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REVIEW ARTICLE

Theme: Delivery of Proteins and Peptides - What is on the Forefront based on our Experience from the COVID-19 Pandemic?

A Comprehensive Investigation Regarding the Differentiation of the Procurable COVID-19 Vaccines

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Abstract

COVID-19 caused by coronavirus SARS-CoV-2 became a serious threat to humankind for the past couple of years. The development of vaccine and its immediate application might be the only to escape from the grasp of this demoniac pandemic. Approximately 343 clinical trials on COVID-19 vaccines are ongoing currently, and almost all countries are motivating ongoing researches at warp speed for the development of vaccines against COVID-19. This review explores the progress in the development of the vaccines, their current status of ongoing clinical research, mechanisms, and regulatory approvals. Many pharmaceutical companies are already in the endgame for manufacturing various vaccines of which some are already being marketed across the globe, while others are yet to get approval for marketing. The primary aim of this review is to compare regulatory accepted vaccines in terms of their composition, doses, regulatory status, and efficacy. The study is conducted by grouping into approved and unapproved vaccines for marketing. Different routes of administration of vaccines along with the efficacy of the routes are also presented in the review. A wide range of database and clinical trial data is reviewed for sorting out the information on different vaccines. Unfortunately, many mutations (alpha, beta, gamma, delta, kappa, omicron etc.) of SARS-CoV-2 have attacked people in very short time, which is the great challenge for investigational vaccines. Moreover, some vaccines like Pfizer's BNT162, Oxford's ChAdOx1, Moderna's mRNA-1273, and Bharat Biotech's Covaxin have got regulatory approval in some countries for its distribution which may prove to stand tall against the pandemic.

KEY WORDS covid-19 · sars-cov-2 · prevention · vaccines · clinical trials

INTRODUCTION

Presently, novel coronavirus disease 2019 or "COVID-19" has a devastating spread in almost all countries with about 30 million positive cases reports and about 9.5 million active patients undergoing treatment so far at the mid of September 2020 (1). COVID-19 has become demoniac day by day and with a present death rate of 4%. This intensified global spread and death rate have caused significant impacts, both short and long term, on society, economy, and politics. The term "COVID-19" is coined by the World Health Organization (WHO) on 11th February 2020 (2). On this account, the WHO reported that COVID-19 is the most recent, which is very infectious disease occurred by currently found coronavirus. There are several characteristic differences between severe acute respiratory syndrome (SARS) and the new CoV. Here in this case, the death rate is lower than that of the reported cases of SARS, but the rate of infected people is greater in the case of COVID-19. This new CoV is different from SARS and is named as "SARS-CoV-2" by the WHO and has the size range of 2.24 μm to 3.58 μm (2, 3). The beginning of it was first reported in Wuhan, Hubei Province, China, in December 2019. The USA first pointed out inceptive symptoms, clinical features, characteristics, detection diagnosis, etc. of COVID-19 from the first hospitalized COVID-19 positive patient (4, 5). In a case of pandemic, the

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term "waves" is used to refer to the number of rising cases or surge of outbreak by WHO. Scientists first used the term for denoting the peaks and valleys of case graph during Spanish flu outbreak in 1918. The first wave denotes the starting of rising of number of cases until the peak is reached. After attaining the peak, the rate of transmission declines gradually as many people get already infected and less people get affected over a period of time (6). These waves of a pandemic are generally calculated by effective reproduction number *R*. This value signifies the number of individuals getting infected in real time during an epidemic outbreak. If the value of *R* is more than 1 over a long period of time, that period of time is denoted as the upward slope of the wave, and if the *R* value is less than 1, the period is denoted as the downward slope of the wave. After the first wave comes the second wave where there is the second surge of rising cases. This wave is deadlier than the initial one as more number of cases and mortality is observed in this period (7). An ICMR study in India from 41 government hospitals concluded that there is 30% hike in mortality rate during the second wave compared to the first. During this period of pandemic, vaccines and treatments start to develop as scientists get more information about the virus, its transmission mechanism, and genetics.

Unfortunately, till date, no vaccines are available for complete prevention of this disease. Several clinical trials are ongoing with the aim to establish the safety and efficacy against this disease. These potential vaccines have different mechanisms of action, i.e., some are targeting the mRNA sequence of the virus or spike (S) glycoprotein of the virus, while others are targeting S2 subunit of it and some are also aiming to reduce the cytokine storm in the human body. While developing these vaccines, it is observed that in the case of mechanism of action, both structural and biological aspects of targeting are playing significant roles. Researchers and pharmaceutical industries of all over the world are involved in preclinical or clinical testing of potential new molecules for the prevention and of COVID-19.

Recently, the WHO published a landscape detail of vaccines in which 23 vaccines are in clinical trial and 140 candidates are in preclinical trial (8). Different departments of the US federal government have established a partnership, namely, Operation Wrap Speed (OWS), in which the US National Institutes of Health (NIH) has collaborated with 18 pharmaceutical industries (9). Under the criteria of OWS, the US federal government is funding for phase 3 trial of 3 investigational vaccines which include mRNA-1273 (Moderna, Inc.) and ChAdOx1 (Oxford University). These are already approved by the USFDA (fast track approval) and the UK regulators and ethical reviewers along with regulatory authority of Brazil (Anvisa), respectively. In India, Zydus Cadila has obtained the approval for its vaccine candidate, namely, "ZyCoV-D" from Drugs Controller General of India (DCGI) to initiate phase 1/2 clinical trials [CTRI/2020/07/026352] (10, 11). After getting positive result in preclinical study on mice, rats, guinea pigs, and rabbits, ZyCoV-D is moving towards phase 1/2 study. This will be a dose-escalation and multicentered study, in which 1000 participants will be taken and is planned to conduct at different locations of India (12–15). This review mainly throws light on the current status of clinical trials of the vaccine candidates against COVID-19 along with their different aspects of targeting, i.e., biological (mRNA-1273 and DAS-181) or structural (Gam-COVID-Vac and Ad5-nCoV) or both (ChAdOx1 and INO-4800), current status of the approval of clinical trial from different regulatory authorities, and the current status of the Russian vaccine Gam-COVID-Vac/Gam-COVID-Vac Lyo, of which the developer has proclaimed that its trial is completed but has not provided any status for phase 3 trial (16). However, there are different SARS-CoV-2 variants like alpha, beta, gamma, delta, and kappa (17). These variants are having different mutations in the S-glycoprotein. These mutations of different variants now become a challenge for approved as well as unapproved vaccines. Among these variants, delta (B.1.617.2) and kappa (B.1.617.1) originated from B.1.617. In today's world, delta becomes the gruesome variant.

APPROVED VACCINES

There are different vaccines, which are now approved for administration in people. All these vaccines are given emergency use authorization from the USFDA, WHO, EMA, and other regulatory bodies of different countries.

mRNA-1273

The National Institute of Allergy and Infectious Diseases (NIAID) and Moderna, in collaboration, are developing mRNA-1273. This mRNA-based vaccine comprises lipid nanoparticle (LNP) encapsulated mRNA which encodes a prefusion stabilized conformation of a full-length spike (S) glycoprotein of SARS-CoV-2 [Clinical Trials Identifier number NCT04470427] (18–20). This LNP is ionizable due to having amine groups in the outer surface of lipids which is protonated. Thereby, endosome membrane is disorganized followed by the stimulation of hexagonal phase structure, for which cellular uptake is initiated and mRNA comes into cytoplasm (21).

An mRNA vaccine acts like a synthetic copy of mRNA-encoding protein, in this case spike protein as an antigen, from the virus. It mainly aims to attack dendritic cells

(DCs) and antigen-presenting cells (APCs). Host body uses mRNA to produce spike protein in it leading to activation of immune response in the body to fight the virus before actual infection takes place (21). The caveat is that the mRNA may code for enough irrelevant proteins that would negatively impact immunogenicity. For adequate protection, it is required to achieve sufficient neutralizing antibody titer levels (22).

In preclinical study conducted in a mouse challenge model, mRNA-1273 gave protection to viral replication in the lungs. The NIAID conducted the phase 1 clinical trial of this vaccine in Kaiser Permanente Washington Health Research Institute (KPWHRI) in Seattle, which is a part of NIAID's Infectious Diseases Clinical Research Consortium. This vaccine was found safe and well tolerated in phase 1 clinical trials [Clinical Trials Identifier number NCT04283461](21, 23, 24). Phase 2 clinical trial [Clinical Trials Identifier number NCT04405076] begun in June 2020 (18, 21). The vaccine elicited sufficient neutralizing antibody titer levels in humans when administered at 25 μg and 100 μg doses. The immunogenicity of the vaccine increased with the increase in dose at three levels, viz., 25 μg, 100 μg, and 250 μg. Phase 2 clinical trials are planned with 50 μg and 100 μg. Moderna received funding from the Biomedical Advanced Research and Development Authority (BARDA), US Department of Health and Human Services (HHS) for the conduct of phase 2 and phase 3 studies and scale-up studies for mRNA-1273 manufacturing (18, 25). Phase 3 study [Clinical Trials Identifier number NCT04470427] was a randomized, stratified, quadruple-blind, placebo-controlled study, which was conducted with 30,000 adult or older participants at 87 locations in USA (26).

Three lipids, viz., distearoylphosphatidylcholine, cholesterol, and polyethylene glycol (PEG2000 DMG), and one proprietary ionizable lipid (SM-102) have been used in encapsulating mRNA. mRNA-1273 was tested in clinical trials as an intramuscular (IM) injectable comprising nanoparticles (26). Doses used in three phases are given here:

- Intramuscular doses of 25 μg, 100 μg, and 250 μg (phase 1 study)
- Intramuscular doses of 50 μg, 100 μg (phase 2 study)
- Intramuscular dose of 100 μg (phase 3 study)

However, it is approved as an investigational vaccine on 11th May 2020 (USFDA fast track approval up to phase 3) (26). Phase 1 clinical trial preliminary data was published on 18th May 2020 phase 2 clinical trial was approved on 7th May 2020 (25, 26). However, it is also approved for emergency use by WHO on 30th April 2021 (27). It has also got emergency use authorization (conditional marketing authorization) by EMA (28).

BNT162

BNT162 is a mRNA-based vaccine candidate developed by Pfizer and BioNTech, and currently, its clinical trial is succeeded, of which the main objective is to examine a single nucleoside-modified messenger RNA (modRNA) candidate from two candidates of BNT162, i.e., BNT162b1 and BNT162b2, along with safety and efficacy against SARS-CoV-2 (29). After an extensive examination, BNT162b2 is selected for the phase 2/3 trial, which already has obtained USFDA fast track approval. BNT162b2 is shown effective delivery of mRNA which is then uptaken by cells and causes synthesis of vaccine antigen rather than that of BNT162b1 (30). Then mRNA induces excellent responses for CD4 + and CD8 + T cell against the receptor binding domain along with the S glycoprotein.

In the preclinical study (approximately for 4 months), both BNT162b1 and BNT162b2 show excellent responses for CD4 + and CD8 + T cell along with suitable antibody neutralizing activity in different animal subjects (29). After proving safe and effective in preclinical study, phase 2/3 study of BNT162 is completed. It was a phase 1/2/3 [Clinical Trials Identifier number NCT04368728], randomized, placebo-controlled, observer-blind, dose-finding study (started form 29th April 2020), conducted by Pfizer and BioNTech SE, which was mainly divided in to two parts, i.e., phase 1 for the detection of suitable candidate for vaccine from BNT162b1 and BNT162b2 along with its dose, which (phase 1/2) was conducted and successfully completed in Germany and phase 2/3 (expanded cohort study) for checking the therapeutic efficacy (31). Developers performed this trial on 29,481 adult and older adult healthy volunteers in 71 different places of the USA, 1 place of Argentina, and 1 place of Brazil. This trial can be summarized as among 43,548 candidates, 21,720 were treated with BNT162b2, and the rest were treated with placebo, in which the confidence interval (CI) for the efficacy of vaccine was obtained 95% along with no chance of adverse events (29).

As per preliminary report, BNT162b1 was administered at a dose of 1 μg, 10 μg, 30 μg, or 50 μg in 4 groups ($n = 12$) on the 1st day and 22nd day and a single dose of 60 μg in 12 other participants through IM route (phase 1/2). Thirty μg of BNT162b2 was administered to participants through IM route in a two-dose regimen (phase 2/3). This vaccine was found less effective against delta variant in comparison to alpha variant (32).

