 10 <sup>10</sup> VP 1× or 2× (i.m.)	Median 1:218 <sup>b</sup> Median 1:51 <sup>d</sup> Range 1:4–1:16 <sup>e</sup>	Median 1:136 <sup>d</sup> Median 1:29 <sup>d</sup>	Yes	NCT04324606
Moderna <sup>59</sup>	mRNA-1273 (mRNA expressing spike protein)	2× 25, 100, 250 µg (i.m.)	Low	1:112.3 (25 µg) <sup>f</sup> , 1:343.8 (100 µg) <sup>f</sup> , 1:332.2 (250 µg) <sup>f</sup> , 1:339.7 (25 µg) <sup>g</sup> , 1:654.3 (100 µg) <sup>g</sup>	Good CD4 <sup>+</sup> and low CD8 <sup>+</sup> response	NCT04283461
Pfizer <sup>60</sup>	BNT162b1 (mRNA expressing a trimeric RBD)	2× 10, 30, 100 µg (i.m.)	Low	1:180 (10 µg) <sup>h</sup> , 1:437 (30 µg) <sup>h</sup>	ND	NCT04368728
Pfizer <sup>81</sup>	BNT162b1 (mRNA expressing a trimeric RBD) and BNT162b2 (mRNA expressing spike protein)	2× 10, 20, 30 µg	Low	Day 28 <sup>h</sup> BNT162b1 (18–55 years): 1:168 (10 µg), 1:267 (30 µg) BNT162b1 (65–85 years): 1:37 (10 µg), 1:179 (20 µg), 1:101 (30 µg) BNT162b2 (18–55 years): 1:157 (10 µg), 1:363 (20 µg), 1:361 (30 µg) BNT162b2 (65–85 years): 1:84 (20 µg), 1:147 (30 µg)	ND	NCT04368728
Novavax <sup>87</sup>	NVX CoV2373 (Matrix-M) spike protein ‘rosettes’	2× 2.5–25 µg (i.m. ± Matrix-M) 1× 25 µg (i.m. + Matrix-M)	1:128 (25 µg + Matrix-M) <sup>i</sup>	1:3,906 (5 µg + Matrix-M) <sup>j</sup> , 1:3,305 (25 µg + Matrix-M) <sup>j</sup> , 1:41 (25 µg unadjuvanted) <sup>j</sup>	CD4 <sup>+</sup>	NCT04368988

<sup>a</sup>Based on microneutralization assay with CPE as readout.<sup>b</sup>Based on PRNT<sub>50</sub> assay with authentic SARS-CoV-2.<sup>c</sup>Neutralization assay based on authentic SARS-CoV-2 but not described in detail.<sup>d</sup>Based on MN assay with authentic SARS-CoV-2 with ID<sub>50</sub> as readout.<sup>e</sup>Based on a virus neutralization assay with CPE as readout.<sup>f</sup>Based on pseudotyped particle entry inhibition ID<sub>50</sub>.<sup>g</sup>Based on PRNT80 with authentic SARS-CoV-2.<sup>h</sup>Based on microneutralization assay with a SARS-CoV-2 reporter virus, ID<sub>50</sub> of relative light units as readout.<sup>i</sup>Based on microneutralization assay with authentic SARS-CoV-2 (ID<sub>99</sub>).

Biological Products, was recently tested in phase I ( $n = 96$ ) and phase II ( $n = 224$ ) trials in adults aged 18–59 years (ChiCTR2000031809)<sup>80</sup>. The phase I trial tested the injection of 2.5, 5 or 10 µg antigen adjuvanted with aluminium hydroxide in a prime–boost–boost regimen in 4-week intervals, whereas phase II tested 5 µg antigen adjuvanted with aluminium hydroxide in a prime–boost regimen in 2-week and 3-week intervals. 50% plaque reduction neutralization titres (PRNT<sub>50</sub>) ranging from 1:121 to 1:316 were reached after the last boost across doses and intervals (see Table 2). Safety results essentially mirrored those of the CoronaVac study. This vaccine candidate is now being evaluated in phase III trials (ChiCTR2000034780).

