ANNUAL REVIEWS

Annual Review of Medicine

Whole Inactivated Virus and Protein-Based COVID-19 Vaccines

Peter J. Hotez[1,2,3,4] and Maria Elena Bottazzi[1,2,3]

[1]Departments of Pediatrics and Molecular Virology, National School of Tropical Medicine, Baylor College of Medicine, Houston, Texas 77030, USA; email: hotez@bcm.edu, bottazzi@bcm.edu

[2]Texas Children's Hospital Center for Vaccine Development, Houston, Texas 77030, USA

[3]Department of Biology, Baylor University, Waco, Texas 76798, USA

[4]Hagler Institute of Advanced Study, Texas A&M University, College Station, Texas 77843, USA

Annu. Rev. Med. 2022. 73:55–64

First published as a Review in Advance on October 12, 2021

The *Annual Review of Medicine* is online at med.annualreviews.org

https://doi.org/10.1146/annurev-med-042420-113212

Keywords

COVID-19, vaccine, virus, protein, immunization

Abstract

The rapid development and deployment of mRNA and adenovirus-vectored vaccines against coronavirus disease 2019 (COVID-19) continue to astound the global scientific community, but these vaccine platforms and production approaches have still not achieved global COVID-19 vaccine equity. Immunizing the billions of people at risk for COVID-19 in the world's low- and middle-income countries (LMICs) still relies on the availability of vaccines produced and scaled through traditional technology approaches. Vaccines based on whole inactivated virus (WIV) and protein-based platforms, as well as protein particle-based vaccines, are the most produced by LMIC vaccine manufacturing strategies. Three major WIV vaccines are beginning to be distributed widely. Several protein-based and protein particle-based vaccines are advancing with promising results. Overall, these vaccines are exhibiting excellent safety profiles and in some instances have shown their potential to induce high levels of virus neutralizing antibodies and T cell responses (and protection) both in nonhuman primates and in early studies in humans. There is an urgent need to continue accelerating these vaccines for LMICs in time to fully vaccinate these populations by the end of 2022 at the latest. Achieving these goals would also serve as an important reminder that we must continue to maintain expertise in producing multiple vaccine technologies, rather than relying on any individual platform.

INTRODUCTION

Coronavirus disease 2019 (COVID-19) vaccines employing new technologies, including mRNA and adenovirus-vectored approaches, have generated intense public and scientific interest. Their relatively quick turnaround, from the time the severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) sequence appeared on a preprint server in January 2020 to production and clinical testing, could revolutionize vaccine development for future pandemic threats. Yet, the penetration of mRNA and adenovirus-vectored COVID-19 vaccines into low- and middle-income country (LMIC) markets is still modest. This is a consequence of many factors, including difficulties in scale-up and scale-out technology transfer and production, costs, uncertain safety profiles, and intense antivaccine aggression on the internet (1, 2). While we anticipate that these important and exciting technologies will ultimately improve and become more adaptable to resource-poor settings in the future, for now we must continue expanding the production and distribution of vaccines that employ conventional technologies.

Two such traditional approaches relevant to COVID-19 vaccines—resulting in vaccines released for emergency use or in advanced clinical (phase III) trials—are whole inactivated virus (WIV) and protein-based vaccines targeting the spike protein or its receptor binding domain (RBD). Protein-based vaccine technology also includes protein-containing nanoparticles, as in virus-like particle (VLP) vaccines.

WIV vaccines are best exemplified by the inactivated polio vaccine (developed by Jonas Salk) and the inactivated rabies vaccine (3). Salk's team during the early 1950s worked carefully to demonstrate the linear kinetics of poliovirus inactivation with formaldehyde (also known as formalin) as a function of time, using defined formalin chemical concentrations and controlled temperature to produce a vaccine that was both safe and effective (2). For rabies vaccine, the inactivating agent is typically β-propionolactone (BPL), an electrophilic compound that binds to amino acid nucleophiles. Unlike formalin, BPL has the advantage of spontaneous hydrolysis to a nontoxic compound and does not require chemical separation from the inactivated vaccine (3).

