COVID-19 vaccine development based on recombinant viral and bacterial vector systems: combinatorial effect of adaptive and trained immunity

**Keywords:** heterologous vaccine, trained immunity, bacterial vector vaccine, viral vector vaccine, COVID-19

**Introduction**

The COVID-19 outbreak began at the end of 2019 due to the sudden appearance of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). As of November 2021, there have been over 250 million cases with over 5 million deaths globally (Worldometer, 2021). To control this pandemic caused by COVID-19, effective vaccines for preventing SARS-CoV-2 infection are urgently needed.

Similar to SARS-CoV, SARS-CoV-2 consists of four major structural proteins: the spike glycoprotein, nucleocapsid, membrane and envelope proteins. Of these, the spike protein contains S1 including the receptor-binding domain (RBD) and S2 subunits, which mediate the entry of the virus into the host cell by binding to the human angiotensin-converting enzyme 2 (hACE2) receptor (Zhu et al., 2020a). Therefore, the S protein plays a pivotal role in eliciting immune responses against SARS-CoV-2 and is a major target for neutralizing antibodies in humans (Huang et al., 2020; Yang and Du, 2021). Moreover, the amino acid sequences of the spike protein were observed to contain a number of CD4+ and CD8+ T-cell epitopes, highlighting their potential roles in eliciting adaptive immune responses (Grifoni et al., 2020, 2021). Therefore, most COVID-19 vaccine candidates have been designed to provide the S protein or RBD as the target antigen, which is responsible for inducing immune responses.

To date, a number of COVID-19 vaccine candidates have been developed on the basis of different platforms, such as non-replicating or replicating viral vectors, protein subunits, conventional whole inactivated or live-attenuated virus, mRNA and DNA (Nagy and Alhatlani, 2021). Of these, a total of 21 vaccines are currently authorized for human use worldwide (Tracker, 2021). Although no significant side effects have been reported for currently used vaccines, the emergence of variants of SARS-CoV-2, including lineages B.1.1.7 and B.1.617.2, and the spread of these variants worldwide pose a serious threat to public health because they compromise the effectiveness of currently developed vaccines (Rambaut et al., 2020; Wibmer et al., 2021; Zhou et al., 2021). Therefore, to overcome the risk posed by variants of SARS-CoV-2 that are less susceptible to protective antibodies, vaccine strategies har-
nessing T cell-mediated immune responses or innate cell-based heterologous effects that less affected by these variants should be developed (Sauer and Harris, 2020).

Compared to other platforms, vaccines that use viral or bacterial delivery systems have a distinct benefit in that they can induce off-target effects to defend against unrelated pathogens via an innate immune memory system termed trained immunity. In epidemiological studies, some live viral or bacterial vaccines have been reported to induce heterologous vaccine effects via trained innate immune cells, resulting in a reduction in all-cause mortality from infectious agents (Agrawal, 2019; Nascimento et al., 2020; Marin-Hernández et al., 2021). The enhanced nonspecific immune response of trained immunity can further enhance both specific humoral and cell-mediated immune responses to defend against SARS-CoV-2, providing better protection against even vaccine escape variants (Covian et al., 2021).

In this review article, we will first review the ability of licensed live vaccines for other infections, including the BCG, oral polio and measles-mumps-rubella (MMR) vaccines, to prevent COVID-19 infections via heterologous vaccine effects. Second, we will discuss the current COVID-19 vaccine platforms based on viral and bacterial delivery systems and discuss the advantages and disadvantages of the different systems used for vaccine delivery.

Table 1. BCG and licensed viral vaccines being tested in clinical trials to evaluate the heterologous protective effects against COVID-19

| Vaccine | Study title                                                                 | Ages for study | Strain / Vaccine          | Clinical stage / Enrollment                      | Sponsor / Country                  |
|---------|-----------------------------------------------------------------------------|----------------|---------------------------|--------------------------------------------------|-----------------------------------|
| BCG     | Use of BCG Vaccine as a Preventive Measure for COVID-19 in Health Care Workers (ProBCG) | 18 years        | Phase 2 (NCT04659941)    | Estimated enrollment : 1000                      | Universidade Federal do Rio de Janeiro / Brazil |
|         | Reducing Health Care Workers Absenteeism in Covid-19 Pandemic Through BCG Vaccine (BCG-CORONA) | 18 years        | Danish strain 1331       | Phase 3 (NCT04328441)   | University Medical Center Utrecht / Netherlands |
| BCG     | Vaccination for Healthcare Workers in COVID-19 Pandemic                       | 18 years        | Danish strain 1331       | Phase 3 (NCT0437936)    | TASK Applied Science / South Africa |
|         | Reducing COVID-19 Related Hospital Admission in Elderly by BCG Vaccination   | 60 years        | Danish strain 1331       | Phase 4 (NCT04417335)   | Radboud University / Netherlands   |
|         | Prevention of Respiratory Tract Infection and Covid-19 Through BCG Vaccination in Vulnerable Older Adults (BCG-PRIME) | 60 years        | Brazilian Moreau sub-strain | Phase 4 (NCT04537663)   | UMC Utrecht / Netherlands          |
| BCG     | Vaccine in Reducing Morbidity and Mortality in Elderly Individuals in COVID-19 Hotspots | 60 to 80 years  | Phase 3 (NCT04475302)    | Estimated enrollment : 2175                      | Tuberculosis Research Centre / India |
|         | Clinical Trial Evaluating the Effect of BCG Vaccination in Aging and/or SARS-CoV-2 Infected Individuals Among Healthcare Professionals During the COVID-19 Pandemic in Poland | 25 years        | Brazilian Moreau sub-strain | Phase 3 (NCT04648800)   | Hana Czajka & Medical Research Agency / Poland |
| BCG     | to Reduce Absenteeism Among Health Care Workers During the COVID-19 Pandemic (EDCT) | 18 years        | Danish strain 1331       | Phase 4 (NCT04641858)   | University of Southern Denmark / Denmark |
|         | Prevention, Efficacy and Safety of BCG Vaccine in COVID-19 Among Healthcare Workers | 18 years        | Danish strain 1331       | Phase 3 (NCT04461379)   | Hospital Universitario Dr. Jose E. Gonzalez / Mexico |
|         | Vaccination to Protect Healthcare Workers Against COVID-19 (BRACE)           | 18 years        | Danish strain 1331       | Phase 3 (NCT04327206)   | Murdoch Children Research Institute / Australia |
|         | COVID-19: BCG as Therapeutic Vaccine, Transmission Limitation, and Immunoglobulin Enhancement (BATTLE) | 18 years        | Danish strain 1331       | Phase 4 (NCT04369794)   | University of Campinas / Brazil    |
|         | Bacillus Calmette-guérin Vaccination to Prevent COVID-19 (ACTIVATEII)       | 50 years        | Moscow strain 361-1      | Phase 4 Completed (NCT04414267) / Actual enrollment : 301 | Hellenic Institute for the Study of Greece / Greece |
|         | Using BCG Vaccine to Protect Health Care Workers in the COVID-19 Pandemic    | 18 to 100 years | Danish strain 1331       | Phase 3 completed (NCT04373291) / Actual enrollment : 1293 | Bandim Health Project / Denmark |
|         | Using BCG to Protect Senior Citizens During the COVID-19 Pandemic            | 65 to 110 years | Danish strain 1331       | Phase 3 (NCT04542330)   | Bandim Health Project / Denmark   |
|         | Efficacy of BCG Vaccination in the Prevention of COVID-19 Via the Strengthening of Innate Immunity in Health Care Workers (COVID-BCG) | 18 years        | Danish strain 1331       | Phase 3 (NCT04384549)   | Assistance Publique - Hôpitaux de Paris / Paris |
| BCG     | Vaccine for Health Care Workers as Defense Against COVID 19 (BADAS)          | 18 to 75 years  | BCG Tice strain          | Phase 4 (NCT04348370)   | Texas A&M University / US          |
|         | Efficacy and Safety of VPM1002 in Reducing SARS-CoV-2 (COVID-19) Infection Rate and Severity (COBRA) | 18 years        | VPM1002a                  | Phase 3 Completed (NCT04390405) / Actual enrollment : 122 | University Health Network, Toronto / Canada |
|         | Study to Assess VPM1002 in Reducing Healthcare Professionals’ Absenteeism in COVID-19 Pandemic | 18 years        | VPM1002a                  | Phase 3 Completed (NCT04387409) / Actual enrollment : 59 | Vakzine Project Management GmbH / Germany |
|         | Study to Assess VPM1002 in Reducing Hospital Admissions and/or Severe Respiratory Infectious Diseases in Elderly in COVID-19 Pandemic | 18 years        | VPM1002a                  | Phase 3 Completed (NCT04435379) / Actual enrollment : 2038 | Vakzine Project Management GmbH / Germany |
Table 1. Continued

