

# **Immunogenicity of clinically relevant SARS-CoV-2 vaccines in non-human primates and humans**

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**Abstract**

Multiple preventive vaccines are being developed to counter the COVID-19 pandemic. The leading candidates have now been evaluated in non-human primates (NHPs) and human Phase 1 and/or Phase 2 clinical trials. Several vaccines have already advanced into Phase 3 efficacy trials, while others will do so before the end of 2020. Here, we summarize what is known of the antibody and T-cell immunogenicity of these vaccines in NHPs and humans. To the extent possible, we compare how the vaccines have performed, taking into account the use of different assays to assess immunogenicity and inconsistencies in how the resulting data are presented. We also summarize the outcome of SARS-CoV-2 challenge experiments in immunized macaques, while noting variations in the protocols used, including but not limited to the virus challenge doses.

## Introduction

The COVID-19 pandemic rages unabated and may continue to do so until there is a safe, effective and widely used protective vaccine. Multiple vaccines to prevent SARS-CoV-2 infection and/or COVID-19 disease are now progressing through pre-clinical testing and Phase 1/2 human trials, while some are already in Phase 2/3 efficacy trials in and outside the USA (<https://www.who.int/who-documents-detail/draft-landscape-of-covid-19-candidate-vaccines>) (1-27) (Table 1). Several of these mid- to late-stage vaccines are part of the US Government's Operation Warp Speed (OWS), which has recently been reviewed elsewhere (27). Multiple vaccine candidates produced in China are also well advanced in the evaluation and approval process (1, 2, 11, 14, 20-22, 27). Phase 1/2 trial data on a Russian vaccine have now been published (26).

All the vaccines are either based solely on the viral Spike (S)-protein, which is administered by various methods including expression from non-replicating adenoviruses and nucleic acid vectors or as recombinant proteins, or are inactivated viruses that include the S-protein together with all other structural viral proteins (Table 1). All the vaccine candidates listed in Table 1 involve full-length S-proteins; variants with truncations of the transmembrane region and/or the cytoplasmic tail were tested as comparators in two studies (4, 5). Some S-protein constructs incorporate two proline substitutions in the S2 region that stabilize the expressed trimer in the pre-fusion structure that is considered to be optimally immunogenic for the induction of NAbs (29). The same method was used to stabilize the respiratory syncytial virus F-protein and improve its immunogenicity (30). In one macaque study, a double proline mutant of the SARS-CoV-2 S-protein was shown to be superior to other constructs that contained or lacked stabilizing changes, truncations or alternative leader sequences for inducing NAbs (4). Comparative experiments in

mice led to the inclusion of the same double proline change (plus a furin cleavage site knockout) in the Sanofi Pasteur mRNA vaccine (12). However, in another macaque experiment, NAb responses to a wild type, full-length S-protein construct were modestly better than to other constructs, including a proline-stabilized soluble S-protein (5). The S-protein expressed in the AstraZeneca/Oxford University ChAdOx1 nCoV-19 vaccine does not include any stabilizing changes (3, 15). The Russian rAd5 and rAd26 adenovirus vectors express a full-length S-protein, but the published report does not mention whether stabilizing mutations were added (26). Similarly, it was not stated whether the S-protein in the CanSinoBio Ad5-nCoV vaccine was stabilized (21, 22). Whether the known or likely absence of stabilizing changes impacts the performance of these various adenovirus vaccines is not known. The Pfizer/BioNTech BNT162b1 vaccine was based on the S-protein's receptor-binding domain (RBD), but did not enter Phase 3 trials (Table 4) (23-25).

It is of considerable scientific and public interest to know the immunogenicity of the leading vaccines in absolute and, to the extent possible, comparative terms. Here, we have reviewed antibody and T-cell immune response data derived from published studies of vaccines that were tested in non-human primates (NHPs) and then progressed into human Phase 1/2 trials, or that are in human trials without a prior NHP experiment (Tables 2-4). We have also evaluated macaque vaccine-challenge experiments, including how they were performed, as the outcomes are relevant to understanding the protective potential of SARS-CoV-2 vaccines (Table 3). The NHP experiments are described in references (1-12), the human trials in references (13-27).

The Chinese government authorized the CanSino Ad5-nCoV vaccine for military use in June 2020, presumably on the basis of the Phase 1/2 trial data (21, 22). In August 2020, the Russian government approved an Ad5 plus Ad26 adenovirus-vector vaccine, Gam-COVID-Vac, after

minimal safety testing and with no evidence of protective efficacy, several weeks before Phase 1/2 trial data were published (26). Reservations have been expressed about the suitability of Ad5 vaccines for use in areas of high HIV-1 incidence, based on the risks of increased HIV-1 acquisition (31). This concern would apply to both the Cansino Ad5-nCoV and the Gam-COVID-Vac vaccines (21, 22, 26). The approval processes that will be applied to the American OWS vaccines are outlined in brief elsewhere (28).

The immunogenicity of some of the >150 vaccine candidates now in pre-clinical development worldwide has been tested in small animals and, in some cases, NHPs. These reports are beyond the scope of this review, although we and others have summarized several previously (32, 33). Small-animal immunogenicity studies that directly relate to the vaccine candidates we review here are described in several of the papers on NHP experiments and human trials, and also in references (34-38).

### **Assessing antibody responses to vaccine candidates**

Antibodies induced by the S-protein based immunogens are generally measured in two ways. Immunoassays, usually but not always ELISAs, quantify antibody binding to the S-protein or fragments thereof, such as the RBD. Neutralization assays assess the abilities of neutralizing antibodies (NAbs) to inhibit SARS-CoV-2 infection of target cells. The binding and neutralizing antibody assays both have value, and titers derived from them generally correlate reasonably well. However, neutralization assays quantify antibodies that block infection while ELISAs and other binding antibody assays also detect antibodies that lack such properties (non-neutralizing antibodies, or non-NAbs). Other assays are sometimes used, for example to detect antibodies that inhibit the binding of the S-protein or its RBD to a soluble form of the angiotensin-converting enzyme 2 (ACE2), which is the entry receptor for SARS-CoV-2. We restrict our discussion to

binding antibodies and NAbs, with some exceptions. A general theme in the papers we summarize is the use of COVID-19 convalescent plasma as comparators for vaccine-induced antibody responses. We have ignored all of these data sets. The serum panels differ among the various studies, and the range of antibody titers seen in COVID-19 patients can span a 5-log range and vary considerably also during convalescence (32). Accordingly, we have not found the convalescent serum panels helpful when gauging the relative immunogenicity of the various vaccine candidates. There is a compelling need to now assemble and use a standard panel containing neutralizing monoclonal antibodies and/or validated convalescent plasma or purified IgG preparations for this purpose.

As we have noted previously, different research groups use different assays and measure antibody binding and virus neutralization differently, which greatly complicates comparisons of datasets (32). While binding antibodies are most often measured using ELISAs, which tend to be fairly similar wherever they are performed, different measurements are recorded. Thus, endpoint titers are presented in some reports but 50%-binding titers ( $ED_{50}$ , effective serum dilution factor giving 50% of maximum binding) in others. Indeed, in some high-profile descriptions of Phase 1 human trials ELISA data are presented in idiosyncratic formats that compromise attempts to cross-compare vaccine immunogenicity. NAbs are measured using either replicating viruses (RV assays) or S-protein pseudotyped viruses that do not complete an infection cycle (PV assays). Usually, but not always, PV assays are a few-fold more sensitive than RV assays and therefore generate higher titer values. The different conditions used to perform RV and PV assays can, however, also affect their sensitivity. The most common practice is to report neutralization data as 50%-neutralizing titers ( $ID_{50}$ , inhibitory serum dilution factor giving 50% infectivity), but this is not always done. Other measurements include the more stringent neutralization  $ID_{80}$ ,  $ID_{>99}$  values, or endpoint

binding titers, as well as areas under the curve (AUC). An additional point is that, in some papers, titer values are not listed in Tables, Figures or the text but can only be estimated from diagrams. When we have had to make such estimates, the values we list in the text and Tables are preceded by an  $\sim$  symbol, which we also use when approximating for succinctness. When multi-digit NAb and binding titer values are reported in the original papers, we repeat those numbers in this review but note that it is rarely justified to use more than two significant figures in such circumstances. Finally, we report median and geometric mean (GM) values for the study groups as was done in the original papers. The range of SARS-CoV-2 vaccine-induced antibody titers seen in groups of NHPs and humans generally exceeds 100-fold and can be as high as 1000-fold. As noted previously, the existence of such a wide range of responses has implications for the proportion of a population that a vaccine can protect (32). The titer spreads reported in the various primary papers are worth comparing from this perspective, which we do where indicated.

Antibody responses in the various studies are usually measured for only a short period after the final immunization or, in the case of some macaque experiments, after the virus challenge. The same constraint applies also to the T-cell data. In one study where evaluations were carried out for longer than is normal, rhesus macaques were immunized twice with the Pfizer/BioNTech BNT162b2 mRNA vaccine. The peak anti-S1 protein antibody titers then declined  $\sim$ 5-fold over a 28 day period while NAb RV IC<sub>50</sub> titers also dropped  $\sim$ 5-fold in the high-dose (100  $\mu$ g) group (10). We estimate that the early-phase half-life of these antibody titers is only 1-2 weeks. The lack of knowledge of the longevity of SARS-CoV-2 vaccine-induced immune responses in humans is a substantial gap that will need filling.

In almost all of the papers we review, antibody responses are measured only in serum. There has been very little attention paid, to date, to mucosal immune responses, which seems

unfortunate given how SARS-CoV-2 is transmitted and where it predominantly replicates. Accordingly, we cannot address mucosal immunity in this review, other than by noting that one recent preclinical study of a chimp adenovirus vaccine (different from the AstraZeneca clinical candidate) in mice highlights how important inducing and characterizing mucosal immune responses might turn out to be (38).

In short, it is often difficult to inspect two different papers on vaccines A and B and conclude with certainty which one induces the superior immune response. Knowledge of how vaccines of different designs generally perform can help form judgements, but there must always be caveats.

### **Assessing T-cell responses to vaccine candidates**

T cell responses to vaccine immunogens are generally measured by quantifying the amount of cytokine expressed by a T cell after specific antigenic stimulation from a peptide, protein or vector delivered antigen. The ELISPOT assay is most often used, or variants thereof, with peripheral blood mononuclear cells (PBMC) being the commonest source for T cells. Interferon gamma secretion is the most commonly chosen cytokine output but other cytokines are sometimes also measured, as is the production of granzyme B. Cytokine flow cytometry (CFC) is often used as a read out, and there is generally a good correlation between ELISPOT and CFC assay results. An advantage of the CFC assay is that it can directly identify the phenotype of responding T cells, which requires depletion procedures when ELISPOT is used. Assays for antigen-specific CD4+ T cells sometimes measure the upregulation of surface activation induced markers (AIM). However, these methods do not usually measure T cell avidity or test the potency of cells in viral inhibition assays (39). Here, we confine our discussion to ELISPOT assays, with some exceptions.

Depending on the vaccine candidate antigen, a T cell assay can use individual peptides, pooled or matrix-pooled peptides, protein or vector-expressed antigen as a source of peptides to bind to the MHC molecules that are expressed on the cell surface and recognized by a specific T cell receptor. As cross-reactive T cells are known to occur, most assays will not specifically identify a response that was elicited by prior exposure to a cross-reactive pathogen (or a different vaccine). For example, an earlier infection with one of the common cold coronaviruses might lead to a secondary memory response that could skew the outcome of the SARS-CoV-2 vaccine trial analysis, unless prior infection by those other coronaviruses is an exclusion criterion (which is rarely if ever the case).