The most widely used recombinant protein vaccine is the hepatitis B vaccine approved as Recombivax in 1986 (4, 5). In the production of Recombivax, the hepatitis B surface antigen (HBsAg) is purified from disrupted yeast cells before it is precipitated with potassium aluminum sulfate (alum). Initially, the HBsAg was produced in baker's yeast—*Saccharomyces cerevisiae*. Since then, HBsAg has been expressed in other yeast expression systems including *Pichia pastoris* or *Hansenula polymorpha* (6, 7). In addition, a widely used and distributed protein-based particle is the human papillomavirus VLP vaccine to prevent cervical cancer and other cancers. Both WIV and recombinant yeast-derived vaccines are produced locally by LMIC manufacturers, many of which are prequalified by the World Health Organization (WHO) (**https://extranet.who.int/pqweb/vaccines/list-prequalified-vaccines**).

During clinical testing of WIV and protein vaccines for COVID-19, there has been careful attention to safety and immunogenicity. Regarding immunogenicity, both humoral and cellular responses are examined, especially virus neutralizing antibodies. For SARS-CoV-2, neutralizing antibodies block virus attachment to the host angiotensin-converting enzyme-2 (ACE-2) receptor (8), which is usually measured by plaque reduction neutralizing tests, pseudotyped virus neutralization, or microneutralization (9). Although virus neutralizing antibody titers are not yet considered a true correlate for COVID-19 vaccine efficacy, vaccines that induce virus neutralizing antibody titers exceeding convalescent antibody titers (antibodies measured in recovered patients) have also induced the highest levels of vaccine immunity in clinical trials (10).

Another concern related to the immunogenicity and safety of COVID-19 vaccines is immune enhancement (11). For human vaccines, this phenomenon was first observed with a formalin WIV vaccine against respiratory syncytial virus, in which immunized children experienced exacerbated

respiratory illness, possibly due to antibodies that promoted virus uptake into host cells and enhanced virus replication, or to infiltration of host inflammatory cells (neutrophils and eosinophils) in the lungs (11). In preclinical studies conducted prior to the pandemic, vaccines for SARS-CoV (the first major serious human coronavirus infection) caused similar cellular infiltrates in the lungs or liver of laboratory rodent models. These included both WIV and recombinant protein vaccines, as well as virus-vectored vaccines (11). Several different underlying mechanisms were postulated, including Th2-associated antibody-dependent enhancement and Th17 cytokines and inflammation (12). However, regarding the former, it was interesting to note that aluminum adjuvants, which are typically associated with Th2 immune responses, actually diminished immune enhancement (13). To date, nonhuman primate immunizations with experimental COVID-19 vaccines have not shown evidence of immune enhancement upon virus challenge, nor has this phenomenon arisen in human clinical trials or following the widespread use of the vaccines approved under emergency use authorization (11).

Some of the WIV and protein-based vaccines are produced in China or India, whose national regulatory authorities are not listed among the stringent regulatory agencies for vaccines (1). However, in most cases, efforts are under way by these vaccine manufacturers to work with the WHO in order to prequalify or validate their vaccines for emergency authorization and export to other nations.

Here we discuss the major WIV and protein-based vaccines currently authorized for emergency use, as well as those completing advanced phase III development with the anticipation that authorization may be imminent.

WHOLE INACTIVATED VIRUS COVID-19 VACCINES

There are three major WIV COVID-19 vaccines authorized for emergency use. Two WIV vaccines are manufactured in China by Sinovac (a private company) and Sinopharm (a state-owned company), respectively, and one in India by Bharat Biotech (a private company) (**Table 1**).

CoronaVac

CoronaVac (Sinovac Biotech Ltd.) was prepared by isolating a CN2 strain of virus from patient bronchoalveolar lavage fluid. Purified virus was adapted to Vero cells (kidney epithelial cells from the African green monkey), where its genetic stability was confirmed by nucleic acid sequencing while its overall intact morphology was confirmed by cryo-electron microscopy (14). Following inactivation with BPL, mouse immunogenicity studies conducted with the WIV vaccine on alum found that the spike protein RBD is the dominant immunogen, inducing significant titers