| Vaccine                                      | Study title                                      | Ages for study | Strain / Vaccine | Clinical stage / Enrollment | Sponsor / Country |
|----------------------------------------------|--------------------------------------------------|----------------|------------------|----------------------------|-------------------|
| Polio Vaccine (IPV) for SARS-CoV-2 and Prevention of Coronavirus Disease (COVID-19) |                     | 18 to 80       | Poliovirus vaccine (Sanofi Pasteur) | Phase 4 (NCT04639375) / Estimated enrollment : 300 | E-MO Biology Inc / US |
| MMR vaccine                                  | CROWN CORONATION: COVID-19 Research Outcomes Worldwide Network for CORONA virus prevention (CROWN CORONA) | 18             | M-M-R II® (Merck) | Phase 3 (NCT04333732) / Estimated enrollment : 30000 | Washington University School of Medicine / US |
| Herpes Zoster vaccine                        | Training the Innate Immune System Against SARS-CoV-2 (COVID-19) Using the Shingrix Vaccine in Nursing Home Residents (NH-Shingrix) | 65 to 100      | Shingrix® (Recombinant varicella zoster) | Early phase 1 (NCT04523246) / Estimated enrollment : 250 | Barbara Carlson Foundation / US |

Heterologous Vaccine Effects of Licensed Bacterial and Viral Vaccines on Trained Immunity

A number of lines of evidence have indicated that childhood vaccination with some live-attenuated vaccines, such as the tuberculosis vaccine, bacilli Calmette-Guerin (BCG), smallpox and polio vaccines, can induce beneficial, nonspecific, heterologous vaccine effects against unrelated pathogens by eliciting innate cell-mediated immune responses (Nascimento et al., 2020; Pasco and Anguita, 2020). These innate cell-based heterologous effects are referred to as trained immunity. For example, BCG vaccination of neonates has been reported to reduce neonatal and infant mortality independent of its effect on tuberculosis (Thysen et al., 2020). In addition, vaccination against smallpox can lead to partial protection against measles, pertussis and scarlet fever, suggesting a long-lasting nonspecific protective effect associated with the vaccine, independent of adaptive immune responses based on T and B lymphocytes (Sánchez-Ramón et al., 2018).

Innate immune cells, such as monocytes and natural killer (NK) cells that are stimulated by inducers imprint memory ability to prepare for a secondary infection through epigenetic modification, metabolic changes and production of pro-inflammatory cytokines, such as IL-6, TNF-α and IL-18 (Neta et al., 2016, 2020a). Since trained immunity provides long-term immunological memory against unrelated pathogens, specifically about three months duration in a mouse, it can be used for the development of effective vaccines to promote host resistance against a broad range of pathogens by training innate immune cells (Sánchez-Ramón et al., 2018; Gyssens and Netea, 2019; Netea et al., 2020b). Accordingly, various clinical trials have been performed to test the protective effects of licensed live vaccines that are already being used for other infections against COVID-19.

BCG

BCG is one of the most widely used live vaccines against Mycobacterium tuberculosis infections globally. It was generated as an attenuated strain via serial subcultures of Mycobacterium bovis to prevent dissemination in the body. BCG has been shown to provide protection against not only tuberculosis but also different infections unrelated to tuberculosis, including leprosy, Buruli ulcer, malaria, respiratory viral infections and yellow fever virus infections (Basak et al., 2021). Given the potential of BCG to prevent infections caused by unrelated pathogens, as described above, the use of BCG for the prevention of SARS-CoV-2 infection has attracted increasing attention. Several epidemiologic studies have demonstrated that nations with active BCG vaccination programs, including Japan and South Korea have lower incidence rates and reduced mortality associated with COVID-19 (Kumar et al., 2020; Jakhmola et al., 2021). Consistently, the data from the WHO immunization monitoring program have also shown an inverse relationship between BCG vaccination and COVID-19 incidence and mortality, suggesting the potential of BCG vaccination to control the COVID-19 pandemic via heterologous vaccine effects (Marín-Hernández et al., 2021). Currently, a total of 21 randomized controlled clinical trials are in progress to evaluate the protective effects of BCG vaccination against COVID-19 at various ages in different countries, including the Netherlands, Brazil, Denmark, Australia, the US, Germany and France (Table 1). In addition, there are three phase 3 clinical trials for a recombinant BCG termed VPM1002 to test its protective effects against COVID-19 (NCT04439045, NCT04387409, and NCT-04435379); this recombinant BCG was originally designed to further potentiate the vaccine effect of BCG against tuberculosis infections (Nieuwenhuizen et al., 2017).