#### AdV5-based vaccine from CanSino

CanSino is developing a replication-deficient, AdV5-based vaccine candidate (Fig. 3i) that expresses the unmodified spike protein. No

NHP data are currently publicly available for this candidate; however, CanSino was the first to publish results from a phase I trial<sup>45</sup> followed by data from a randomized, double-blind, placebo-controlled phase II trial (NCT04341389)<sup>46</sup>. This vaccine is currently licensed for use in the Chinese military. It was tested as a one-shot vaccine in two doses— $5 \times 10^9$  virus particles and  $1 \times 10^{11}$  virus particles—in 508 healthy adults aged 18 and above (Table 2). Both cellular responses and neutralizing antibody responses were assessed 28 days after vaccination. Neutralization assays were performed with authentic SARS-CoV-2, but no details about the assay procedure were given; T cell responses were evaluated with an interferon- $\gamma$  enzyme-linked immunospot assay with overlapping spike peptides on peripheral blood mononuclear cells (PBMCs). Antibody responses to the RBD were also monitored. Neutralizing antibody responses were low, with GMTs between 1:19.5 (59% seroconversion) and 1:18.3 (47% seroconversion) for the high and the low doses,



respectively. T cell responses were below the limit of detection in 506 out of 508 individuals on day 0, but increased to 11 (90% response) and 10 (88% response) spot-forming units (SFU) per  $10^5$  PBMCs in the high- and the low-dose groups after vaccination. Notably, it was found that both pre-existing immunity to AdV5 and age—older individuals have a higher likelihood of having immunity to AdV5—correlated with lower immune responses to the vaccine. In terms of safety, the vaccine seemed to be relatively reactogenic, especially at the higher dose. Fever, fatigue and headache were common, and pain at the injection site was reported in more than 50% of individuals. Grade 3 adverse reactions (mostly fever) were reported in 9% of individuals in the high-dose group and 1% in the low-dose group. This vaccine candidate is currently being evaluated in phase III clinical trials at a dose of  $5 \times 10^5$  virus particles (NCT04526990, NCT04540419, among others).

## ChAdOx1 nCoV-19 from AstraZeneca

On the basis of their longstanding experience with the replication-incompetent ChAdOx1 vector (Fig. 3i), the University of Oxford—together with AstraZeneca and the Serum Institute of India—is developing the vaccine candidate ChAdOx1 nCoV-19, which expresses a full-length, wild-type version of the spike protein. They recently reported preliminary results from a phase I/II, single-blind, randomized control trial in 1,077 participants aged 18–55 years (NCT04324606)<sup>47</sup>. The vast majority of participants in the vaccine group received a single dose of  $5 \times 10^{10}$  virus particles, but a small cohort of 10 individuals also received a booster dose 28 days after the prime dose (Table 2). A meningitis vaccine was administered to the placebo control group, enabling comparisons of the safety profile with that of a licensed vaccine. Antibody responses were tracked using several binding assays as well as three different neutralization assays, all performed with authentic SARS-CoV-2. Cellular immune responses were measured using an interferon- $\gamma$  enzyme-linked immunospot assay in which PBMCs were stimulated using a peptide pool that spans the spike protein. To determine neutralizing-antibody responses, a subgroup of 35 individuals was analysed. Using a PRNT<sub>50</sub> assay, a microneutralization assay with 80% inhibitory concentration (IC<sub>80</sub>) as readout and a CPE-based virus neutralization assay, 28-day post-vaccination titres were found to be 1:218 (median titres, 100% seropositivity), 1:51 (median titre, 91% seropositivity) and in the 1:4–1:16 range (62%; this assay measures potentially an equivalent to IC<sub>100</sub>), respectively. A booster dose increased the median titres in the latter two assays to 1:136 (100%) and 1:29 (100%). It is noteworthy that pre-existing immunity to SARS-CoV-2 was found in a small number of participants (4%). Cellular immunity peaked at day 14, with 856 SFU per  $10^6$  cells, and decreased to 424 SFU by day 56. Background cellular immunity was found mostly in the range of 50–100 SFU per  $10^6$  PBMCs. The most common side effects were fatigue (more than 70% of participants) and headache (more than 60% of participants); an increased temperature or feeling feverish was also relatively common. The booster dose seemed to be better tolerated; however, because it was given to only 10 individuals, further data would be required before conclusions can be drawn. Overall, ChAdOx1 nCoV-19 had a poorer safety profile than the licensed meningitis vaccine that was used in the placebo group, independently of whether paracetamol was given to alleviate side effects. This vaccine candidate is currently being evaluated in phase III clinical trials in several countries as a one-dose or a two-dose regimen (ISRCTN89951424, NCT04516746).