Table 1 Major whole inactivated virus COVID-19 vaccines

Vaccine	Notes (including mode of preparation)	Organization (country)	Current status
CoronaVac (formerly PiCoVacc)	Vero cell cultivation, β-propionolactone inactivation, and aluminum hydroxide formulation	Sinovac (China)	Approved in China Emergency use in other countries Emergency use validation from the World Health Organization
BBIBP-CorV	Vero cell cultivation, and aluminum hydroxide formulation	Sinopharm (China)	Approved in China, United Arab Emirates, Bahrain Emergency use in other countries Emergency use validation from the World Health Organization
Covaxin	β-Propionolactone inactivation, Algel-IMDG formulation	Bharat Biotech (India)	Emergency use in India, other countries

of virus neutralizing antibodies (14). Immunization of rhesus macaques with an intermediate dose of 3 μg induced the highest levels of virus neutralizing antibodies, and after intratracheal SARS-CoV-2 challenge, both low- and high (6 μg)-dose vaccines significantly decreased viral loads compared to negative controls (14). There was also no evidence of immune enhancement-related lung pathology.

In phase I and II studies conducted in Hebei Province, China, during May and June of 2020, both one- and two-dose regimens of the WIV vaccine adjuvanted with aluminum hydroxide (Alhydrogel®) were evaluated in a range of three doses: 1.5, 3, and 6 μg. The adverse reactions were mild or moderate, with pain at the site of injection the most commonly reported event (15). High rates of seroconversion were observed, but geometric mean neutralizing antibody titers were low, typically in the 40–50 range (15). In phase III randomized double-blind placebo-controlled case-driven clinical trials (16), ultimately conducted in Brazil, Chile, Indonesia, and Turkey, Sinovac reported levels of protective efficacy exceeding 90% (17). However, widely divergent protection levels were subsequently reported, with more than 80% efficacy in Turkey but only 50% in Brazil, possibly due to differences in clinical trial design (18, 19). Subsequently, Chilean authorities indicated that the vaccine induces 65.9% protection against COVID-19 (19). In these cases, as with many of the WIV vaccines, it is unclear whether the heterogeneity seen in different countries reflects diminished protection against emerging variants, waning immunity, or some combination of these factors.

Sinovac announced that its CoronaVac WIV vaccine was given conditional approval in China and that the company had negotiated vaccine supply arrangements with at least 11 countries. The European Medicines Agency (EMA), a stringent regulatory body, is reviewing company data, and in June 2021, the WHO validated the Sinovac CoronaVac vaccine for emergency use.

BBIBP-CorV

BBIBP-CorV (Sinopharm) is a Chinese WIV vaccine similar to CoronaVac. It was developed by the Beijing Institute of Biological Products in collaboration with Sinopharm, a state-owned pharmaceuticals company. For this vaccine, an HB02 strain was passaged through Vero cells, and its genetic and structural integrity was confirmed (20). Like CoronaVac, it is formulated with aluminum hydroxide and was shown to be safe and immunogenic in laboratory animals and protective against challenge infections in nonhuman primates (20). In randomized double-blind placebo-controlled phase I and II trials, using 2, 4, and 8 μg doses of antigen, the vaccine was shown to elicit only mild or moderate adverse reactions, except for one individual who developed high fever (21). Overall, BBIBP-CorV elicited higher virus neutralizing antibody titers than CoronaVac, but it is difficult to compare these clinical trial results directly due to variability in the methods used to measure virus neutralization (10).

BBIBP-CorV became the first WIV COVID-19 vaccine to receive emergency use authorization by the WHO. In addition, several nations have approved it for emergency release, beginning with the United Arab Emirates and Hungary. Although publication of the phase III clinical trial results is pending, information released by the WHO indicates that two doses protect at a high level, between 70–80% (18), with studies pending to determine its efficacy against variants of concern. In May 2021, the WHO listed BBIBP-CorV for emergency use enabling the vaccine to be rolled out globally.

Covaxin

Covaxin (Bharat Biotech) is a WIV vaccine created in collaboration with the Indian Council of Medical Research and the National Institute of Virology in India. Originally known as BBV152,

it is also prepared by BPL inactivation. It is adjuvanted with Algel-IMDG, which is an imidazoquinoline molecule absorbed on alum. The IMDG component is a Toll-like receptor 7/8 agonist (22) that induces a Th1-type immune response in order to balance the Th2-inducing properties of alum. The vaccine was shown to be highly protective in Syrian hamsters (23) and rhesus macaques (24). It was the first vaccine developed in India to enter clinical testing. In a double-blind multicenter (11 hospitals in India) randomized controlled phase I trial, Covaxin was associated with mild and moderate adverse events, but with high rates of seroconversion and persistent virus neutralizing antibodies (25, 26). These antibodies also neutralize some variants of concern including B.1.1.7 and B.1.1.28 P2 (27, 28). Covaxin was released for emergency use in India at the beginning of 2021, although publication of the phase III trial data is pending. However, a preliminary analysis suggests that the vaccine exhibits high levels of protective immunity, especially against severe infection (18). The results of clinical trials in children are also pending.