However, there is currently no clear evidence demonstrating that vaccination with BCG or the rBCG VPM1002 can protect against COVID-19 infections. In Taiwan, whether neonatal BCG vaccination could alleviate severe COVID-19 symptoms in the 4–24 year age group was investigated. There was no significant difference in COVID-19 symptoms between the groups that received and did not receive BCG vaccination (Su et al., 2021). Nonetheless, the potential of BCG as a COVID-19 vaccine has not been completely eliminated. More in-depth studies with more cases with reliable vaccination records grouped by different types of BCG strains and different periods after vaccination might be needed.

Live-attenuated viral vaccines

Some live-attenuated viruses could be strong inducers of trained immunity. Respiratory adenovirus infection has been
reported to induce trained immunity in the lungs by generating long-lasting memory alveolar macrophages, resulting in protective effects against bacterial infection (Yao et al., 2018). This suggests the potential of adenosivirus to be effectively used as a new vaccine strategy for protecting against respiratory disease.

The MMR vaccine has been used globally since 2001 and can provide effective protection against measles, mumps and rubella. It has been hypothesized that the MMR vaccine could confer cross-protection against or reduce the severity of COVID-19 infection (Anbarasu et al., 2020). Consistently, a recent study showed that there is a significant inverse correlation between mumps IgG titers and COVID-19 severity in individuals who had received the MMR vaccine in childhood (Gold et al., 2020). A negative case-control study using a recent measles outbreak with MMR vaccination was conducted among healthcare workers in Sweden to investigate the potential protective effect of the MMR vaccine against SARS-CoV-2. The results indicated that while no substantial protective effect of the MMR vaccine was observed in the whole study population, significant effectiveness in preventing symptomatic disease was seen in men, suggesting that there may be a protective effect of the MMR vaccine against SARS-CoV-2 in males but not females (Lundberg et al., 2021). In addition, the MMR vaccine has been reported to exert protective effects against COVID-19 in adults in a retrospective cohort study in Turkey (Yengil et al., 2021). In line with these studies, two clinical trials (NCT04333732 and NCT0464623) are currently underway to evaluate the efficacy of the vaccine in preventing COVID-19 infection based on MMR-induced trained immunity (Table 2).

Oral polio vaccination (OPV) has also been reported to exert beneficial nonspecific effects, particularly against respiratory infections. Of note, it has recently been reported that OPV can lead to a 62% reduction in deaths caused by respiratory infections during the post-neonatal period (1–35 months) in Bangladesh (Andersen et al., 2018; Nielsen et al., 2021a, 2021b). Hence, there are two phase 4 clinical trials (NCT-04445428 and NCT04639375) to evaluate whether OPV can ameliorate COVID-19 severity (Table 2). Furthermore, a retrospective study provided additional evidence for COVID-19 protection via cross-protective humoral immunity induced by OPV. Sera from a series of vaccinated people also inhibited SARS-CoV-2 infection in vitro via the production of cross-protective antibodies that were induced by OPV and capable of binding the RNA-dependent RNA polymerase.

Table 2. Licensed viral vaccines being tested in clinical trials to evaluate the heterologous protective effects or antigen-specific protective effects against COVID-19

| Vaccine          | Study title                                                                 | Strain / Vector | Ages for study | Administration / Dose | Clinical stage / Enrollment | Sponsor / Country             |
|------------------|------------------------------------------------------------------------------|-----------------|----------------|------------------------|-----------------------------|-----------------------------|
| Adenovirus       | ADZ122 Phase III Double-blind, Placebo-controlled Study of ADZ1222 for the Prevention of COVID-19 in Adults | ChAdOx1         | 18 to 130      | IM / Two dose          | Phase 3 (NCT04516746) (Falsy et al., 2021) Actual enrollment : 52459       | AstraZeneca / UK             |
|                  | National Cohort Study of Effectiveness and Safety of SARS-CoV-2/COVID-19 Vaccines (ENFORCE) | ChAdOx1         | 18             | IM / T wo dose         | Phase 4 (NCT04760132) Estimated enrollment : 10000                     | Jens D Lundgren, MD / Denmark |
|                  | JNJ-78436735 (Ad26.COV.S) Participants With or Without Stable Co-morbidities Associated With Progression to Severe COVID-19 at Different Stages of the Protocol | Ad26            | 18             | IM / Single dose       | Phase 3 (NCT04505722) Actual enrollment : 44325                       | Johnson & Johnson / US       |
| Gam-COVID -Vac   | Clinical Trial of Efficacy, Safety, and Immunogenicity of Gam-COVID-Vac Vaccine Against COVID-19 (RESIST) | Ad26 / Ad5      | 18 to 111      | IM / Two dose          | Phase 3 (NCT04530396) (Logunov et al., 2021) Actual enrollment : 33758    | Gamaleya Research Institute / Russia |
|                  | Study of Gam-COVID-Vac in Adolescents (OLSTAD)                               | Ad26 / Ad5      | 12 to 17       | IM                      | Phase 2, 3 (NCT04954092) Estimated enrollment : 350                    | Gamaleya Research Institute / Russia |
|                  | Clinical Trial of Efficacy, Safety, and Immunogenicity of Gam-COVID-Vac Vaccine Against COVID-19 in Belarus | Ad26 / Ad5      | 18 to 60       | IM / Two dose          | Phase 3 (NCT04564716) Actual enrollment : 100                           | Gamaleya Research Institute / Russia |
| Sputnik Light    | Study to Evaluate Efficacy, Immunogenicity and Safety of the Sputnik-Light (SPUTNIK-LIGHT) Vaccine | Ad26            | 18 to 111      | IM / Single dose       | Phase 3 (NCT04741061) Estimated enrollment : 6000                      | Gamaleya Research Institute / Russia |
|                  | An Open Study on the Safety, Tolerability, and Immunogenicity of “Sputnik-Light” Vaccine | Ad26            | 18 to 111      | IM / Single dose       | Phase 1, 2 (NCT04713488) Estimated enrollment : 110                     | Gamaleya Research Institute / Russia |
| Ad5-nCoV (Convidecia) | Phase III Trial of COVID-19 Vaccine of Ad5-nCoV in Adults 18 Years Old and Above | Ad5             | 18             | IM / Single dose       | Phase 3 (NCT04526990) Estimated enrollment : 40000                     | CanSino Biologics Inc. / China |
|                  | A Clinical Trial of a COVID-19 Vaccine Named Recombinant Novel Coronavirus Vaccine (Adenovirus Type 5 Vector) | Ad5             | 6–17, 18–49, Over 56 | IM / Two dose          | Phase 2 (NCT04566770) Estimated enrollment : 481                      | CanSino Biologics Inc. / China |
|                  | Phase I/II Clinical Trial of Recombinant Novel Coronavirus (COVID-19) Vaccine (Adenovirus Type 5 Vector for Inhalation) | Ad5             | 18             | IM, Inhalation / Single or two dose | Phase1, 2 (NCT04840992) Estimated enrollment : 840   | CanSino Biologics Inc. / China |
|                  | Phase I/II Clinical Trial of Recombinant Novel Coronavirus Vaccine (Adenovirus Type 5 Vector) in Canada | Ad5             | 18 to 55       | IM / Single or two dose | Phase 1, 2 (NCT04398147) Estimated enrollment : 696                  | CanSino Biologics Inc. / China |
|                  | Phase I Clinical Trial of a COVID-19 Vaccine in 18–60 Healthy Adults (CTCOVID-19) | Ad5             | 18 to 60       | IM / Single dose       | Phase 1 (NCT04313127) Actual enrollment : 108                         | CanSino Biologics Inc. / China |
(RdRp) of both poliovirus and SARS-CoV-2 (Comunale et al., 2021). There is a clinical trial to investigate the protective effects of the Shingrix vaccine against COVID-19 (NCT-04523246); this vaccine is composed of recombinant varicella zoster virus and the adjuvant AS01B (Table 2). Its safety has been already proved, as the herpes zoster vaccine was approved by the U.S. FDA in 2017 (FDA, 2021) and the clinical trial focuses on the induction of protection against SARS-CoV-2 by training the innate immune system.

