Vaccines' Candidates Against SARS-CoV-2

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Scientists, health organizations, and pharmaceutical companies are making a large global effort to develop vaccines against SARS-CoV-2, the virus of COVID-19 since the outbreak began. Until now, we have more than 150 candidates. However, 19 vaccine candidates have entered clinical trials in phase 2 and 3 trials (31 July 2020). In this article we aimed to present the platforms for COVID-19 vaccine, the types of vaccines (live, attenuated, inactivated, DNA/RNA, proteins subunits, viral vector), the antigen selection, adjuvants, and we focused on the phase 2/3 trial vaccines at this point (Sinopharm, Coronavac, Moderna, Oxford, Biontech). We searched the data in the main database (PubMed/Medline, Elsevier Science Direct, Scopus, Isi Web of Science, Embase, Excerpta Medica, UptoDate, Lilacs, Novel Coronavirus Resource Directory from Elsevier), in the high-impact international scientific Journals (Scimago Journal and Country Rank - SJR - and Journal Citation Reports - JCR), such as The Lancet, Science, Nature, The New England Journal of Medicine, Physiological Reviews, Journal of the American Medical Association, Plos One, Journal of Clinical Investigation, and in the data from Center for Disease Control (CDC), National Institutes of Health (NIH), National Institute of Allergy and Infectious Diseases (NIAID) and World Health Organization (WHO). We prior selected meta-analysis, systematic reviews, article reviews, and original articles in this order. We reviewed 216 articles and used 106 from March to June 2020, using the terms coronavirus, SARS-CoV-2, novel coronavirus, Wuhan coronavirus, severe acute respiratory syndrome, 2019-nCoV, 2019 novel coronavirus, n-CoV-2, covid, n-SARS-2, COVID-19, corona virus, coronaviruses, vaccine, platform, antigen, subunit, live and attenuated vaccine, RNA vaccine, live vaccine, inactivated vaccine, types of vaccines, adjuvants, replication, viral vector, phase 1-3, trial, with the tools MeSH (Medical Subject Headings), AND, OR, and the characters [,",.; /., to ensure the best review topics. We concluded that although vaccines have shown safety in phase 1 and efficacy in phase 2 and the beginning of phase 3 is starting, the most renowned scientists believe that a vaccine will be available only in the middle of next year.
Keywords: COVID-19. SARS-CoV-2. Types of Vaccines. Phase 3. Immunity.

Introduction

Since the pandemic started on December,2019, the researchers of all over the world are trying to find a vaccine against the SARS-CoV-2. The development of a vaccine normally takes 10 to 15 years. However, because of the COVID-19 pandemic, many centers of research are working together to develop a vaccine within one year. A total of more than 100 different vaccines for SARS-CoV-2 are under development, but a small number of them have reached the stage of development that the vaccines can be tested in humans (Phase 3). Until now, we have some promised phase 3 vaccines.

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This article aims to compile the update of the potential vacines against SARS-CoV-2 as well as the platforms for COVID-19 vaccine development and the types of the vacines.

Biochemical and Molecular Roadmap of SARS-CoV-2 [1]

The terminology for the RNA virus that causes COVID-19, SARS-CoV-2, has been established by the International Committee on Taxonomy of Viruses (ICTV) [2], due to its extensive homology with the 2003 SARS coronavirus. The SARS-CoV-2 coronavirus belongs to the subfamily of Coronavirinae, with a genomic structure of (+)ss-RNA of 30kb in length that includes a 5'-cap structure and 3'-poly-A tail [3]. From the viral RNA, polyprotein 1a/1ab (pp1a/pp1ab) is synthesized in the host to form 16 non-structural proteins (nsps) that organize the replication-transcription complex (RTC) in double membrane vesicles

(DMVs). The nRTC synthesizes a set of minus-strand subgenomic RNAs (sgRNAs) discontinuously [4]. Between open reading frames (ORFs), transcription terminates, and then a subsequent acquisition of a leader RNA occurs. During this process, subgenomic mRNAs need these sgRNAs as the templates [5, 6]. At least six ORFs exist for a typical CoV, including SARS-CoV-2. The first ORFs (ORF1a/b) with over 65% of the whole genome length encode 16 nsps. Of note, two polypeptides (pp1a and pp1ab) come from a -1 frameshift between ORF1a and ORF1b. For the other ORFs on the 35% of the genome close to the 3'-terminus encode at least four main structural proteins, including spike (S), membrane (M), envelope (E), and nucleocapsid (N). All these structural and non-structural proteins are translated from the sgRNAs [4-6]. Currently, more than 200 complete and partial genome sequences of SARS-CoV-2 have been decoded and deposited in the Global Initiative on Sharing All Influenza Data (GISAID) database (https://www.gisaid.org/) [7] and in the National Institutes of Health (NIH) GenBank database (https://www.ncbi.nlm.nih.gov/nuccore/?term=COVID-19) [8].

Phylogenetic analysis showed that SARS-CoV-2 was closely related to two SARS-like coronaviruses present in bats, including bat-SL-CoVZC45 and bat-SL-CoVZXC21, with 88% identity, and showed 79% homology with SARS-CoV, and 50% with MERS-CoV [9]. However, homology modeling disclosed that SARS-CoV-2 had a similar RBD structure to that of SARS-CoV, despite amino acid variation at some key residues [10, 11]. These findings suggest that SARS-CoV-2 emerged from a single animal source within a short period [12]. However, because the sequence similarity between SARS-CoV-2 and its close relatives bat-SL-CoVZC45 and bat-SL-CoVZXC21 is less than 90%, the bat-derived viruses may not be the direct origins of SARS-CoV-2.

Platforms for COVID-19 Vaccine Development [13]

Whole Virion Vaccines (Live Attenuated Virus and Inactivated Virus)

Attenuated Virus Vaccine

Live attenuated vaccines (LAV) are viruses that are rendered replication-incompetent through repeated passage in cell culture, and inactivated vaccines utilise whole pathogen which has typically been killed by exposure to chemicals (e.g. formaldehyde) or heat inactivation [14]. LAV are immunogenic and reproduce the breadth of the humoral and cellular immune protection that would be generated by live viral infection [15, 14] however inactivated vaccines are generally less immunogenic and require more than one dose or an additional adjuvant [16]. Safety issues regarding the generation and subsequent attenuation of the virus, with potential for reactivation in vaccinated individuals, means LAV are not a tenable vaccine strategy for highly pathogenic viruses [14, 17]. This also prevents immunisation of individuals with weakened immune systems who are at further risk of illness if the pathogen reverts [18]. From the perspective of vaccine distribution, LAV are generally kept refrigerated to preserve immunogenicity, which may be problematic in countries that cannot sustain cold chain distribution [16, 18]. LAV for SARS-CoV-1 were tested in pre-clinical trials [19]. There is currently one company, Codagenix, proposing a computationally designed, lab-made SARS-CoV-2 'virus' that is immunogenic but not pathogenic [20]. SinoVac demonstrated safety and immunogenicity of an inactivated SARS-CoV-1 vaccine in a Phase 1 trial [21], and have determined efficacy of a formalininactivated SARS-CoV-2 vaccine in rhesus macaques [22]. Although this vaccine did not demonstrate any ADE-derived pathogenesis, previous whole virus SARS-CoV-1 vaccines trialled in mice induced eosinophil-derived immunopathology upon viral challenge [23], and Th2-driven histopathological changes in the lungs [24].