Other WIV Vaccines

Other WIV vaccines are in development in China, France, Iran, Kazakhstan, Russia, and Turkey. Of these, the furthest along is from the French company Valneva, which has a vaccine known as VLA2001 with a CpG oligonucleotide from Dynavax. The vaccine is entering phase III trials in the United Kingdom comparing immunogenicity with the Oxford AstraZeneca vaccine.

RECOMBINANT PROTEIN COVID-19 VACCINES

Several different approaches are under way for protein-based vaccines, including those produced through microbial fermentation or mammalian cells. At least three of these vaccines are either completing phase III clinical trials or have been released through an emergency use authorization (**Table 2**).

Corbevax

The Texas Children's Hospital Center for Vaccine Development at Baylor College of Medicine has maintained a recombinant protein coronavirus vaccine program for SARS-CoV and MERS-CoV (Middle East respiratory syndrome coronavirus) since 2011. This approach is based on the protein expression of the RBD of the spike protein of SARS-CoV, which was shown to induce

Table 2 Major recombinant protein COVID-19 vaccines

Vaccine	Notes (including mode of preparation)	Organization (country)	Current status
Corbevax	Receptor binding domain expressed in yeast (*Pichia pastoris*) formulated with aluminum hydroxide and CpG (Dynavax)	Biological E (India)	Phase III with emergency use authorization in India pending
Soberana 2	Receptor binding domain expressed in CHO cells, conjugated to tetanus toxoid, and formulated on aluminum hydroxide	Finlay Vaccine Institute (Cuba)	Emergency use in Cuba and Iran
ZF2001	Receptor binding domain expressed in CHO cells and formulated on aluminum hydroxide	Anhui Zhifei Longcom and Institute of Medical Biology of the Chinese Academy of Medical Sciences (China)	Emergency use in China and Uzbekistan
MVCCOV1901	S-2P stabilized prefusion spike protein ectodomain in CHO cells, formulated on aluminum hydroxide and CpG (Dynavax)	Medigen Vaccine Biologics Corporation (Taiwan)	Emergency use in Taiwan

virus neutralizing antibodies at high levels while minimizing the immune enhancement seen in WIV or full spike protein–based vaccines. At the beginning of 2020, the program pivoted to developing a recombinant protein COVID-19 vaccine candidate produced in yeast (*P. pastoris*). An initial construct combined deglycosylation by deleting the first N-terminal asparagine with a cysteine mutagenesis to prevent intermolecular disulfide bond formation. Therefore, through genetic modification, a more stable and better-controlled vaccine candidate was developed (29). The yeast-expressed protein was shown to induce high levels of binding and virus neutralizing antibodies in mice (30) and was protective in a rhesus macaque challenge model. A process for scale-up production was developed (31), and the production cell bank was transferred to Biological E, where the recombinant protein candidate is advancing with a formulation including adsorption to aluminum hydroxide, together with a CpG oligonucleotide from the Dynavax Corporation. The vaccine, Corbevax (Biological E and Baylor College of Medicine), is completing phase III clinical trials following a highly favorable safety and immunogenicity profile in a combined phase I/II clinical trial in India.

Soberana 2

Soberana 2 (Finlay Vaccine Institute) is also an RBD candidate but one expressed in mammalian (CHO) cells and conjugated through a free cysteine with a tetanus toxoid carrier protein, followed by formulation on aluminum hydroxide (32, 33). The vaccine is immunogenic in mice and induces virus neutralizing antibodies. The vaccine has been authorized for emergency use in Cuba, where it is also approved for children over the age of 2. In addition, the vaccine has received emergency use authorization in Iran (where it is known as PasteurCovac, in collaboration with the Institut Pasteur, Iran).