However, phase 1/2 trial was approved by the USFDA (fast track approval) and Paul-Ehrlich-Institut (German regulatory agency) (29, 33). Phase 2/3 trial approved by the USFDA (fast track approval) (33). The UK regulatory agency issued emergency use authorization for this vaccine on 2 December 2020 and the vaccination is started in the UK (34). The USFDA also issued emergency use authorization

for this vaccine on 11 December 2020, for which distribution of this vaccine is already started on the candidates aged 16 years and onwards in the USA (33). Recently, on 23rd August 2021, the USFDA approved this vaccine, for which this has become the world's first USFDA-approved vaccine now (35). It has also got emergency use authorization (conditional marketing authorization) by EMA (36).

Covaxin

Bharat Biotech and ICMR-National Institute of Virology, Pune (India), in collaboration are developing Covaxin (BBV152), which comprises inactivated or killed parts of Indian strain of SARS-CoV-2 and alum as adjuvant. Specifically, it contains aluminum hydroxide gel, imidazoquinolinone, and 2-phenoxyethanol. Drug Controller General of India (DCGI) approval has been obtained for starting phase 1/2 clinical trial, and this trial has been registered into the Clinical Trials Registry of India (Bhuyan, 2021). Since it is an inactivated vaccine, despite of getting replicated, it will stimulate B cells, which take it to T cells. T cells release cytokines by which B cells are activated for producing antibodies against SARS-CoV-2 (37).

After showing excellent efficacy in safety and immunogenicity in mice and hamsters (3 months study), developers decided to begin phase 1/2 randomized, active-controlled, double-blind, and multicenter trial [CTRI/2020/07/026300] in July 2020 for assessing safety, immunogenicity, tolerability, and reactogenicity (38, 39). This trial was planned to conduct this study for 1 year, 3 months with 1125 healthy adult and older adult volunteers in 12 different sites of India (40). IM dose of 0.5 ml BID (on the first day and 14th day) was administered in phase 1/2 (39). Phase 1/2 trial was approved and got emergency use authorization by DCGI (38). Recently, it is also observed in ICMR study that Covaxin is more effective against delta plus variant, while ChAdOx1 is found more effective against delta variant (41).

Gam-COVID-Vac/Gam-COVID-Vac Lyo

Gam-COVID-Vac and Gam-COVID-Vac Lyo are the DNA of SARS-CoV-2-type adenovirus-based vaccines, of which both vaccines mainly contain rAd5 and rAd26 expressing SARS-CoV-2 S glycoprotein, but in case of the later one, it is present in lyophilized form and is developed by the Gamaleya Research Institute of Epidemiology and Microbiology, Health Ministry of the Russian Federation (42). The exact mechanism of actions of these vaccines is currently not known, but according to the report of the Indian Express, these vaccines will utilize the weakened virus to deliver the small material of SARS-CoV-2, i.e., rAd5 and rAd26, which express S glycoprotein of SARS-CoV-2, and finally, adaptive immunity will be stimulated for producing antibodies against SARS-CoV-2 in response of originated S glycoprotein (16).

Both vaccines proved themselves as non-toxic, safe, and effective in preclinical studies, and these have shown effective immunogenic activities in small and large animals at the 48th Central Research Institute of Russian Defense Ministry (16). According to the report of US Clinical Trial Registry and the draft landscape of clinical trial status for the vaccine provided by the WHO, phase 1/2 trials of Gam-COVID-Vac [Clinical Trial Identifier number NCT04436471] and Gam-COVID-Vac Lyo [Clinical Trial Identifier number NCT04437875] were started from 17th June 2020 (43). Both trials were non-randomized, parallelly assigned, open-label study, where 76 healthy adult volunteers in each trial are taken. The trials for Gam-COVID-Vac [Clinical Trial Identifier number NCT04436471] and Gam-COVID-Vac Lyo [Clinical Trial Identifier number NCT04437875] were planned to conduct in Main Military Clinical Hospital named after academician N. N. Burdenko andSechenov First Moscow State Medical University at Moscow in Russia, respectively. Despite the status in US Clinical Trial Registry and not providing the report of phase 3 trial, Gamaleya Research Institute of Epidemiology and Microbiology proclaimed that the clinical trials of their vaccines were successfully completed and the volunteers were discharged on 15th and 20th July 2020 (44). For this reason, question came about the safety and efficacy of these vaccines. For both cases, IM dose of vaccines was administered to volunteers along with a follow-up period (180 days) (phase 1/2 for both cases) (16). Phase 1/2 trial (of both vaccines) and phase 3 (as per media report) were approved by the Health Ministry of Russia (Sputnik V) in the month of June.

Ad5-nCoVs

Ad5-nCoV is the first vaccine for which clinical trial started in China for examining for the prophylaxis against SARS-CoV-2. It is a genetically engineered vaccine comprising adenovirus type 5 vector (Ad5), which is a replication defective to express spike protein of SARS-CoV-2. It is being developed by CanSino Biologics (Tianjin, China) with the collaboration of the Institute of Biotechnology of Academy of Military Medical Sciences (PLA of China), Tongji/Zhongnan Hospital, and Province Centers for Disease Control and Prevention at Jiangsu and Hubei (43, 45).

This vaccine utilizes weak adenovirus, a common cold virus to deliver genetic material, recombinant Ad5 (rAd5), for coding the spike protein (S glycoprotein) of SARS-Cov-2 to the cells. The shaft, a distinct segment of the rAd5 fiber with a repetitive heparin-binding motif, KKTK, identifies and interacts with a heparin sensitive receptor leading to

Ad5 targeting to dendritic cells and subsequent stimulation of adaptive immunity in the human body to produce antibodies to kill coronavirus in response to the generated spike protein (45).

Phase 1 non-randomized, open-label, single-center trial of this vaccine established its safety and immunogenicity. A specific T cell response was observed after 14 days of its administration and humoral responses; peak was observed on day 28 post-vaccination (46). This trial was conducted in Wuhan, Hubei province, which was the center of the COVID-19 epidemic. At three different dose levels studied, it was noted that half of the subjects at low and middle dose levels and about 3/4 subjects in the high dose level developed antibodies capable of neutralizing live SARS-CoV-2 virus. It's phase 2 [Clinical Trial Identifier number NCT04341389] clinical trial was a randomized, placebo-controlled, crossover, double-blind, wherein immunogenicity and safety of this vaccine in 508 volunteers were assessed on the 14 and 28 days, and after 6 months, it was assessed in healthy adults and older through IM injection (47, 48). Intramuscular dose of 1.0 mL was administered in the deltoid muscle (phase 2) (47, 48). Phase 2 clinical trial was approved on 16th March 2020 (48). The trial was approved by Central Military Commission (CMC), China and Ethics Committee of Jiangsu Provincial Center for Disease Control and Prevention (CDC) (43).

ChAdOx1

This vaccine uses chimpanzee adenovirus vector developed by the Jenner Institute of the Oxford University. It can produce firm immunogenicity in COVID-19 patients with only one dose. It cannot be transmitted because this chimpanzee adenovirus is non-replicating virus vector for which reason it was selected against COVID-19 infection. It is safe for every aged patient, i.e., children, adults, and older adults, and even the patients having any pre-existing disease including diabetes. Currently, it is in clinical trial, and phase 3 is being run for it (49, 50).

SARS-CoV-2 has spike (S) glycoprotein, present on its outer cell wall, which is targeted by this novel vaccine. The Oxford University proclaimed that this novel vaccine is containing the same genetic sequence as that contained in the SARS-CoV-2, and after vaccination, the S glycoprotein will be generated; i.e., after vaccination, cells will express S glycoprotein because of having the same genetic sequence, and the host defense mechanism in human body will then be generated against it (51, 52). The mechanism of action is illustrated in Fig. 3. Existing indications for this vaccine include COVID-19 [Clinical Trial Identifier number NCT04324606] and along with or without human immunodeficiency virus (HIV) infection [Clinical Trial Identifier number NCT04444674] and malaria [Clinical Trial Identifier number NCT03203421] (52–54).

The Oxford University proclaimed that this vaccine showed excellent therapeutic efficacy against SARS-CoV-2 in preclinical studies which were performed with the collaboration of Rocky Mountain Laboratories (NIH/NIAID); the "CSIROxbridge Consortium"; Public Health England; the Pirbright Institute; Professor Dr. Stephen Becker at the Institutfür Virologie; and Philipps Universität Marburg and can establish safety, immunogenicity, and therapeutic efficacy against SARS-CoV-2 (55). Phase 1 (phase 1/2) trial [Clinical Trial Identifier number NCT04324606] of this vaccine, started on 23rd April 2020, was a randomized, single-blinded, sequential model, multicenter study on 1090 healthy adults at 6 different locations of the UK (56, 57). Phase 2 (phase 2/3) study, started on 28th May 2020 [Clinical Trial Identifier number NCT04400838], was a randomized, single-blind, sequential model which includes 10,260 child, adults, and older adult healthy volunteers. The Oxford University is continuing the trial at 20 different places in the UK (58). The Oxford University and AstraZeneca conducted phase 3 trial (randomized and single-blinded) together. In this case, at first, 2000 health workers from Sao Paulo and 1000 participants from Rio de Janeiro were taken. According to their current report, the first dosing of this vaccine (ChAdOx1 nCoV-19/AZD1222) in 1077 participants under phase 3 [Clinical Trial Identifier number NCT04324606] was found more successful and safer along with reduced reactogenic than the group taking paracetamol and humoral, and cellular immune response in their body was also found originated. Recently, the efficacy data for the clinical trial of this vaccine is published, where the confidence interval for efficacy was found 95% and there was no any adverse effects (59).

The Oxford University and AstraZeneca collaborated with the Serum Institute of India (SII) for conducting the phase 3 trial of their vaccine in India, where the vaccine is named as "Covishield" (mainly contains L-histidine ethanol, L-histidine hydrochloride monohydrate, magnesium chloride hexahydrate, polysorbate 80, sucrose, sodium chloride, disodium edetate dihydrate, water for injection). This vaccine is approved by DCGI for this trial and got emergency use authorization from the same Indian regulatory agency for distribution in India (58, 59).

Side by side, the University of Witwatersrand, South Africa, and the Medical Research Council, South Africa; the Bill and Melinda Gates Foundation, and the Oxford University are conducting another one phase 1/2 [Clinical Trial Identifier number NCT04444674], double-blinded, placebo-controlled, randomized trial on 2000 healthy adults having or not having HIV at 6 different locations of South Africa (59).

A recent study in Germany has shown that heterologous priming with ChAdOx1 followed by a booster dose of mRNA vaccine like mRNA-1273 or BNT162b2 is much more efficacious rather than that of the homologous priming (60). In this study, 216 participants were taken and out of which 97 participants were administered heterologous priming dose (ChAdOx1 with booster dose of mRNA-1273), and 55 from 64 participants were administered homologous priming, i.e., ChAdOx1 or mRNA-1273, respectively. It was observed that the levels of spike specific IgG, helper T cells (CD4 T cells and CD8 T cells), and neutralizing antibodies are increased in case of this heterologous priming than the homologous priming. Even the reactogenicity for heterologous priming with ChAdOx1 followed by booster dose of mRNA-1273 was found negligible in contrast to the homologous priming.

A dose of 5×10^{10} viral particles (vp) was administered through IM route, and for some groups, a maintenance dose of 2.5×10^{10} vp with the previous dose was administered in phase 1 [Clinical Trial Identifier number NCT04324606] (56). A dose of 5×10^{10}vp was administered through IM route, and for some groups, a maintenance dose of 2.2×10^{10} vp with the previous dose was administered in phase 2 [Clinical Trial Identifier number NCT04400838] (58). A single dose of 5×10^{10} vp was administered through IM route in phase 3 (first dose) [Clinical Trial Identifier number NCT04324606] (58). However, phase 1and 2 clinical trial was approved by UK regulators and ethical reviewers (57). Phase 3 clinical trial was approved by Anvisa (Health regulatory authority of Brazil) which approved it earlier of June 2020 (59). This vaccine got approval and emergency use authorization from the renowned Indian regulatory agency, namely, DCGI (58, 59). The WHO also listed this vaccine for the emergency use in this pandemic situation on 15th February 2021 (61). This vaccine (named as Vaxzevria in this case) has also got emergency use authorization (conditional marketing authorization) in European territories by EMA (62).