## mRNA-1273 from Moderna

Moderna and the VRC recently reported preliminary data from a phase I, open-label, dose-escalation trial of their mRNA-based vaccine candidate mRNA-1273 (NCT04283461) (Fig. 3l) in 45 healthy individuals aged between 18 and 55 years<sup>59</sup>. As discussed above, mRNA-1273 is delivered via LNPs and expresses the full-length spike protein containing two stabilizing mutations. Three doses (25  $\mu$ g, 100  $\mu$ g and

250  $\mu$ g) of RNA were evaluated in a prime–boost regimen with a 4-week interval (Table 2). Readouts included full-length spike-protein ELISA, pseudovirus and virus-neutralization assays, as well as the assessment of different T cell populations via intracellular cytokine staining using a spike peptide pool for stimulation. Less than 50% of participants induced antibodies that could neutralize pseudotyped particles after the prime dose. However, at day 43 (15 days post-boost), 50% inhibitory dilution (ID<sub>50</sub>) GMTs of 1:112.3, 1:343.8 and 1:332.2, respectively, were recorded for the three dose groups. More informative, PRNT<sub>80</sub> values with authentic SARS-CoV-2 reached 1:339.7 and 1:654.3 in the 25  $\mu$ g and 100  $\mu$ g groups (data for the 250  $\mu$ g group was not provided), which is within the range seen in convalescent samples from patients who have recovered from COVID-19. T cell responses were analysed in detail, and good CD4<sup>+</sup> responses were detected in the 25  $\mu$ g and 100  $\mu$ g groups, with T helper 1 cell (T<sub>H</sub>1) polarization. CD8<sup>+</sup> T cell responses were detected but were low, as expected for the SARS-CoV-2 spike protein. Adverse events were dose-dependent and were most common at the highest dose. Solicited systemic events were reported in 33%, 67% and 53% of individuals after the prime dose and in 54%, 100% and 100% of individuals after the booster for doses of 25  $\mu$ g, 100  $\mu$ g and 250  $\mu$ g, respectively. Although fever was not detected after the prime dose, it was reported in 40% and 57% of individuals after the booster at doses of 100  $\mu$ g and 250  $\mu$ g. This vaccine candidate is currently being evaluated at the 100  $\mu$ g dose in phase III clinical trials in adults, including those in older age groups (NCT04470427).