MVCCOV1901

The MVCCOV1901 vaccine (Medigen Vaccine Biologics Corporation in Taiwan) is the stabilized prefusion spike protein ectodomain known as S-2P, produced in CHO cells. It is adjuvanted with aluminum hydroxide and CpG1018 from Dynavax (34). The vaccine has received emergency use authorization from the Taiwan FDA.

ZF2001

ZF2001 (Anhui Zhifei Longcom and Institute of Medical Biology of the Chinese Academy of Medical Sciences) is also an RBD candidate expressed in CHO cells and formulated on aluminum hydroxide, now undergoing phase III clinical testing in China (NCT04646590). This vaccine has been released for emergency use in China.

Sanofi and GlaxoSmithKline plc

Major multinational companies Sanofi and GlaxoSmithKline plc (GSK) partnered to develop a vaccine based on recombinant spike protein expressed in insect cells, formulated with the squalene oil-based adjuvant AS03. The vaccine induced low immunogenicity in initial trials. The two companies have since announced that an improved formulation produced strong immune responses and have initiated phase III clinical trials with a goal to have a vaccine authorized for emergency use by the end of 2021.

VIRUS-LIKE PARTICLE AND OTHER PROTEIN PARTICLE VACCINES

A VLP is typically a recombinant protein-based vaccine in which the proteins self-assemble in a three-dimensional particle, or they embed in an outer lipid envelope. At least three VLP vaccines are either completing phase III clinical trials or have been released for emergency use (**Table 3**).

CoVLP

Medicago is a Canada-based vaccine producer that has developed processes for protein expression in tobacco plants (*Nicotiana benthamania*) in which the protein spontaneously forms a trimer and assembles into a VLP (35). In collaboration with GSK, the company produced a plant-derived protein particle vaccine formulated with AS03 (35, 36). In two doses, the CoVLP vaccine (Medicago and GSK) was found to be immunogenic and exhibit a strong safety profile. Health Canada, the Canadian national regulatory authority, is conducting rolling review of the Medicago trials, with the possibility that CoVLP will be released under emergency use in Canada.

SCB-2019

Clover Pharmaceuticals in Chengdu, Sichuan Province, China, has developed a trimer-tag system of protein expression based on a self-assembling C-terminus of human type I procollagen (37, 38). Expression of recombinant spike protein fused to this tag in mammalian (CHO) cells results in a trimer-like particle that is subjected to an affinity column purification method (37, 38). The trimer also binds to the ACE-2 receptor and induces neutralizing antibodies in both rodents and nonhuman primates, as well as protective immunity in the latter to challenge infections (38). Formulated either with AS03 or CpG on alum, the SCB-2019 (Clover Pharmaceuticals) vaccine is well tolerated and induces significant levels of virus neutralizing antibodies and other human immune responses (37). For phase III studies of SCB-2019, Clover announced it will partner with Dynavax to focus on the CpG adjuvant.

NVX-CoV2373

The NVX-CoV2373 (Novavax) vaccine was created by cloning the spike protein gene into a baculovirus and expressing it in *Spodoptera frugiperda* Sf9 cells. The recombinant protein is then formulated with a Matrix-M adjuvant (39). Matrix-M particles contain saponin, combined with cholesterol and phospholipids (39). The vaccine induces high levels of virus neutralizing antibodies and protective immunity in nonhuman primates (40) and was shown in phase I and II clinical trials to also induce among the highest levels of virus neutralizing antibodies seen to date for any vaccine (41, 42), resulting in >90% protective immunity in the United Kingdom. Against the B.1.351 variant from South Africa, the vaccine induces cross-protective virus neutralizing

Table 3 Major recombinant protein particle COVID-19 vaccines

Vaccine	Notes (including mode of preparation)	Organization (country)	Current status
CoVLP	Spike protein trimer virus-like protein expressed in tobacco plants (*Nicotiana benthamania*) formulated with AS03 (GlaxoSmithKline)	Medicago (Canada)	Phase III with emergency use authorization in Canada pending
SCB-2019	Trimer tag expression in CHO cells formulated on alum with CpG (Dynavax)	Clover Pharmaceuticals (China)	Phase III
NVX-CoV2373	Particle vaccine created through insect cell expression and formulation with Matrix-M	Novavax (United States)	Phase III

antibodies and lower but still substantial protection, around 50% (42). The NVX-CoV2373 vaccine also has an excellent safety profile, with rare serious adverse events (41, 42). Novavax has entered into an agreement with the Serum Institute of India for scale-up production, but the company is also entering into agreements with additional manufacturers. Recently, the company announced production and other delays, which will also delay emergency use authorization requests (43).