AD26.COV2.S

AD26.COV.S is a recombinant adenovirus serotype 26, which cannot be replicated (63). This vector encodes the full-length S glycoprotein of the SARS-CoV-2. It was originated from the Wuhan strain, clinically isolated first. Janssen Pharmaceuticals (under Johnsons & Johnsons) have developed it and sponsored the clinical trial along with the Biomedical Advanced Research and Development Authority of the Department of Health and Human Services for this vaccine. The report of clinical trial phase 1/2a (COV1001) [Clinical Trial Identifier number NCT04436276] is published in a recent article. This trial was a randomized, multicenter, placebo-controlled, double-blinded, cohort study, where 1085 adults and older adults were taken. In this case, participants (18–55 years old) were included in cohort 1a group, participants (65 years and older) were included in cohort 1b, and further older age groups were divided in cohort 3. Enrollment process was started for cohort 2 to obtain the prolong period data and to compare single-dose effect with two-dose effect of the vaccine. Participants were administered 0.5 ml of vaccine through IM route. More specifically, cohort 1 and 3 groups were administered 5×10^{10} vp/ ml or 1×10^{11} vp/ml through IM route either for one time or two times with a gap of 56 days (63) (Clinical Trials Identifier number NCT04436276). This trial was conducted in 12 different centers of Belgium and the USA. The aim of this trial was to examine the safety, efficacy, reactogencity, and immunogenicity of the vaccine. As a result of this study, it was observed that single dose elicited better humoral immunity response rather than that of the two-dose and was found safe and effective against SARS-CoV-2. Two phase 3 trials [Clinical Trial Identifier number NCT04505722 (ENSEMBLE) and Clinical Trial Identifier number NCT04614948 (ENSEMBLE 2)] were conducted for this vaccine. One (NCT04505722) was randomized, multicentered, double-blinded, placebo-controlled trial, where 44,325 adults and older adults were taken (Clinical Trials Identifier number NCT04505722). This was a single-dose study where the participants obtained 5×10^{10} vp on the 1st day through IM route. This study was conducted in 213 different centers of the USA, Argentina, Brazil, Chile, Colombia, Mexico, Peru, and South Africa. In case of the next study (NCT04614948), it was a randomized, multicentered, double-blinded, placebo-controlled trial, where 31,836 adults and older adults were taken (Clinical Trials Identifier number NCT04614948). It was a double-dose trial, where participants obtained two doses (one is on the 1st day and other is on the 57th day) through IM route. This study was conducted in 125 locations of the USA, Brazil, Belgium, Colombia, France, Germany, the Philippines, South Africa, Spain, and the UK. This vaccine is already approved (emergency use authorization/ conditional marketing authorization) by the USFDA, EMA, and WHO (28, 64, 65).

EpiVacCorona

EpiVacCorona is another potent vaccine formulated by Vector State Research Center of Virology and Biotechnology of Russia. It contains peptide immunogens corresponding to chosen epitopes of S glycoprotein, which is conjugated with N protein recombinant SARS-CoV-2 (66). In this formulation aluminum hydroxide is used as an adjuvant. In one clinical trial, the safety, efficacy, reactogenicity, and immune response were studied after two shots of this vaccine. Phase 1 and phase 2 studies [Clinical Trials Identifier number NCT04527575] for this vaccine were conducted simultaneously (Clinical Trials Identifier number NCT04527575). Phase 1 trial was an open, randomized, parallel, placebo-controlled study, in which

14 adult volunteered. Phase 2 trial was a single-blinded randomized, comparative, placebo-controlled study, in which 86 adults were recruited. Normal saline was used as the placebo. Vaccination was conducted through IM route in both of these trials. Developers proclaimed that after the second dose, the level of neutralizing antibody was significantly improved rather than that of the placebo group. Phase 3 trial [Clinical Trials Identifier number NCT04780035] for this vaccine was a double-blinded randomized, parallel, placebo-controlled study, in which 3000 adults and older adults participated (Clinical Trials Identifier number NCT04780035). Out of 3000 participants, 2250 were administered 0.5 ml of the vaccine twice through IM route, and the rest 750 were administered placebo. This study examined the humoral and cell-mediated immunity of volunteers along with prophylactic efficacy of the vaccine. Clinical trials in different phases (Phase 1/2/3) were conducted at different locations of the Russian Federation. According to the WHO's draft landscape of the COVID-19 vaccines, this vaccine is approved in Russia (67).

All the details of some approved vaccines are summarized in the Table I.

UNAPPROVED VACCINES

Some vaccines are there, which have not been given approval for vaccination, but their clinical trials are ongoing, and till date, they proved themselves as potent vaccine candidate against SARS-CoV-2.

DAS-181(Fludas)

DAS-181, a recombinant fusion protein, contains *Actinomyces viscosus* derived sialidase catalytic domain and a cell surface-anchoring domain named amphiregulin glycosaminoglycan (68, 69). This antiviral agent is effective against multiple sialic acid-dependent viruses. It has a broad-spectrum activity for several viral infections like COVID-19 infections, influenza virus, parainfluenza virus, and metapneumovirus, as well as human enterovirus-68 (70–72).

DAS181 works by cleaving the α(2,6)- and α(2,3)-linked sialic acid receptors on the epithelial cell surface lining of respiratory tract of the human body (72). Sialic acid receptors are used by several viruses to infect the epithelial cells of human respiratory tract. In the absence of sialic acid on the epithelial cell surface, the virus cannot be internalized, leading to the preventive action on the replication of virus in respiratory tract. The cell surface-anchoring domain facilitates the attachment of sialidase to the respiratory epithelium for longer retention leading to enhancement of potency (73). Existing indications for this vaccine include COVID-19 infections, influenza virus, parainfluenza virus, and metapneumovirus, as well as human enterovirus-68.

Study for the compassionate use of DAS-181 as inhalation in COVID-19 infections was completed as a phase 2 trial in April 2020 by Ansun Biopharma in collaboration with Renmin Hospital of Wuhan University [Clinical Trials Identifier number NCT04324489] (74). DAS-181 is (July 2020) in phase 3 and is multicenter, worldwide trial which is tested on the adult or older adult hospitalized patient having COVID-19 pneumonia. According to the National Cancer Institute, its phase 3 trial is being continued in 7 different places (75). A multicenter, phase 2/3 placebo-controlled, double-blind randomized study (Inhalation) has been planned in Italy in July 2020 to ascertain COVID-19 clinical status scale [Clinical Trials Identifier number NCT04354389] (76).

DAS-181 is a nebulized formulation delivered in patient body using Aerogen® soloaerosol drug delivery system (76). Nebulized DAS181 of dose 4.5 mg BID/day that is a total 9 mg/day for 10 days (Phase 2 and Phase 3 clinical trial study). DAS181 is permitted as investigational new drug (IND) for clinical studies in humans, and now its clinical trial (phase 2/3) is being continued in 7 different places of THE USA. The USFDA has granted both fast track and breakthrough therapy designation to DAS181. A two-stage randomized, double-blind, placebo-controlled clinical study is planned to confirm the early results and evaluate the safety and efficacy of DAS181 for the prevention of severe COVID-19 pneumonia (76). A severely immunocompromised patient was treated with Fludase, under the Emergency Investigational New Drug Application (eIND) granted by the USFDA, after undergoing an allogeneic stem cell transplantation. However, sponsors halted the trial for this vaccine (Clinical Trials Identifier number NCT04354389).

CD24Fc

CD24Fc, a recombinant fusion protein for potential immunomodulation, contains non-polymorphic domains of CD24 linked with the Fc receptor domain of IgG1. It has potential anti-inflammatory, anticancer activity with immune checkpoint inhibitory activity (77, 78). CD24Fc binds to both Siglec G/10 and DAMPs. CD24Fc effectively binds to the components of the respective damaged cell which are referred to as damage-associated molecular patterns (DAMPs) leading to inhibition of interaction between TLRs and DAMPs resulting in leading to hampered cytokine production (responsible for inflammation) (77, 79). The innate immunogenicity is stimulated by DAMPs. To cure the conditions created by several immunogenic diseases, the interaction between CD24 and Siglec G/10 also plays pivotal role. CD24Fc binds to the sialic acid-binding immunoglobulin-type lectin, namely, Siglec G/10, and stimulates it along with the activation of Src homology region 2 domain-containing

Table I Summary of the Details of Some Approved Vaccines

Vaccine name	Constituent	Developer	Examined route of administration	Examined dose in current phase	Identifying number of current phase
mRNA-1273	mRNA encoding the conformation of S-glycoprotein	NIAID and Moderna	IM	100 µg	[NCT04470427]
BNT162	Nucleoside-modified mRNA	Pfizer and BioNTech	IM	30 µg	[NCT04368728]
Covaxin	Inactivated or killed parts of Indian strain of SARS-CoV-2 and alum	Bharat Biotech and ICMR-National Institute of Virology	IM	0.5 mL BID	[CTRI/2020/07/026300]
Gam-COVID-Vac/ Gam-COVID-Vac Lyo	rAd5 and rAd26 expressing SARS-CoV-2 S glycoprotein	Gamaleya Research Institute of Epidemiology and Microbiology, Health Ministry of the Russian Federation	IM		[NCT04436471] and [NCT04437875]
Ad5-nCoV	Harmless Ad5	CanSino Biologics	IM	1 mL	[NCT04341389] (CTII-nCoV)*
ChAdOx1	Chimpanzee adenovirus vector	Jenner Institute of Oxford University	IM	0.5 mL	[NCT04324606] (COV001)# and [NCT04516746]
AD26.COV.S	Recombinant adenovirus serotype 26 expressing SARS-CoV-2 S glycoprotein	Janssen Pharmaceuticals (under Johnsons & Johnsons)	IM	Single dose of 0.5 mL	[NCT04505722] (ENSEMBLE)@ and [NCT04614948] (ENSEMBLE 2) $
EpiVacCorona	Peptide immunogens corresponding to chosen epitopes of S glycoprotein, conjugated with N protein recombinant	Vector State Research Center of Virology and Biotechnology of Russia	IM	0.5 ml	[NCT04780035]

CTII-nCoV: phase 2 trial of Ad5-nCoV

#COV001: phase 2 trial of ChAdOx1*

@ENSEMBLE: phase 3 (first) trial of AD26.COVS*

$ENSEMBLE 2: phase 3 (second) trial of AD26.COVS*

phosphatase-1 (SHP-1)-mediated inhibitory signaling and blocks the stimulation of nuclear factor-kappa B (NFκB). It is also reported that in preclinical studies of HIV and simian immunodeficiency virus (SIV) infections, loss of T cells was altered, and functional T cells were exhausted by CD24Fc leading to diminished leukocyte infiltration of multiple organs. The aforesaid mechanism of action is illustrated in Fig. 1.

Existing indications for this vaccine include prophylaxis of COVID-19 [Clinical Trials Identifier number NCT04317040], prophylaxis of acute (grade 3–4) graft-*versus*-host disease (GVHD) in leukemia patients (undergoing hematopoietic stem cell transplantation or HCT) [Clinical Trials Identifier number NCT02663622], multiple sclerosis, and rheumatoid arthritis (79, 80).

In a preclinical study, the administration of CD24Fc to a SIV-infected Chinese rhesus monkey diminished the rate of pneumonia from 83 to 33%. In phase 1 clinical trial, it has shown safety and biological activity to suppress the expression of multiple inflammatory cytokines in healthy volunteers (81–83). Phase 2 study [Clinical Trials Identifier number NCT02663622] was performed to establish the desirable efficacy in prophylaxis of acute (grades 3–4) GVHD in leukemia patients (undergoing HCT), which was promising. Currently, OncoImmune Inc. has obtained USFDA approval for a phase 3 [Clinical Trials Identifier number NCT04317040] trial (started on 8th April 2020), which is a randomized, controlled, double-blinded, placebo-controlled, parallel mode study in 230 adult or older adult hospitalized COVID-19 patients. Recently, the developer released the result of this trial, which clearly indicated high efficacy of the vaccine (84, 85). The recovery rate of COVID-19 patients of intervention group was faster than the patients taken placebo or standard of care.