## BNT162b1 and BNT162b2 from Pfizer

Pfizer, in collaboration with the German company BioNTech, has recently published data from an ongoing phase I/II randomized, placebo-controlled, observer-blind dose-escalation study of BNT162b1 in 45 healthy adults, 18–55 years of age (NCT04368728)<sup>60</sup>. BNT162b1 is an mRNA-based vaccine candidate that is delivered in LNPs (Fig. 3l), and it expresses a trimeric version of the RBD that is held together by a T4 foldon. Three doses—10  $\mu$ g, 30  $\mu$ g and 100  $\mu$ g of RNA—were tested in a prime–boost vaccination regimen with a 3-week interval (Table 2). ELISA binding to the RBD and neutralization of a SARS-CoV-2 reporter virus (IC<sub>80</sub>) was tested. Three weeks after dose 1, neutralization titres were generally low (similar to those of the vaccine candidate mRNA-1273). Seven days after dose 2, GMTs of 1:168 and 1:267 were detected for the two different doses; the 100  $\mu$ g group was not given the booster dose owing to an unfavourable safety profile. At 14 days post-boost, titres reached 1:180 and 1:437, respectively. Convalescent serum was also tested and reached titres of 1:94. However, it is unknown how representative these sera were. Systemic adverse events after the prime dose seemed to be dose-dependent and included fever—especially in the 100  $\mu$ g group, for which it was seen in 50% of individuals—fatigue, headache and chills. Similar to mRNA-1273, side effects were more common after the booster dose, with more than 70% of participants reporting fever in the 30  $\mu$ g group. One participant reported grade 3 fever in the 30  $\mu$ g group, and sleep disturbance was reported as a severe adverse event by one participant in the 100  $\mu$ g group. Participants in the 100  $\mu$ g group did not receive a booster dose due to tolerability profiles of the 100  $\mu$ g dose post-prime and the 30  $\mu$ g dose post-boost. In an additional study, Pfizer recently reported a direct comparison between BNT162b1 and BNT162b2 (NCT04368728). BNT162b2 is similar to BNT162b1 but encodes a full-length spike protein with the two stabilizing proline residues. Whereas antibody titres between the two candidates were comparable, BNT162b2 showed a more favourable safety profile. The trial also included a group of older individuals (65–85 years). Reactogenicity for both vaccines was lower in this group compared to that in younger individuals; however, antibody titres were also lower, with GMTs of approximately 40% those of younger individuals<sup>81</sup> (Table 2). BNT162b2 was selected to move forward and is now in a phase III trial in healthy adults and older age groups (NCT04368728).

## NVX-CoV2373 from Novavax

Novavax has recently published a primary analysis of the results from their randomized, observer-blind, placebo-controlled phase I trial with NVX-CoV2373 in 131 healthy adults aged 18–59 (NCT04368988)<sup>42</sup>. This vaccine candidate uses a recombinant version of the full-length spike protein (Fig. 3e), in which the polybasic cleavage site is deleted and the two stabilizing proline residues are present, which is expressed in insect cells and purified by membrane extraction. The spike protein exhibits rosette formation via its hydrophobic tails—similar to the FluBlok recombinant haemagglutinin-based vaccine from Sanofi—which has been termed as a ‘nanoparticle’ by Novavax. The antigen was formulated with or without the saponin-containing adjuvant Matrix-M and was given at doses of 5 µg or 25 µg in a prime–boost regimen with a 3-week interval (Table 2). A prime-only scenario was also tested. Immunogenicity was assessed by ELISA and by using a microneutralization assay with authentic SARS-CoV-2 (ID<sub>50</sub> as readout) as well as by intracellular cytokine staining for CD4<sup>+</sup> stimulated with spike peptides. The group receiving the unadjuvanted vaccine showed essentially no response after the prime dose and barely responded after the boost, with a GMT neutralization titre of 1:4114 days post-boost. Both the adjuvanted 5 µg and 25 µg doses elicited intermediate responses after the prime dose, and reached very high GMT titres of 1:3,906 and 1:3,305, respectively, with 100% seroconversion after the boost. The adjuvanted prime-only 25 µg dose group reached a titre of 1:128 at the same time point (35 days post-prime), with two individuals showing no seroconversion. These data show the value both of including an adjuvant and of a prime–boost regimen, in which there was no appreciable difference in response to low and high doses. CD4<sup>+</sup> responses were evaluated 7 days post-boost, and both adjuvanted groups showed a robust, T<sub>H</sub>1-polarized response. Local reactogenicity and systemic events were milder after the first dose than after the second dose and were mostly driven by the adjuvant. Malaise, fatigue and headache were the most common systemic side effects, but fever was rare. Two participants had severe events after the first vaccination (malaise, fatigue, headache) and eight after the second vaccination (tenderness at injection site, muscle pain, nausea/vomiting, joint pain, malaise, fatigue and headache). This vaccine candidate has now advanced into phase II and III trials (NCT04533399).