FUTURE DIRECTIONS

The WIV and protein-based vaccines offer enormous promise for filling the COVID-19 vaccine access gaps, especially for the world's LMICs in Africa, Latin America, and Southeast Asia. An attractive feature of these vaccines is their capacity for production in LMICs, especially the Developing Country Vaccine Manufacturer Network producers in Brazil, Cuba, Mexico, South Africa, China, India, Indonesia, and elsewhere (1). Vaccine producers will need to be responsible for manufacturing up to 6 billion doses of vaccine or more, considered a global priority for bringing the Southern Hemisphere up to the high vaccination rates already achieved in North America, Europe, and Israel (1). In many cases, the costs to produce and scale these vaccines could be far lower than those of the newer mRNA or other vectored technology vaccines.

Capacity for developing, manufacturing, and delivering WIV and protein vaccine technologies must be maintained even as mRNA, vesicular stomatitis virus, and adenovirus-vectored vaccine technologies accelerate. Each pandemic threat or neglected disease pathogen has its own unique vulnerability to acquired immunity, and it is not yet possible to predict which technology might achieve the highest levels of protective immunity or can be produced easily at scale. Therefore, maintaining expertise in WIV and protein-based technologies remains important for pandemic preparedness.

DISCLOSURE STATEMENT

The authors are inventors on a COVID-19 vaccine technology owned by Baylor College of Medicine that was licensed nonexclusively to the LMIC vaccine producers Biological E in India (where it is known as Corbevax) and BioFarma in Indonesia. The technology is owned and licensed by Baylor College of Medicine and it would not be considered a Significant Financial Interest per PHS regulations.

LITERATURE CITED

1. Hotez PJ, Narayan KMV. 2021. Restoring vaccine diplomacy. *JAMA* 325(23):2337–38
2. Hotez P, Batista C, Ergonul O, et al. 2021. Correcting COVID-19 vaccine misinformation. Lancet Commission on COVID-19 Vaccines and Therapeutics Task Force members. *EClinicalMedicine* 33:100780
3. Sanders B, Koldijk M, Schuitemaker H. 2014. Inactivated viral vaccines. In *Vaccine Analysis: Strategies, Principles, and Control*, ed. BK Nunnally, VE Turula, RD Sitrin, pp. 45–80. Berlin/Heidelberg: Springer
4. Adkins JC, Wagstaff AJ. 1998. Recombinant hepatitis B vaccine: a review of its immunogenicity and protective efficacy against hepatitis B. *BioDrugs* 10(2):137–58
5. Bucci M. 2020. First recombinant DNA vaccine for HBV. *Nat. Portf.* **https://www.nature.com/articles/d42859-020-00016-5**
6. Liu R, Lin Q, Sun Y, et al. 2009. Expression, purification, and characterization of hepatitis B virus surface antigens (HBsAg) in yeast *Pichia pastoris*. *Appl. Biochem. Biotechnol.* 158(2):432–44
7. Kumar R, Kumar P. 2019. Yeast-based vaccines: new perspective in vaccine development and application. *FEMS Yeast Res*. 19(2):foz007
8. Jiang S, Zhang X, Yang Y, et al. 2020. Neutralizing antibodies for the treatment of COVID-19. *Nat. Biomed. Eng*. 4(12):1134–39