However, the two doses of Sinovac Biotech's COVID-19 vaccine candidate, dubbed CoronaVac, induced neutralizing antibodies 14 days after vaccination. More than 90% of the 600 healthy volunteers in the phase 2 part of the phase 1/2 study showed that immune response [13]. Now, the Chinese vaccine is in the phase 3 in which Brazil, in a partnership with Butantan Institute and Sinovac Biotech's, a Chinese company, is participating with health volunteers.

Inactivated Virus Vaccine [25]

Multiple SARS-CoV-2 vaccine types are under development, such as DNA- and RNA-based formulations, recombinant subunits containing viral epitopes, adenovirus-based vectors, and purified inactivated virus [26, 27]. Purified inactivated viruses have been traditionally utilized for vaccine development, and such vaccines are secure and efficient for the prevention of diseases caused by viruses such as influenza virus and poliovirus [28, 29]. No antibody-dependent enhancement (ADE) of infection was recognized for the vaccinated macaques despite the observation that a relatively low NAb titer existed within the medium-dose group before infection, giving partial protection. The chance of manifestation of ADE after antibody titers wane could not be ruled out in this study. Although T cell responses elicited by multiple vaccines have been shown to be vital for acute viral clearance, protection from subsequent coronavirus infections is largely mediated by humoral immunity [30-33]. The "cytokine storm" provoked by excessive T cell responses has really been exhibited to accentuate the pathogenesis of COVID-19 [34, 35].

Consequently, T cell responses elicited by any SARS-CoV-2 vaccine(s) would have to be well controlled to withdraw immunopathology. In this context, the safety of PiCoVacc applied in macaques by recording a number of clinical observations was systematically evaluated. Although it is still too early to define the best animal model for studying SARS-CoV-2 infections, it was evidenced by the safety of PiCoVacc in macaques, and no infection intensification or immunopathological exacerbation were observed in this study.

Nucleic Acid: DNA and RNA [36]

Similar to subunit vaccines, specific proteins from the target pathogen are chosen for their immunogenic epitopes, however these proteins are delivered as either plasmid DNA or RNA sequences [37, 38]. Upon vaccination, the host cell manufactures the pathogen protein, which is recognised by the immune system as foreign and generates an immune response [37]. Non-capsulated RNA vaccines are readily removed by the host cell upon injection, so advances in delivery technology, including encapsulation of RNA in liposomes, have been developed to avoid degradation [39].

RNA vaccines have been shown to induce antigen-specific antibody and polyfunctional T-cell responses in phase I clinical trials of cancer vaccines [39], and functional antibodies against Rabies virus glycoprotein [40], however there are currently no licensed RNA vaccines for humans. Although DNA vaccines are immunogenic in small animal models, they show less immunogenicity in human clinical trials and require adjuvants or multiple doses [16, 41]. Four DNA vaccines are available for animal use [39], however there are currently none licensed for humans [42].

There are several nucleic acid vaccines in development for COVID-19 prophylaxis. Nucleic acid vaccines are relatively cheap and rapid to manufacture, with the possibility to mass-produce large-scale GMP product [43].

Replicating Viral Vectors (e.g. Measles) [44]

Replicating viral vector vaccines use a replicating viral vector that has been altered to produce coronavirus proteins in the body. They provide a strong immune response and have long been applied successfully in poultry, using herpesvirus and poxvirus backbones to immunize against Newcastle disease [45] and

infectious bursal disease [46]. In human vaccine development, the attenuated measles virus can be used as a replicating vector [47]. A recent example is a vaccine that is being developed against chikungunya fever [48]. One potential limitation is that previous immunity to the vector may render the vaccine useless in some cases.

Non-replicating Viral Vector (e.g. Adenoviral Vectors and Modified Vaccinia Ankara, MVA) or Recombinant Viral-Vectored Vaccines [13]

Recombinant viral-vectored vaccines utilise the host's innate immunity to generate self-adjuvanted immunogenicity, whilst eliciting a targeted immune response against genetically-encoded pathogen antigens [49]. The viral vector 'backbone' is constructed from a genetically-modified virus [50], examples including adenoviruses, poxviruses, and Vesicular stomatitis virus [50, 51]. This vector typically has insertion sites for gene(s) of the target pathogen, which are expressed intracellularly in the host upon vaccination [52].

Important considerations for development of virus vectored vaccines is the generation of immunity towards the vector, which could hinder the antigen specific response upon a boost vaccination. However reports from preclinical and clinical studies show sufficient protection can be elicited from a single dose [53, 54].

Human adenoviruses (hAds) are a frequently used viral vector, however circulate at high frequency in most populations [55], contributing towards demographically variable yet significant pre-existing immunity that can reduce vaccine efficacy [52]. Vectors constructed from chimpanzee adenovirus (ChAd) were developed to elicit similar or superior immunogenicity as hAd vectors, whilst having significantly reduced seroprevalence and hence neutralising antibodies in most populations [56]. In pre-clinical studies, ChAd vectors have demonstrated up to 100% efficacy with a single vaccination against several emerging pathogens [54, 57]. Clinical trials have established that ChAd vectors also have a good safety profile and immunogenicity for Influenza A [58], ebolavirus [59], and MERS [60].

Adenovirus vectors can be rapidly made to GMP at large scale, and a single vaccination can be sufficient to provide rapid immunity in individuals [61]. This rapid production and distribution pipeline was tested during the 2013-2016 ebolavirus (EBOV) outbreak in Guinea, Liberia, and Sierra Leone, where five viral-vectored vaccines were rapidly escalated to clinical trials [61]. A recombinant vesicular stomatitis virus vector expressing the EBOV glycoprotein (rVSV-ZEBOV) progressed to phase III trials in Guinea and Sierra Leone and provided 100% efficacy across 4,359 individuals vaccinated with a single dose [54]. Following the second ebolavirus outbreak in the Democratic Republic of the Congo (DRC) in 2018, the WHO allowed compassionate use of rVSV-ZEBOV in the DRC, which has now been licensed in the DRC, Burundi, Ghana and Zambia [62]. An Ad26-vectored ebola virus vaccine has also been developed by Janssen and tested extensively in a prime-boost regimen in sub-Saharan Africa for efficacy and immunogenicity [63].

Protein Subunit Vaccines [13]

Protein subunit vaccines include antigenic proteins thought to induce a protective immune response. This vaccine type is produced *in vitro* and circumvents handling highly pathogenic live viruses [14, 64]. Subunit vaccines predominantly elicit a humoral antibody response, and most are administered with an adjuvant, which is a prerequisite to stimulate a strong immune response and generate a higher quality immune memory in humoral and cellular compartments. However, the inclusion of adjuvants can increase the reactogenicity and production costs, which are important considerations [64]. Virus-like particles (VLP)

are a type of subunit vaccine that present many copies of the relevant antigen in a 3D virus-like structure, and may be immunogenic enough to not require the inclusion of adjuvants [64].