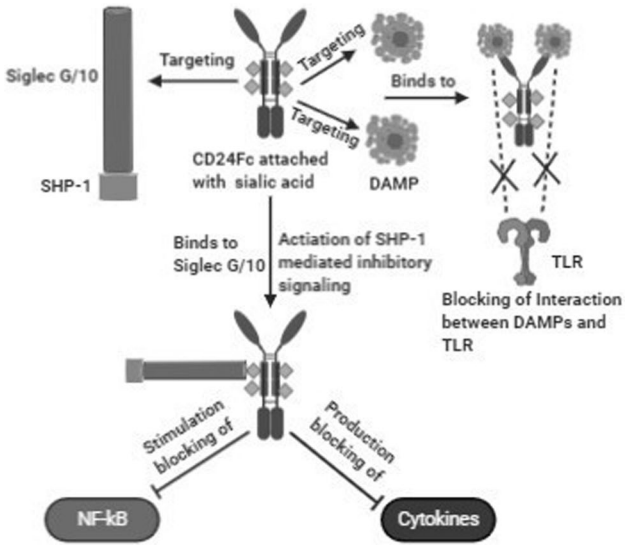

Fig. 1 Mechanism of action of CD24Fc

IV infusion of 480 mg (1st day), 240 mg (14th day), and 240 mg (28th day) of vaccine was administered along with 0.03 mg/kg/day IV of tacrolimus (from 3rd day) or 0.045 mg/kg/dose PO and 15 mg/m^2/dose IV once daily methotrexate (1st day after HCT) were administered and at a dose of 10 mg/m^2/dose on 3rd, 6th, and 11th days after HCT) (phase 2) (79, 86, 87). IV infusion of 480 mg CD24Fc was diluted to 100 ml with normal saline in 1 h (phase 3). Phase 2 trial was approved by the USFDA (77). Phase 3 trial was approved by the USFDA in April 2020.

PUL-042

PUL-042 is a solution containing Pam2CSK4 acetate (Pam2) and a synthetic diacylated lipopeptide (LP), which serves as a toll-like receptor (TLR) 2 agonist and TLR6 agonist and the oligodeoxynucleotide (ODN) M362 containing unmethylated CpG-based dinucleotides which is a TLR9 agonist possessing potential immunostimulating activity. Administration of PUL-042 via inhalation route has shown potential therapeutic activity against various major classes of pathogens like bacteria, fungus, and virus (88–90). It is currently in clinical trial, and phase 2 [Clinical Trial Identifier number NCT04312997] of it will be run by Pulmotect Inc. the manufacturer of PUL-042 (79).

PUL-042 is a combination product of Pam2 and ODN, which are TLR agonists. TLRs are proteins present on the endosomes and surface of lung epithelial cells, which can identify pathogens and subsequently activate the innate immune system of the human body. PUL-042 agonist action leads to the production of reactive oxygen species (ROS) and peptides against pathogens. M362 (CpG) binds to and activate TLR9-mediated immunostimulatory effect leading to activation of natural killer cells (NK cells), macrophages, B cells, and plasmacytoid dendritic cells (pDCs) and stimulation of interferon alpha production and induction of T helper 1 (Th-1) cell-mediated immune response. Pam2 binds to the TLR 2 and TLR 6 for activating the production of T helper 2 cells (Th-2) with subsequent production of Th-2-specific cytokines. These immunostimulatory effects collectively work together for killing pathogens entering the lungs and thereby prevent infection in the respiratory tract (88, 89). Existing indications of this vaccine include COVID-19 [Clinical Trials Identifier number NCT04312997 and NCT04313023], stem cell transplant [Clinical Trials Identifier number NCT03097796], and chronic obstructive pulmonary diseases [Clinical Trials Identifier number NCT03794557] (79, 88, 91, 92).

An inhaled single dose of PUL-042 had shown excellent therapeutic efficacy against CoVs in mouse model. Phase 2 clinical investigation (May 2020) of PUL-042 [Clinical

Trials Identifier number NCT04312997] is a randomized, quadruple-blinded, placebo-controlled parallel study in which 100 adults and older adults SARS-CoV-2 positive patients, who do not require supplemental oxygen, receive intervention three times in a week (79). An another phase 2 clinical randomized, placebo-controlled, parallel, quadruple-blind study is planned to assess the prevention of infection with four doses of PUL-042 or placebo via inhalation over a 10-day period to be administered to 200 subjects who have repeated exposure to individuals with SARS-CoV-2 infection and are asymptomatic at enrolment [Clinical Trials Identifier number NCT04313023] (89). In both of these studies, included subjects are to be examined for their clinical condition on a nine-point ordinal scale (0–8) for over 28 days to check the efficacy of PUL-042 inhalation solution for nebulization of dose 50 µg which contains 20.3 µg Pam2 and 29.8 µg ODN/mL (Phase 2 Clinical trial) [Clinical Trials Identifier number NCT04312997] (Lythgoe & Middleton, 2020). Phase 2 clinical trial approved on 5th May 2020 Pulmotect Inc. declared that the vaccine has obtained USFDA approval to undergo in phase 2 trial (79).

BLD-2660

BLD-2660 is a synthetic and novel molecule which is orally active and acts as the selective and reversible blocker of dimeric-calpain (CAPN) 1, 2, and 9, which belong to the family of CAPN proteases (93, 94). Along with CAPN, it also acts as a selective blocker of some other protease families and also possess inhibitory action on cytochrome P450 (CYP). The molecule is claimed to have excellent permeability and metabolic stability. At present, this molecule is under development for the prevention of COVID-19 pneumonia and post-recovery lung dysfunction.

CAPNs belong to the non-lysosomal cysteine protease family and are involved in diverse cellular processes which play a pivotal role in the replication of SARS-CoV-2. Along with the ACE-2, CAPNs are also expressed on the epithelial cells of the respiratory tract, which is very much prone to the entry of SARS-CoV-2 and the lung damage due to the same cause. BLD-2660 binds reversibly, covalently and selectively to the CAPN 1, 2, and 9. IL-6 aids in the process of lung damage due to COVID-19 infection, and for this, SARS-CoV-2 can be able to express itself. That is why BLD-2660 diminishes both nonproductive host-response to infection and viral replication, which are involved in mortality rate and morbidity rate produced in COVID-19. Though there are different inhibitors of CAPN, higher selectivity for CAPN was observed for BLD-2660 rather than other inhibitors which also block the replication of SARS-COV-2 (93). Existing indications for this vaccine include prevention of COVID-19 pneumonia [Clinical Trial Identifier Number NCT04334460], idiopathic pulmonary fibrosis [Clinical Trial Identifier Number NCT04244825], and non-alcoholic steatohepatitis (93, 95, 96).

In preclinical trial, this vaccine has shown excellent efficacy in reducing the expression of IL-6 in damaged lungs, which was significantly higher than the other prototype inhibitors of CAPNs (94). Currently, Blade Therapeutics is conducting the phase 2 [Clinical Trial Identifier Number NCT04334460] trial of this vaccine (started on 4th May 2020) with the collaboration of Clinipace Worldwide, which is a multicenter, randomized, quadruple-blinded, placebo-controlled, parallel mode study where 120 adult or older adult patients are being enrolled at different centers of the USA. This study aims to establish this vaccine as an additional therapy with the standard of care along with the safety and tolerability (93). Developers have conducted phase 1 (phase 1a/1b)[Clinical Trial Identifier Number NCT03559166] clinical trial (dose-escalation and placebo-controlled study) and now reached to phase 2 trial [Clinical Trial Identifier Number NCT04244825] of this vaccine to establish its anti-fibrotic effect after obtaining fruitful result in multiple preclinical disease models (97). It has also shown effective results in mouse model having lung injury in the dose of 100 mg/kg BID. Currently, this study [Clinical Trial Identifier Number NCT04244825] is suspended due to patient safety during COVID-19 situation. Single or multiple oral dose of vaccine was administered (phase 1) (94). Currently, the vaccine is being administered through the oral route (phase 2) (97). IND and phase 2 trial was approved by the USFDA as per the declaration of sponsor in clinical trial registry of the USA (94).

LV-SMENP

LV-SMENP is a bioengineered, novel, cellular vaccine, which contains the vector system of lentivirus (LV) and SMENP minigene for immunomodulation of dendritic cells (DCs). This LV-DC presenting COVID-19-specific antigens stimulate the cytotoxic T cells (98). This vaccine is modified by the development of DCs, and transduction process is taken place between these DCs and spike (S), membrane protein (M), envelope protein (E), nucleoprotein or nucleocapsid protein (N), and protease protein (P). The developer has not yet been disclosed its detailed mechanism of action against COVID-19, but on the account of clinical trial, they proclaimed that the vaccine is composed of multiple viral genes which is performed by effective system of LV vector for the expression of genes for immunomodulation and for the modification of DCs leading to stimulation of T cells (99).

Shenzhen Geno-Immune Medical Institute (City, China) along with Shenzhen Third People's Hospital and

Shenzhen Second People's Hospitals (Guangdong, China) conducted the phase 1/2 [Clinical Trial Identifier number NCT04276896] trial of this vaccine (started on 24th March 2020), which is an open-label, single-group assignment in 100 child/ adult/older adult healthy volunteers and COVID-19 infected patients having white blood cells (WBC) ≥ 3,500/ µl, lymphocytes ≥ 750/µl, and negative result in test of HIV, HBV, HCV, or TB. This trial assesses safety and therapeutic efficacy of this novel vaccine and antigen-specific cytotoxic T cell vaccines (13, 99, 100). In this trial, LV-SMENP vaccine is being administered through SC injection, and the antigen-specific cytotoxic T cell vaccine is being administered through the IV infusion to the study participants (101, 102). SC dose of 5×10^6 of LV-SMENP vaccine and IV infusion dose of 1×10^8 of antigen-specific cytotoxic T cell vaccine were followed weekly for 1 month, monthly for 3 months, and then every 3 months.

aAPC Vaccine

It is an artificial antigen-presenting cell (aAPC) vaccine, which is principally developed by Shenzhen Geno-Immune Medical Institute, China, by applying synthesized biomaterials and engineered cells. It is developed through immunomodulatory genetic modification and the viral minigene modification of the lentivirus and thereby converted to the aAPCs. Finally, its efficacy safety are investigated against COVID-19 (13). Although the mechanism of action of aAPC vaccine against COVID-19 is not disclosed yet, it is reported that for the stimulation of T cells, aAPCs are specially bioengineered domains, after entering the body, which mainly simulates the T cells along with their interactions DCs (103). To stimulate and differentiate T cells, it is conjugated with three signals, i.e., signals 1, 2, and 3. At first, signal 1 is generated for the conjugation of peptide—major histocompatibility complex (MHC) which is required for the specification of T cell receptor (TCR). These TCR agonists (including antibodies or recombinant peptide-MHC) are governed to cluster of differentiation 3 (CD3) along with upregulated co-stimulatory molecules (including anti-CD28 monoclonal antibodies), for which TCR is ligated, and simultaneously, signal 2 is generated for the conjugation of programmed death-ligand 1 (PD-L1) and programmed cell death protein 1 (PD-1). Thus, T cell is stimulated. Finally, signal 3 is generated by aAPC vaccine (or by T cells) to cause T cell differentiation and cytokines (like IL-2, IL-7, IL-15, and IL-21) expansion. This mechanism of action is illustrated in Fig. 2.

Shenzhen Geno-Immune Medical Institute (Guangdong City, China) funded this trial with the collaboration of Shenzhen Third People's Hospital and Shenzhen Second People's Hospital for assessing safety, potential efficacy, and immunogenicity of this vaccine for COVID-19. Before going to clinical trial, sponsors proclaimed that the vaccine has been examined on rhesus macaques, while phase 1 [Clinical Trial Identifier number NCT04299724] trial, conducted at Shenzhen Geno-immune Medical Institute of Guangdong in China, started on 15th February, 2020, was an open-model, single-group assignment on 100 child, adult, or older adult healthy volunteers and COVID-19 positive volunteers having white blood cells ≥ 3,500/ µL, lymphocytes ≥ 750/µL, and negative HIV, HBV, HCV, or TB test (13, 102, 104). Three subcutaneous (SC) injections having a dose of 5×10^6 of the vaccine (0, 14th, and 28th day) (102, 104).

bacTRL-Spike

bacTRL-Spike vaccine is developed from a genetically modified probiotic bacteria, *Bifidobacterium longum*, which colonize in the gut of the human body (105). This oral vaccine is composed of bacterial medium with either 1 billion or 3 billion or 10 billion colony-forming units (cfu) of live *Bifidobacterium longum*. Bacteria are bioengineered to delivery plasmids which contain synthetic DNA-encoding S glycoprotein from SARS-CoV-2.