ELISPOT results are usually expressed as spot forming cells (SFC) per  $10^6$  input PBMC, but this is not a uniform practice. For example, some investigators use SFC/ $10^5$  cells as their read out; we multiply their values by 10 and report them as SFC/ $10^6$  cells. We also use the abbreviation SFC rather than SFU when the latter is used in the original paper. There are also variations in methodologies, including the length of time between blood draw and cryopreservation, the method used for thawing, the peptide concentration used, the duration of peptide incubation with the cells, the time taken to complete the assay, and whether responding T cells are separated. All of these factors can affect an ELISPOT result, and need to be considered when comparing different studies. A general feature of the papers we have summarized is a lack of detail on how the assays were performed. It would also be useful if images of key ELISPOT plates were provided as raw data, to allow the spots to be re-counted. The timing of when cell samples are collected after a vaccine prime or boost is also relevant. Thus, the time-dependent decay of circulating T-cells affects the

magnitude of the responses measured *in vitro*, to a greater extent than applies to the more stable antibody responses.

Concerns have been expressed that SARS-CoV-2 vaccines may cause enhanced disease in infected animals, based on data arising in earlier animal model experiments with vaccines against other coronaviruses (32, 33, 40-42). One particular potential problem is referred to as Vaccine-Associated Enhanced Respiratory Disease (VAERD; 41). While it is not possible to determine whether VAERD will be a problem with SARS-CoV-2 vaccines prior to the outcome of efficacy trials and post-licensure safety assessments, the pulmonary dysfunctions are associated with increased production of IL-4, -5 and -13, eosinophil recruitment and impeded CD8+ T-cell responses (41-43). This pattern of immune responses is indicative of Th2-polarization. Accordingly, some of the NHP and human experiments include analyses of *in vitro* cytokine release profiles, to seek signs of unwanted, Th2-biased responses. To date, Th2 responses have rarely been seen. We briefly note the outcomes of such analyses when they were performed.

### **Immunogenicity of vaccine candidates in NHPs**

Immunogenicity studies have been performed in rhesus or cynomolgus macaques or, in one case, baboons (1-12). The immunogens were generally tested beforehand in small animals, often but not always mice, to provide initial assessments of their performance and to provide some indication of the dose or dose range to then evaluate in NHPs. Here, we focus only on the NHP studies themselves; the primary papers should be consulted for the small animal data. In general, the NHP experiments also involved safety assessments. The outcomes were unexceptional in that no significant problems were reported in the primary papers, which should, again, be consulted for details. Key serum antibody titer values recorded in this section are summarized in Table 2 and, for data obtained at the time of closest to challenge, also in Table 3. T-cell response data are

similarly summarized and tabulated, although these assays were not performed in several of the studies. In all cases, the vaccines were administered intramuscularly (i.m.), which also applies to the human clinical studies (see below). However, small-animal studies of a chimp adenovirus vaccine and an Ad5 vaccine (not the AstraZeneca/Oxford and CanSino clinical candidates, respectively) suggests that viral vectors might be very fruitfully delivered by the intranasal route instead (38, 44).

Usually, one or more sub-groups of macaques were rolled over into a SARS-CoV-2 challenge study, or the optimal regimen was tested in a *de novo* experiment. Some details and the outcomes of the virus challenges are summarized separately below and in Table 3.

The first macaque immunogenicity paper to appear described PiCoVacc, the Sinovac  $\beta$ -propiolactone-inactivated, Vero cell-produced virus vaccine (1). (Note that this vaccine was renamed CoronaVac for human clinical trials (Table 1) (13). Two vaccine doses ( $3 \mu\text{g}$  and  $6 \mu\text{g}$  of viral protein) with an Alum adjuvant were tested on groups of 4 rhesus macaques via three immunizations on days 0, 7 and 14. The  $6 \mu\text{g}$  dose elicited slightly the stronger antibody responses on day 21, when the anti-S-protein GM ELISA endpoint titers were  $\sim 12,800$  and NAb GM ID<sub>50</sub> values were  $\sim 50$  in an RV assay (Table 2). Antibodies specific for the RBD dominated the antibody response to the inactivated virus vaccine, which is relevant to understanding the outcome of the challenge experiment (see below).

The Sinopharm/BIBP inactivated virus vaccine, BBIBP-CorV, was also produced in Vero cells and inactivated with beta-propiolactone. Mixed with Alum adjuvant, three different doses ( $2 \mu\text{g}$ ,  $4 \mu\text{g}$  and  $8 \mu\text{g}$  of viral protein) were administered to groups of 10 cynomolgus macaques on days 0, 7 and 14 (2). The resulting NAb titers, measured in an RV assay, were dose-dependent, with a GM ID<sub>50</sub> value of  $\sim 210$  reported for the highest dose group on day 21 (Table 2).

The ChAdOx1nCoV-19 recombinant virus vector expresses a non-stabilized form of the SARS-CoV-2 S-protein (3). Groups of 6 rhesus macaques received this vaccine ( $2.5 \times 10^{10}$  particles) either once (day 0) or twice (days 0 and 28) in a prime-boost protocol. In the single-dose group, the anti-S-protein median endpoint titer on day 14 was  $\sim 600$  and the median NAb ID<sub>50</sub> value was  $\sim 20$  in an RV assay. The second dose boosted these responses to  $\sim 28,000$  and  $\sim 280$ , respectively, on day 42 (Table 2). The animals were challenged with SARS-CoV-2 on day 28 (one-dose group) and day 56 (prime-boost group), as summarized below.

Rhesus macaques were used to identify and evaluate the optimal design of the Janssen Ad26.COV2.S vaccine candidate (4). First, antibody responses to seven different S-protein variants were compared using a range of assays, leading to the selection of the optimal Ad26 S.PP design. After a single dose of this immunogen, median RBD-ELISA endpoint titers at week 4 were  $\sim 4000$ , while the PV and RV NAb median ID<sub>50</sub> values were 408 and 113, respectively (Table 2). In an IFN-gamma ELISPOT, at week 4, the median response elicited by the S.PP vaccine was only  $\sim 80$  SFC/ $10^6$  cells. The data were insufficient to confidently assess the Th1 vs. Th2 bias, as only IFN-gamma and IL-4 responses were measured. T-cell response data were presumably not factored into the decision to choose the S.PP construct as the clinical candidate, as this virus was the least immunogenic of the seven variants from the perspective of inducing CD4+ and CD8+ T cell immunity. The antibody responses were clearly prioritized.

DNA vaccines expressing 6 different SARS-CoV-2 S-protein variants, including the full-length S-protein and the RBD, were tested, without adjuvant, in rhesus macaques (5). Median endpoint anti-S-protein titers at week 5 varied moderately with the immunogen but were 140-180 for the full-length S-protein and RBD immunogen groups. Midpoint NAb titers at week 5 also

varied by immunogen, with median ID<sub>50</sub> values of ~50-200 and ~30-40 in PV and RV assays, respectively (Table 2). The full-length S-protein construct elicited somewhat stronger NAb responses than its RBD counterpart. At week 5, T-cell responses were detectable in ELISPOT assays with pooled S peptides (~80 SFC/10<sup>6</sup> cells in the S Group, Table 2). Intracellular staining showed IFN-gamma responses both in CD4+ and CD8+ subpopulations; the responses to full-length S were stronger than to S1 and RBD. Finally, IL-4 responses were barely detectable, which is compatible with a Th1 bias of the cellular immune responses.

Inovio's INO-4800 S-protein based DNA vaccine was given to 5 rhesus macaques in 1 mg doses at weeks 0 and 4 by an i.m. electroporation device that provides a mild electric shock to open membrane channels in muscle cells (6). The peak anti-S-protein GM endpoint titer (week 6) was ~130,000, but dropped ~40-fold by the time of challenge at week 17. The binding antibody endpoint titers against the RBD were ~5-fold lower than against the S-protein at week 6. In the PV NAb assay, the peak GM ID<sub>50</sub> titers were ~1000 but had declined to ~250 by week 12 (i.e., a 4- or 5-fold titer decrease over 6 weeks against the two PVs tested). Low titers of anti S-protein IgG (~10) were also detected in bronchoalveolar lavage (BAL) samples from vaccinated animals. An IFN-gamma ELISPOT was used to measure T-cell responses triggered by 5 peptide pools at week 6. Signals were seen with PBMC from 4 of the 5 animals, with a range of 0-518 SFC/10<sup>6</sup> cells and an arithmetic mean (AM) value of ~140. By week 12, the mean value had declined to only ~30.

The Moderna S-protein based vaccine candidate, designated mRNA-1273, was also tested in rhesus macaques (7) The lipid-encapsulated mRNA formulation was given i.m. at doses of 10  $\mu$ g or 100  $\mu$ g to each of two groups of 8 animals, at weeks 0 and 4 anti-S-protein ELISA data were presented only in the form of AUC values, precluding direct comparison with other studies. For

the high-dose group at 4 weeks after the second dose, the NAb GM ID<sub>50</sub> values in PV and RV assays were 1862 and 3481, respectively. In a CFC assay, Th1 responses were dose-dependent, while Th2 and CD8+ T-cell responses were at most minimal. Although all animals responded according to pre-specified criteria, the T-cell assay signals were generally weak, even at the highest vaccine dose. There were no differences in Th1- or Th2-associated cytokines or chemokines in BAL samples from the vaccine and control animals.

A paper describing the immunogenicity of the Pfizer/BioNTech BNT162b2 S-protein expressing mRNA vaccine in mice and rhesus macaques appeared several weeks after one that reported on the performance of the same vaccine in humans (Tables 2-4) (10, 23). Either 30 or 100  $\mu$ g doses of the mRNA were given intramuscularly to the macaques on days 0 and 21. Serum anti-S1 antibodies were quantified by ELISA on days 21, 28, 35, 42 and 56 and presented as ELISA units per ml (derived from comparison with a standard curve). On day 28 these values were 30,339 and 34,668 for the 30 and 100  $\mu$ g groups, respectively, but had declined ~5-7- fold to 4,236 and 6,317 by day 56. The pattern of NAb data, assessed by an RV assay, was similar; the peak NAb GM ID<sub>50</sub> values were 962 on day 35 and 1,689 on day 28 for the two dosing groups, but by day 56 they had dropped 4-5-fold to 285 and 310, respectively. The inferred half-life for the early-phase decline is approximately 1 week for the binding antibodies (ELISA) and 2 weeks for NAb, although the latter titers declined more rapidly at the later time points. Antibody decay rates this short are a potential concern for the longevity of any protection seen in humans, particularly if they are also seen in trials of the other vaccines. T-cell responses, measured by IFN-gamma ELISPOT on days 28 and 42, were ~750 SFC/10<sup>6</sup> PBMC for both dosing groups, with IL-4 responses below 250 SFC/10<sup>6</sup> PBMC (10).

A third S-protein based mRNA vaccine has now been described, MRT5500 from Sanofi Pasteur (12). Initial studies in mice led to the choice of a full-length S-protein construct, 2P/GSAS, that contains the commonly used two proline mutations (2P) and a furin cleavage site knock-out mutation (GSAS). The 2P/GSAS mRNA, formulated as lipid nanoparticles, was tested in a cynomolgus macaque dose-ranging study. Thus, 15, 45 or 135  $\mu\text{g}$  of the mRNA were given on days 0 and 28, leading to NAb responses on day 35 that trended upwards in a dose-dependent manner. For the 135  $\mu\text{g}$  dose group, the NAb GM IC<sub>50</sub> titers were 2871 (PV assay) and 1877 (RV assay). PBMC T-cell responses, assessed using a cytokine release (ELISPOT) assay, were Th1-biased (IFN-gamma release but not IL-13). The magnitude of the macaque IFN-gamma response was very low, around 30-40 SFC/10<sup>6</sup> PBMCs (Table 2). No virus challenge was performed (12).