Subunit vaccines are an attractive vaccine technology for rapid vaccine development, and multiple institutions worldwide are developing protein subunit-based vaccines. They can be upscaled for mass production at good manufacturing practice (GMP) standards [65], and distribution has less reliance on cold chain systems [16]. However, they can require bespoke manufacturing processes, which can increase cost, and may require specific mammalian cell expression and optimisation [28, 66].

Adjuvant

Also, to live attenuated vaccines and live vector vaccines, adjuvants are demanded to improve the immune response in the development of other types of vaccines. In order to stimulate the development of a SARS-CoV-2 vaccine, the favored adjuvant should be those that have been broadly used in other marketable vaccines, including (1) classic aluminum adjuvant, aluminum adjuvants improve the immune response by helping phagocytosis and reducing the diffusion of antigens from the injection site. It can efficiently stimulate Th2 immune response upon injection [67]; (2) MF59, MF59 is an oil-in-water emulsion composed of Tween 80, sorbitol trioleate, and squalene, and it has already been adopted in flu vaccines in Europe and in the United States. The mechanism of MF59 is to produce a transient immune environment at the injection site, then to recruit immune cells to cause antigen-specific immune responses [68]; (3) Adjuvant system (AS) series adjuvants, which are a series of adjuvants produced by GlaxoSmithKline (GSK), including AS01, AS02, AS03, and AS04. Among them, AS01 is a liposome adjuvant containing 3-O-desacyl-4'-monophosphoryl lipid A (MPL) and saponin QS-21 [69], which has been adopted in malaria vaccines [70]. AS02 is an oil-in-water emulsifier that has MPL and QS-21 [71]. AS03 is an oil-in-water emulsifier containing alpha-tocopherol, squalene, and Tween 80. It has been adopted in influenza vaccines [72]. AS04 is an aluminum adjuvant containing MPL and has been used in a human papillomavirus vaccine and the hepatitis B virus vaccine [73].

Because adjuvants were capable to manage the type of immune response, the optimal adjuvant should be chosen according to the design of the vaccine. In order to provoke a more adequate immune response, a combination of different types of adjuvants could be used to enhance the immune efficacy.

Antigen Selection [74]

Whole Cell Antigens

The whole-cell antigens (WCA) carry all the components of the virus, including proteins, lipids, polysaccharide, nucleic acids, and some other elements. WCA has been utilized for developing whole-cell killed and live-attenuated vaccines [75]. Since the complex structures of WCA, it is inevitable to face more issues in quality control and compatibility evaluation. So far, several companies have successfully isolated the virus of SARS-CoV-2 and began whole-cell killed or live-attenuated vaccine progress. Nevertheless, research on the type of vaccine demands rigorous screening for reaching strains with undoubted low or no pathogenicity [76].

Spike Protein (S Protein)

S protein is currently the most hopeful antigen formulation for SARS-CoV-2 vaccine investigations. Primary, it is surface exposure and thus is capable to be directly identified by the host immune system [77]. Secondary, it mediates the binding with the host cell by attaching to the receptor ACE2, which is imperative for succeeding virus entrance to target cells and

causes subsequent pathogenicity [77, 78]. Lastly, the homolog proteins were already applied for vaccine development against SARS-CoV and MERS-CoV and were proved to be effective [79, 80]. The monomer of S protein from SARS-CoV-2 has 1,273 amino acids, with an approximately 140 kDa. Self-association naturally assembles the S protein into a homo-trimer, typically alike to the first class of membrane fusion protein (Class I viral fusion protein). The S protein includes two subunits (S1 and S2). The S1 subunit can be determined with two domains with the N-terminal domain (NTD) and the C-terminal domain (CTD). The receptor-binding domain (RBD) is placed in the CTD. S2 subunit contains the basic elements needed for membrane fusion, including an internal membrane fusion peptide (FP), two 7-peptide repeats (HR), a membrane-proximal external region (MPER), and a transmembrane domain (TM) [81]. Lately, the structure of the SARS-CoV-2 S trimer in the pre-fusion conformation and the RBD domain in complex with ACE2 has been successfully determined [77, 78], which has contributed to relevant data for vaccine design based on this protein. So far, the potential fragments of S protein for application as antigens in vaccine development include the full-length S protein, the RBD domain, the S1 subunit, NTD, and FP.

The Full-Length S Protein

Full-length proteins are ordered to hold the correct form of the protein, capable of providing more epitopes and presenting higher immunogenicity. Pallesen and colleagues [82] showed that higher titer of neutralizing antibodies in BALB/c mice immunized with recombinant prefusion MERS-CoV S protein. Another study confirmed that S protein produced in baculovirus insect cells was capable to assemble into nanoparticles. Mice immunized with these nanoparticles formulated with alum adjuvant that produced a high titer of neutralizing antibodies [83]. Muthumani and colleagues [84] described that DNA vaccine encoding MERS-CoV S protein was immunogenic in mice, camels, and rhesus macaques. Animals immunized with the DNA vaccine exhibit reduced typical clinical symptoms including pneumonia during the infection. So far, Clover Biopharmaceuticals had declared that they have created a SARS-CoV-2 S protein trimer vaccine (S-Trimer) by using its patented Trimer-Tag© technology, and this vaccine will be produced via a fast mammalian cell-culture based expression system.

RBD

Since the RBD of S protein directly interacts with the ACE2 receptor on host cells, RBD immunization provoked specific antibodies that may obstruct this identification and thus limit the invasion of the virus. Most SARS-CoV-2 subunit vaccines currently under development use RBD as the antigen. Furthermore, the RBD domain was also applied in the development of SARS-CoV and MERS-CoV vaccines. For instance, studies have shown that recombinant RBD is multiple conformational neutralizing epitopes that can cause a high titer of neutralizing antibodies against SARS-CoV [85]. Lan and colleagues [86] described that Rhesus macaques immunized with the recombinant RBD formulated with alum adjuvant could provide neutralizing antibodies, in association with observed mitigation of the clinical symptoms during MERS-CoV infection. Nyon and colleagues [87] also described that hCD26/DPP4 transgenic mice immunized with RBD fused to Fc elicited neutralizing antibodies and were able of protecting against MERS-CoV infection. Moreover, the RBD domain is relatively conserved as associated with the S1 subunit and was described to have multiple conformational neutralizing epitopes [88], making it more proper for vaccine development.