**COVID-19 Vaccines Based on a Recombinant Bacterial Vector System**

Bactofection, using bacterial vectors to deliver foreign genes into host cells, is a promising vaccine platform that allows the introduction of antigens into specific target cells, including antigen-presenting cells (Motin and Torres, 2009; Chamekh, 2015). There are several advantages of using recombinant bacterial vector systems for vaccine development. First, most bacterial vectors can easily incorporate large target sequences via plasmid or phage systems, and their generation requires relatively little labor and inexpensive production procedures (Lin et al., 2015). Second, live bacterial vectors themselves can act as vaccine adjuvants via innate cell activation, resulting in the induction of both cell-mediated immune responses and humoral immune responses against the delivered target antigens, unlike protein-based subunit vaccines, which can induce mainly humoral immune responses (Detmer and Glentning, 2006; Silva et al., 2014). Third, bacterial vectors can lead to further enhanced vaccine effects by inducing trained immunity, as described above (Goodridge et al., 2016; Covían et al., 2019). Despite the overt advantages of this approach in vaccine development, there is a safety concern that these bacteria might become activated and cause disease in the body, which makes it difficult to recruit participants for clinical trials (WHO, 2021b). Herein, we have introduced the current status of COVID-19 vaccines based on recombinant bacteria, focusing on the published literature (Table 5).

**Francisella tularensis**

*Francisella tularensis* is a Gram-negative aerobic bacterium that can infect both invertebrates and mammals, causing sepsis, fever, pneumonia and possibly death in humans (Feldman et al., 2001; Steiner et al., 2014). Although the *F. tularensis* live vaccine strain has been reported to provide some protection against tularemia, it shows high toxicity and reduced protective effects against aerosolized *F. tularensis* infections (Jia et al., 2009). Hence, its virulence has been attenuated for application as a reliable vaccine platform mainly through deletion of the capsule synthesis gene (*capB*) (Jia et al., 2010). In a preclinical study, plasmids for overexpressing the pathogenicity island proteins of *F. tularensis* were modified to develop the COVID-19 vaccine by incorporating different regions of SARS-CoV-2. Each recombinant *F. tularensis* strain was administered intradermally or intranasally with a prime-boost vaccination regimen. Consequently, co-expression of the SARS-CoV-2 membrane and nucleocapsid protein ameliorated the severity of lung pathology by SARS-CoV-2 infection in a golden Syrian hamster model by inducing anti-nucleocapsid antibody production (Jia et al., 2021) (Table 5).

**Mycobacterium paragordonae**

There are more than 150 species within the genus *Mycobacterium*, including strict human pathogens such as *M. tuberculosis* and *M. leprae*, and nontuberculous mycobacteria (NTM) with lower pathogenic potential, most of which exist in the environment (Pereira et al., 2020). Mycobacteria have a thick lipid-rich cell wall, and the cell wall components can act as strong vaccine adjuvants to elicit innate cell activation. *Mycobacterium paragordonae* (Mpg) is a slow-growing NTM that exists in the environment and rarely causes diseases in humans and animals. Mpg is temperature sensitive and shows an optimal growth rate at 30°C but cannot grow at 37°C, indicating its potential as a safer vaccine for tuberculosis infection than BCG due to its inability to survive in the human body (Kim et al., 2019). Indeed, it has been reported that compared to BCG, Mpg showed higher safety in both in vivo and in vitro studies and elicited a stronger protective effect against both *M. tuberculosis* and *M. abscessus* infections (Kim et al., 2017). Moreover, recombinant Mpg expressing HIV-1 p24 (rMpg-p24) can induce enhanced p24 specific immune responses in vaccinated mice as evidenced by increased p24-specific T lymphocyte proliferation, gamma interferon induction, antibody production and cytotoxic T lymphocyte (CTL) responses (Kim et al., 2019) demonstrating the potential of recombinant Mpg (rMpg) as a recombinant bacteria-based vaccine platform. Hence, in a recent study, SARS-CoV-2 receptor-binding domain (RBD)-expressing rMpg (rMpg-RBD-7) was generated to elicit RBD-specific immune responses in a mouse model with single- or two-dose vaccination regimens. Moreover, rMpg-RBD-7 led to enhanced cell-mediated immune responses as well as humoral immune responses, supporting its feasibility as a COVID-19 vaccine candidate (Kim et al., 2021) (Table 5).