The Novavax NVX-CoV3273 vaccine is an insect cell-derived S-protein that is mixed with detergent to form what are described as nanoparticles (8, 9, 19, 45). That formulation is combined with the Matrix M adjuvant. The immunogenicity study in baboons compared 1, 5 and 25  $\mu\text{g}$  doses of the S-protein in a two-dose (days 0, 21) regimen, while a fourth group received 25  $\mu\text{g}$  with no adjuvant. Antibody assays on days 21, 28 and 35 showed that the optimal dose was 5  $\mu\text{g}$  with adjuvant, with the peak response reached by day 28. The highest GM anti-S-protein GM EC<sub>50</sub> and NAb ID<sub>>99</sub> values were 174,000 and 17,000, respectively (8). The same protein/adjuvant combination was then tested in cynomolgus macaques (9). The animals were immunized on days 0 and 21 at different doses, with both the protein and adjuvant amount varying (protein at 2.5, 5 and 25  $\mu\text{g}$  doses). At the highest dose (25  $\mu\text{g}$  S-protein, 50  $\mu\text{g}$  adjuvant, mirroring one of the human study groups, see below), the anti-S-protein GM EC<sub>50</sub> value on day 35 was 469,739. Note that binding antibody data were presented as EC<sub>50</sub> values, not the more usual endpoint titers, which

would be substantially higher (perhaps 10-100 fold). In the RV NAb assay, the CPE<sub>100</sub> (inhibition of ~100% of the cytopathic effect, approximately equivalent to ID<sub>>99</sub>) was measured, with GM values ranging from 17,920-23,040 in the different dosing groups. It should be noted that the neutralization titers for near complete efficacy of neutralization measured in these studies (CPE<sub>100</sub> or ID<sub>>99</sub>) will be substantially lower than the more conventional IC<sub>50</sub> values, although we cannot estimate by how much. In summary, the NAb titers in these papers are based on highly stringent assessments of virus neutralization, which should be borne in mind to avoid underestimating the clearly strong antibody immunogenicity of NVX-CoV3273 (8, 9).

Another recombinant S-protein vaccine has been tested in rhesus macaques, in this case from the Chinese company, Clover Biopharmaceuticals (11). The S-protein contains a C-terminal “TRIMER-Tag”, to promote trimer formation and stability, and was produced in a high-yielding, stable CHO cell line. After pilot experiments in mice, rhesus macaques were given 30  $\mu$ g of the S-protein on days 0 and 21 in either AS03 or CpG/Alum adjuvant, or vehicle as a control (Table 2). On day 35, the strongest anti-S-protein antibody responses were in the AS03 group (GMT endpoint titers of ~17,497 vs. ~3,157 in the CpG/Alum). At the same time, the GM NAb IC<sub>50</sub> titers in the AS03 group were 5,227 and 20,234 in a PV and a RV assay, respectively. The D614G mutation in the recombinant trimer did not affect ACE2 binding or the competition therewith by murine immune sera. The partial protection observed in a virus-challenge experiment is summarized below (Table 3). A Phase 1 clinical trial of the S-protein with either AS03 or CpG/Alum adjuvant is now in progress in China (11).

### **Outcome and interpretation of macaque challenge experiments**

When interpreting the outcome of macaque challenge experiments, it should be borne in mind that SARS-CoV-2 does not cause a lethal COVID-19 like disease in these animals. The macaques do become sick, rhesus perhaps more so than cynomolgus, but the disease course is generally mild, self-limiting and overcome within ~2 weeks (46-48). In general, the various vaccines reduce the severity of this mild disease, including by reducing or even preventing the transient lung damage that can be seen in post-mortem samples taken from control animals. No signs of vaccine-mediated enhancement of infection, including VAERD, were reported. The most common way in which vaccine efficacy is assessed is by determining the viral load (VL) in samples from various locales and tissues at short intervals during the week after challenge. In some experiments, both viral RNA copies per ml and subgenomic RNA copies per ml are determined, the latter avoiding problems associated with the presence of residual challenge virus and more unambiguously demonstrating *de novo* replication in the infected animal (5, 49) (Table 3). Lung pathology was also generally assessed, although the criteria chosen tends to vary among the different experiments.

Antibody titers in the animals on or very close to the day of challenge were reported in some of the papers and are summarized below and also in Table 3. In the other papers, the antibody data were derived at an earlier time point (Table 3). The inconsistencies in how the different studies were conducted and/or reported is another factor that blurs attempts to compare and interpret the performances of the different vaccines. Only three of the reports include data on T-cell responses at any time point prior to challenge, which limits understanding of any role they may play (Table 2) (4-6). In a separate section, we discuss what, if any, correlates of protection (CoP) can be inferred from some of the challenge experiments.

Most of the experiments involved SARS-CoV-2 challenges within a few weeks of the final (or only) vaccine dose, i.e., at a time when the immune response is likely to be close to its peak. The exception is the Inovio DNA vaccine study in which the challenge was delayed by 13 weeks (6). The next longest delays are 55 days after the second dose of the BNT162b2 mRNA vaccine and 42 days after the delivery of a single dose of the Ad26.COV2.S vaccine (Table 3) (4, 10). Thus, it is not yet known whether these various vaccines would be as effective against challenges conducted many months after the immunization protocol was completed. Extrapolating to what might happen when vaccinated humans become exposed to SARS-CoV-2 over the subsequent months or years is not possible.

Another issue when considering these macaque experiments is the SARS-CoV-2 challenge itself. There is no generally accepted standard, and various different challenge virus stocks (in several cases, of unspecified origin) were used. The challenge dose also varies by 100-fold and the route of challenge is another variable (Table 3). In one experiment, the virus was even administered by four different routes (3). All of these protocol variations constitute yet another factor hindering cross-study comparisons. As a general principle, it will be easier to protect against a low dose of a challenge virus than a higher one, all other things being equal. Thus, would a vaccine that protected against a relatively low challenge dose be as protective against the 100-fold higher dose used in other experiments? Or would its protection break down under those conditions? We return to this point at the end of this section. Challenge doses for vaccine experiments are traditionally predetermined in naïve animals, to identify an inoculum size that is neither too low to be consistently infectious nor too high to protect against. It is rarely clear from the papers whether such titrations were performed. In one report, nasal swab VLs taken from SARS-CoV-2-infected humans and from the virus-challenged macaques soon after infection were said to be comparable ( $\sim 1 \times 10^6$

RNA copies/ml) (7). However, the initial infection and subsequent replication efficiencies are likely to differ substantially between the two species so it is not clear that this comparison is useful.

In the Sinovac PiCoVacc study, groups of 4 rhesus macaques were immunized with either 3  $\mu$ g or 5  $\mu$ g of the inactivated virus vaccine on days 0, 7 and 14 and challenged intratracheally with the CN1 strain of SARS-CoV-2 on day 22 (1). At this time, the anti-S-protein GM endpoint titer was  $\sim$ 12,800 while the NAb GM ID<sub>50</sub> titer was  $\sim$ 50 when measured 7 days earlier. All the vaccinated animals became infected after challenge, but disease severity was reduced compared to the control group (adjuvant-only) as judged by lung pathology assessments. VLs (i.e., viral RNA) was frequently detected at high levels in lung samples from control animals but in none of the high-dose vaccine recipients and only sporadically at significantly lower levels in the low-dose group. Viral RNA levels in throat swabs were also lower and declined more rapidly, particularly in the higher vaccine-dose group (Table 3). The observed increases in NAb titers after day 7 post-infection may be consistent with an anamnestic antibody response (1).

The Sinopharm/BIBP inactivated virus vaccine experiment involved two groups of 4 cynomolgus macaques that were immunized with different doses (2  $\mu$ g or 8  $\mu$ g of viral protein) on days 0 and 14 (2). Binding antibodies were not measured. The NAb GM ID<sub>50</sub> values in an RV assay were  $\sim$ 200 and 230 in the low and high dose groups when the animals were challenged on day 24 with a SARS-CoV-2 isolate from the Chinese Center for Disease Control and Prevention, by the tracheal route (Table 3). There were no changes in body temperature in either the vaccine or placebo groups over the next 7 days, which is indicative of a mild disease course. Viral RNA in all lung lobes was analyzed post mortem, but none was detected in any lobe taken from vaccine recipients (in either dosing group). In contrast, the RNA copy number per ml ranged from  $\sim$ 30,000 to 3,000,000 in the lower lobes of the control animals. Lung pathology was also prevented or

reduced in the vaccine groups. Although viral RNA in throat swabs became undetectable 7 days after challenge in the high-dose group, other evidence suggests these animals did become infected, albeit to a much lesser extent than the control and low-dose vaccine groups. Thus, gastrointestinal virus (detected in anal swabs) remained stable in the high-dose at ~ 100 RNA copies per ml from day 3-7, whereas the corresponding values in the two other groups fluctuated in a range around 100,000. While this study only analyzed viral, not subgenomic, RNA, it seems highly unlikely that gastrointestinal viral RNA could simply represent residual challenge virus. Thus, the higher-dose animals were at least strongly, but apparently not completely, protected from infection, and in both dosing groups the vaccine reduced the extent of virus replication post-infection (2).

All 12 of the ChAdOx1-vaccinated macaques became infected when they were challenged 28 days after their final immunization (they received either 1 or 2 vaccine doses, see above) (3). The SARS-CoV-2 challenge strain was WA1-2020 (MN985325.1). At the time of challenge, the median binding antibody endpoint titers were ~6,300 with median NAb ID<sub>50</sub> values of ~60 in a RV assay (Table 3). The vaccinated animals had fewer symptoms than the control group, less lung damage and lower VLs (measurements included subgenomic RNA) in BAL and lung samples. No virus was detected in BAL samples from the vaccinated animals on day 5, but subgenomic RNA could be detected in lung samples from some animals in both groups. No antibody or T-cell data post-challenge were reported, so it is unknown whether there were anamnestic responses to the infecting virus (3).

Seven different Ad26-based vectors were given once to groups of 4-6 rhesus macaques before challenge 6 weeks later with an unspecified isolate of SARS-CoV-2 (4). Compared to a control group of 20 animals, VLs in BAL and nasal swabs were significantly reduced in each of the 7 Ad26 vector groups, by >5-log in the case of BAL samples. The best performing vector, from

this perspective, was the one designated S.PP; it was chosen to become the Ad26.COVID.S clinical vaccine candidate. Overall, the authors assessed that 17 of the 32 vaccinated macaques were protected from infection, judged by the VL data. There was no evidence for anamnestic B- and T-cell responses in the protected Ad26.COVID.S vaccinated animals, although NAb titer increases were seen in other vaccine groups. The strongest, and perhaps complete, protection was seen in the S.PP group. Thus, virus (subgenomic RNA) could not be detected in BAL from 6/6 and in intranasal swabs from 5/6 animals (4). More recently, hamsters immunized once with the S.PP-expressing Ad26 clinical vaccine candidate were protected from severe disease when challenged with SARS-CoV-2 nasally 4 weeks later (37).

In another study, rhesus macaques were immunized i.m. with S-protein-expressing DNA plasmids at weeks 0 and 3, and challenged at week 6 with an unspecified SARS-CoV-2 isolate (5). All of the 10 control animals became infected, with BAL and nasal swab peak subgenomic RNA levels copies in the range  $10^4$ - $10^7$  per ml. However, 8 of the 25 vaccine recipients were RNA-negative in BAL and nasal swab samples while median subgenomic RNA levels in the other 17 macaques were 3-4 logs lower than the median values from the 10 control animals. Even when subgenomic RNA was undetectable in vaccinated animals, the observation of anamnestic antibody and T-cell responses does imply that the animals were not completely protected from infection. Instead, initially replicating virus may have been suppressed by vaccine-mediated immunity (5).

The Inovio INO-4800 DNA vaccine was given to 5 rhesus macaques at weeks 0 and 4 (6). The SARS-CoV-2 challenge (USA-WA1/2020 strain) was then delayed until week 17 (i.e., 13 weeks after the second immunization), a substantially longer period than applies in the other studies summarized here. Upon challenge, all the macaques became infected, judged by VLs in various samples. However, VLs in the vaccinated group were lower and declined more quickly

than in 5 control animals, the reduction in medians being in the <10-300-fold range depending on the sample site and the time point assayed; the difference was significant for BAL but not intranasal swab (INS) samples. Antibody and T-cell recall responses were quantified in the animals after virus challenge. Thus, by 14 days post-challenge anti-S-protein antibody and NAb measurements were higher (~10-30 fold) in the vaccinated than control animals, while there was an ~2-fold increase in INF-gamma signals. Overall, the vaccine-mediated reduction in viremia post-challenge was attributed to recall responses (i.e., T- and B-cell memory) (6).