NTD

Similar to RBD, the N-terminal domains (NTD) of S protein from many coronaviruses were related to show carbohydrate receptor-binding

activity. For example, the NTD of spike protein from transmissible gastroenteritis virus (TGEV) was described to attach sialic acid via NTD [89]. The carbohydrate-binding characteristics of IBV M41 strain are also correlated to the NTD of the S protein [90]. So, this domain is also a candidate antigen for vaccine development. One study reported that rNTD of S protein from MERS-CoV led to strong cellular immunity and antigen-specific neutralizing antibodies in mice and was protective against the viral challenge [91]. There is a study that a mAb that attaches to the N-terminal domain (NTD) of the MERS-CoV S1 subunit revealed efficient neutralizing action against the wild-type MERS-CoV strain EMC/2012 [92]. This result revealed that NTD specific antibodies are useful in neutralization. Nevertheless, as the genomes of coronaviruses are extremely variable, it is better to use antibodies targeting different epitopes to avoid the immune evasion of the virus. Although the function of S1-NTD of SARS-CoV-2 has not been clarified, it may also be implicated in the union of certain receptors and can also be a candidate antigen.

S1 Subunit

The S1 subunit, which has both RBD and NTD, is principally involved in the S protein attachment to the host receptor. It is also extensively applied in vaccine development. Wang and colleagues [93] demonstrated that MERS-CoV S1 protein formed with MF59 adjuvant protected hDPP4 transgenic mice against lethal virus challenge, and the protection related well with the neutralizing antibody titer. Adney and colleagues [94] reinforced that immunization with adjuvanted S1 protein decreased and delayed virus shedding in the upper respiratory tract of dromedary camels and complete protection was seen in alpaca against MERS-CoV challenge.

FP

The FP domain of the S2 subunit is involved in the membrane fusion of the virus, which is also a principal step in viral pathogenicity [95]. Hence, it may also serve as a vaccine candidate antigen. Tianjin University has constructed an RBD-FP fusion protein, and a high titer of antibodies was identified in mice immunized with this fusion protein, and the effectiveness is under evaluation.

Nucleocapsid Protein (N Protein)

The N protein is the most abundant protein in coronavirus, and it is normally deeply conserved. N protein has multiple roles including the development of nucleocapsids, signal transduction virus budding, RNA replication, and mRNA transcription [96]. This protein was described to be extremely antigenic, 89% of patients who developed SARS, formed antibodies to this antigen [97]. DNA vaccine encoding SARS-CoV N protein produced strong N-specific humoral and cellular immune responses in vaccinated C57BL/6 mice and was able to significantly decrease the titer of challenging vaccine virus [98]. Till, some other researchers published that the N protein of avian infectious bronchitis virus is related to the induction of CTLs that are associated with a reduction in clinical signs and viral clearance from lungs, proposing that cellular response is essential in N protein-mediated protection [99, 100]. In opposition, another research showed that the N protein immunization did not provide a significant contribution to neutralizing antibody response and provided no protection to infection in hamsters [101]. These results insinuate that there is controversy about whether this protein could be accepted for vaccine development. But, there is no doubt that it can be applied as a marker in diagnostic assays due to its high immunogenicity.

Membrane Protein (M Protein)

M protein is a transmembrane glycoprotein and is implicated in virus attachment, and this protein is the most abundant protein on the surface of SARS-CoV [102]. It was described that immunization with the full length of M protein is capable to evoke efficient neutralizing antibodies in SARS patients

[103]. Immunogenic and structural analysis also showed that the transmembrane domain of the M protein has a T cell epitope cluster that is capable to induce a strong cellular immune response [104]. M protein is also highly conserved in evolution among different species [102], consequently, it may be adopted as a candidate antigen for developing the SARS-CoV-2 vaccine.

Envelope Protein (E Protein)

Compared with S, N, and M protein, E protein is not proper for use as an immunogen, because it consists of 76–109 amino acids in different coronaviruses with channel activity, thus the immunogenicity is restricted. Studies have determined that SARS-CoV E protein is an important virulence factor, and the secretion of inflammatory factors IL-1β, TNF, and IL-6 are significantly decreased after knocking out E protein [105].

Summary Contents

Table 1 summarizes the vaccine platforms developed against SARS-CoV-2, indicating the advantage and disvantage of each one [17]. Table 2 presents the 1/2-3 clinical-trial-phases of the vaccines against COVID-19 by Regulatory Affairs Professionals Society (RAPS) [106], and the Figures 1-8 sumarize the main types being tested range from those containing the whole virus, either in a weakened or inactivated form, or those that contain part of the viral structure, to those that depend on our own cells to produce viral proteins that the immune system can recognise. All of them rely on the same basic principle of mimicking a real viral infection and inducing a protective immune response [76].

Vaccines' Candidates Against SARS-CoV-2

Researchers worldwide are working around fastly to discover a vaccine against SARS-CoV-2, the virus causing the COVID-19 pandemic. Specialists expect that a fast-tracked vaccine development process could speed a successful candidate to market in approximately 12-18 months.

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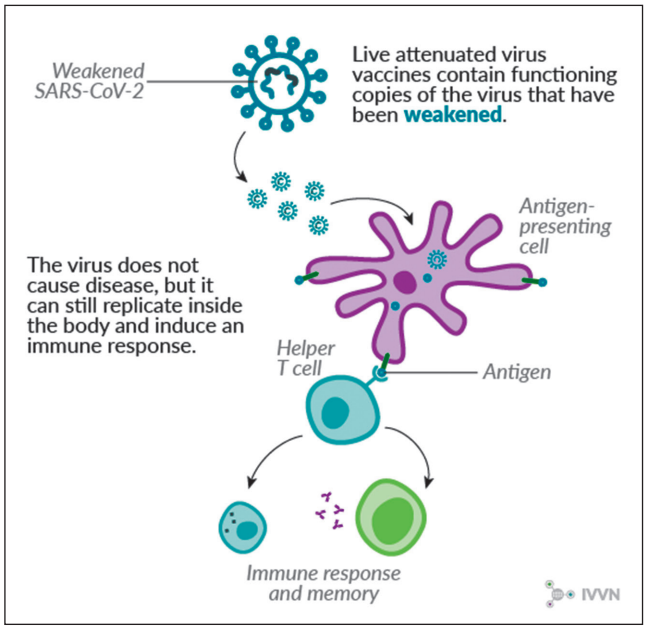
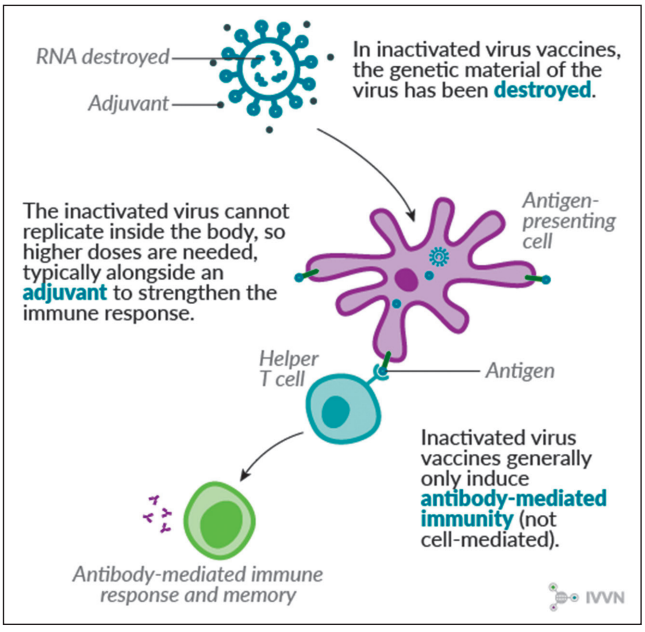
Figure 1. Live attenuated virus vaccine.