The genetically modified probiotic bacteria colonize the gut of the human body and link immediately with epithelial cell of the intestine. There, it begins to replicate, and nanobodies are neutralized leading to the release of plasmid DNA molecules encoding the antigenic transgene. Translation of the transgenic antigens into proteins occurs in COVID-19 infection. The translated proteins localize to their natural cellular or extracellular locations along with the recycling and providing the MHC-class I activity. Thereby, the cell-mediated immunity and mucosal and systemic humoral

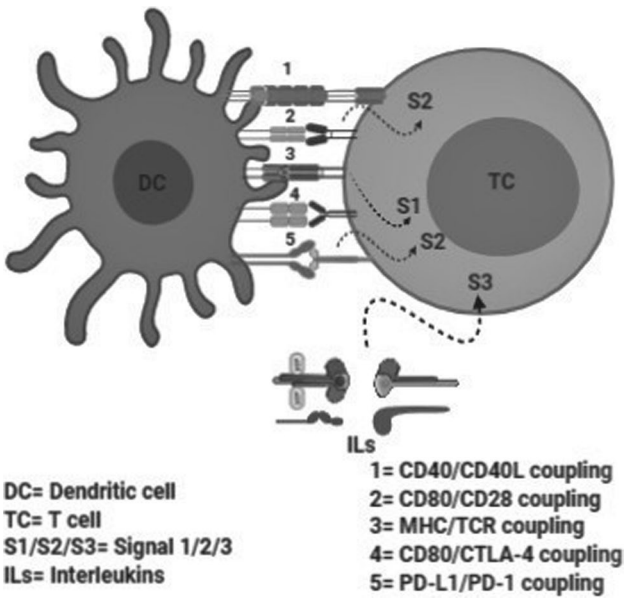

Fig. 2 Mechanism of action of aAPCs

immunity activate. On the other hand, the neutralization of nanobodies furnishes passive immunity immediately against the COVID-19 infection. Another great advantage of this approach is that the vaccine contains living probiotic bacteria for which rate of gene delivery will be sustained all over the life of the bacterial colony and for which the transgene will be expressed extensively throughout the epithelial lining (106).

Symvivo Corporation (Burnaby, Canada) is conducting the phase 1 [Clinical Trial Identifier number NCT04334980] clinical trial (started on July, 2020) of this vaccine with the collaboration of University of British Columbia, Dalhousie University, which will start soon. This is a randomized, triple-blinded, placebo-controlled, parallel model study. It is planned to test the tolerability, safety, and capability of immunomodulation of this vaccine on 112 healthy adult volunteers which will be conducted in two separate places of Canada (107). In this clinical trial, the vaccine will be administered orally (probiotic capsule) to the participants, who will be grouped into 8 (4 groups for vaccine and 4 groups for placebo) (106, 107). For active comparator groups ($n=21$ for each group), single oral dose of vaccine equivalent to 1, 3, and 10 billion cfu of *Bifidobacterium longum* and single oral dose was defined in Data and Safety Monitoring Board (DSMB) (1-day intervention phase and 12-month follow-up phase). Phase 1 trial was approved by the USFDA as per the declaration of sponsor in clinical trial registry of the USA (106).

LY-CoV555 (LY3819253)

Eli Lilly and AbCellera have collaborated for developing antibody-based therapies against COVID-19. AbCellera and NIAID initially identified this antibody in the blood sample of a COVID-19 patients who recovered from it. In just a short span of 3 months, scientists at Lilly developed this antibody, namely, LY-CoV555 (LY3819253), which is a robust and neutralizing IgG1 monoclonal antibody (108). LY-CoV555 is directed against the S-glycoprotein of SARS-CoV-2. This antibody neutralizes virus by blocking viral attachment and thereby inhibiting entry into human cells (109).

In preclinical studies, this vaccine has shown effective result in preventing SARS-CoV-2 from infecting human cells (108, 109). Phase 1 trial [Clinical Trial Identifier number NCT04411628] involved a randomized, parallel, single ascending dose, placebo-controlled, double-blind study for assessing safety and tolerability of an intravenous administered single dose to 40 COVID-19 hospitalized patients. This study, planned at five different locations in the USA, also aims for pharmacokinetics and pharmacodynamics assessment of LY-CoV555. Phase 2 trial [Clinical Trial Identifier number NCT04427501] is a randomized, double-blind, placebo-controlled study in 400 non-hospitalized patients having mild to moderate COVID-19 symptoms. This study is planned to be conducted at 34 different centers of the USA (110, 111). Single intravenous dose was administered in this case (phase 1 and phase 2). Phase 1 and 2 trial was approved by the USFDA as per the declaration of sponsor in clinical trial registry of the USA) (109).

INO-4800

Inovio Pharmaceutical's INO-4800 is a DNA vaccine for prophylactic control of SARS-CoV-2. Currently, its phase 1 [Clinical Trial Identifier number NCT04336410] clinical trial is being conducted by Inovio Pharmaceuticals with the collaboration of Coalition for Epidemic Preparedness Innovations (CEPI). This vaccine is administered intradermally with a USFDA-approved CELLECTRA® 2000 electroporation device (112, 113).

CELLECTRA® 2000, a handheld electroporation device, uses electrical pulses for inducing temporary pores for facilitating permeation of DNA plasmids across skin in a reversible and transient manner (113). After going into the cells, the machineries of those respective cells use the entered plasmids for which the coded antigens are produced and blocked S glycoprotein by attaching ACE2 receptor. Thus, the immunogenicity (T cell immune response and neutralizing antibody) of the body is activated, and the inbuilt host defense mechanism of the body is revamped, for which SARS-CoV-2 is neutralized (114). The precedence of this system is to ensure that the DNA vaccine is delivered directly into the correct cells of the human body, and after entering, this DNA vaccine performs the task of revamping the immunogenicity immediately without interfering with the DNA of that respective person.

The preclinical study of this vaccine was found successful in mice and guinea pigs where it shown effective T cell immune response and strong ability to neutralize SARS-CoV-2 with antibody (114). Currently, the phase 1 trial [Clinical Trial Identifier number NCT04336410] for this vaccine is being conducted by Inovio Pharmaceuticals (started on 3rd April 2020), which is funded by the Coalition for Epidemic Preparedness Innovations (CEPI). It is a non-randomized, open-label, sequential model of study being conducted on 40 healthy adult participants at two locations of the USA, which are the Center for Pharmaceutical Research, Missouri, and the University of Pennsylvania, Pennsylvania. This study aims to find out the safety, efficacy, tolerability, and immunogenicity boosting capability of this novel vaccine with this novel approach of intradermal delivery (115). It is reported that phase 2/3 trial (INNOVATE) may be begun in the month of August after getting necessary regulatory approval (116, 117). Currently, in this trial,

participants are being administered 1 mg or 2 mg dose of the vaccine through an intradermal (ID) injection on 0 day and 28th day through CELLECTRA® 2000 device (115). IND was approved by the USFDA on 6th April 2020 (115).

Vidprevtyn

Vidprevtyn is a recombinant protein vaccine, which mainly consists of S glycoprotein of SARS-CoV-2. Researchers of Sanofi and GlaxoSmithKline developed this spike glycoprotein in the highly specialized laboratory. This vaccine also consists of an adjuvant. This adjuvant gives synergistic activity for boosting up the immunity in human body. This vaccine is prepared based on the recognition activity of the body, i.e., after its administration, the immunity system recognizes the spike glycoprotein contained in this vaccine. After vaccination, if the person is infected with SARS-CoV-2 virus, then the immunity system of the body recognizes the virus and protects the body from this virus. Currently, phase 3 trial for this vaccine is running.

In phase 1 trial [Clinical Trial Identifier number NCT04537208], two different adjuvants were used for preparing the formulation (Clinical Trials Identifier number NCT04537208). This trial was randomized, parallel, quadruple-blinded, and cohort study, where 440 adults and older adults were taken. In this case, this vaccine was administered through IM route. In phase 1 study, two cohort groups were prepared, and they received either 1 or 2 IM injections of vaccines or placebo. The aim of this phase was to check safety of this vaccine up to 1 year from the last dose. Phase 1 study was conducted in different places of the USA, i.e., New York, Alabama, California, Florida, Massachusetts, Nebraska, Ohio, Pennsylvania, and South Carolina. Its phase 2 trial [NCT04762680] was randomized, parallel, quadruple-blinded, and cohort study, where 722 adults and older adults were taken (Clinical Trials Identifier number NCT04762680). The aim of this phase was to check the safety of this vaccine, profile of neutralizing antibody, and describe synergistic effect of a booster dose along with this vaccine. Phase 2 was conducted in 23 different places, i.e., Arizona, Connecticut, California, District of Columbia, Florida, Georgia, Illinois, Maryland, Massachusetts, Nebraska, New York, Pennsylvania, South Carolina, and Texas in USA and Honduras and Panama. Phase 3 trial [Clinical Trial Identifier number NCT04904549] was also a randomized, parallel, quadruple-blinded, and cohort study, where 37,430 adults and older adults were taken (Clinical Trials Identifier number NCT04904549). In both phase 2 and 3, monovalent and bivalent vaccines were administered along with the adjuvants against placebo. The aim of this phase is to check the occurrence of COVID-19 after the second dose. The vaccine was administered through IM route in both phase 2 and 3 studies. The study was conducted in 31 different locations. This vaccine is approved by the USFDA.

SCB-2019

SCB-2019 is a protein subunit vaccine developed by Clover Biopharmaceuticals AUS Pty Ltd. It mainly contains the trimeric form of S glycoprotein (S-trimer) along with two adjuvants (ASO3 and CpG 1018/Alum). After administration of this S-trimer containing vaccine, the S-trimer will bind to the ACE2 receptor and elicit its efficacy against SARS-CoV-2 (118). The phase 1 [Clinical Trial Identifier number NCT04405908] trial for this vaccine was a double-blinded, randomized, placebo-controlled study. In this study, 150 adults and older adults were taken, and the study was conducted in Linear Clinical Research Ltd, Australia (118) (Clinical Trials Identifier number NCT04405908). In this case, two groups were prepared, of which, one contains younger adults (18–54 years) and older adults (55–75 years). The treatment group was administered 2 shots of the vaccine in 21-day gap. The doses of vaccine were 3 µg, 9 µg, and 30 µg, which were administered through IM route. In case of placebo group, they received 0.9% sodium chloride solution. After vaccination, it was observed that 9 µg vaccine with ASO3 adjuvant and CpG 1018/Alum adjuvant have shown pain. Adverse events were more in case of the vaccine with ASO3 adjuvant, i.e., approximately 44% to 69%. The adverse events were 38% in case of younger adults and 17% in case of older adults after first dose, which was further increased after the second dose, i.e., 34% and 30%, respectively. However, both formulations (having ASO3 or CpG 1018/Alum) shown significant efficacy in improving cellular and humoral immunity along with helper T cell activity. Phase 2/3 [Clinical Trial Identifier number NCT04672395] trial was randomized, parallel, quadruple-blinded, and placebo-controlled study (Clinical Trials Identifier number NCT04672395). In this study, 22,000 adults and older adults were taken who were administered 30 µg dose through IM route. Placebo group was administered 0.9% saline as placebo. It was conducted in 48 different locations of Belgium, Brazil, Columbia, Germany, the Dominican Republic, Nepal, Panama, the Philippines, Poland, and South Africa. The aim of phase 1/2/3 trial was to check the efficacy, immunogenicity, and safety of recombinant SARS-CoV-2 S-trimer protein-based vaccine against SARS-CoV-2.

However, details of some unapproved vaccines are summarized in the Table II.