Two dosing groups of 8 rhesus macaques were immunized with the Moderna mRNA-1273 vaccine at weeks 0 and 4 and then challenged with the USA-WA1/2020 strain of SARS-CoV-2 at week 8 (7). Judged by VLs, most (~7 of 8) of the animals in the higher dose group were protected, but most (~5 of 8) of the lower dose group became infected (the exact numbers vary per time point, and depend on the VL sample site). There were indications of anamnestic responses in some animals, including in BAL fluids. Post-mortem analyses of the lungs found little or no signs of inflammation in the higher-dose group, but some indications of pathology in the lower dose animals that became viremic. Neutralization titers in both PV and RV assays correlated inversely with INS viral loads; virus-specific IgG and IgA levels in BAL were elevated in the high dose group on days 2-7 post-challenge, an anamnestic response that may perhaps have contributed to VL reduction. In the high-dose, high-protection group, the GM ID<sub>50</sub> values from PV or RV NAb assays were >900 in 7/8 animals, whereas the corresponding values were <900 for 7/8 animals in the low-dose, low-protection group. The data pattern implied that NAbs were protective in the high-dose group (7).

In the BNT162b2 vaccine study, 6 rhesus macaques immunized on days 0 and 21 with 100  $\mu$ g of the mRNA and 3 naïve controls were challenged on day 76 overall with  $1.05 \times 10^6$  PFU of

the USA-WA1/2020 strain of SARS-CoV-2 by the IN-IT routes (10). Antibody titers on the day of challenge were not reported, but measurements made on day 56 are summarized above and in Tables 2 and 3. Infection was monitored by viral RNA copies in BAL, nasal swabs and oropharyngeal swab on days 3, 6 and 10. No viral RNA was detected at any time in the BAL samples from the immunized macaques but was present control samples. A similar data pattern, after day 1, was seen when the nasal swabs were analyzed, and in the oropharyngeal swabs, 2/6 were positive on day 3. The GM reductions in VL measured as viral RNA copies  $\sim 3.0$  log for BAL, 1.5 log for intranasal swabs and 2.5 log for oropharyngeal swabs (the first and last of which were statistically significant). One unusual aspect of the experiment was that the complete absence of disease symptoms in the control animals (and, of course, also in the vaccine recipients). As noted above, SARS-CoV-2 infection generally causes moderate disease in rhesus macaques, including in other vaccine experiments. Why no such symptoms were seen in the present study was not explained, but could perhaps be rooted in the challenge virus stock or the origins of the macaques (10).

The adjuvanted Novavax NVX-CoV2373 recombinant protein vaccine was given to cynomolgus macaques on days 0 and 21 before the animals were challenged with the WA1 strain of SARS-CoV-2 via the nasal and tracheal routes on day 35 (9). Judged by VLs (subgenomic RNA) in BAL and nasal swabs, performed 2 and 4 days later, every animal was virus-negative except for one in the lowest dose group that had a weakly positive BAL sample. Post-mortem lung samples in the vaccine groups showed no sign of the pathologies that were visible in the control animals. To the extent that can be judged, the vaccinated animals may have been completely protected from infection. This outcome may reflect the very high GM antibody titers on the day of challenge (anti-S-protein  $EC_{50}$  469,739, NAb  $CPE_{100}$ , 23,040) (9).

Clover Biopharmaceuticals tested their recombinant “TRIMER-Tag” S-protein plus AS03 or CpG/Alum adjuvants in rhesus macaques (11). After two vaccine doses on days 0 and 21, the animals were challenged on day-35 using  $2.6 \times 10^6$  TCID<sub>50</sub> of SARS-CoV-2 by the IN+IT routes (Table 3). Both of the trimer/adjuvant groups of animals were partly protected as measured by body mass and temperature and VL in throat, anal and tracheal but less so in nasal swab samples. However, VLs in the lungs indicated complete protection in both of the trimer groups, compared with the vehicle controls. Serum Ab responses dropped a little in the week post-challenge, which the authors suggested reflected the formation of immune complexes with the incoming virus and hence Ab-mediated virus clearance (11). Whether this explanation is correct remains to be determined.

In summary, all of the vaccines tested to date have conferred a substantial degree of protection to the immunized macaques. In some cases, there is reasonable evidence for complete protection (i.e., ‘sterilizing immunity’), but the more common outcome is a reduction in the severity of the already mild disease course seen in control animals. We discuss in the next section what immune factors and other variables may have influenced the outcomes of the different experiments. In respect of what the outcomes may mean for vaccine efficacy in humans, we note that it is generally easier to protect animals against mild infections than severe ones. Hence, it is hard to assess whether and how any of the present findings in macaques might translate to the subset of humans who need protection from severe and lethal COVID-19. Moreover, as noted above, it is not known whether the various vaccines would still protect macaques, and by extrapolation humans, after a substantial period (multiple months) has elapsed.

### **Towards correlates and mechanisms of protection**

It is striking that in the various macaque immunization studies, similar outcomes were associated with substantial (~2000-fold) differences in serum antibody titers to the S-protein, the NVX-CoV2373 recombinant protein vaccine being the strongest immunogen for inducing binding antibodies (8, 9) or NABs (8,9,11) (Table 2). It can also reasonably be concluded the ChAdOx1 vaccine is not a strong inducer of antibody responses to the S-protein, particularly when given only once (Table 2) (3). The same inference can be made about the DNA plasmid vaccines (Table 2) (5, 6). Are the serum antibody responses induced by the weaker vaccines solely responsible for any protection that was conferred? Perhaps cellular immune responses or some other unmeasured factor, such as mucosal IgA, were contributory? The potential protective role of mucosal immunity is highlighted by the outcomes of experiments involving a ChAd virus vector in mice (38). These inferences are similar to what has been seen in studies of other vaccines, such as HIV-1 Env, where only protein-based immunogens induce very strong antibody titers (50).

What protected the vaccinated animals from SARS-CoV-2 infection and/or disease? CoPs are important in vaccine development, because they can serve as robust predictors of future vaccine efficacy whether they are derived from animal experiments or clinical trials and whether the endpoints involve protection from infection or a reduction in disease severity. There are nuances to the identification of CoPs in population-wide studies that we cannot address here (51, 52). The present macaque-challenge studies are not sufficiently powered and are not wide-ranging enough in scope to allow the identification of CoPs with high confidence. The few attempts to identify CoPs have pointed towards a predominant role for NABs, which is not unexpected, but it is premature to conclude that NAB titers at the time of challenge (i.e., in humans, virus exposure) tell the entire story. The possible role of recall responses (i.e., T- and B-cell memory) in clearing a transient infection has only been addressed in the Inovio DNA vaccine study, which involved the

longest delay between immunization and challenge (Table 3). Even in that experiment, the amount of information available is quite limited (6).

Despite the limitations of the available data, we sought hints of CoPs. Thus, we analyzed the relationships between, on the one hand, binding antibody and NAb titers at the pre-challenge peak or within two weeks before challenge and, on the other hand, VL reductions in vaccinated animals compared with controls (summed for two locales of sampling and based only on subgenomic RNA; Tables 2 and 3). We found no tangible non-parametric (Spearman) positive correlations of any significance between VL reduction and any antibody parameters, which is not surprising given the number of variables between the different experiments. We were also unable to identify any consistent relationship between the challenge virus dose or delivery route and the degree of protection. The challenge dose was not a consistent predictor of the magnitude or duration of high viral loads in the control animals, but we should bear in mind that the challenge virus stocks represent another variable, as does the sub-species (and sources) of the animals involved. Nonetheless, the wide variation in challenge dose between experiments should not be ignored. Would a vaccine that protected against a relatively low challenge dose be as protective against the 100-fold higher dose used in other experiments? Or would its protective capabilities degrade under those conditions? Experimental conditions yielding high VLs in control animals may impede complete protection while giving the potential for greater VL reductions. The smallest VL decrease in the vaccine group compared to control was seen in the Inovio DNA vaccine experiment which involved one of the lowest challenge doses (Table 3) (6). In the report on the Ad26.COVS2 vaccine, there were indications of sterilizing immunity when an intermediate challenge dose was used (4).

In four studies, some groups of vaccine recipients seem to be completely protected, or nearly so (4, 5, 9, 10). In one experiment, no anamnestic antibody or cellular immune responses were detected in the protected animals, which suggested that immunity was sterilizing (4). However, in another study there were anamnestic immune responses in animals with undetectable VLs, which is more indicative of incomplete but aborted infection (5). Anamnestic responses were not analyzed in the third report (9). In two other cases, lung lobes in the vaccine groups where protection was strongest were free of viral RNA 7 days after infection, which contrasted to the high levels found in the lower lobes of control animals (1, 2). The criteria for sterilizing immunity, or at least complete protection against persistent infection, are neither defined nor standardized in the SARS-CoV-2 animal model field, which also extends into other protection-challenge systems involving small animals and both vaccines and antiviral antibodies (31, 46-48).

Within individual studies there are fewer confounding factors than in a cross-study meta-analysis. Some evidence was presented that antibodies were the CoP in the Moderna mRNA vaccine study (7). In the report on the Janssen Ad26.COVID2 vector vaccine, comparing various antibody and T-cell responses with infection outcomes (as judged by VLs) identified NAb as the strongest CoP, with some possible contribution from Ab-effector functions such as antibody-dependent cellular phagocytosis and antibody-dependent activation of natural killer cells. In contrast, T-cell responses (measured by ELISPOT or intracellular cytokine staining; ICS) did not correlate with protection (4). Similar inferences about an antibody but not a T-cell CoP emerged from the experiments involving DNA plasmid immunizations (5). But even when significant differences were identified, the ranges of the various measurements were generally overlapping between completely and partially protected animals (4, 5). This degree of variation compromises attempts to identify the threshold response required for protection, particularly in the study of the

Ad26 vector vaccine (4). Overall, the available evidence from macaque-challenge experiments does point towards a protective role for antibody-based immunity (probably NAbs), but not to the extent that a protective titer can be inferred and then extrapolated to the outcome of human efficacy trials.

### **Immunogenicity of vaccine candidates in humans**

Key antibody and T-cell response data summarized below for individual trials are presented in Table 4. As with the NHP studies, the primary papers and reviews should be consulted for additional details of the human trials, which are variously described as Phase 1, Phase 2 or combined Phase 1/2a trials (13-27). Vaccine safety assessments were a key component of these trials; in all cases, reported side effects and adverse events were considered to be minor or moderate; the primary papers contain the details, which we have not attempted to summarize. A grade 3 serious adverse event (SAE) due to a neurological complication happened in the AstraZeneca/Oxford vaccine Phase 3 trial in the U.K., leading to a now-concluded pause while the case was investigated. A placebo recipient in the Brazilian arm of the Phase 3 trial of this vaccine reportedly died of COVID-19. An SAE, also triggering a temporary clinical hold, occurred in the Janssen Phase 3 trial, although no details have been reported.

The initial human trials have predominantly involved young/middle-aged, healthy adults (see primary papers for details). Some information is becoming available on age-dependent decreases in immunogenicity. In the CanSino Ad5-nCoV vaccine, participants aged older than 55 responded with weaker antibody responses than their younger counterparts. However, that outcome could reflect either the aging process or time-dependent increases in exposure to other Ad5 viruses that compromise expression of the immunogen from by the vector (22). An ~2-3-fold reduction in antibody responses was seen in older adults (aged 65-85) compared to younger ones

(aged 18-55) in a Pfizer/bioNTech mRNA vaccine trial (25). Moderna has now reported similar findings for their mRNA vaccine; in a small-scale (40 volunteer) extension to their original Phase 1 trial, antibody and NAb responses were comparable in volunteers aged 56-60 and over 71 and similar to what was reported for those in the 18-55 age range (17, 18). NAb responses were slightly lower in volunteers aged over 60 than in ones in the 18-59 range, during the BBIBP-CorV inactivated Phase 1 trial. The ratio was ~2-fold, but varied with the time point and vaccine dose, and group sizes were small (14). The Sinovac inactivated virus vaccine trial only involved volunteers under 60, but an analysis of the 18-29 vs. 50-59 age groups suggested that NAb responses were ~2-fold higher in the younger people. Overall, there was a modest trend towards weaker immunogenicity with age (13). A limited amount of preliminary data on the Janssen Ad26-COV2 vaccine also indicates that immunogenicity in volunteers aged over 65 is only modestly reduced (16).