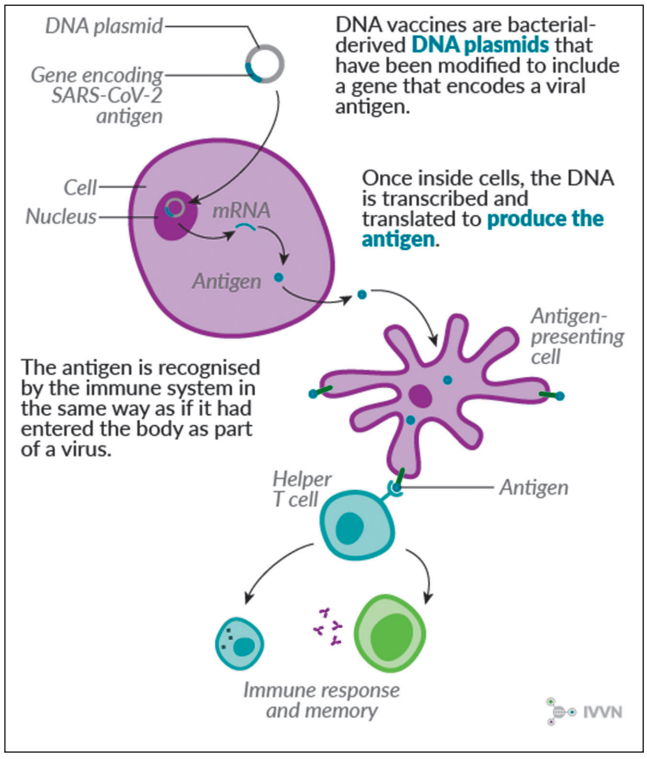
Credit/Source: International Veterinary Vaccinology Network (IVVN) [44].

Figure 2. Inactivated virus vaccine.



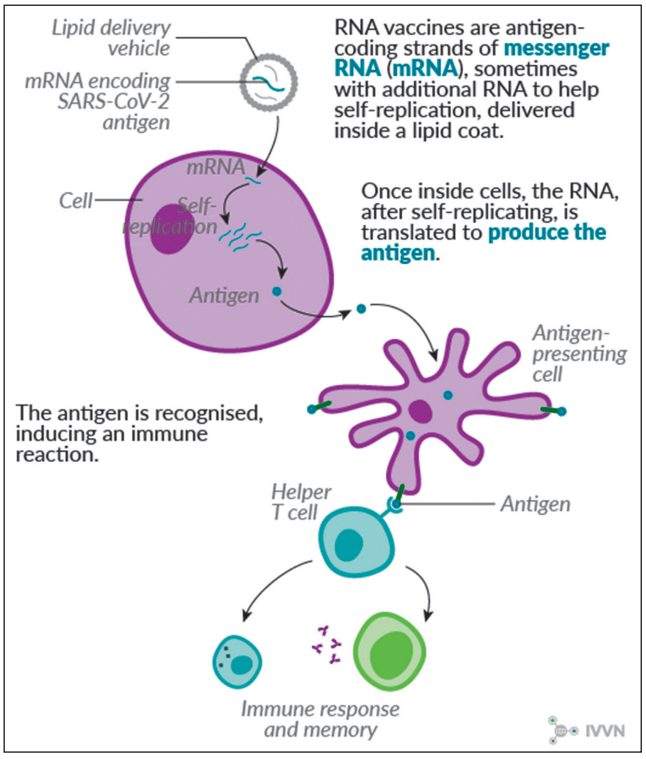
Credit/Source: International Veterinary Vaccinology Network (IVVN) [44].

Figure 3. DNA vaccines.



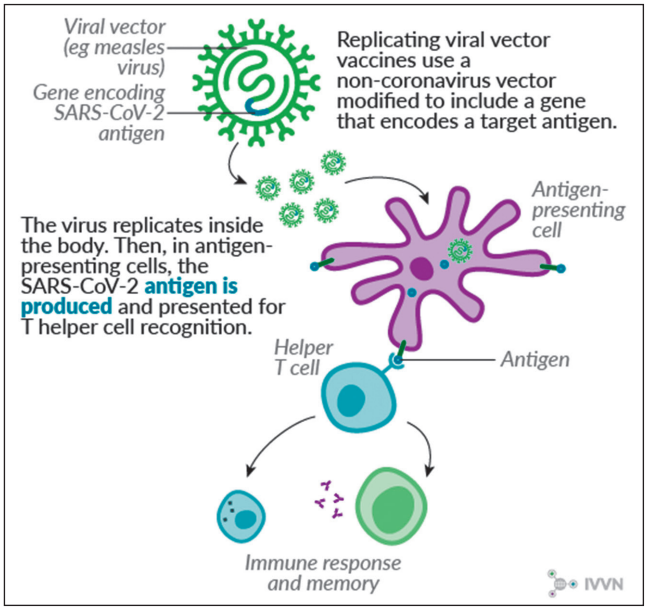
Credit/Source: International Veterinary Vaccinology Network (IVVN) [44].

Figure 4. RNA vaccines.



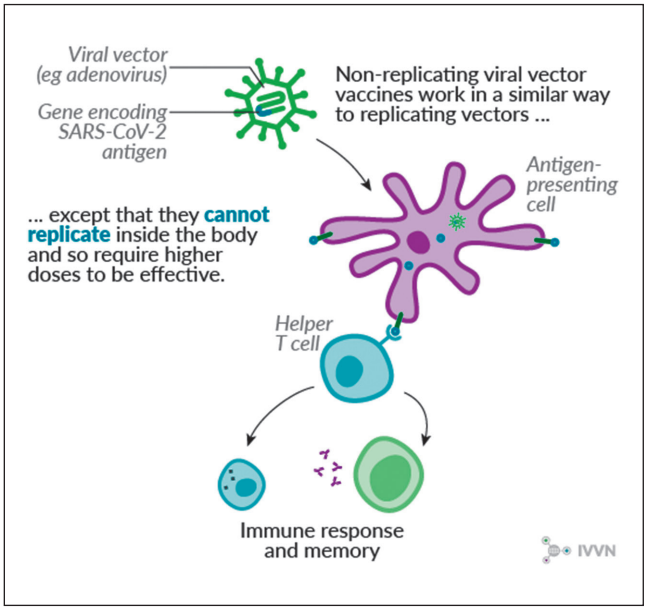
Credit/Source: International Veterinary Vaccinology Network (IVVN) [44].

Figure 5. Viral vector vaccines (replicating).