**Salmonella typhimurium**

*Salmonella* is one of the most common causes of food poisoning and can infect various types of cells, including epithelial cells, macrophages and dendritic cells. *Salmonella typhimurium* is a Gram-negative bacterium that is usually found in the intestinal lumen and has been intensively investigated as a vaccine delivery system (Chin’ombe, 2013; Roland and Brenneman, 2013; Clark-Curtiss and Curtiss, 2018). Deletion of the virulence transcriptional regulatory protein phoP enabled the generation of an attenuated *S. typhimurium* vaccine platform (Groisman et al., 1989; Mether et al., 2004). In a recent study, oral administration of recombinant attenuated *S. typhimurium*, which contains the full-length spike gene of SARS-CoV-2, exerted a protective effect against SARS-CoV-2 infection, mainly by inducing SARS-CoV-2-specific humoral immunity, in a rat model, suggesting the feasibility of a COVID-19 oral vaccine (Zhu et al., 2020b) (Table 5).

**Probiotics**

Since SARS-CoV-2 is a respiratory disease that is transmitted through the upper respiratory mucosa, mucosal immunity
Table 3. Vaccine candidates based on non-replicating viral vectors being tested in clinical trials to evaluate the protective effects against COVID-19

| Vaccine | Study title | Vector | Ages for study | Administration / Dose | Clinical stage / Enrollment | Sponsor / Country |
|---------|-------------|--------|----------------|-----------------------|-----------------------------|------------------|
| Adenovirus | AdCLD-CoV19 | hAd5/35 | 19 to 64 | IM / Single dose | Phase 1, 2 (NCT04666012) Actual enrollment: 150 | Celllid Co., Ltd. / South Korea |
| | Safety and Immunogenicity Study of AdCLD-CoV19: A COVID-19 Preventive Vaccine in Healthy Volunteers | hAd5/35 | 19 to 64 | IM / Single dose | Phase 1 (NCT05047692) Estimated enrollment: 40 | Celllid Co., Ltd. / South Korea |
| Modified Vaccina Virus | SC-Ad6-1 | Ad5 | 18 to 70 | Oral Tablet / Two dose | Phase 2 (NCT0567933) Estimated enrollment: 896 | Vaxart / US |
| | A Phase 1, First-In-Human Study of the Investigational COVID-19 Vaccine SC-Ad6-1 in Healthy Volunteers | Ad5 | 18 to 70 | Oral Tablet / Two dose | Phase 2 (NCT0504791423) Actual enrollment: 10300 | Reithera Srl. / Italy |
| | COVITAR (GRAd-COV2) | GRAd | 18 | IM / Single or two dose | Phase 2, 3 (NCT04954287) Estimated enrollment: 90 | CyanVac LLC / US |
| | Study of GRAd-COV2 for the Prevention of COVID-19 in Adults (COVITAR) | GRAd | 18 | IM / Single or two dose | Phase 2, 3 (NCT04954287) Estimated enrollment: 90 | Reithera Srl. / Italy |
| Modified Vaccina Virus | COH04S1 | MVA | 18 to 55 | IM / Two dose | Phase 1 (NCT04639466) Estimated enrollment: 129 | City of Hope Medical Center / US |
| | A Synthetic MVA-based SARS-CoV-2 Vaccine, COH04S1, for the Prevention of COVID-19 | MVA | 18 to 55 | IM / Two dose | Phase 1 (NCT04639466) Estimated enrollment: 129 | City of Hope Medical Center / US |
| | SARS-CoV-2 Vaccine (COH04S1) Versus Emergency Use Authorization SARS-CoV-2 Vaccine for the Treatment of COVID-19 in Patients With Blood Cancer | MVA | 18 | IM / Two dose | Phase 2 (NCT04977024) Estimated enrollment: 240 | City of Hope Medical Center / US |

Table 4. Vaccine candidates based on replicating viral vectors being tested in clinical trials to evaluate the protective effects against COVID-19

| Vaccine | Study title | Vector | Ages for study | Administration / Dose | Clinical stage / Enrollment | Sponsor / Country |
|---------|-------------|--------|----------------|-----------------------|-----------------------------|------------------|
| Vesicular stomatitis virus | IIBR-100 | VSV | 18 to 90 | IM / Two dose | Phase 2, 3 (NCT04990466) Estimated enrollment: 20000 | NeuroRx, Inc. / Israel |
| | Phase 2b/3 Trial of VSV-AG SARS-CoV-2 Vaccine (BRILIFE) Against Approved Comparator Vaccine (BRILIFE002) | VSV | 18 to 90 | IM / Two dose | Phase 2, 3 (NCT04990466) Estimated enrollment: 20000 | NeuroRx, Inc. / Israel |
| Measles virus | TMV-083/V-591 | MeV | 18 to 55 | IM / Two dose | Phase 1 (NCT0497298) Actual enrollment: 90 | Institute Pasteur / France |
| | Clinical Trial to Evaluate the Safety and Immunogenicity of The COVID-19 Vaccine (COVID-19-101) | MeV | 18 to 55 | IM / Two dose | Phase 1 (NCT0497298) Actual enrollment: 90 | Institute Pasteur / France |
| Influenza Virus | DelNS1-nCoV-RBD LAIV | MVA | 18 to 55 | IM / Two dose | Phase 1 (NCT04809389) Estimated enrollment: 115 | The University of Hong Kong / Hong Kong |
| | Study to Evaluate Safety and Immunogenicity of DelNS1-nCoV-RBD LAIV for COVID-19 | MVA | 18 to 55 | IM / Two dose | Phase 1 (NCT04809389) Estimated enrollment: 115 | The University of Hong Kong / Hong Kong |
| Newcastle Disease Virus | rNDV | NDV | 18–55 | IM / Two dose | Phase 1 (NCT04871737) Estimated enrollment: 90 | Laboratorio Avi-Mex / Mexico |
| | Study of a Live rNDV Based Vaccine Against COVID-19 | NDV | 18–55 | IM / Two dose | Phase 1 (NCT04871737) Estimated enrollment: 90 | Laboratorio Avi-Mex / Mexico |

COVID-19 Vaccines Based on a Recombinant Viral Vector System

Viral vectors have been considered reliable vehicles for gene delivery into host cells. Since the initial attempt in the 1970s, the application of this approach has been widely extended, mainly for vaccine development and gene therapy (Ura et al., 2014; Bull et al., 2019). Although viral vectors originate from pathogenic viruses, they have typically been engineered to be attenuated by deleting genes that are necessary for replication or pathogenicity (Bull et al., 2019). Since viral vector-based vaccines can generate endogenous antigens in a broad range of host cells, they can effectively induce both humoral and cell-mediated immune responses (Vrba et al., 2020). In...
addition, large-scale manufacturing is allowed, and they do not require freezing for transport or storage (Li et al., 2021b). However, pre-existing immunity to the viral vector can impair the use of the vector as a vaccine delivery platform (Shirley et al., 2020). Herein, we discuss two categories of viral vector-based vaccines: non-replicating and replicating viral vector vaccines.