Taken together, the studies summarized above are encouraging for the efficacy of the various vaccines in older adults, if that is the outcome of the Phase 3 trials being conducted in mostly younger people.

There has also been an under-representation of minority groups in the American and European trials, so again information on how immunogenicity might vary in different populations is lacking. These various lacunae will need to be filled in Phase 3 trials, given that COVID-19 is more severe in older people and in African-American and Latinx populations.

The Sinopharm/WIBP inactivated virus vaccine was delivered in Alum adjuvant. It was first tested in 96 volunteers in a Phase 1 trial and then in 224 more people in a Phase 2 study (20). The study cohorts were based on healthy individuals aged from 18-59. The Phase 1 trial was dose-ranging (2.5, 5, 10  $\mu$ g of viral protein) and involved i.m. injections on days 0, 28 and 56, while in

Phase 2 only the 5  $\mu\text{g}$  dose was tested in two sub-studies that involved immunizations on days 0 and 14 or on days 0 and 21. Immune responses were measured by ELISA using inactivated virus as the detecting antigen, which does not allow a comparison with other vaccines, and by a RV neutralization assay. For sera collected 14 days after the final dose, the NAb titers (GM ID<sub>50</sub> values) in the Phase 1 trial were 316, 206 and 297 in the low, medium and high dose groups respectively. Allowing for the titer ranges among participants, the three doses induced similar antibody responses. In the Phase 2 trial, the corresponding NAb titer values were 121 and 247 for the 0, 14 day and 0, 21 day groups, respectively. Anti-virus ELISA endpoint GM titers were also similar among the different test groups in the two trials, and were ~200-300 in Phase 1 and ~90-200 in Phase 2 (Table 4). There were no T-cell data in the paper. Phase 3 trials are now in progress in South America, although the vaccine dose and delivery regimen (i.e., the number and spacing of doses) was not specified in the report on the Phase 1 and 2 trials (20).

Sinovac's PiCoVacc inactivated vaccine was renamed CoronaVac prior to Phase 1 and 2 human trials (Table 1, 3) (1, 13). The production process used to make the vaccine was stated to be changed between the Phase 1 and 2 trials to yield a product with an ~2-fold higher S-protein content, which the authors suggest improved its immunogenicity (13). However, only data from the Phase 1 trial have been reported to date, the Phase 2 results still pending. For Phase 1, the Alum-adjuvanted vaccine (or a placebo) was given to 600 adults aged 18-59 years in a two-dose regimen on days 0 and 14 or days 0 and 28. For each of the two regimens, two vaccine doses, 3  $\mu\text{g}$  and 6  $\mu\text{g}$ , were tested in 120 volunteers, compared to a placebo group of 60. The safety profile was unexceptional. Antibody responses were measured on days 28 and 42 in an anti-RBD ELISA and NAbs in an RV assay with a CPE readout (the cutoff for neither assay was reported). Anti-RBD GM titers were ~1000 in all the four vaccine groups on day 28. The NAb GMT values for all the

groups at all the time points were generally in the 32-64 range (Table 4). The age-dependency of the antibody responses was noted above. No T-cell data were reported (13).

The BBIBP-CorV Alum-adjuvanted inactivated virus vaccine also advanced from macaque studies into Phase 1 and 2 human trials (14). The Phase 1 trial involved 192 volunteers aged 18-59 and 60 or older, who received vaccine doses of 2, 4 and 8  $\mu\text{g}$  on days 0 and 28. Only volunteers in the younger age-range participated in the Phase 2 trial, in which 448 people received a single vaccine dose of 8  $\mu\text{g}$  or two 4  $\mu\text{g}$  doses given first on day 0 and then on day 14, 21 or 28. Taken together, the trials involved multiple small sub-groups, which limits the statistical power of any comparisons. The paper should be consulted for details of how the different dosing regimens performed. The vaccine was generally safe, with only minor adverse events reported. Immunogenicity was assessed only in an RV NAb assay. The resulting NAb titers were modestly dose-dependent, were much stronger after two doses than one, and were slightly lower in the younger than older age groups. In Phase 1, the 8  $\mu\text{g}$  on day 0 and 28 regimen gave a GM titer of 228.7; in Phase 2, the highest NAb titers were seen in the group given an 8  $\mu\text{g}$  dose on day 0 and 21, for which the GM titer was 282.7 (Table 4). No data on ELISA anti-virus or anti-S-protein titers, or on T-cell responses, were reported. Phase 3 trials are underway, using a two-dose regimen, but no details of the doses and scheduling chosen were provided (14).

A fourth Chinese Alum-adjuvanted, inactivated virus vaccine, from IMB/CAMS/PUMC, has also entered human trials, although no prior preclinical data were reported (27). This vaccine virus was also produced in Vero cells but, unlike the other three, it was inactivated first with formaldehyde before beta-propiolactone treatment. Another distinguishing feature is that the virus strain used contains the D614G substitution in the S-protein. In the Phase 1 trial, the IMB/CAMS/PUMC vaccine was given twice to 192 people aged 18-59 on days 0 and either 14 or

28, at doses of 50, 100 or 150 EU (the stated unit of antigen content). There were no significant adverse events and sera from selected volunteers did not trigger antibody-dependent enhancement *in vitro*. Immunogenicity was assessed at several time points using an RV NAb assay, via various ELISAs including anti-S-protein and anti-virion, and by IFN-gamma ELISPOT. The paper should be consulted for data on the 9 individual sub-groups in the trial, but in general the GMT NAb titers (CPE with unspecified cutoff) were all <100 and often <50. Anti-S-protein GM titers (with no cut-off specified) were 2000-4000 for the two highest doses of immunogen (Table 4). Only limited data were presented for the day 0, 28 group after the second dose. Thus, on day 90, the GMT NAb titer was <10 and the anti-S-protein titer was ~500, which indicate a time-dependent reduction in the initial antibody levels. IFN-gamma ELISPOT assays using S peptides gave AM values of 30-250 per  $10^6$  cells for the two vaccine doses, indicative of a generally weak T-cell response (27).

In a Phase 1 trial, the immunogenicity of the CanSino Ad5-nCoV vaccine candidate was found to be dose-dependent (21). Doses of  $5 \times 10^{10}$ ,  $1 \times 10^{11}$  or  $1.5 \times 10^{11}$  virus particles were given once to three different sub-groups. In the highest dose group, the anti-S-protein and anti-RBD GM titers on day 28 were 596.4 and 1445.8, respectively (in references 12 and 13, the cut-offs for titer determinations are not specified; we refer to them as “titers”). The GM NAb titers were 34.0 and 45.6 in RV and PV assays, respectively, and were strongly correlated with anti-S and -RBD titers. A Phase 2 trial was then conducted on 508 participants, of which 126 received a placebo (22). The protocol again involved a single administration of the Ad5 virus, which was tested at doses of  $1 \times 10^{10}$  or  $1.5 \times 10^{10}$  in sub-groups. The anti-RBD GM titers on day-28 was 656.5 range, which is a ~2-fold lower than in the Phase 1 trial. NAb titers in the RV and PV assays were 19.5 and 61.4, respectively, and hence similar to the Phase 1 trial data. T cell responses were

measured by ELISPOT on samples taken before vaccination and then on days 14 and 28. Freshly drawn PBMC were incubated with S-protein peptide pools for >12 h, with the data expressed as SFC/10<sup>5</sup> cells after subtraction of background values derived from unstimulated control cells. (Note that the data in Table 4 have been adjusted to SFC/10<sup>6</sup> cells to facilitate comparison to other datasets). There was no mention of a positive control method, nor of the number of replicates. An ELISPOT result was stated to be positive if the number of IFN-gamma secreting T cells responding to the S-protein peptides was increased 2 times above baseline post vaccination. TNF-alpha, IL-2 and IFN-gamma responses to the vaccine were also assessed by CFC. T cell responses peaked at day 14 post vaccine, and ranged from 200 SFC/10<sup>6</sup> cells in the low dose group to 580 SFC/10<sup>6</sup> cells in the high dose group. In CFC assays, both CD4+ and CD8+ T cells were found to be responsive (22).

AstraZeneca's ChAdOx1 nCoV-19 recombinant virus vaccine (also known as AZD1222) was tested in a randomized Phase 1/2 trial involving 543 people; another 544 participants were given a meningococcal control vaccine (15). The original protocol involved a single dose of 5 x 10<sup>10</sup> virus particles, which is twice the amount given to macaques (Tables 2 and 4). However, a decision was taken during the trial to give ten participants a second dose of ChAdOx1 on day 28 in a non-randomized boosting protocol. It is assumed that the decision was taken because of the limited immunogenicity of the single-dose regimen (a modest boosting effect of a second dose was seen in the NHP study, see above; 3). Anti-S-protein binding was measured at single dilutions and converted to "ELISA Units", an approach that complicates comparisons with anti-S-protein responses to other vaccine candidates in humans and that represents an unexplained change from how the macaque sera were analyzed by titration in ELISA (3). By day 14 and 28, the responses

in most of the participants were in the 100-1000 Units range (medians 102.7 and 157.1, respectively), with little change by day 56 in the sub-group that was assayed at that time point (median 119). After the second vaccine dose in the prime-boost protocol, a ~5-fold increase in median anti-S-protein ELISA Units was measured 14 days later (median 997.5), and the levels were largely maintained by day 56 (median 639.2). NAb were measured 14 days after the booster immunization using one PV and three different RV assays. NAb data from the PV assay and from the only RV assay that reported ID<sub>50</sub> values are given in Table 4. The primary paper should be consulted for other aspects of the neutralization data generated in various assays (15). Overall, the seemingly modest NAb responses to the single-dose vaccine were increased a few-fold by the day 28 boosting immunization, at least in the short term (until day 42). The median titers for the prime-boost group on day 42 were 372-450.9 (Table 4). ELISPOT assays were performed on freshly isolated PBMC at days 0, 7, 14, 28 and 56, and at day 35 for the participants who received 2 doses. Pooled peptides were used as antigens, and data were excluded if the assay background response rate was deemed to be too high. The measured responses peaked at day 14 at a value of 856 SFC/10<sup>6</sup> PBMC in the prime group and 1642.3 SFC/10<sup>6</sup> PBMC in the prime-boost group (i.e., after one dose in either group). The results for other time points are given in Table 4. Of note is that ~10% of recipients of this vaccine appear to generate no measurable T cell response after the first dose. Furthermore, the booster dose given to ten trial participants did not further increase their T-cell responses (15). This vaccine has now advanced into Phase 3 trials in several international locations, including Brazil and South Africa. It is thought that these trials were initiated as a single-dose regimen but were later changed to incorporate the second, boosting dose. A two dose Phase 3 trial started in the USA at the beginning of September.