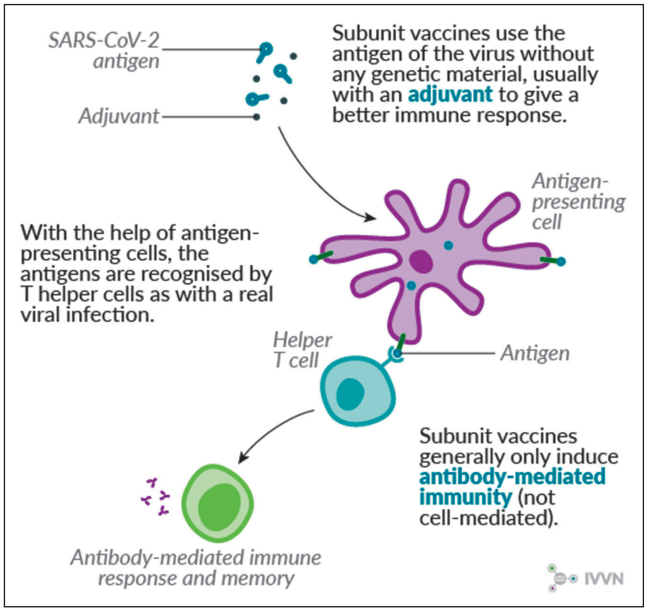
Credit/Source: International Veterinary Vaccinology Network (IVVN) [44].

Figure 6. Viral vector vaccines (non-replicating).



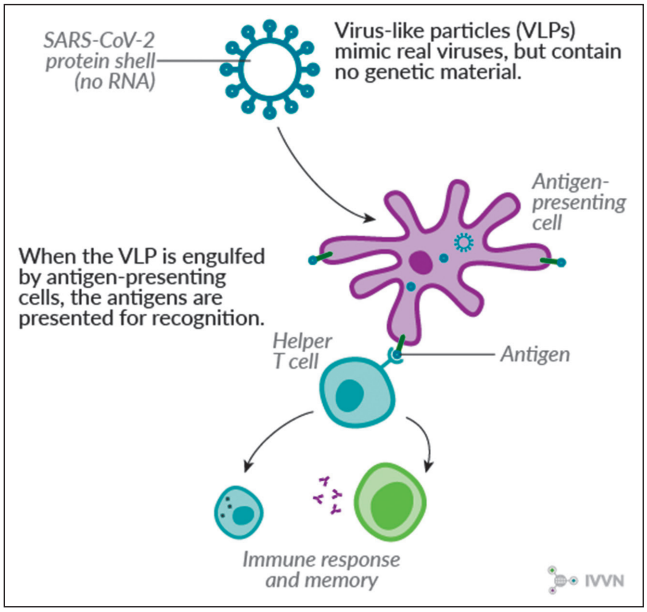
Credit/Source: International Veterinary Vaccinology Network (IVVN) [44].

Figure 7. Viral vector vaccines (non-replicating).



Credit/Source: International Veterinary Vaccinology Network (IVVN) [44].

Figure 8. Virus-like particles vaccines.



Credit/Source: International Veterinary Vaccinology Network (IVVN) [44].

Table 1. Selected antigens and vaccine platforms against SARS-CoV-2.

Platform	Target	How It Works	Advantages	Disadvantages	Examples	Group Against COVID-19
RNA vaccines	S protein	They use the RNA to lead the immune system to target the key viral proteins	Easy design. No infectious virus needs to be handled, vaccines are typically immunogenic, rapid production possible.	Safety issues with reactogenicity have been reported.	None	Moderna
DNA vaccines	S protein	They use the DNA to lead the immune system to target the key viral proteins	Easy design. No infectious virus needs to be handled, easy scale up, low production costs, high heat stability, tested in humans for SARS-CoV-1, rapid production possible.	Vaccine needs specific delivery devices to reach good immunogenicity.	None	Inovio
Viral vector-based vaccines	S protein	They use a harmless virus and use it to deliver viral genes to build immunity	Live virus tends to lead stronger immune responses than dead virus or subunit vaccines. Excellent preclinical and clinical data for many emerging viruses, including MERS-CoV.	Vector immunity might negatively affect vaccine effectiveness (depending on the vector chosen). And it is important to choose a truly safe viral vector.	Ebola Veterinary medicine	University of Oxford and Astrazeneca CanSino Biologics Johnson & Johnson
Live attenuated vaccines	Whole virion	It uses a weakened version of the virus	Stimulates a robust immune resonse without serious disease or adverse events. Straightforward process used for several licensed human vaccines, existing infrastructure can be used.	May not be safe for immuno-compromised immune systems. Creating infectious clones for attenuated coronavirus vaccine seeds takes time because of large genome size. Safety testing will need to be extensive.	Measles, Munps and Rubella, Chicken pox	Codagenix Indian Immuno-logicals Inc.
Virus innactivated	Whole virion	It uses the whole virus after it has been killed with heat or chemicals	It is easy to make and safe because the virus is already dead.	This vaccine is not more effective than the live virus. Some previous inactivated virus have made the disease worse. The safety for the novel coronavirus needs to be shown in clinical trials.	Polio	Sinovac Sinopharm (Coronavac)
Subunit	S protein	It uses a pieces of a virus' surface to focus your immune system as a single target.	Focuses the immune response on the most important part of the virus for protection and cannot cause infection.	May not stimulates a strong response, other chemicals may need to be added to boost long-term immunity.	Pertussis Hepatitis B HPV	Novavax AdaptVac

Credit/Source: Adapted from Amand and Kramer [17].

Table 2. Vaccine candidates against COVID-19 in 1/2-3 clinical trial phase.