## Summary of clinical trials

In summary, there is a gradient of immunogenicity in terms of neutralizing antibodies elicited by the vaccine candidates: inactivated and AdV5 vaccine candidates are at the lower end, ChAdOx1 nCoV-19 and the mRNA candidates are in the medium range and the recombinant protein vaccine candidate is at the high end, eliciting the greatest titres of neutralizing antibodies. Of course, different assays and readouts (ID<sub>50</sub>, ID<sub>80</sub>, ID<sub>90</sub>, ID<sub>100</sub>, different assay set-ups and different time points) were used and therefore the results are difficult to compare. In terms of tolerability, the inactivated and recombinant protein vaccines seem to perform relatively well, followed by the mRNA vaccines—which show increased reactogenicity after the second dose—and then the AdV-vectored vaccines. In addition to the data discussed above, phase II data for a vaccine candidate from the Gamaleya Institute has also been published recently<sup>50</sup>: this candidate comprises non-replicating AdV5 and AdV26 vectors expressing the spike protein, and was administered in a prime–boost regimen (Fig. 3i).

## Outlook

With 10 SARS-CoV-2 vaccine candidates in phase III trials already, and encouraging data from many candidates in NHPs and phase I, II or I/II trials, the situation can be described as cautiously optimistic. However, there are many unknowns moving forward. Phase III trials need to demonstrate that any potential vaccine is effective and safe in a larger population. Currently, on the basis of data from NHPs and from

a small study on a fishing vessel<sup>25,26</sup>, it is speculated that neutralizing antibodies could be a correlate of protection. However, this still needs to be verified in humans, and other factors—including cellular immune responses—might also have a protective role.

Importantly, all of the vaccine candidates that are currently in clinical trials are administered intramuscularly. Although this administration route induces strong IgG responses that are thought to protect the lower respiratory tract, unlike natural infection it does not drive the secretory IgA responses that are thought to protect the upper respiratory tract. Small amounts of IgG can also be found in the upper respiratory tract, but only in the case of very high serum titres. It is therefore conceivable—and this is supported by evidence from experiments with NHPs—that most vaccines will protect only against infection of the lower respiratory tract, and might not induce sterilizing immunity in the upper respiratory tract. This could lead to vaccines that, although protecting from symptomatic disease, might still enable transmission of the virus. In this case, the amount of virus shed and the duration of shedding might be reduced. However, a vaccine that could induce sterilizing immunity in the upper respiratory tract would be preferable. Live attenuated vaccines or viral vectors that can be applied intranasally would probably also lead to a strong mucosal immune response as well as an IgG response. Unfortunately, very few vaccines that are suitable for intranasal administration are undergoing development, and none are currently in clinical trials.

In addition, we do not know how long vaccine immunity will persist. Currently, after natural infection we see what looks like a ‘normal’ immune response, with some—but not a severe—reduction in the titre of antibodies over time. At this time, it is not known whether vaccine-induced immune responses are longer- or shorter-lived than immune responses induced by natural infection. However, booster doses every few years are given for many vaccines, and a reduction in immunity over longer periods of time would therefore not be a major obstacle.

Another unknown is how well older individuals, who are most at risk from COVID-19, will respond to the vaccine. From trials with Sinovac’s inactivated vaccine candidate and from Pfizer’s two mRNA vaccine candidates it has already become clear that such individuals respond less well than younger adults, and different vaccine formulations—or even special prime–boost regimens—might be required in order to increase immune responses in individuals from this age group. Notably, older individuals often need to achieve higher neutralization titres than younger individuals, at least for protection from influenza virus<sup>82,83</sup>. Potentially, a vaccine with higher reactogenicity that could induce a stronger interferon/antiviral response (mRNA vaccines, AdV vectors or even VSV-vectored vaccines) might improve titres in this age group. In addition, high-dose vaccines<sup>84</sup> or heterologous prime–boost regimens (for example, a virus-vectored prime followed by an adjuvanted protein vaccine boost)<sup>85</sup> have been successfully used to increase immune responses to vaccines against influenza virus, and could be used in this case.