The Phase 1 trial of the Moderna mRNA-1273 vaccine involved 45 volunteers in three dosing groups who were given 25, 100 or 250  $\mu\text{g}$  of the immunogen by the i.m. route on days 1 and 29 (17). Antibody immunogenicity was dose-dependent and much stronger after the second dose than the first. Anti-S-protein GM endpoint titers in the 100  $\mu\text{g}$  group on day 57 (28 days after the second dose) were 782,000, while the corresponding anti-RBD endpoint titers were  $\sim 30,000$ . Most of the NAb data were derived from a PV assay; on day 43, the GM ID<sub>50</sub> titer for the 100  $\mu\text{g}$  group was 344. An RV assay was also used on a subset of day 43 samples. The resulting ID<sub>80</sub> titers were 654 for the 100  $\mu\text{g}$  group. Note that these are not ID<sub>50</sub> values, which would be higher numbers. No detailed data on the longevity of the antibody responses were reported, but inspection of the graphs suggest that the antibody titers on a downward trend at the day 57 time point compared to days 36 and 43. T cell responses were measured only by CFC, and no data on their magnitude was reported. For both vaccine dose groups, the peptide pools activated specific Th1 responses from  $<0.3\%$  of the CD4<sup>+</sup> T cells, and no Th2 responses were detectable. CD8<sup>+</sup> T cell activity was, at most, minimal (17). In an extension of the trial that involved older adults (56-70 and over 71), the magnitudes of the anti-S-protein, anti-RBD and NAb responses to the two-dose regimens (25  $\mu\text{g}$  or 100  $\mu\text{g}$ ) were similar to those reported for the 18-55 age groups (18) (Table 4). The 100  $\mu\text{g}$ , two dose regimen was chosen for the Phase 3 studies that began in the USA during August 2020.

The Pfizer/BioNtech consortium has conducted three Phase 1 trials of lipid nanoparticle-encapsulated mRNAs that eventually led to the selection of the clinical candidate for now ongoing Phase 2/3 studies (23-25). In the first trial, the BNT162b1 mRNA expressing a soluble, trimerized version of the RBD was given at two doses (10  $\mu\text{g}$  and 30  $\mu\text{g}$ ) on days 1 and 21 to groups of 12

participants, and once at 100  $\mu\text{g}$  on day 1 to a third group of 12. There were also 9 placebo recipients (23). Immunogenicity was assessed by anti-RBD (15,16) or anti-S1 (18) binding Abs on days 7, 21, 28 and 35, although the data were reported in a non-traditional format that does not allow for cross-study comparison (23-25). All recipients in the two lower dose groups developed anti-RBD antibodies by day 21 that were boosted  $\sim$ 10-20-fold by the second immunization when measured on day 28 and unchanged by the end of the study on day 35. The 30  $\mu\text{g}$  group was more immunogenic than 10  $\mu\text{g}$  by  $\sim$ 3-fold. The pattern of the NAb data was similar, although fewer time points were studied. In all three groups, the NAb responses to the initial immunization were low, but were boosted by the second dose. On day 28, the GM ID<sub>50</sub> values in an RV assay for the 10 and 30  $\mu\text{g}$  groups were 168 and 267, respectively (23).

A second Phase 1 trial, conducted in Germany, also explored dosing regimens (24). Multiple doses of BNT162b1 mRNA, in the range 1-50  $\mu\text{g}$ , were tested, as were single doses and a prime-boost protocol involving two doses on days 0 and 21. Overall, and as expected, the immunogenicity data were comparable to what was seen in the first trial. Higher immunogen doses and the prime-boost format were associated with stronger responses, as expected. The anti-RBD ELISA data were again presented in a non-traditional format. After the second dose, NAb ID<sub>50</sub> GM titers in the higher dose groups were 578 in a RV assay and  $\sim$ 3100 in a PV assay. In an additional analysis, selected sera were tested in the PV-NAb assay against RBD and S-protein sequence variants (including the D614G change); no significant sensitivity differences were observed. T-cell responses were measured by a modified ELISPOT in which either CD4+ or CD8+ T cells were depleted from the effector population, or by CFC. An unpublished 'normalization' method was applied to enable direct comparison of spot counts/strength of response to anti-CD3 stimulation between individuals. Because PBMC were separated into either CD4+ or CD8+

subpopulations in the ELISPOT assay, no direct comparison can be made with ELISPOT data on the other vaccines reviewed here due to differences in methodology. CD4+ and CD8+ T-cell responses were analyzed immediately before vaccination and then on day 29, i.e., 7 days after the booster immunization. The magnitudes of both T-cell responses were dose-dependent. At the highest dose, the majority of participants had T-cell responses  $>1500$  SFC/ $10^6$  cells. The magnitudes of the CD4+ and CD8+ responses were comparable. Approximately equal proportions of the CD4+ responders fell into groups with  $<500$ , 501-1500 and  $>1500$  SFC/ $10^6$  cells. Cytokine secretion profiles showed that CD4+ T cells producing only IL-2 were the most abundant subset, while IL-4 release was minimal. This pattern is of potential concern, as CD4+ cells secreting IL-2 can polarize CD4+ T cells towards the Th2 phenotype that may be associated with VAERD (34-30=6). The responding CD8+ T cells mostly produced IFN-gamma (24).

The third Pfizer/BioNTech Phase 1 trial compared the BNT162b1 RBD-based construct with BNT162b2, an mRNA expressing a full-length, membrane-anchored S-protein (25). The two constructs were comparably immunogenic but BNT162b2 was associated with lower reactogenicity levels. Accordingly, BNT162b2 at a 30  $\mu$ g dose was selected to progress into Phase 2/3 trials. The Phase 1 trial had two principal sub-components, involving adults aged 18-55 and ones aged 65-85. For each of the two mRNAs, 3 or 4 different doses (10, 20, 30  $\mu$ g and in one case 100  $\mu$ g of mRNA, plus placebo) were tested in a two-immunization protocol (days 0, 21), so the 195 participants were split among 13 different groups in all. Here, we list the immunogenicity data only for the clinical candidate (BNT162b2, 30  $\mu$ g), on day 28. NAb ID<sub>50</sub> GM titers in a RV assay were 361 and 149 for the 18-55 and 65-85 age groups respectively, while the corresponding GM antibody endpoints to the S1 protein in a Luminex assay were 9136 and 7985 (see also Table 4).

Thus, for the clinical candidate, the NAb titers for the older group were 41% of those in their younger counterparts. Visual inspection of other antibody data sets suggests that the age-related reduction is generally ~2-3 fold, a decline that is perhaps meaningful but not catastrophic. No longer term antibody data and no information on T-cell responses were presented (25). The BNT162b2 vaccine candidate is now in Phase 3 trials in the USA and Europe, which involve a 2 dose regimen.

The first report on how a recombinant S-protein performs in humans described the Novavax NVX-CoV2373 vaccine candidate (19). The immunogen is an insect cell-derived soluble S-protein. When mixed with detergent, 5 or 6 S-proteins become attached non-covalently via their bases to the resulting micelles (45). This component of the immunogen was co-administered with Matrix-M adjuvant. Two formulations (5  $\mu\text{g}$  and 25  $\mu\text{g}$  of S-protein) were tested in 106 people with or without adjuvant, in a two-dose regimen on days 0 and 21. In the absence of adjuvant, antibody responses were, as expected, very weak, while the 5 and 25  $\mu\text{g}$  doses performed comparably when the adjuvant was present. Anti-S-protein ELISA data were presented as Units, which again prevents cross-study comparison. The highest values recorded, on day 35, were 63,160. NAbS were measured in an RV assay and reported as  $\text{ID}_{>99}$  values. Here, the peak GM values were 3906 and 3305 for the 5 and 25  $\mu\text{g}$  groups, respectively, on day 35. As noted above when discussing the corresponding macaque experiment, when NAb data are presented as  $\text{ID}_{>99}$  or  $\text{CPE}_{100}$  values the reported numbers are likely to be several-fold lower than the more commonly used  $\text{ID}_{50}$  values. CFC was used to measure  $\text{CD4}^+$  T-cell responses at days 0 and 28, but in only 4 participants per group. There were no responses in the placebo or protein with no-adjuvant recipients, but  $\text{CD4}^+$  T cell signals could be measured in the adjuvanted protein groups at day 28,

with two protein doses inducing similar but moderate responses. Both Th1 and Th2 cytokines were released although the Th1 signals were more consistent, particularly at the lower protein dose (19). Phase 3 trials of the Novavax vaccine are thought to commence towards the end of 2020.

Taken together, and allowing for caveats about comparing data from different studies, two features of the binding-antibody and NAb data stand out (Table 4). The seemingly strongest responses were induced by the NVX-CoV2373 adjuvanted recombinant S-protein. This judgement takes into account the presentation of ID<sub>>99</sub> values, rather than the more commonly used ID<sub>50</sub> titers (19). The relationship between ID<sub>>99</sub> and ID<sub>50</sub> values depends on the shape of the titration curve, but ID<sub>>99</sub> are by necessity lower, often by >10-fold. The superior immunogenicity of the recombinant S-protein mirrors its performance in macaques (Table 2) (8, 9). The second conclusion we can draw is that binding antibody and NAb responses to the single dose adenovirus vector vaccines are quite weak, although a second dose does improve their performance, as judged by the limited data set available (Table 4) (15, 21, 22). The T-cell response data are too limited, and the protocols used too variable, for us to draw any conclusions about relative immunogenicity.

Several weeks after the Russian government approved the widespread use an adenovirus-vector based vaccine, Gam-COVID-Vac, a report appeared on how it had performed in Phase 1/2 trials (26). The vaccine involves the sequential delivery of rAd5 and rAd26 vectors that each express a full-length S-protein, which are given intramuscularly at  $1 \times 10^{11}$  particles per dose. Two sub-trials, each involving 38 volunteers aged 18-60, compared frozen/thawed (Gam-COVID-Vac), or lyophilized/reconstituted (Gam-COVID-Vac-Lyo) vaccine formulations, which performed similarly (Gam-COVID-Vac was chosen for widespread use on convenience grounds). In the combination trial (n = 20), the first dose was rAd26 on day 0 followed by rAd5 on day 21. Smaller

sub-groups (n = 9) received only rAd5 or only rAd26. Phase 2 trials began a mere 5 days after Phase 1 ended, based on a successful interim safety assessment. Safety studies over 42 days (maximum) revealed nothing other than the generally mild reactions reported in other Ad-vector studies. Immunogenicity assessments involved determining endpoint titers in anti-RBD and antiS1 IgG ELISAs and in an RV NAb assay, at weekly intervals. T-cell response data were derived from an INF-gamma ELISPOT and CD4+ and CD8+ T-cell proliferation assays. Anti-rAd5 antibodies were also measured to assess the possible influence of pre-existing immunity. Anti-RBD endpoint titers peaked at ~2000 by day 21 in the single-vaccine groups, and were boosted to 10,000-15,000 in the combination vaccine groups by day 42. On day 42, the anti-S1 GM endpoint titer in the Phase 2 trial combination group was 53,006. NAb CPE<sub>67</sub> titers at on day 28 in the single vaccine groups were in the range 5-10, but rose to GM values of 45.95-49.25 by day 42 in the combination groups. The authors themselves note that these titers are lower than were seen in the AstraZeneca/Oxford ChAdOx1 and mRNA vaccine trials, a difference to which the different measurements of NAb titers (ID<sub>50</sub> vs. CPE<sub>67</sub>) may contribute (see Table 4). Cell-mediated immunity was measured via a T-cell proliferation assay that is rarely used in the trials of the other vaccines reviewed here. Proliferative responses were detected in all participants but seem weak in magnitude and were not boosted by the second dose. On day 28, after the boosting immunization, median T-cell proliferation values were 2.5% vs. 1.3 % for CD4+ and 1.3% vs. 1.1% for CD8+ cells in the groups receiving the frozen and lyophilised formulations, respectively. INF-gamma ELISPOT data were presented only as fold-increase from baseline values, so cannot be compared with other studies. However, there was again no boosting effect of the second immunization. A Phase 3 trial of the Gam-COVID-Vac combination vaccine began on August 26, and is planned to involve 40,000 volunteers of various ages and risk groups (26).