Candidate	Sponsor	Phase	Institution
Inactivated vaccine	**Wuhan Institute of Biological Pharmaceutical Group (Sinopharm)**	**Phase 3**	**Henan Provincial Center for Disease Control and Products; China National Prevention**
Study Design & Details **Background:** Researchers at Sinopharm and the Wuhan Institute of Virology under the Chinese Academy of Sciences are developing an inactivated COVID-19 vaccine candidate. They have initiated a randomized, double-blind, placebo parallel-controlled Phase 1/2 clinical trial (ChiCTR2000031809) of healthy individuals starting at 6 years old. **Outcomes:** The vaccine has shown a "strong neutralizing antibody response" in Phase 1/2 trials, according to a release from China National Biotec Group. It appeared to be working best at the middle strength when given 28 days apart, as all participants in that dosing schedule developed neutralizing antibodies that can defend a cell from infection. Until now, all 1,120 volunteers in the phase 1/2 trial have received two injections of the vaccine at low, middle or high dosing strengths—or placebo—either 14 days, 21 days or 28 days apart, according to CNBG. The seroconversion rate for the 14-day and 21-day schedule of the mid-dose was 97.6%. At 28 days, it was 100%. The company didn't specify the neutralizing antibody response rates for the low dose or the high one. It also didn't elaborate on the exact levels of immune response, only saying the antibody titers were "high." No serious adverse event was observed. **Status:** A Phase 3 trial is underway conducted in the United Arab Emirates.			
CoronaVac	**Sinovac**	**Phase 3**	**Sinovac Research and Development Co., Ltd.**
Study Design & Details **Background:** CoronaVac (formerly PiCoVacc) is a formalin-inactivated and alum-adjuvanted candidate vaccine. Results from animal studies showed "partial or complete protection in macaques" exposed to SARS-CoV-2, according to a paper published by researchers in the journal Science. **Study Design:** A Phase 1/2 trial enrolled 743 healthy volunteers (18-59 years old) who received two different dosages of the vaccine or placebo. There were 143 participants in Phase 1 (NCT04352608) and 600 participants in Phase 2 (NCT04383574). **Outcomes:** The phase I/II clinical trials were designed as randomized, double-blind and placebo-controlled studies. In total, 743 healthy volunteers, aged from 18 to 59 years old, enrolled in the trials. Of those, 143 volunteers are in phase I and 600 volunteers are in phase II. There have been no severe adverse event reported in either the phase I or phase II trials. The phase II clinical trial results show that the vaccine induces neutralizing antibodies 14 days after the vaccination with a 0,14 day schedule. The neutralizing antibody seroconversion rate is above 90%, indicating a positive immune response. **Status:** Sinovac said a Phase 3 trial in collaboration with Butantan Institute in Brazil is underway, and the company plans to enroll around 9,000 patients in the healthcare industry.			
mRNA-1273	**Moderna**	**Phase 3**	**Kaiser Permanente Washington Health Research Institute**
Study Design & Details **Background:** mRNA-1273 was developed by Moderna based on prior studies of related coronaviruses such as those that cause severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS). A Phase 1 trial (NCT04283461) of 105 healthy participants provided the basis for Moderna's investigational new drug application (IND), which was successfully reviewed by the FDA and set the stage for Phase 2 testing. A Phase 2 trial of 600 healthy participants evaluating 25 μg, 100 μg, and 250 μg dose levels of the vaccine was completed, and mRNA-1273 has advanced to a Phase 3 trial (NCT04405076. **Study Design:** A Phase 3 trial of 30,000 participants at high risk for SARS-CoV-2 infection who will receive a 100 μg dose of mRNA-1273 or placebo and then followed for up to 2 years (COVE trial; NCT04470427). **Outcomes:** Phase 1 data published in the New England Journal of Medicine showed mRNA-1273 successfully produced neutralizing antibody titers in 8 participants who received either 25 μg or 100 μg doses. The response was dose dependent in 45 participants across 25 μg, 100 μg, and 250 μg dose levels. In participants with available antibody data, neutralizing antibody titers were on par with what has been in seen in convalescent sera from people who have successfully fought off COVID-19. Results from a challenge in a mouse model showed mRNA-1273 prevented viral replication in the lungs, and neutralizing titers in the mouse model were similar in participants receiving 25 μg or 100 μg doses of the vaccine. Moderna said mRNA-1273 was "generally safe and well tolerated."A study of nonhuman primates challenged with SARS-CoV-2 published in the New England Journal of Medicine had neutralizing activity, and limited inflammation and lung activity after being administered the vaccine. **Status:** On 12 May, the FDA granted Fast Track designation to mRNA-1273. A Phase 3 trial of the vaccine is underway, which is being funded by Operation Warp Speed.			

Bacillus Calmette-Guerin (BCG) live-attenuated vaccine	**University of Melbourne and Murdoch Children's Research Institute; Radboud University Medical Center; Faustman Lab at Massachusetts General Hospital**	**Phase 2/3**	**University of Melbourne and Murdoch Children's Research Institute; Radboud University Medical Center; Faustman Lab at Massachusetts General Hospital**

Study Design & Details

Background: The BCG vaccine is indicated to prevent tuberculosis in those who have a higher risk of the disease. It has been implicated in helping to combat other infections outside TB by boosting the immune system to fight similar infections. In 2017, the World Health Organization (WHO) reported the BCG vaccine may be effective against leprosy and other nontuberculous mycobacteria such as buruli ulcer disease. Other papers have posited the vaccine is effective in preventing acute respiratory tract infections in elderly patients, other respiratory infection and sepsis. A non-peer reviewed paper posted in March 2020 on the preprint server medRxiv has suggested countries with BCG vaccination programs at childhood are faring better in the fight against COVID-19 compared with countries that do not require BCG vaccination. BCG vaccines are being studied in the randomized, controlled, Phase 3 BRACE trial, which aims to recruit 4,170 healthcare workers in hospitals in Australia (NCT04327206). Researchers in The Netherlands launched the randomized, parallel-assignment, phase 3 BCG-CORONA trial on 31 March and plan to enroll 1,500 healthcare workers to receive the BCG vaccine or placebo (NCT04328441). The Faustman Lab is currently evaluating the BCG vaccine's effectiveness in type 1 diabetes and is seeking funding to launch trial to assess whether the vaccine helps prevent COVID-19 in healthcare workers, according to independent reporting from the New York Times.

AZD1222	**The University of Oxford; AstraZeneca; IQVIA**	**Phase 2/3**	**The University of Oxford, the Jenner Institute**

Study Design & Details

Background: The Oxford Vaccine Group at the University of Oxford are developing a new vaccine candidate for COVID-19, a chimpanzee adenovirus vaccine vector called AZD1222 (previously ChAdOx1). The team has previously developed a MERS vaccine. Preclinical data in a paper on the pre-print server bioRxiv that showed a significantly reduced viral load and "humoral and cellular immune response." The vaccine candidate also showed an immune response in mice and pigs, according to information in a pre-print paper.

Study Design: A Phase 1/2 (NCT04324606) single-blinded, multi-center study of 1,090 healthy adult volunteers aged 18-55 years with four treatment arms. Participants in two treatment arms will receive a single dose of AZD1222 or MenACWY, a meningococcal vaccine. A third treatment arm will receive AZD1222 and a booster at 4 weeks. In a fourth arm, participants will receive AZD1222 or MenACWY together with 1 g of paracetamol (acetaminophen) every 6 hours for 24 hours. The trial is active, but not currently recruiting.

Outcomes: Preliminary results from the trial published in The Lancet showed the vaccine candidate had an "acceptable safety profile" with most patients demonstrating an antibody response after one dose and all patients showing a response after two doses.

Status: On 21 May, AstraZeneca announced it has received $1 billion in funding from the Biomedical Advanced Research and Development Authority (BARDA) for "development, production and delivery of the vaccine," beginning in September 2020. The agreement between AstraZeneca and BARDA includes a minimum of 400,000 doses of the vaccine, an upcoming Phase 3 trial of 30,000 participants, and a pediatric trial. On 22 May, Oxford researchers announced that they had begun recruitment for a Phase 2/3 trial of approximately 10,000 healthy adult volunteers to assess how well people across a broad range of ages could be protected from COVID-19. A Phase 3 trial of AZD1222 is being funded by Operation Warp Speed. IQVIA announced they are partnering with AstraZeneca to advance clinical trials for the vaccine Brazil will participate of the Phase III trial in São Paulo.