Another important point is tolerability, especially when considering the vaccination of children, because they usually show greater reactogenicity than adults. Given that many of the vaccine candidates have relatively strong side effects, low-dose vaccines might be needed for this age group, especially for AdV and mRNA vaccines. However, the reactogenicity of Pfizer’s BNT162b1 and BNT162b2 vaccine candidates was reduced in older adults, making them more suitable for this age group.

Furthermore, it is not clear how vaccines will be rolled out and distributed globally after they are licensed. Even within countries, distribution and rollout are not yet clear. It is likely that, in many countries, the first doses will be used to immunize high-risk groups and healthcare workers; however, this will need to be discussed and established. At the beginning of September 2020, the US National Academies of Sciences, Engineering, and Medicine published a draft document for public comment in order to discuss this important topic<sup>86</sup>.

Assuming that two doses per person are needed, it will be necessary to produce 16 billion doses of vaccine in order to meet the global demand. It is encouraging to see that many vaccine producers have good candidates in development and that there is considerable diversity in terms of vaccine platforms and geographical location of the producers, because no single company will be able to produce the amount of vaccine that will be required. Even the supply of syringes, glass vials and related equipment might become a bottleneck when considering such a large number of doses. Specific concerns here are vaccine producers that have never before had a vaccine licensed and produced it at large scale for the market (for example, Moderna or Novavax), and vaccines based on platforms that have never been produced at large scale for the market (mRNA and DNA vaccines). During scale-up, manufacturing and distribution of these vaccine candidates, unforeseen challenges might arise owing to limited experience with technologies or organizational structures. In the case of mRNA vaccines, the need for frozen storage and distribution already presents challenges, especially in low-income countries in which even regular cold chains are difficult to maintain.

For the vaccines in clinical trials for which phase I/II data are available, we observe both an immunogenicity and a reactogenicity gradient. In terms of immunogenicity, inactivated and AdV5-based vaccines seem to rank the lowest, followed by ChAdOx1-based vaccines and mRNA vaccines, and finally adjuvanted, protein-based vaccines, which show the best performance. Reactogenicity seems to be lowest in inactivated and protein-based vaccines, followed by mRNA vaccines, with vectored vaccines having the highest rate of side effects. It is highly likely that the vaccine candidates from AstraZeneca, Moderna and Pfizer—which have progressed the furthest in clinical trials in the USA and Europe—will all show sufficient efficacy and will be licensed if they are shown to be sufficiently safe. However, it might also be the case that these vaccines will be replaced at a later date by newer candidates that show similar efficacy but have more tolerable reactogenicity profiles. In addition, it is difficult to predict how availability and production capacity will shape the global landscape of SARS-CoV-2 vaccines. Although they might not be licensed in the USA and Europe, it is very likely that AdV5-based and inactivated vaccines produced in China—as well as other vaccine candidates produced in India and elsewhere—will have a major role in satisfying the global demand for vaccines against SARS-CoV-2.

Despite all the challenges discussed here, we are in the process of developing vaccines as a countermeasure against SARS-CoV-2 at an unprecedented speed, and it is certainly possible that vaccines with safety and efficacy that have been proven in phase III trials might enter the market in 2020.

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**Competing interests** The Icahn School of Medicine at Mount Sinai has filed patent applications relating to SARS-CoV-2 serological assays and NDV-based SARS-CoV-2 vaccines. I would also like to note the following, which could be perceived as a conflict of interest: I have previously published work on influenza virus vaccines with S. Gilbert (University of Oxford), have consulted for Merck and Pfizer (before 2020), my laboratory is collaborating with Pfizer on animal models of SARS-CoV-2, my laboratory is collaborating with N. Pardi at the University of Pennsylvania on mRNA vaccines against SARS-CoV-2, my laboratory was working in the past with GlaxoSmithKline on the development of influenza virus vaccines and one of my mentees has recently joined Moderna.

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