Uniquely among the vaccines in Phase 3 trials, the Janssen Ad26.COV2 adenovirus vector is being given only once. Some of the human immunogenicity data on which this scheme was reportedly based have now been described in an 'interim report' (16). The vaccine was tested at two doses ( $5 \times 10^{10}$  and  $1 \times 10^{11}$  viral particles) that were given i.m. either once (day 0 only) or twice (days 0 and 56) to healthy adults aged 18-55 ( $n = 402$ ) or  $>65$  ( $n = 394$ ). Safety data were generally unexceptional, although two SAEs were reported and deemed, after investigation, to be either not vaccine-related or not problematic (a high fever that was resolved). Antibody immunogenicity was measured by S-protein ELISA, with data reported as Units/ml and an RV NAb assay with an  $IC_{50}$  endpoint. T-cell responses to the S-protein were measured by ICS, and cytokine release profiles were used to gauge Th1 vs. Th2 bias. The paper should be consulted for data on the multiple individual sub-groups. Here, we will summarize what was reported for the Phase 3 trial regimen, a single dose of  $5 \times 10^{10}$  virus particles (Table 4). Moreover, although the two-dose groups are mentioned in the paper, no data were presented for the antibody and T-cell responses to the second dose. And only a small subset of some one-dose groups were included in several immunogenicity analyses. For the Phase 3 regimen, the GMT anti-S-protein ELISA values on day 29 were 528 and 507 Units for 15 of the younger and older volunteers, respectively. The corresponding NAb GMT  $IC_{50}$  titers were 214 and 196, although some of the samples were said to need re-assaying and additional data from a PV NAb assay are reportedly pending. The weak T-cell response data show the expected Th1 bias. The ICS percentages of CD4+ and CD8+ T-cells expressing IFN-gamma and IL-2 at day 15 for the Phase 3 regimen were 0.08% and 0.07%, respectively for the younger adults, and 0.36% and 0.05% for the older group (with large confidence intervals). The T-cell response rate varied depending on the sub-group and assay, and

was anything from 33% to 100%, although given the low number of samples in many cases the meaning of these data is not clear (16).

### **Summary and conclusions**

Assuming that serum NAb titers are indeed the principal CoP for a human SARS-CoV-2 vaccine, we do not know what a protective NAb titer will be, we know even less about the role of T-cells in protection, there is almost no information available about mucosal immunity or immune memory, and the duration of any vaccine-induced immunity is yet another unknown. In an earlier review, we suggested that NAb titers in the low hundreds might be sufficient for protection (32). In a recent study, when 122 Seattle seamen sailed seawards, 104 of them had become SARS-CoV-2-RNA-positive from a single source of virus by the time their boat returned to port 18 days later. However, three sailors who had NAb responses at the time of departure were not re-infected while at sea. Their serum PV ID<sub>50</sub> NAb titers were 174, 161 and 3082 (53). Thus, while certainly far from definitive, this study further suggests that a protective serum NAb titer may lie in the low hundreds. Once more, however, the use of NAb assays with different properties and sensitivities blurs most attempts to cross-compare the datasets from the different trials (32). Now that robust, reliable and potentially high throughout assays are becoming available, perhaps these major knowledge gaps can be addressed (54, 55). While emphasizing the above constraints, our judgement is that an approximate rank-order (low to high) for the initial, peak NAb responses to the different vaccine classes is: inactivated virus < adenovirus vectors < mRNA < adjuvanted S-proteins. It is not possible to assess antibody decay-rates or the role of immunological memory. Hard evidence on the comparative immunogenicity and relative worth of the leading SARS-CoV-2 vaccines will most likely emerge from ongoing and planned Phase 3 trials. If these vaccines turn out to be inadequate, one option is to combine some of them in prime-boost formats as we have

suggested previously (31). Alternatively, next-generation immunogens now at the pre-clinical stage of development may solve the problem. In that scenario, greater national and international coordination on how best to assess vaccine immunogenicity and, in NHP models, efficacy would be strongly advised.

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## TABLES

Table 1: SARS-CoV-2 vaccines under evaluation in NHPs and Phase 1/2 human trials

Vaccine name <sup>a</sup>	Sponsor <sup>b</sup>	Design principle <sup>c</sup>	NHP studies (citation)	Human trials (citation)
PiCoVacc/CoronaVac <sup>d</sup>	Sinovac	Inactivated virus	(1)	(13)
BBIBP-CorV	Sinopharm/BIBP	Inactivated virus	(2)	(14)
ChAdOx1 nCoV-19	<b>AstraZeneca</b>	ChAdeno virus – S-protein	(3)	(15)
Ad26.COV2	<b>Janssen</b>	Ad26 virus – S-protein	(4)	(16)
Various constructs	Not applicable <sup>e</sup>	DNA –S-protein	(5)	
INO-4800	Inovio	DNA –S-protein	(6)	
mRNA-1273	<b>Moderna</b>	mRNA –S-protein	(7)	(17, 18)
NVX-CoV2373	<b>Novavax</b>	Recombinant S-protein	(8, 9)	(19)
Unnamed	Sinopharm/WIBP	Inactivated virus		(20)
Ad5-nCoV	CanSinoBIO	Ad5 virus – S-protein		(21, 22)
BNT162b1 <sup>f</sup> BNT162b2	<b>Pfizer/BioNTech</b>	mRNA – RBD mRNA - S-protein	(10)	(23, 24, 25)
Gam-COVID-Vac	Gamaleya Center	Ad26 + Ad5 virus S-protein		(26)
S-Trimer	Clover Biopharm.	Recombinant S-protein	(11)	
Unnamed	IMB, CAMS, PUMC	Inactivated virus, D614G		(27)
MRT5500	Sanofi Pasteur	mRNA-S-protein	(12)	

<sup>a</sup> Some vaccines have alternative names or corporate designations. We use the same names as in the papers cited. The entries in this column are arranged in approximate order of appearance of the first relevant paper on a preprint server or journal website. The citations are arranged such that the papers on the NHP studies are all numbered before those on human trials.

<sup>b</sup> The five companies highlighted in bold in this and subsequent tables are part of the US government's Operation Warp Speed program or, in the case of Pfizer/BioNTech, have close ties to it. As this program rapidly evolves, readers should consult appropriate websites (e.g., <https://medicalcountermeasures.gov/app/barda/coronavirus/COVID19.aspx>) for updated information. In some cases, the companies have academic partners. For example, Moderna is the corporate partner of the NIH's Vaccine Research Center, where the mRNA vaccine was designed,

while the AstraZeneca vaccine (also known as AZD1222) similarly involves the U.K.'s Oxford University. BIBP = Beijing Institute of Biological Products; WIBP = Wuhan Institute of Biological Products. Both these organizations are part of the Sinopharm consortium. The Gamaleya Center in Moscow has multiple partners within the Ministry of Health of the Russian Federation. IMB, CAMS, PUMC = Institute of Medical Biology, Chinese Academy of Medical Sciences, Peking Union Medical College.

<sup>c</sup> The SARS-CoV-2 components of these vaccines are all based on the S-protein or, in the case of the Pfizer/BioNTech BNT162b1 vaccine, the S-protein's RBD. All the constructs listed here express or involve the full-length S-protein. Truncated variants have been studied as comparator immunogens (4, 5). The inactivated virus vaccines all include S-proteins together with other viral components. For details of the immunogens, including modifications made to the S-proteins, the primary papers should be consulted.

<sup>d</sup> The Sinovac vaccine was named PiCoVacc at the preclinical stage and then renamed CoronaVac when it moved into human trials.

<sup>e</sup> The DNA vaccines tested in the macaque study are not known to be part of a clinical development program; we include this paper in the review because it has a macaque challenge component and is therefore relevant to the comparison with other such studies.

<sup>f</sup> Although both vaccines were studied at Phase 1, only BNT162b2 was advanced into Phase 2/3.

**Table 2. Vaccine immunogenicity in NHP studies**

<b>Vaccine name (citation)</b>	<b>Vaccine dose<sup>a</sup></b>	<b>Binding antibody titer<sup>b</sup></b>	<b>NAb titer<sup>c</sup></b>	<b>T-cell response<sup>d</sup></b>
Sinovac PiCoVacc (1)	6 $\mu$ g x 3	GM EP ~12,800	RV GM ID <sub>50</sub> ~50	ND <sup>e</sup>
Sinopharm/BIPP BBIBP-CorV (2)	8 $\mu$ g x 2	ND	RV GM ID <sub>50</sub> ~230	ND
<b>AstraZeneca</b> ChAdOx1 nCoV-19 (3)	2.5 x 10 <sup>10</sup> VP x 2	Median EP ~28,000 f	RV median ID <sub>50</sub> ~280 <sup>f</sup>	ND
<b>Janssen</b> Ad26.COV 2S.PP (4)	1 x 10 <sup>11</sup> VP x 1	Median EP ~ 4,000	PV median ID <sub>50</sub> 408 RV median ID <sub>50</sub> 113	Median ~80 (day 28)
DNA, full-length S-protein (5)	5 mg x 2	Median EP ~ 140	PV median ID <sub>50</sub> ~200 RV median ID <sub>50</sub> ~40	Median ~80 (day 35)
Inovio INO-4800 (6)	1 mg x 2	GM EP ~130,000	PV GM ID <sub>50</sub> ~1000	AM ~140 (day 42) AM ~30 (day 84)
<b>Moderna</b> mRNA-1273 (7)	100 $\mu$ g x 2	Log AUC = 4-5	PV GM ID <sub>50</sub> 1862 RV GM ID <sub>50</sub> 3481	ND
<b>Novavax</b> NVX-CoV2373 (8)	5 $\mu$ g x 2	GM EC <sub>50</sub> 174,000	RV GM ID <sub>&gt;99</sub> 17,000	ND
<b>Novavax</b> NVX-CoV2373 (9)	25 $\mu$ g x 2	GM EC <sub>50</sub> 469,739	RV GM CPE <sub>100</sub> 23,040	ND
<b>Pfizer/BioNTech</b> BNT162b2 (10)	100 $\mu$ g x 2	GM EU 34,668	RV GM ID <sub>50</sub> 1689	GM ~750 (days 28, 42)
<b>Clover Biopharmaceuticals</b> S-Trimer (11)	30 $\mu$ g x 2	GM EP 17,497	PV GM ID <sub>50</sub> ~ 5227 RV GM CPE <sub>50</sub> ~ 20,234	ND
<b>Sanofi Pasteur</b> MRT5500 (12)	135 $\mu$ g x 2	GM EP ~ 200,000	PV GM ID <sub>50</sub> ~ 2871 RV GM ID <sub>50</sub> ~ 1877	GM 30-40 (day 42)

<sup>a</sup> Only results for the optimal dose, i.e., the strongest responses without unacceptable side effects, are recorded. When the number of immunizations differed between groups, the one inducing the strongest response was chosen. VP = virus particle.

<sup>b</sup> Antibody binding was measured in S-protein IgG ELISA 2 weeks after the last immunization and the values are listed as EC<sub>50</sub>, endpoint (EP) or ELISA units derived from comparison with a standard curve (EU); GM = geometric mean; AUC = area under the curve.

<sup>c</sup> Neutralization was quantified in PV or RV assays, as indicated. The potency was measured as ID<sub>50</sub> or ID<sub>>99</sub> values (CPE<sub>100</sub> in ref. 9 is the approximate the equivalent of ID<sub>>99</sub> in ref. 8).

<sup>d</sup> T-cell responses measured in ELISPOT IFN-gamma assays SFC/10<sup>6</sup> cells or intracellularly stained cells /10<sup>6</sup> (PBMC unless specified) after stimulation with different SARS-CoV-2 S-derived peptides. The days between immunization (day 0) and sampling are also listed (in brackets). AM = arithmetic mean.

<sup>e</sup> ND = not done (no data were presented in the paper).

<sup>f</sup> Data are for the 2 dose (prime boost) group.