SUITABLE ROUTES OF ADMINISTRATION FOR COVID-19 VACCINES

The route of administration for the vaccines is very much important because based on the route, immunity will be provided to some specific organs. From the traditional period, parenteral vaccines are common. Parenteral route provides stimulation of helper T cells and for which immune system will be boosted up. In a study, it was observed that parenteral vaccines are very much effective and provide long-term effect (119). Although maximum vaccines are administered through IM route, there are also options for intranasal route, subcutaneous route, and intradermal route. Sometimes, intradermal routes may be effective due to its low dose requirement (120). Intramuscular route only provides systemic immune response only in lower respiratory tract.

Comparison Between Intranasal, Intramuscular, and Intradermal Route

Intranasal route provides local immunity in lower respiratory tract with systemic immunity, for which nasal shedding of the virus can be overcome (121). This point can be proved by a study. In this study, adenovirus (Ad5-S-nb2) was administered through the intranasal route against SARS-CoV-2 in rhesus macaques, and there the point of local and systemic immunity was proved (122). Since SARS-CoV-2 attacks through the upper respiratory tract, it is very important to protect the nasal area. Although intranasal vaccination provides tissue resident memory T cells in the lung, which gives protection against SARS-CoV-2, T cell immune responses are lesser in the spleen, lymph node, and brain rather than that of IM route. On the other hand, subcutaneous vaccination provides tissue resident memory T cells in airway passage and memory T cells in the spleen (121, 123). Mucosal site, especially respiratory mucosa route, is considered as one of the potential routes for entry of pathogens causing local effect and systemic effect by entering the blood circulation. Coronavirus is one of the major example, which enters the body through respiratory mucosa (124). All approved vaccines for SARS-CoV-2 are injected through intramuscular (IM) route, although a number of researches are going across the globe for the development of intranasal vaccines for the same. Ideally, vaccine against SARS-CoV-2 acting against the mucosal pathogens should induce mucosal immune responses, but it is considered that most of the IM vaccines are not much effective against the mucosal pathogens. However, many studies proved that vaccines developed for influenza and polio virus show mucosal immunity even after systemic administration, but this is not fully evaluated in case of coronavirus. Studies revealed that AstraZeneca vaccine protected against pneumonia but did not show any effect in the upper respiratory tract. Intranasal vaccine delivery was tested in both hamsters and rhesus monkeys. Researchers compared the effectiveness between intramuscular and intranasal vaccines in hamsters by dosing them with each of the type. Likely, in rhesus monkeys, 2 doses of intranasal vaccine were administered and were subjected to SARS-CoV-2 where it was observed that the monkeys developed antibodies equivalent to human recovered from COVID-19 (125). Thus, the administration of intranasal vaccine results in the formation of the memory lymphocytes in the upper airway tract and prevents the virus going further into the lower airways and lungs.

Bharat Biotech's nasal COVID-19 vaccine, BBV154, got its permission for phase 1 human clinical trial from Central Drug Standard Control Organisation. BBV154 is an intranasal replication-deficient chimpanzee adenovirus SARS-CoV-2 vectored vaccine. The Hyderabad-based company, the maker of Covaxin is working on nasal vaccination shot, which can bring more immunity against the demoniac pathogen. As per developers of the vaccine, Bharat Biotech is leading its trials using the nasal vaccination as one of the booster shot after the administration of 2 doses of Covaxin. This could not only boost the mucosal immunity but also strengthen the IgG and IgA antibody production, further improving the immunity against the virus (126). Recently, in the month of August, BBV154 got regulatory green light for phase 2 trial, which was carried in healthy volunteers of 18 to 60 years of age. Moreover, the intranasal vaccine possibly has the ability to activate other immunological pathways and should be taken as booster dose after 2 doses of Covaxin. Live adenovirus vectored vaccine is easy to manufacture and is estimated the nasal vaccine can meet the shortage issue across the country. The hope of light is the data regarding the ongoing trial might be available by the end of December 2021. In case of preclinical trial of ChAdOx1, it was observed that through the IM route, it can give protection against the virus but could not provide nasal shedding of the virus (49). On the other hand, another study reveals a single dose of intranasal vaccination can provide better upper and lower respiratory tract protection rather than that of IM route (127). However, the efficacy of intranasal vaccination depends up on the dosage, because this route prevents the transmission and better herd immunity (128).

Beside, intradermal route is also a potent route for vaccination. Inovio's INO-4800 was administered intradermally through a USFDA-approved handheld electroporation device, namely, CELLECTRA® 2000 (113). It binds to ACE2 receptor and blocks S glycoprotein for boosting the T cell and neutralizing antibody and improved the immunity system in body.

Table II Summary of the Details of Some Unapproved Vaccines

Vaccine name	Constituent	Developer	Examined route of administration	Examined dose in current phase	Identifying number of current phase
DAS-181	*Actinomyces viscosus* derived sialidase and amphiregulin glycosaminoglycan	Ansun Biopharma	Inhalation	9 mg/day	[NCT04354389] (trial halted by sponsors)
CD24Fc	Non-polymorphic domains of CD24 linked with the Fc receptor domain of IgG1	OncoImmune, Inc	IV infusion	480 mg CD24Fc, diluted to 100 ml with normal saline in 1 h	[NCT04317040] (SAC-COVID)*
PUL-042	Pam2CSK4 acetate and oligodeoxynucleotide M362	Pulmotect, Inc	Inhalation	50 μg	[NCT04312997]
LV-SMENP	Vector system of lentivirus and SMENP minigene	Shenzhen Geno-Immune Medical Institute	SC for LV-SMENP vaccine and IV infusion for antigen-specific cytotoxic T cell vaccine	5×10^6 of LV-SMENP and 1×10^8 of antigen-specific cytotoxic T cell vaccine	[NCT04276896]
BLD-2660		Blade Therapeutics	Oral		[NCT04334460]
bacTRL-Spike	Bacterial medium with either 1 billion or 3 billion or 10 billion cfu of live *Bifidobacterium longum*	Symvivo Corporation	Oral		[NCT04334980]
aAPC	Viral minigene of lentivirus	Shenzhen Geno-Immune Medical Institute	SC	5×10^6 of the vaccine	[NCT04299724]
LY-CoV555 (LY3819253)	Antibody from COVID-19 survived patient	Eli Lilly and AbCellera	IV		[NCT04427501] (BLAZE-1)#
INO-4800	DNA plasmids	Inovio Pharmaceutical	ID	1 mg or 2 mg	[NCT04336410] (INNO-VATE)@ and [NCT04642638]
Vidprevtyn	Recombinant protein containing S-glycoprotein of SARS-CoV-2	Sanofi and GlaxoSmithKline	IM		[NCT04904549] (VAT00008) $
SCB-2019	Trimeric form of S glycoprotein (S-trimer) along with two adjuvants (ASO3 and CpG 1018/Alum)	Clover Biopharmaceuticals AUS Pty Ltd	IM	30 μg	[NCT04672395]

SAC-COVID: phase 3 trial of CD24Fc

#BLAZE-1: phase 2/3 trial of LY-CoV555*

@INNOVATE: phase 1 trial of INO-4800*

$VAT00008: phase 3 trial of Vidprevtyn*

VACCINATION FOR CHILDREN

The rapid development and deployment of SARS-CoV-2 vaccines reached a historical milestone. Children, on a large-scale, got affected by the deadly virus leading to negative consequences directly and indirectly. These consequences forced the vaccine testing drive in young age groups. Canada on 5th May 2021 became the first country to approve vaccination for emergency use in children aged 12 to 15 (129). In fact, most of the vaccination drive still remains dormant in case of children above 12 years of age, and only limited vaccination campaigns are carried for age group of below 20 years. The lack of attention on vaccination of young age group is due to the fact that children are getting less affected by the virus, developing symptoms like low to moderate body temperature, influenza, and gastrointestinal manifestations. According to the WHO data, from 30 December 2019 to 6 September 2021, only 8% of total cases was made up by children of age below 15 years. However, in rare cases, children get severely affected by COVID-19 and need intensive care (130, 131). One of the adverse outcome is termed as multisystem inflammatory syndrome in children (MIS-C) which further develops multisystem disorders in the heart, eyes, kidneys, lungs, brain, and gastrointestinal tract (132).

In March 2021, Pfizer and BioNtech announced the results of phase 3 trial of BNT162b2 on more than 2200 children aging from 12 to 18 years (133, 134). The result showed that 18 children who were found out to have symptoms of COVID 19 were all treated with placebo, hence proving 100% effectiveness of the vaccine. The data was submitted to FDA requesting emergency use authorization for children between 12 and 15 years, which was granted later on October 2021. On the other hand, Moderna has submitted a similar data regarding the COVID vaccine and got permission from the European Medicine Agency for its use in children between 12 and 17 years. Moreover, granting of permission from the FDA for the same is still awaited. Covaxin (Bharat Biotech), one of the three leading vaccine manufacturer in India, Bharat Biotech, submitted the result of phase 3 trial and requested granting of permission for its emergency use in children 2 to 18 years of age. The phase 3 trial was conducted in 525 child volunteers and was found that the safety and efficacy of the vaccine in child volunteer were 77.8%. Bharat Biotech has submitted all the data regarding safety and efficacy of the vaccine to Central Drug Standard Control Organisation (CDSCO), which is already thoroughly reviewed, by CDSCO and Subject Expert Committee (SEC) with positive recommendations (Fig. 3).

CHALLENGES FOR VACCINE TO OVERCOME SARS-COV-2 MUTATIONS

At present, different variants of SARS-CoV-2 have come. It is a great challenge for the present as well as upcoming vaccines to combat against them. Maximum present vaccines are developed based on the S glycoprotein of SARS-CoV-2 virus. The recurrent mutation of the SARS-CoV-2 is observed in this S glycoprotein. Phase 3 trials of the AD26.COV2.S and ChAdOx1 have been conducted in different locations. Among these, it is observed in South Africa trial that the efficacy of both vaccines is significantly lower against B.1.351 variant (135). Neutralizing efficacy of another potential vaccine BNT162b2 for B.1.351 (beta) variant is found 2/3 lower than its normal efficacy reported. In the clinical trial of mRNA-1273, it was observed that after both doses, the serum level of neutralizing antibody against B.1.351 variant is 6 times lower than the normal. A recent study also supports the statement. In a cohort batch of 417 participants, everybody is given the second dose of BNT162b2 or mRNA-1273 (136). Among them, 2 women are found infected again. They have been found with some new variants, which include, E484K, T95I, del142-144, and D614G. These results indicate that there is potential risks of SARS-CoV-2 infection even after full vaccination. In case of variants, deadlier one is B.1.617.2 (delta), which is spreading very fast in different countries like the UK, India etc. (137). Different mutations under delta are very much dangerous which include L452R, T19R, del157-158, G142D, D614G, P681R, T478K, and D950N (138, 139). Another variant is B.1.617.1 (kappa). Different mutations of kappa variant are G142D, T95I, E154K, E484Q, L452R, D614G, P681R, and Q1071H. Among these mutations, P681R is very much harmful. Apart from that, some more new variants B.1.1.7 or alpha (first detected in the UK in 26.2% COVID situations), B.1.526 (first detected in New York in 42.9% COVID cases), P.1, or gamma (first detected in Brazil) are now responsible for 72% COVID cases worldwide (137). Some harmful mutations of alpha variant against which vaccine efficacy is very less are N501Y, del69-70, del144, A570D, P681H, D1118H, T716I, and S982A (139). In a recent study, the efficacy of BNT162b2 and ChAdOx1 is compared against alpha (B.1.1.7) and delta (B.1.617.2) variants (138). The variants were detected by the sequencing of gene and status of S glycoprotein. It was a test negative case–control study, i.e., the participants had COVID-19 symptoms and tested (RTPCR) negative. These participants were given two doses of BNT162b2 or ChAdOx1 to compare their efficacy against alpha and delta variant. After the first dose of both vaccines, it was observed that the efficacy is very much lower against delta variant (30.7% and 95% CI) than that of alpha variant (48.7% and 95% CI). After two