9. Bewley KR, Coombes NS, Gagnon L, et al. 2021. Quantification of SARS-CoV-2 neutralizing antibody by wild-type plaque reduction neutralization, microneutralization and pseudotyped virus neutralization assays. *Nat. Protoc.* 16(6):3114–40
10. Khoury DS, Cromer D, Reynaldi A, et al. 2021. Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. *Nat. Med.* 27:1205–11
11. Haynes BF, Corey L, Fernandes P, et al. 2020. Prospects for a safe COVID-19 vaccine. *Sci. Transl. Med.* 12(568):eabe0948
12. Hotez PJ, Corry DB, Bottazzi ME. 2020. COVID-19 vaccine design: the Janus face of immune enhancement. *Nat. Rev. Immunol.* 20(6):347–48
13. Hotez PJ, Corry DB, Strych U, et al. 2020. COVID-19 vaccines: neutralizing antibodies and the alum advantage. *Nat. Rev. Immunol.* 20(7):399–400
14. Gao Q, Bao L, Mao H, et al. 2020. Development of an inactivated vaccine candidate for SARS-CoV-2. *Science* 369(6499):77–81
15. Wu Z, Hu Y, Xu M, et al. 2021. Safety, tolerability, and immunogenicity of an inactivated SARS-CoV-2 vaccine (CoronaVac) in healthy adults aged 60 years and older: a randomised, double-blind, placebo-controlled, phase 1/2 clinical trial. *Lancet Infect. Dis.* 21(6):803–12
16. Akova M, Unal S. 2021. A randomized, double-blind, placebo-controlled phase III clinical trial to evaluate the efficacy and safety of SARS-CoV-2 vaccine (inactivated, Vero cell): a structured summary of a study protocol for a randomised controlled trial. *Trials* 22(1):276
17. Sinovac Biotech Ltd. 2021. *Sinovac announces phase III results of its COVID-19 vaccine*. News release, Feb. 5, Sinovac Biotech Ltd., Beijing, China. **https://www.businesswire.com/news/home/20210205005496/en/Sinovac-Announces-Phase-III-Results-of-Its-COVID-19-Vaccine**
18. Zimmer C, Corum J, Wee S-L. 2020. Coronavirus vaccine tracker. *N. Y. Times*. **https://www.nytimes.com/interactive/2020/science/coronavirus-vaccine-tracker.html**. Accessed Sep. 20, 2021
19. Jara A, Undurraga EA, González C, et al. 2021. Effectiveness of an inactivated SARS-CoV-2 vaccine in Chile. *N. Engl. J. Med.* 385(10):875–84
20. Wang H, Zhang Y, Huang B, et al. 2020. Development of an inactivated vaccine candidate, BBIBP-CorV, with potent protection against SARS-CoV-2. *Cell* 182(3):713–21.e9
21. Xia S, Zhang Y, Wang Y, et al. 2021. Safety and immunogenicity of an inactivated SARS-CoV-2 vaccine, BBIBP-CorV: a randomised, double-blind, placebo-controlled, phase 1/2 trial. *Lancet Infect. Dis.* 21(1): 39–51
22. Colak E, Leslie A, Zausmer K, et al. 2014. RNA and imidazoquinolines are sensed by distinct TLR7/8 ectodomain sites resulting in functionally disparate signaling events. *J. Immunol.* 192(12):5963–73
23. Mohandas S, Yadav PD, Shete-Aich A, et al. 2021. Immunogenicity and protective efficacy of BBV152, whole virion inactivated SARS- CoV-2 vaccine candidates in the Syrian hamster model. *iScience* 24(2):102054
24. Yadav PD, Ella R, Kumar S, et al. 2021. Immunogenicity and protective efficacy of inactivated SARS-CoV-2 vaccine candidate, BBV152 in rhesus macaques. *Nat. Commun.* 12(1):1386
25. Ella R, Vadrevu KM, Jogdand H, et al. 2021. Safety and immunogenicity of an inactivated SARS-CoV-2 vaccine, BBV152: a double-blind, randomised, phase 1 trial. *Lancet Infect. Dis.* 21(5):637–46
26. Ella R, Reddy S, Jogdand H, et al. 2021. Safety and immunogenicity of an inactivated SARS-CoV-2 vaccine, BBV152: interim results from a double-blind, randomised, multicentre, phase 2 trial, and 3-month follow-up of a double-blind, randomised phase 1 trial. *Lancet Infect. Dis.* 21(7):950–61
27. Sapkal GN, Yadav PD, Ella R, et al. 2021. Inactivated COVID-19 vaccine BBV152/COVAXIN effectively neutralizes recently emerged B.1.1.7 variant of SARS-CoV-2. *J. Travel Med.* 28(4):taab051
28. Sapkal G, Yadav PD, Ella R, et al. 2021. Neutralization of B.1.1.28 P2 variant with sera of natural SARS-CoV-2 infection and recipients of inactivated COVID-19 vaccine Covaxin. *J. Travel Med.* **https://doi.org/10.1093/jtm/taab077**
29. Chen W-H, Wei J, Kundu RT, et al. 2021. Genetic modification to design a stable yeast-expressed recombinant SARS-CoV-2 receptor binding domain as a COVID-19 vaccine candidate. *Biochim. Biophys. Acta Gen. Subj.* 1865(6):129893