Based on published research articles, 9 types of viral vectors have been developed for the COVID-19 vaccine, including 4 non-replicating viral vectors (Tables 2 and 3), namely, the adenovirus vector (AdV), modified vaccinia virus Ankara (MVA) vector, parainfluenza virus vector (PIV), and lenti-virus (LV), and 5 replication competent viral vectors (Table 4), namely, the single-cycle adenovirus (SC-Ad), the vesicular stomatitis virus (VSV) vector, the measles virus vector (MeV), influenza virus (IV), and Newcastle disease virus (NDV) (Bezbaruah et al., 2021).

### Non-replicating viral vector vaccines

Replication-deficient viral vector vaccines had not been approved before the COVID-19 pandemic. However, a total of 5 types of AdV-based vaccines, AZD1222 (Oxford/Astra-Zeneca), JNJ-78436735 (Johnson & Johnson), Ad5-nCoV (CanSino Biologics of Chinese), Sputnik V and Sputnik Light (Gamaleya Research Institute of Russia), currently have emergency approval and are in clinical use in one or more nations (Abdulla et al., 2021; Francis et al., 2021) (Table 6). Non-replicating viral vectors are typically generated by genetic deletion of replication ability. Through genome engineering, a larger space is created in the genome as many genes are removed, and longer inserts can be incorporated (Choi and Chang, 2013). Although high doses can be administered to elicit sufficient immune responses due to the lack of replication capacity, this is considered a safer vaccine strategy than replicating viral vector vaccines (Robert-Guroff, 2007).

### Adenovirus (AdV) vectors

For the generation of non-replicating viral vector vaccines, adenovirus (AdV) vectors have been used most frequently. The AdV genome is 26–45 kb of linear double-stranded DNA, and the virus can be classified into different groups and serotypes. AdV vectors can be modified to eliminate replication ability by deleting the E1 genes that are necessary for replication. In addition, E3 region genes can also be deleted to further create space for larger insert genes (Ura et al., 2014). However, although AdV vectors can provide an efficient gene delivery system in host cells, they are not appropriate for repeated vaccination, especially in the case of human AdV. Since more than 80% of people have been exposed to human AdV, they may already possess pre-existing neutralizing antibodies that bind to injected AdV vectors, interfering with entry into target cells. To avoid pre-existing neutralizing antibodies, rare types of human AdV vectors or nonhuman vectors, such as chimpanzee adeno-
viruses, are being used for vaccine delivery (Li et al., 2021a; Mendonça et al., 2021).

Among the 5 vaccines approved for emergency use, AZD-1222 (ChAdOx1 nCoV-19, NCT04516746, NCT04760132) is based on a chimpanzee adenovirus vector that contains the SARS-CoV-2 spike protein (Table 2). It showed 70.4% vaccine efficacy after clinical testing in 23 countries and received approval in over 120 countries (Falsey et al., 2021). JNJ-78436735 (NCT04505722) is an adenovirus serotype 26-based vaccine that showed 66% efficacy after 4 weeks with a single dose (WHO, 2021a). In addition, a two-vector vaccine termed Gam-COVID-Vac (SputnikV, NCT04741061, NCT04530396, NCT04954092, NCT04713488, and NCT04564716) has been developed and are currently authorized in 72 countries (Table 2). This vaccine is a combination of recombinant human Ad26 and Ad5 vectors containing the same spike gene of SARS-CoV-2 incorporated (Jones and Roy, 2021). According to recent reports, Gam-COVID-Vac showed 91.6% vaccine efficacy, with 45 severe adverse events in 16,427 patients (Logunov et al., 2021). Unlike Sputnik V, Sputnik Light is composed of only the Ad26 vector and has been applied in a single-dose regimen (NCT04741061, NCT04713488) (Table 2). It showed 75.28% vaccine efficacy against delta variants in the group between the ages of 18 and 59 years, suggesting that it has even higher efficacy than other two-shot vaccines (Dolzhikova et al., 2021). Additionally, an oral tablet COVID-19 vaccine termed VXA-COV2-1.1-S expressing two different SARS-CoV-2 proteins, spike (S) and nucleoprotein (N) was designed to protect against both prevalent and emerging strains. A phase I study revealed that VXA-COV2-1 is generally well tolerated and could have broader activity against variants with low coverage by first-generation vaccines incorporated (Mascellino et al., 2021).

Currently, its safety, immunogenicity and efficacy are being evaluated in a phase 2 clinical trial (NCT05067933) (Table 3). Two clinical trials for AdCLD-CoV19, a novel COVID-19 vaccine based on the Ad5/35 chimeric adenoviral vector, are underway to evaluate their immunogenicity and protective effects against COVID-19 in healthy volunteers (NCT04666012, NCT05047692) (Table 3). Moreover, a preclinical study of a gorilla adenovirus-based COVID-19 vaccine called COVITAR (GRAd-COV2) in both mouse and macaque models indicated that GRAd-COV2 could induce neutralization of SARS-CoV-2 infection and elicit a robust cell-mediated immune response (Capone et al., 2021). Therefore, a phase 2/3 trial is also underway for COVITAR to assess its safety and efficacy in protecting against COVID-19 (NCT04791423) (Table 3).

**Modified vaccinia virus Ankara (MVA) vectors:** MVA is an attenuated vaccinia virus that has a replication-incompetent phenotype due to loss of approximately 15% of the vaccinia virus genome. Since its biological safety in vivo has been proven, recombinant MVA viruses can be handled at biosafety level 1 (Altenburg et al., 2014). Despite its high safety, this type of vector can also lead to elicit immune responses comparable to those induced by replication competent vaccinia virus vectors. However, there are some environmental risks of dissemination of the recombinant MVA vector through excreta and blood from the treated patient when high-risk foreign genes are inserted (Verheust et al., 2012).