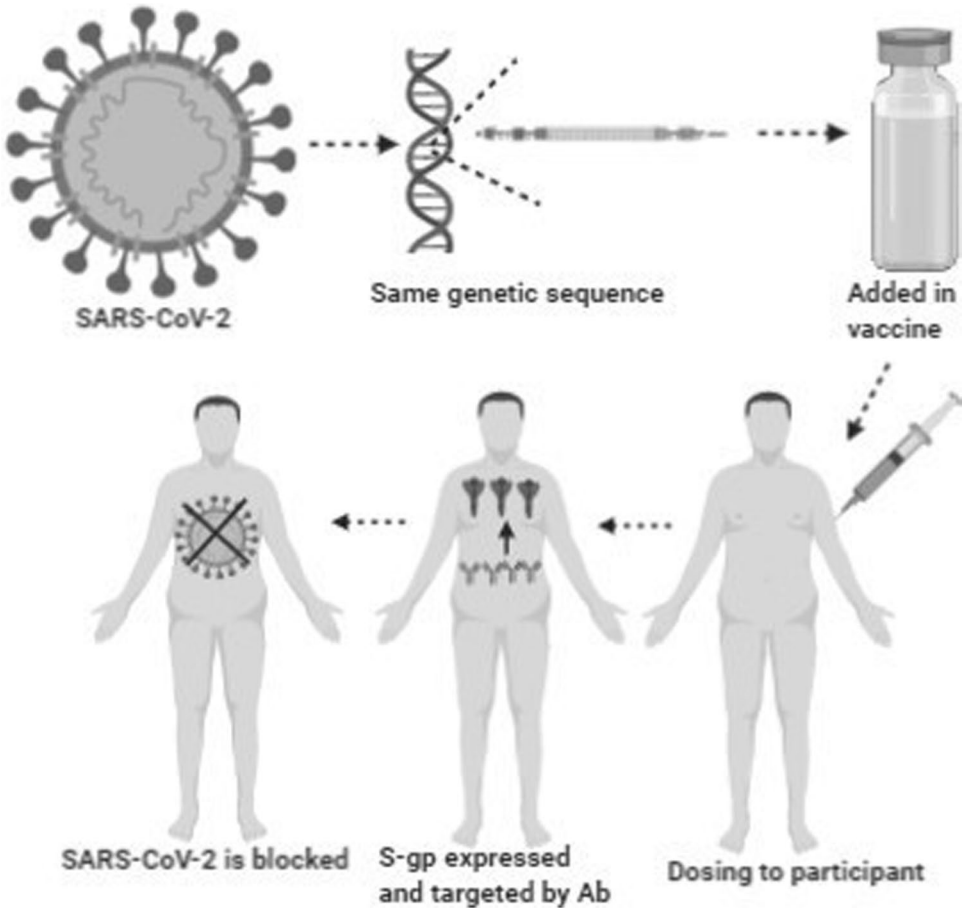

Fig. 3 Mechanism of action of ChAdOx1 (S-gp = S glycoprotein, Ab = antibodies)

doses of BNT162b2, the efficacy against the delta variant was found to be 88% (95% CI), and against the alpha variant, it was found to be 93.7% (95% CI). On the other hand, after two doses of ChAdOx1, the efficacy against delta variant was found to be 67% (95% CI), and against alpha variant, it was 74.5% (95% CI). The results clearly indicate that the delta variant is more dominant rather than the other variants, and it is a great challenge for the researchers to develop any vaccines against the delta variant. In another recent study, the efficacy of BNT162b2 against the alpha and beta variant was checked (140). In this case, at first test negative case–control study was conducted, and later, that data was further evaluated using a cohort study. In the former study, after 14 days or more of the second dose, the efficacy of the vaccine against the alpha variant was found to be 89.5% (95% CI), and against the beta variant, it was 75% (95% CI). In this study, it was also observed that BNT162b2 was having highly combatting ability (97.4% efficacy with 95% CI) against severe or critical infection related to SARS-CoV-2. However, these values of efficacy were found slightly lower in the latter study. In the cohort study, the efficacy of the vaccine against the alpha variant was found to be 87% (95% CI), and against the beta variant, it was 72.1% (95% CI). Another study reveals the lower efficacy of ChAdOx1 against the alpha variant, specifically found in the UK (141). The study was a multicentered, single-blinded, randomized, cohort, phase 2/3 trial (COV002), which was conducted in the UK. In this case, a total 8534 adult male and female participants were taken. Their COVID-19 test has been done by nucleic acid amplification test and after collection of swab genome sequencing was performed, which confirmed that it was alpha variant. In this study, it was observed that the efficacy of the vaccine against alpha variant in adults was 78% (95% CI) and specifically in women was 59% (95% CI). The result clearly indicates that ChAdOx1 is not very much effective against the alpha variant. After vaccination, it is necessary to release the neutralized antibodies. Unfortunately, some mutations like E484K mutation of the beta variant inhibits the neutralized antibodies (142). Recently, another new variant is reported in South Africa on 24 November 2021. This B.1.1.529 or *Omicron* variant is found with a number of concerned mutations. The susceptibility of spreading in case of omicron is found higher rather than delta or delta plus variant. In South Africa, it is mainly found in the bodies of youngsters. The most dangerous thing is that the conventional process of RTPCR cannot detect this variant (143). Therefore, it is a great challenge for the developer

to develop any vaccine against these emerging variants of SARS-CoV-2.

The efficacy of some approved and unapproved vaccines against different SARS-CoV-2 variants are given in the Table III.

CHALLENGES IN PRODUCTION OF VACCINES

The production of a vaccine is a very challenging task in industries, which mainly aimed at safety, therapeutic efficacy, and most importantly consistency of action over its whole life cycle (can be ranged from 1 to 3 years) (148). The production time varies for different vaccines, for example, the production time of vaccines for influenza virus can be up to 3 years. Permissions from regulatory bodies should be taken for that vaccine along with all the processes adopted for its production. Both in *vitro* and in *vivo* studies are required for checking its action, for which clinical trials should be done. The modification of these can be a cause of lower potency of the vaccine, which is the biggest challenge for vaccine production. In case of in *vivo* study, suitable animal model selection and safety monitoring (role of clinical research officer or CRO) in the clinical trial as well as conducting it for a pandemic disease (mainly the less time) are one of the biggest challenges for researchers. It's difficult to predict where and when outbreaks will occur and to prepare trial sites to coincide with vaccine readiness for testing. Another big challenge for vaccine production is to respond against different pandemic diseases. In these cases, the time for vaccine production is generally expedited than the normal course. Several pandemic diseases have come worldwide, of which the responsible virus belonged to the coronavirus family like SARS and MERS (149). In these cases, there were no any single vaccines which can combat effectively against any one of them. For example, there was vaccine for influenza in world, but this pandemic threat had different strains for which a single vaccine was not effective (150). For this reason, it was important to produce new vaccines within a short period during that emergency condition, which was a very big challenge for researchers. However, it took 4 to 6 months to produce a new vaccine for this disease. In all the pandemic situations, researchers took several months to find new vaccines along with their safety and efficacy. In pandemic situations, regulatory authorities play a vital role in case approval of these vaccines. When the US Department of Health and Human Services (HHS) declares the condition as an emergency, then the USFDA goes for fast track approval process or emergency use authorization. In this case, they approve the product which was not approved yet or the product which is approved for different case but is having action against the pandemic threat. As an example, the pandemic situation due to A/H1N1 can be taken which was occurred in 2009 (151). In this case, the USFDA approved a respirator, antiviral treatment, and polymerase chain reaction (PCR) diagnostic test as emergency use authorization. In the present day, SARS-CoV-2 becomes demoniac day by day of which the vaccine is not available, but several are in phase 3 clinical trial. This virus is having different strains, and it can undergo into the mutation, which is another

Table III Efficacy of Some Approved and Unapproved Vaccines Against Different Variants

Vaccine	SARS-CoV-2 variant/mutation	Efficacy	Safety information	CI	Reference
BNT162b2	Delta	88.0%	Low chance of serious adverse effects and mild to moderate pain at the site of injection, fatigue, and headache were observed	95%	(29, 138)
	Alpha	93.7%		95%	
BNT162b2	Beta	75% and 72.1%		95%	(29, 140)
	Alpha	89.5% and 87.0%		95%	
ChAdOx1	Delta	67.0%	Considerable safety profile	95%	(59, 138)
	Alpha	74.5%		95%	
mRNA-1273	Gamma	94.0%	Considerable adverse events	95%	(144)
	Alpha	83.0%		95%	(25, 145)
Ad26.COV2.S	Beta (moderate to severe disease)	64.0%	Considerable safety and reactogenicity profile	95%	(63, 146)
	Beta (severe-critical disease)	81.7%		95%	
Covaxin	Gamma	310.6 (GMT)	Confirmed safety profile	95%	(38, 147)
	Delta	241.6 (GMT)		95%	
	Delta AY.1	209.1 (GMT)		95%	
	B.1.617.3	165.3 (GMT)		95%	
INO-4800	Alpha	295 (ID_{50} titer)	No serious adverse events are observed	95%	(115, 117)
	Beta	105 (ID_{50} titer)		95%	
	Gamma	664 (ID_{50} titer)		95%	

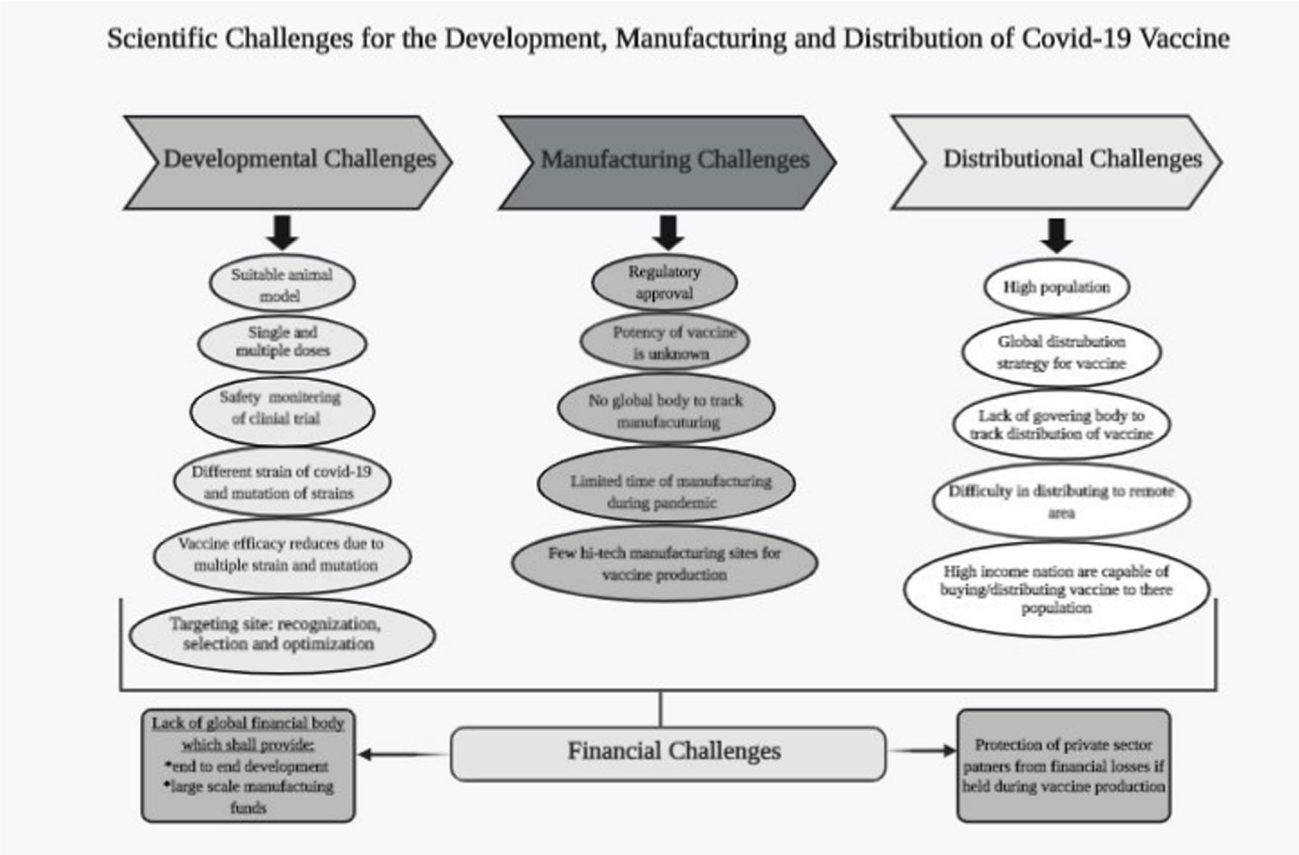

Fig. 4 Scientific challenges for the development, manufacturing, and distribution of COVID-19 vaccine