30. Pollet J, Chen W-H, Versteeg L, et al. 2021. SARS-CoV-2 RBD219-N1C1: a yeast-expressed SARS-CoV-2 recombinant receptor-binding domain candidate vaccine stimulates virus neutralizing antibodies and T-cell immunity in mice. *Hum. Vaccin. Immunother.* 17(8):2356–66
31. Lee J, Liu Z, Chen W-H, et al. 2021. Process development and scale-up optimization of the SARS-CoV-2 receptor binding domain-based vaccine candidate, RBD219-N1C1. *Appl. Microbiol. Biotechnol.* 105(10):4153–65
32. Valdes-Balbin Y, Santana-Mederos D, Quintero L, et al. 2021. SARS-CoV-2 RBD-tetanus toxoid conjugate vaccine induces a strong neutralizing immunity in preclinical studies. *Chem. Biol.* 16(7):1223–33
33. Gorry C. 2020. SOBERANA, Cuba's COVID-19 vaccine candidates: Dagmar García-Rivera PhD. *MEDICC Rev.* 22(4):10–15
34. Hsieh SM, Liu WD, Huang YS, et al. 2021. Safety and immunogenicity of a recombinant stabilized prefusion SARS-CoV-2 spike protein vaccine (MVC-COV1901) adjuvanted with CpG 1018 and aluminum hydroxide in healthy adults: a phase 1, dose-escalation study. *EClinicalMedicine* 38:100989
35. Ward BJ, Gobeil P, Séguin A, et al. 2021. Phase 1 randomized trial of a plant-derived virus-like particle vaccine for COVID-19. *Nat. Med.* 27:1071–78
36. Gobeil P, Pillet S, Séguin A, et al. 2021. Interim report of a phase 2 randomized trial of a plant-produced virus-like particle vaccine for Covid-19 in healthy adults aged 18–64 and older adults aged 65 and older. *medRxiv* 21257248. **https://doi.org/10.1101/2021.05.14.21257248**
37. Liang JG, Su D, Song T-Z, et al. 2021. S-Trimer, a COVID-19 subunit vaccine candidate, induces protective immunity in nonhuman primates. *Nat. Commun.* 12(1):1346
38. Richmond P, Hatchuel L, Dong M, et al. 2021. Safety and immunogenicity of S-trimer (SCB-2019), a protein subunit vaccine candidate for COVID-19 in healthy adults: a phase 1, randomised, double-blind, placebo-controlled trial. *Lancet* 397(10275):682–94
39. Bengtsson KL, Song H, Stertman L, et al. 2016. Matrix-M adjuvant enhances antibody, cellular and protective immune responses of a Zaire Ebola/Makona virus glycoprotein (GP) nanoparticle vaccine in mice. *Vaccine* 34(16):1927–35
40. Guebre-Xabier M, Patel N, Tian J-H, et al. 2020. NVX-CoV2373 vaccine protects cynomolgus macaque upper and lower airways against SARS-CoV-2 challenge. *Vaccine* 38(50):7892–96
41. Keech C, Albert G, Cho I, et al. 2020. Phase 1–2 trial of a SARS-CoV-2 recombinant spike protein nanoparticle vaccine. *N. Engl. J. Med.* 383(24):2320–32
42. Shinde V, Bhikha S, Hoosain Z, et al. 2021. Efficacy of NVX-CoV2373 Covid-19 vaccine against the B.1.351 variant. *N. Engl. J. Med.* 384(20):1899–909
43. Parsons L. 2021. Novavax announces further delays for regulatory filings of COVID-19 vaccine. *PMLiVE*, May 12. **https://www.pmlive.com/pharma_news/novavax_announces_further_delays_for_regulatory_filings_of_covid-19_vaccine_1369844**