**Table 3. Antibody responses at the time of challenge and degree of protection in NHP studies**

Vaccine name (citation)	Binding antibody titer near time of challenge <sup>a</sup>	NAb titer near time of challenge <sup>b</sup>	Dose and route of challenge <sup>c</sup>	Time from last immunization to challenge	Viral load reductions <sup>d</sup>
Sinovacc PiCoVacc (1)	GM EP ~12,800 1 day BC	RV GM ID <sub>50</sub> ~50 1 day BC	1 x 10 <sup>6</sup> TCID <sub>50</sub> IT	22 days	TS AM ~1.8 AS AM ~4.7
Sinopharm/BIBP BBIBP-CorV (2)	ND <sup>e</sup>	RV GM ID <sub>50</sub> ~230 Day of challenge	1 x 10 <sup>6</sup> TCID <sub>50</sub> IT	14 days	TS AM ~5.0 <sup>f</sup> AS AM ~2.9
<b>AstraZeneca</b> ChAdOx1 nCoV-19 (3)	Median EP ~6300 <sup>g</sup> 28 days BC	RV median ID <sub>50</sub> ~60 28 days BC	(1.6+0.8+0.8+0.2) x 10 <sup>6</sup> TCID <sub>50</sub> IT-IN-OR-OC <sup>h</sup>	14 days	BAL median ~1.7 INS median ~1.5
<b>Janssen</b> Ad26.COV 2S.PP (4)	Median EP ~ 4000 14 days BC	PV median ID <sub>50</sub> 408 RV median ID <sub>50</sub> 113 14 days BC	1 x 10 <sup>5</sup> TCID <sub>50</sub> IT-IN	42 days	BAL median 3.2 0/6 detectable INS median 3.9 1/6 detectable
Full-length S-protein (5)	Median EP ~ 160 7 days BC	PV median ID <sub>50</sub> ~40 RV median ID <sub>50</sub> ~200 7 days BC	1.2 x 10 <sup>8</sup> VP = 1.1 x 10 <sup>4</sup> PFU IT-IN	21 days	BAL median 3.1 INS median 3.7
<b>Inovio</b> INO-4800 (6)	GM EP ~ 3200 14 days BC	PV GM ID <sub>50</sub> ~260 14 days BC	1.1 x 10 <sup>4</sup> PFU IT-IN	77 days	BAL median ~1.5 INS median ~0.20
<b>Moderna</b> mRNA-1273 (7)	Log AUC = 4-5 14 days BC	PV GM ID <sub>50</sub> 1862 RV GM ID <sub>50</sub> 3481 14 days BC	7.6 x 10 <sup>5</sup> PFU 1 x 10 <sup>6</sup> TCID <sub>50</sub> IT-IN	28 days	BAL median ~4.0 INS median ~3.0
<b>Novavax</b> NVX-CoV2373 (9)	GM EC <sub>50</sub> 469,739 Day of challenge	RV GM CPE <sub>100</sub> 23,040 Day of challenge	1.04 x 10 <sup>4</sup> PFU IT-IN	35 days	BAL median ~2.6 (0/4 detectable) INS median ~2.6 (0/4 detectable)
<b>Pfizer/BioNTech</b> BNT162b2 (10)	GM EU 6317 20 days BC	RV GM ID <sub>50</sub> 310 20 days BC	1.05 x 10 <sup>6</sup> PFU IT-IN	55 days	BAL GM ~3.0 (0/6 detectable)

					INS GM ~1.5 (0/6 detectable) OPS GM ~2.5 (2/6 detectable)
Clover Biopharm. S-Trimer (11)	GM EP 17,497 Day of challenge	PV GM ID <sub>50</sub> ~ 5227 RV GM CPE <sub>50</sub> ~ 20,234 Day of challenge	2.6 x 10 <sup>6</sup> TCID <sub>50</sub> IT (60%) IN (40%)	14 days	TS GM ~1.7 AS GM ~ 1.5 ITS GM ~ 1.7 INS GM ~ 0.5

<sup>a</sup> Antibody binding was measured in S-protein IgG ELISA and the values are listed as EC<sub>50</sub>, endpoint (EP) or ELISA units derived from comparison with a standard curve (EU); GM = geometric mean; AUC = area under the curve. The data are derived from the timepoint (listed in days) closest to the time of challenge. BC = before challenge

<sup>b</sup> Neutralization was quantified in PV or RV assays, as indicated and the potency measured as ID<sub>50</sub> or CPE<sub>100</sub> values.

<sup>c</sup> Challenge dose (in plaque-forming units (PFU) or tissue culture infectious dose yielding infection in 50% of wells (TCID<sub>50</sub>) and route of challenge; only in ref. 7 were both PFU and TCID<sub>50</sub> given. IT = intratracheal; IN = intranasal; OR = oral; OC = ocular. VP = virus particles.

<sup>d</sup> Protection was measured as median log reductions in subgenomic RNA copies/ml (except for references 2 and 10 where viral RNA data are listed). The viral load (VL) data were derived from bronchoalveolar lavages (BAL), intranasal swabs (INS), throat swabs (TS),

oropharyngeal swabs (OPS) or anal swabs (AS), at times when VLs were approximately at their peak levels post-challenge. In some studies, more substantial protective effects could be detected after the peak values began to decline (see the primary papers for details).

AM = arithmetic mean of the VL log values, which is equivalent to the GM (geometric means) of the antilog values.

<sup>e</sup> ND = not done (no data were presented in the paper).

<sup>f</sup> Since viral RNA declined without any discernable peak in the control animals, only RNA measurements for day 7 (the last time point sampled) are listed.

<sup>g</sup> Data are for the 2 dose (prime boost) group.

<sup>h</sup> The macaques were challenged simultaneously via 4 different routes (IT-IN-OR-OC) with the various doses listed in the same order in the brackets.

Table 4. Vaccine immunogenicity in human Phase 1 and/or Phase 2 trials

Vaccine name (citation)	Design	Vaccine dose <sup>a</sup>	Binding antibody titer <sup>b</sup>	NAb titer <sup>c</sup>	T-cell response <sup>d</sup>
Sinovac CoronaVac (13)	Inactivated virus	6 $\mu\text{g}$ x 2	GM titer ~2500	RV GM CPE titer ~64	ND <sup>f</sup>
Sinopharm/WIBP Unnamed, Phase 1 (14)	Inactivated virus	8 $\mu\text{g}$ x 2 (day 0 and 28)	ND	RV GM titer 228.7	ND
Sinopharm/WIBP Unnamed, Phase 2 (14)	Inactivated virus	8 $\mu\text{g}$ x 2 (day 0 and 21)	ND	RV GM titer 282.7	ND
Sinopharm/WIBP Unnamed, Phase 1 (20)	Inactivated virus	10 $\mu\text{g}$ x 3 <sup>e</sup>	GM EP (whole virus) 311	RV GM ID <sub>50</sub> 297	ND
Sinopharm/WIBP Unnamed, Phase 2 (20)	Inactivated virus	5 $\mu\text{g}$ x 2	GM EP (whole virus) 215	RV GM ID <sub>50</sub> 247	ND
CanSinoBIO Ad5-nCoV (21)	Ad5 virus	1.5 x 10 <sup>11</sup> VP x 1	GM titer 596.4	RV GM titer 34 PV GM titer 45.6	GM ~580 (day 14)
CanSinoBIO Ad5-nCoV (22)	Ad5 virus	1.0 x 10 <sup>11</sup> VP x 1	GM titer (RBD) 656.5	RV GM titer 19.5 PV GM titer 61.4	ND
<b>AstraZeneca</b> ChAdOx1 nCoV-19 (15)	ChAdeno virus	5 x 10 <sup>10</sup> VP x 1	Median EU 157.1	RV median ID <sub>50</sub> 201 PV median ID <sub>50</sub> 87.9	Median 856 (day 14) Median 424 (day 56)
<b>AstraZeneca</b> ChAdOx1 nCoV-19 (15)	ChAdeno virus	5 x 10 <sup>10</sup> VP x 2	Median EU 997.5	RV median ID <sub>50</sub> 372 PV median ID <sub>50</sub> 450.9	Median 1642.3 (day 14) Median 528.7 (day 35) Median 614 (day 56)
<b>Janssen</b> Ad26.COV2 (16)	Ad26 virus	1 x 10 <sup>11</sup> VP x 1	GM EU 695	RV GM ID <sub>50</sub> 243	Median CD4+ ~0.1% <sup>i</sup> (day 15) Median CD8+ ~0.09% <sup>i</sup> (day 15)
<b>Moderna</b> mRNA-1273 (17, 18)	mRNA	250 $\mu\text{g}$ x 2 (17) 100 $\mu\text{g}$ x 2 (18)	GM EP 1,262,975 (17) ~1000,000 RBD (18)	PV GM ID <sub>50</sub> 373.5 (17) ~300 (18)	Median CD4+ ~800 Median CD8+ ~40 (day 43) (17) Median CD4+ ~160 (day 43) (18)
<b>Pfizer/BioNTech</b> BNT162b1 (23)	mRNA RBD	30 $\mu\text{g}$ x 2	GM EU (RBD) 16,166	RV GM ID <sub>50</sub> 267	ND
<b>Pfizer/BioNTech</b> BNT162b1 (24)	mRNA RBD	50 $\mu\text{g}$ x 2	GM EU (RBD) 25,006	RV GM ID <sub>50</sub> 578 PV GM ID <sub>50</sub> 3100	CD4+ median ~2000 CD8+ median ~2600 (day 29)
<b>Pfizer/BioNTech</b> BNT162b1 <sup>g</sup> (25)	mRNA RBD	30 $\mu\text{g}$ x 2	GM EU (S1) 6580- 23,516	RV GM ID <sub>50</sub> 101- 267	ND
<b>Pfizer/BioNTech</b> BNT162b2 <sup>g</sup> (25)	mRNA S-protein	30 $\mu\text{g}$ x 2	GM EU (S1) 7895- 9136	RV GM ID <sub>50</sub> 149- 361	ND

<b>Novavax NVX-CoV2373 (19)</b>	S-Protein	5 $\mu$ g x 2	GM EU 63,160	RV GM ID <sub>&gt;99</sub> 3906	ND
Gamaleya Center Gam-COVID-Vac (26) <sup>h</sup>	Ad26 + Ad5 virus	1 x 10 <sup>11</sup> VP of each virus	GM EP (S1) 53,006	RV GM CPE <sub>67</sub> 49.25	NA <sup>j</sup>
IMB, CAMS, PUMC Unnamed (27)	Inactivated virus <sup>k</sup>	100-150 unspecified units	GM titer 2000-4000	RV GM CPE titer ~20	AM 250 (middle dose) AM 50 (high dose)

<sup>a</sup> The number of immunizations is also given. VP = viral particles.

<sup>b</sup> Antibody binding was measured in S-protein (except when RBD, S1 protein, or inactivated, purified whole virus was used instead, as stated) in IgG ELISAs and the values are listed as EC<sub>50</sub>, endpoint (EP) or ELISA units derived from comparison with a standard curve (EU); titer = unspecified method of the titer determination. The samples were obtained at a time corresponding approximately to the peak response after the final (or only) immunization. GM = geometric mean.

<sup>c</sup> Neutralization was quantified in PV or RV assays as indicated. The potency was measured as ID<sub>50</sub>, ID<sub>>99</sub> or CPE<sub>67</sub> values 2 weeks after the final (or only) immunization, which corresponds approximately to the peak response. The values reported for references 14, 21 and 22 are given simply as GM titers, as in the original text where the cut-off was not defined.

<sup>d</sup> T-cell responses were measured in ELISPOT IFN-gamma assays and recorded as SFC/10<sup>6</sup> cells (PBMC except where subpopulations of CD4+ and CD8+ are indicated) after stimulation with different SARS-CoV-2 S-protein-derived peptides. The days between immunization (day 0) and sampling are also listed (in brackets).

<sup>e</sup> The binding antibody and NAb titer ranges were similar in the lower dose (2.5 and 5.0  $\mu\text{g}$ ) groups 14 days after the third dose. In other studies one dose stood out as giving stronger responses and was chosen for tabulation.

<sup>f</sup> ND = not done (no data were presented in the paper).

<sup>g</sup> The ranges listed for BNT162b1 and BNT162b2 are the GM values for the age groups 65-85 years (lower value) and 18-55 years (higher value).

<sup>h</sup> Data are for the frozen/thawed stock sub-component of the Phase 2 combination vaccine trial.

<sup>i</sup> IFN-gamma or IL-2 measured by intracellular cytokine staining (ICS).

<sup>j</sup> NA = not applicable (data were not presented in the relevant quantitative format; see text).

<sup>k</sup> Uniquely among the inactivated virus vaccines, this one is based on a virus with the D614G substitution in the S-protein.

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