COH04S1 is a synthetic MVA vector that contains both the spike and nucleocapsid genes of SARS-CoV-2. A phase 1 clinical trial is ongoing to evaluate the safety and optimal dose of COH04S1, and the next phase of the clinical trial is planned to examine vaccine efficacy by comparison with vaccines that have been given an Emergency Use Authorization

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**Table 6. Different COVID-19 vaccines currently authorized for human use worldwide**

| Vaccine type | Vaccine name | Number of approved countries | Clinical trials | Development |
|-------------|-------------|-------------------------------|-----------------|-------------|
| Non-replicating viral vector | JNJI-78436735 (Ad26.COV.S) | 75 | 14 trials in 18 countries | Johnson & Johnson |
| | AZD1222 | 124 | 47 trials in 23 countries | Oxford/AstraZeneca |
| | Sputnik V | 73 | 22 trials in 7 countries | Gamaleya |
| | Sputnik Light | 19 | 4 trials in 2 countries | Gamaleya |
| | Ad5-nCoV (Convidecia) | 9 | 11 trials in 6 countries | CanSino |
| mRNA | mRNA-1273 | 76 | 32 trials in 8 countries | Moderna |
| | BNT162b2 | 103 | 42 trials in 21 countries | Pfizer / BioNtech |
| Protein subunit | ZF2001 | 3 | 8 trials in 5 countries | Anhui Zhifei Longcom |
| | CLGB-66 | 4 | 5 trials in 1 country | Center for Genetic Engineering & Biotechnology |
| | EpiVacCorona | 2 | 3 trials in 1 country | FBRI |
| | MVC-COV1901 | 1 | 7 trials in 2 countries | Medigen |
| | COOVAX | 1 | 2 trials in 1 country | Serum Institute of India |
| | COVAX-19 | 1 | 4 trials in 2 countries | Vaxine / Cinnagene Co. |
| Inactivated | Covaxin | 9 | 7 trials in 1 country | Bharat Biotech |
| | BBIBP-CorV | 68 | 15 trials in 10 countries | Sinopharm |
| | CoronaVac | 42 | 24 trials in 8 countries | Sinovac |
| | QozVac | 2 | 3 trials in 1 country | Kazakhstan RIBSP |
| | SARS-CoV-2 Vaccine | 1 | 5 trials in 1 country | Minhai Biotechnology Co. |
| | Kovivac | 1 | 2 trials in 1 country | Chumakov Center |
| | COVID-19 Inactivated Vaccine | 1 | 4 trials in 1 country | Shifa Pharmed Industrial Co. |
| DNA | ZyCoV-D | 1 | 5 trials in 1 country | Zydus Cadila |
respiratory infections, including influenza virus, influenza A vaccine has been used as an efficient viral vector for protection against Mycobacterium tuberculosis (Garcia-Arriaza et al., 2021). Another preclinical study showed that MVA-SARS-CoV-2 S vaccination led to reduction of viral loads in lung against SARS-CoV-2 infection in human ACE2-transduced mice (Tscherne et al., 2021) (Table 5).

Parainfluenza virus vectors (PIVs): Parainfluenza virus belongs to the Paramyxoviridae family, which consists of negative single-stranded RNA viruses. It has been reported as the second most common pathogen that causes respiratory diseases in children under 5 years of age (Álvarez-Argüelles et al., 2018). Parainfluenza virus type 5 (PIV5) has been effectively used as an efficient viral vector for protection against respiratory infections, including influenza virus, influenza A H5N1 virus, rabies virus, respiratory syncytial virus and Mycobacterium tuberculosis (Chen et al., 2015; Xiao et al., 2021). Unlike positive single-stranded RNA viruses, the PIV5 vector is stable and has a low frequency of mutation in host cells. Furthermore, the serum of only approximately 30% of people was found to have neutralizing antibodies at low titers prior to exposure to PIV5 (Wang et al., 2017).

A COVID-19 vaccine, CVXGA1 (PIV5-SARS-CoV-2), based on a non-replicating PIV5 vector has been developed, and it showed no weight loss and 100% survival rate by inhibiting SARS-CoV-2 replication in the upper respiratory tract in a mouse model (An et al., 2021) (Table 5). A phase 1 clinical study is also investigating its safety and immunogenicity in humans (NCT04954287) (Table 3).

Lentivirus vector (LV): Since the non-replicative lentiviral vector (LV) can elicit powerful adaptive immunity, it represents a promising vaccine platform for delivering target antigens (Ku et al., 2021b). In an HIV-1 vaccine trial, the safety of this vector has also been proven in humans (2011006260-52 EN), and it has been used for gene therapy studies. Furthermore, LV has a broad host cell range and a low risk of reduced vaccine efficacy due to pre-existing immunity when it is enveloped with vesicular stomatitis virus G glycoprotein (VSV-G) (Ku et al., 2021a). In a recent study, it was reported that intranasal vaccination with an LV vector encoding SARS-CoV-2 spike protein reduces viral loads in lungs by inducing mucosal immunity in rodents, suggesting the feasibility of LV-based intranasal vaccination against SARS-CoV-2. Additionally, non-integrative version of this vector for human clinical trials also showed less severe pulmonary lesions with low copies of SARS-CoV-2 RNA in lungs (Ku et al., 2021b) (Table 5).

Replicating viral vectors

Unlike non-replicating viral vector vaccines, replicating viral vectors are already being manufactured for worldwide use. rVSV-ZEBOV was the first approved vaccine for Ebola based on a replication-competent viral vector in 2019 (Ku et al., 2021a). Replication competent vectors can induce robust and persistent immune responses by producing many copies of antigen at low doses in host cells. Due to its strong immune induction ability, this system is also considered an effective mucosal delivery platform (Robert-Guroff, 2007). However, compared to replication-deficient viral vectors, these vectors have limited space for inserted genes and still have some risk of genotoxic events caused by excessive or mutated antigen production (Choi and Chang, 2013).

Adenovirus vector: A “single-cycle” Ad (SC-Ad) vector has been used for the development of some vaccines, including vaccines against influenza A, HIV-1 or Clostridium difficile (Barry et al., 2020). In the best currently available SC-Ad format, since key late genes of SC are deleted, the virus can replicate its genome but cannot produce progeny adenovirus virions. It has been reported that SC-Ad can elicit higher and more persistent transgene-specific IgA production than non-replicating Ad after a single intranasal immunization in hamsters (Crosby et al., 2015). Therefore, a COVID-19 vaccine using an “SC-Ad6 vector” is also being evaluated in a phase I clinical study (NCT04839042) (Table 3).