challenge for the researchers in case of vaccine development because due to the mutation the desired therapeutic efficacy of the vaccine will be reduced. It reported three types of genome of this virus, i.e., type A, B, and C (152). They proclaimed that the variant of SARS-CoV-2 found from the bats and pangolin is very much similar to type A, but this was not found predominant in Wuhan, China. The variant found in the USA and Australia is the mutated version of type A. Type B mainly predominates the region of Wuhan, China, and the East Asia because it is immunologically or according to environment adapted by the people live there, and type C is facing by the European countries like England, Sweden, France, and Italy. Mainly, variant C is derived from variant B. For this reason, a single vaccine will not be effective against this virus. Another challenge is the potential duration of the immunity, which is unknown. A recent study in *Nature Medicine* shown that the COVID-19 vaccines can save people up to a few days (153). Researchers of China conducted this study on some COVID-19 patients, of whom some were symptomatic and some were asymptomatic. After vaccination, researchers were consistently observing the immunoglobulin G (IgG) and IgM level, where they observed that IgG level decreased significantly after 8 weeks sharp of recovery in asymptomatic patients (71% drop) and in symptomatic patients (76% drop) and levels of antibody dropped in 40 and asymptomatic and 13% symptomatic patients. Despite having a smaller number of patients, the result of this experiment raises a question regarding the lasting of the effect of COVID-19 vaccines. The dose of the vaccine is also an important factor for the researchers, i.e., whether it is single dose or multiple doses. Although the virus's spike protein is a promising immunogen for protection, optimizing antigen design is critical to ensure optimal immune response. Though some high-income countries may pay for the development and manufacture with their own populations in mind, there is no global entity responsible for financing or ordering vaccine manufacture. On this account globally, fair vaccine-allocation system also creates challenge for researchers. A global financing system that supports end-to-end development and large-scale manufacturing and deployment that ensures fair allocation and protect private sector partners from significant financial losses will be a critical component of future pandemic preparedness. However, the scientific challenges for the

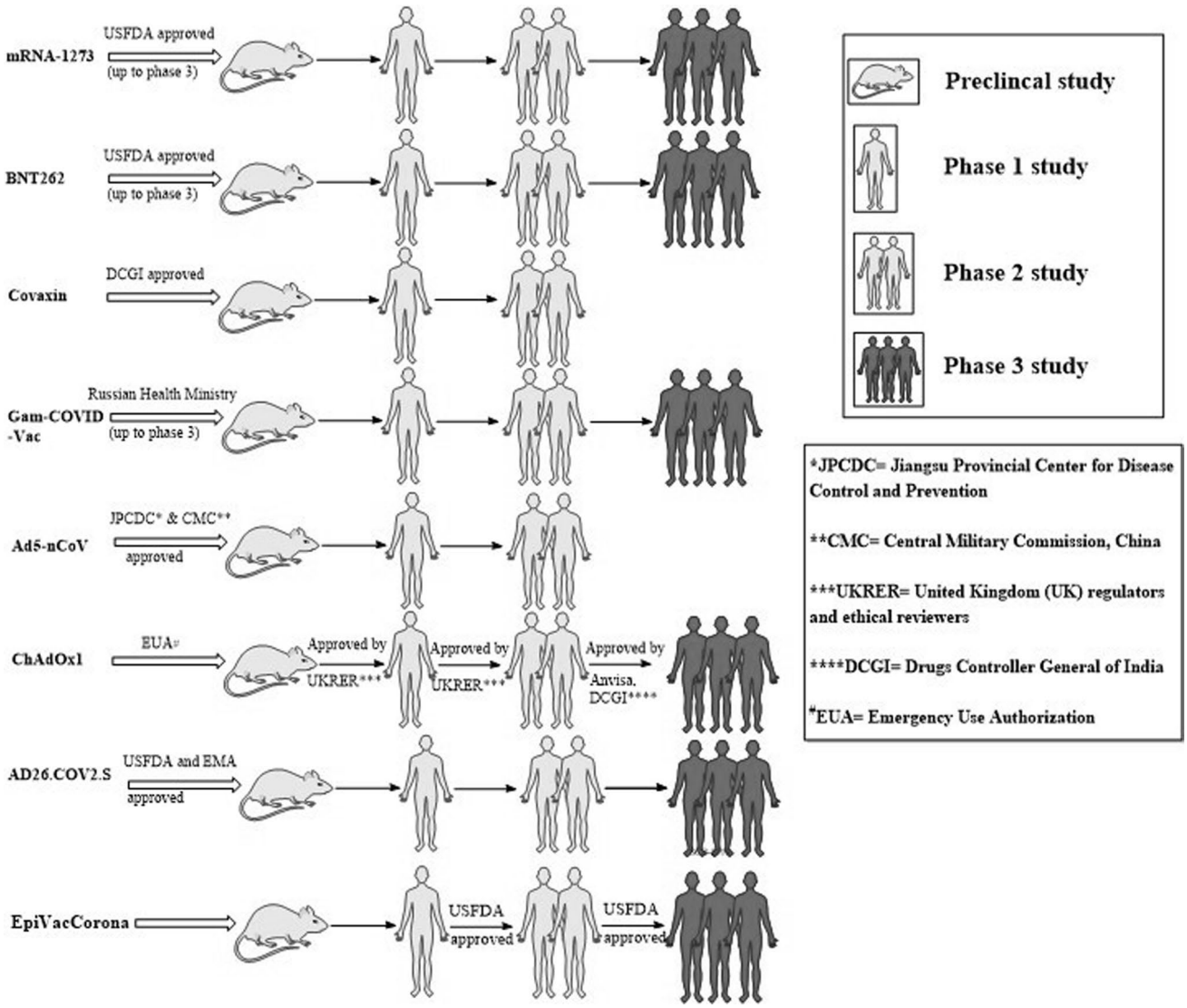

Fig. 5 Clinical trials of approved vaccines at a glance

development, manufacturing, and distribution of COVID-19 vaccine are described in Fig. 4.

CONCLUSIONS

The current status of all the aforesaid vaccines is summarized in Figs. 5 and 6. Recently, the WHO published a landscape detail of vaccines in which 35 vaccines are in clinical trial and 194 candidates are in preclinical trial. The hope still remains as 21 vaccines are already in use for general public and 10 vaccines are in phase 4 of clinical trial worldwide. ChAdOx1, backed by the Oxford University and AstraZeneca as novel coronavirus vaccine, is one of the leading candidates reached global pharmaceutical market by the beginning of 2021 and was available to general public by the mid of 2021. Another potential candidate for corona virus vaccine, BNT162b2, backed by Pfizer and BioNTech, and Moderna's mRNA-1273, are already approved in the UK and the USA for distribution. The regulatory authorization is obtained, and initial 800,000 doses began to roll out in the beginning of December 2020. Presently, a good number of vaccines across the globe have got approval for Investigational New Drug (IND). According to clinicaltrials.gov 343, clinical trials are ongoing for COVID-19 vaccine, of which some notable vaccines are Novavax's NVX-CoV2373 (phase 3), CureVacc's CVnCoV (phase 2b/3), University of Melbourne and Murdoch Children's Research Institute's Bacillus Calmette-Guerin (BCG) vaccine (phase 2/3), and many more. As per clinical trial registration, the potent vaccines are likely to be launched in global market by 2021 to

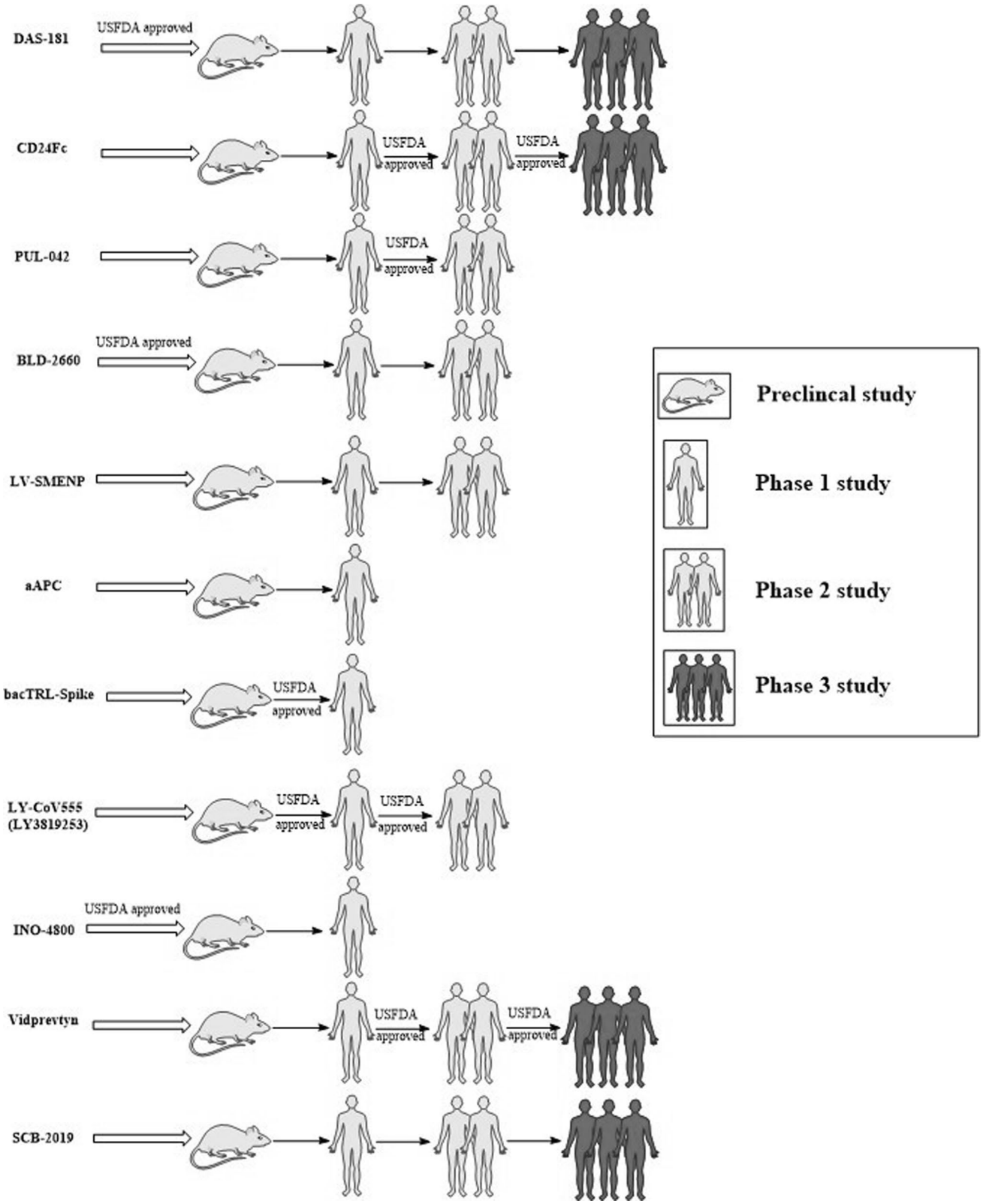

Fig. 6 Clinical trials of unapproved vaccines at a glance

2024. The good among the odds is that being an emergency drug, the infectious disease vaccine candidates pass the drug regulatory approval process at consistently higher rates than other drug types. Further, the race to develop COVID-19 vaccine has led a debate on minimum efficacy of vaccines among scientists, regulators, and pharmaceutical companies as 50% being the minimum efficacy recommended by the WHO for COVID-19 vaccine. Russian vaccine has entered the market with emergency authorizations even before the mandatory phase 3 clinical trials leading to alarming safety and efficacy issues. There are different variants, and a few specific vaccines are found to be effective against them. Oxford's ChAdOx1 (double-dose) and Bharat Biotech's Covaxin (double-dose) are found effective against delta and delta plus variants, respectively. With some constraints, most of the vaccines have been found safe and effective against SARS-CoV-2 in phase 2 clinical trials, and some of these are able to produce robust immunomodulatory effect, which shows a light of hope for their successful phase 3 trials followed by speedy launching of vaccines in global market.

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Author Contribution All authors contributed to the design, acquisition, analysis, interpretation, drafting, and revision of the paper.

Declarations

Conflict of Interest The authors declare no competing interests.

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