BNT162	**Pfizer, BioNTech**	**Phase 2/3**	**Multiple study sites in Europe and North America**

Study Design & Details

Background: Pfizer and BioNtech are collaborating BNT162, a series of vaccine candidates for COVID-19. BNT162 was initially four vaccine candidates originally developed by BioNTech, two candidates consisting of nucleoside modified mRNA-based (modRNA), one of uridine containing mRNA-based (uRNA), and the fourth candidate of self-amplifying mRNA-based (saRNA). The companies have selected the modRNA candidate BNT162b2 to move forward in a Phase 2/3 trial.

Study Designs: A Phase 1/2 trial in the US and Germany of 200 healthy participants between aged 18-55 years, with a vaccine dose range of 1 µg to 100 µg is currently recruiting (NCT04380701). A Phase 2/3 trial of about 32,000 healthy participants is active, but not currently recruiting (NCT04368728).

Outcomes: Results of one study of BNT162b1, a modRNA candidate, were reported 1 July on the non-peer-reviewed preprint server medRxiv. Robust immunogenicity was seen after vaccination at all three doses (10 μg, 30 μg and 100 μg). Adverse events were elevated at the highest dose; therefore, participants did not receive a second dose at that level.
Status: Pfizer and BioNTech received FDA Fast Track designation for two of the BNT162 candidates, BNT162b1 and BNT162b2. The companies have published results from a study that suggests one of the BNT162 candidates, BNT162b1, produced a neutralizing antibody response in participants who received the vaccine. However, they selected BNT162b2 as a candidate to advance to a Phase 2/3 safety study "based on the totality of available data from our preclinical and clinical studies, including select immune response and tolerability parameters." A candidate could be ready for regulatory approval as early as December.

Ad5-nCoV	**CanSino Biologics**	**Phase 2**	**Tongji Hospital; Wuhan, China**

Study Design & Details
Background: China's CanSino Biologics has developed a recombinant novel coronavirus vaccine that incorporates the adenovirus type 5 vector (Ad5). Preliminary safety data from a Phase 1 (ChiCTR2000030906; NCT04313127) clinical trial of 108 participants between 18 and 60 years old who will receive low, medium, and high doses of Ad5-nCoV has allowed the company to plan to initiate a Phase 2 trial, according to an announcement. The Phase 2 (ChiCTR2000031781) trial has identical inclusion criteria.
Outcomes: Results from Phase 1 of the trial show a humoral and immunogenic response to the vaccine, according to a paper published in The Lancet. Adverse reactions such as pain (54%), fever (46%), fatigue (44%), headache (39%), and muscle pain (17%) occurred in 83% of patients in the low and medium dose groups and 75% of patients in the high dose group. In Phase 2 of the trial, neutralizing antibodies and specific interferon γ enzyme-linked immunospot assay responses were observed at all dose levels for most participants.
Status: On 25 June, China's Central Military Commission announced the military had been approved to use Ad5-nCoV for a period of 1 year, according to reporting in Reuters.

Adjuvant recombinant vaccine candidate	**Anhui Zhifei Longcom Biopharmaceutical, Institute of Microbiology of the Chinese Academy of Sciences**	**Phase 2**	**-**

Study Design & Details
Background: China's National Medical Products Administration has approved a Phase 1 trial of a COVID-19 vaccine candidate developed by the Anhui Zhifei Longcom Biopharmaceutical and the Institute of Microbiology of the Chinese Academy of Sciences. A Phase 2 trial is underway, with results from Phase 1 expected in September, according to Reuters.

BBIBP-CorV	**Beijing Institute of Biological Pharmaceutical Group (Sinopharm)**	**Phase 1/2**	**Henan Provincial Center for Disease Control and Products; China National Prevention**

Study Design & Details
Background: Sinopharm is developing a second inactivated COVID-19 vaccine candidate, BBIBP-CorV, with the Beijing Institute of Biological Products. BBIBP-CorV is currently being evaluated in a Phase 2 trial (ChiCTR2000032459).
Outcomes: Results from a paper published in the journal Cell appear to show BBIBP-CorV provides "highly efficient protection" against SARS-CoV-2 in rhesus macaques who underwent challenge against the virus.
Status: More than 2,000 vaccines administered between Sinopharm's two inactivated vaccine trials. Both vaccine candidates could be ready for market by the end of the year, according to reporting from Reuters.

GX-19	**Genexine**	**Phase 1/2**	**GenexineGenexine**

Study Design & Details
Background: Genexine, a biotechnology based in South Korea, is testing GX-19, a DNA vaccine candidate for COVID-19. The company has been approved for a Phase 1/2a clinical trial of 190 healthy participants randomized to receive the vaccine or placebo (NCT04445389). The company aims to complete Phase 1 in 3 months before moving to a multinational Phase 2 trial.

Gam-COVID-Vac	**Gamaleya Research Institute, Acellena Contract Drug Research and Development**	**Phase 1/2**	**Various**
Study Design & Details **Background:** The Gamaleya Research Institute in Russia is testing their non-replicating viral vector COVID-19 vaccine candidate, Gam-COVID-Vac, in a Phase 1/2 trial. The trial is expected to recruit about 38 participants to receive the vaccine candidate (NCT04436471) (NCT04437875). **Status:** The institute reportedly plans to test the candidate on a small section of the public in August, which would be the equivalent of a Phase 3 trial.			
Self-amplifying RNA vaccine	**Imperial College London**	**Phase 1/2**	**Imperial College London**
Study Design & Details **Background:** Imperial College London researchers are developing a self-amplifying RNA vaccine for COVID-19. They developed a vaccine candidate within 14 days of receiving the sequence from China. Animal testing is underway. The investigators have received two rounds of funding from the United Kingdom's government – one on 22 April and another on 17 May. **Study Design:** The Phase 1/2 COVAC1 trial will enroll approximately 300 healthy participants between 18 and 75 years old, with an efficacy trial for 6,000 participants planned for October. **Status:** On 7 June, Imperial College London announced it had partnered with Morningside Ventures to establish VacEquity Global Health, an initiative that would help keep costs down for their COVID-19 vaccines down for citizens in the UK and internationally.			
LUNAR-COV19	**Arcturus Therapeutics and Duke-NUS Medical School**	**Phase 1/2**	**Duke-NUS Medical School, Singapore**
Study Design & Details **Background:** Arcturus and Duke-NUS Singapore are partnering to develop a COVID-19 vaccine candidate that uses Arcturus' self-replicating RNA and nanoparticle non-viral delivery system. Pre-clinical data from the company indicates LUNAR-COV19 provides an adaptive cellular (CD8+ cells) and balanced (Th1/Th2) immune response. Arcturus said a Phase 1/2 clinical trials will proceed in Singapore.			
ZyCoV-D	**Zydus Cadila**	**Phase 1/2**	**Zydus Cadila**
Study Design & Details **Background:** India's Zydus Cadila is researching ZyCoV-D, a plasmid DNA vaccine candidates for COVID-19 that targets the viral entry membrane protein of the virus. The company has launched an adaptive Phase 1/2 dose escalation trial and plans to enroll about 1,000 healthy volunteers.			

Credit/Source: RAPS [106].

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