Vesicular stomatitis virus (VSV) vector: VSV contains a negative single-stranded RNA genome and belongs to the Rhabdoviridae family with rabies virus. It can infect a broad range of hosts and often causes mild illnesses in humans. VSV vectors have been examined as efficient delivery platforms in vaccine development studies (Tober et al., 2014). Since humans are rarely exposed to this vector, there are low titers of pre-existing antibodies, and the vector is less pathogenic due to modification of the VSV vector via replacement of its glycoprotein with other proteins. Additionally, the authorization of recombinant VSV for the Ebola vaccine in 2019 demonstrated its safety (Heppner et al., 2017; Bache et al., 2020). A research group in Israel showed that the replacement of VSV glycoprotein with the spike protein of SARS-CoV-2 (VSV-ΔG-spike), termed IIBR-100, leads to the production of SARS-CoV-2 neutralizing antibodies after single-dose vaccination in a hamster model (Yahalom-Ronen et al., 2020). Furthermore, a phase 2/3 clinical trial was performed in 2021 to evaluate the protective efficacy of this approach after massive immunization (NCT04990466) (Table 4). It has been reported that another VSV vector-based COVID-19 vaccine from a Washington University research group, which is also generated by replacing the native glycoprotein gene with the SARS-CoV-2 spike gene, could elicit production of antibodies against the RBD associated with human angiotensin-converting enzyme 2 (ACE2) at high titers. Two doses of VSV-eGFP-SARS-CoV-2 vaccination showed a reduction of lung inflammation and transferring of the sera from vaccinated mice to SARS-CoV-2-challenged mice reduced viral burdens, supporting its protective effect (Case et al., 2020) (Table 5).

Measles virus vector (MeV): Measles is one of the most contagious diseases, and it undergoes aerosol transmission. It has a negative single-stranded RNA genome and belongs to the Paramyxoviridae family. Live-attenuated MeV vaccines have been proven to be safe and effective since the 1960s, and MeV vectors have been a prominent delivery platform in studies of vaccines against HIV, HBV, HCV, influenza virus, and dengue virus (Zuniga et al., 2007; Frantz et al., 2018).

In recent studies, MeV vectors were engineered with different SARS-CoV-2 spike genes and RBD genes to compare their vaccine efficacy in vivo. Notably, it was found that recombinant MeV with the target antigen inserted into the pre-
fusion S (PreS) region had the strongest protective effects with reduced severity of lung pathology by preventing the cytokine storm and viral replication in the lungs (Lu et al., 2021). When the full-length spike gene was incorporated, recombinant MeV elicited Th1-biased immune responses, inducing not only a neutralizing antibody response but also S protein-specific clearance ability (Hörner et al., 2020) (Table 5). Furthermore, a novel MeV-based COVID-19 vaccine termed TMV-083/V-591 was tested for safety and immunogenicity in a phase 1 clinical trial with 90 participants divided into high- and low-dose groups (NCT04497298) (Table 4).

**Influenza virus (IV) vector:** Influenza vaccines are updated frequently because of the fast evolution of the virus, and most authorized vaccines use inactivated or attenuated forms. A quadrivalent inactivated influenza vaccine that contains killed H1N1, H3N2 and influenza B virus strains was developed in 2012 and is often modified with the annual circulating strains (Nuwarda et al., 2021). Influenza virus contains a negative single-stranded RNA genome large enough to express long foreign gene sequences and leads to efficient induction of mucosal immune responses. Influenza A, one of the most common types of influenza viruses, has been developed as a vaccine platform to protect against respiratory diseases (Barria et al., 2013; Pérez-Girón et al., 2014).

Replacement of the neuraminidase (NA)-coding region of IV with the SARS-CoV-2 spike RBD sequence was assessed for its protective effect in a mouse model. It could generate a neutralizing antibody against SARS-CoV-2 with a single intranasal immunization in a mouse model (Loes et al., 2020). Another preclinical study used a recombinant influenza A virus, which expresses the SARS-CoV-2 spike RBD incorporated into the hemagglutinin ORF. It can generate effective neutralizing antibodies and provide protection against SARS-CoV-2, especially after boosting post-immunization (Koonpaew et al., 2021) (Table 5). Furthermore, a clinical trial of live-attenuated recombinant IV (DelNS1-nCoV-RBD LAIV), based on a nonstructural NS1 protein-deficient influenza A virus vector, was undertaken to evaluate its safety and immunogenicity after two doses administered intranasally (NCT04809389) (Table 4).

**Newcastle disease virus (NDV) vector:** Newcastle disease virus (NDV) belongs to the **Paramyxoviridae** family and was first identified in Indonesia in 1926. Although its zoonoses are associated with birds, this virus rarely causes influenza-like symptoms in humans (Xiao et al., 2021). Since there is no pre-existing immunity against NDV in humans, the NDV vector...
has been considered a safe gene delivery platform due to host range restriction. The first attempt to generate recombinant NDV virus was made in 2000, and an NDV vector vaccine expressing the hemagglutinin (HA) gene of influenza virus was tested in mammals (Hu et al., 2020). Recently, a SARS-CoV-2 vaccine based on NDV virus expressing the spike protein of SARS-CoV-2 was introduced as a live virus vaccine candidate. NDV vector vaccines elicit high levels of neutralizing antibodies and show no detectible viral load in the lung when the vaccine is given intramuscularly in rodents (Sun et al., 2020a, 2020b) (Table 5). The safety and immunogenicity of a SARS-CoV-2 vaccine based on NDV virus has been evaluated in a phase 1 clinical trial (NCT04871737) (Table 4).

**Conclusion**

The recent emergence of new variants of SARS-CoV-2 and their rapid worldwide expansion may compromise the efficacy of COVID-19 vaccines currently authorized for human application by interfering with currently used vaccines. To minimize the risk posed by SARS-CoV-2 variants, vaccine strategies that modulate T cell-mediated immune responses or induce innate cell-based heterologous effects that are less affected by mutated variants are needed. Compared to other platforms, vaccines using viral or bacterial delivery systems have several distinct merits. First, since the delivery vector itself possesses various pattern-associated molecular patterns (PAMPs), adjuvant therapy is not needed to activate innate cells. Second, these vaccines can induce both T cell-mediated and humoral immune responses against SARS-CoV-2 infection. Third, they can induce both broad-spectrum off-target vaccine effects via the unrelated vector itself, which are mediated partly by trained immunity, and specific humoral and cell-mediated immune responses against delivered COVID-19 antigens, which can provide a better protective effect, even against vaccine escape variants (Fig. 1). Since more promising new vaccines using viral or bacterial delivery systems are under development, these approaches are expected to broaden the repertoire of COVID-19 vaccine regimens to potentiate the efficacy of current vaccines via combination with other currently available vaccine platforms.

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**Conflicts of Interest**

The authors declare that the article have no potential conflicts of interest to disclose.

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