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REVIEW

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

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Severe acute respiratory syndrome coronavirus 2 virus (SARS-CoV-2) infection, which causes coronavirus disease 2019 (COVID-19), has led to many cases and deaths worldwide. Therefore, a number of vaccine candidates have been developed to control the COVID-19 pandemic. Of these, to date, 21 vaccines have received emergency approval for human use in at least one country. However, the recent global emergence of SARS-CoV-2 variants has compromised the efficacy of the currently available vaccines. To protect against these variants, the use of vaccines that modulate T cell-mediated immune responses or innate immune cell memory function, termed trained immunity, is needed. The major advantage of a vaccine that uses bacteria or viral systems for the delivery of COVID-19 antigens is the ability to induce both T cell-mediated and humoral immune responses. In addition, such vaccine systems can also exert off-target effects via the vector itself, mediated partly through trained immunity; compared to other vaccine platforms, suggesting that this approach can provide better protection against even vaccine escape variants. This review presents the current status of the development of COVID-19 vaccines based on recombinant viral and bacterial delivery systems. We also discuss the current status of the use of licensed live vaccines for other infections, including BCG, oral polio and MMR vaccines, to prevent COVID-19 infections.

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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; Marín-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 (Covián *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 Vaccine					
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) / Estimated enrollment : 1500	University Medical Center Utrecht / Netherlands
	BCG Vaccination for Healthcare Workers in COVID-19 Pandemic	18 years		Phase 3 (NCT04379336) / Estimated enrollment : 500	TASK Applied Science / South Africa
	Reducing COVID-19 Related Hospital Admission in Elderly by BCG Vaccination	60 years	Danish strain 1331	Phase 4 (NCT04417335) / Estimated enrollment : 2014	Radboud University / Netherlands
	Prevention of Respiratory Tract Infection and Covid-19 Through BCG Vaccination in Vulnerable Older Adults (BCG-PRIME)	60 years	Danish strain 1331	Phase 4 (NCT04537663) / Estimated enrollment : 5200	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 on the Incidence and Severity of SARS-CoV-2 Infections Among Healthcare Professionals During the COVID-19 Pandemic in Poland	25 years	Brazilian Moreau sub-strain	Phase 3 (NCT04648800) / Estimated enrollment : 1000	Hanna Czajka & Medical Research Agency / Poland
	BCG to Reduce Absenteeism Among Health Care Workers During the COVID-19 Pandemic (EDCTP)	18 years	Danish strain 1331	Phase 4 (NCT04641858) / Estimated enrollment : 1050	University of Southern Denmark / Denmark
	Prevention, Efficacy and Safety of BCG Vaccine in COVID-19 Among Healthcare Workers	18 years	Tokio 172 strain	Phase 3 (NCT04461379) / Estimated enrollment : 908	Hospital Universitario Dr. Jose E. Gonzalez / Mexico
	BCG Vaccination to Protect Healthcare Workers Against COVID-19 (BRACE)	18 years	Danish strain 1331	Phase 3 (NCT04327206) / Estimated enrollment : 10078	Murdoch Children Research Institute / Australia
	COVID-19: BCG as Therapeutic Vaccine, Transmission Limitation, and Immunoglobulin Enhancement (BATTLE)	18 years		Phase 4 (NCT04369794) / Estimated enrollment : 1000	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) / Estimated enrollment : 1900	Bandim Health Project / Denmark
	Efficacy of BCG Vaccination in the Prevention of COVID19 Via the Strengthening of Innate Immunity in Health Care Workers (COVID-BCG)	18 years	Danish strain 1331	Phase 3 (NCT04384549) / Estimate enrollment : 1120	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) / Estimate enrollment : 1800	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 (NCT04439045) / 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
Viral vaccine (Live vaccine)					
Polio vaccine	OPV as Potential Protection Against COVID-19	50		Phase 4 (NCT04445428) / Estimated enrollment : 3400	Bandim Health Project / Africa
	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
	Biomarkers of Trained Immunity Following MMR Vaccination	18	M-M-R II® (Merck)	Phase 1 (NCT04646239) / Estimated enrollment : 140	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-1β (Netea *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 NCT04435379); 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 adenovirus 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						
AZD1222	Phase III Double-blind, Placebo-controlled Study of AZD1222 for the Prevention of COVID-19 in Adults	ChAdOx1	18 to 130	IM / Two dose	Phase 3 (NCT04516746) (Falsey *et al.*, 2021) Actual enrollment : 32459	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 (Sputnik V)	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)	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 A COVID-19 Vaccine of Adenovirus Vector 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 55 to 85	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 Glenting, 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; Covián *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; Methner *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	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, 2 (NCT04666012) Actual enrollment : 150	Cellid Co., Ltd. / South Korea
	Safety and Immunogenicity Study of AdCLD-CoV19-1: A COVID-19 Preventive Vaccine in Healthy Volunteers	hAd5/35	19 to 64	IM / Single dose	Phase 1 (NCT05047692) Estimated enrollment : 40	Cellid Co., Ltd. / South Korea
VXA-COV2-1.1-S	A Phase 2 Trial With an Oral Tableted COVID-19 Vaccine	Ad5	18 to 75	Oral Tablet / Two dose	Phase 2 (NCT05067933) Estimated enrollment : 896	Vaxart / US
SC-Ad6-1	A Phase 1, First-In-Human Study of the Investigational COVID-19 Vaccine SC-Ad6-1 in Healthy Volunteers	SC-Ad6	18 to 60	IM, IN / Single or multiple dose	Phase 1 (NCT04839042) Estimated enrollment : 80	Tetherex Pharmaceuticals Corporation / US
COVITAR (GRAd-COV2)	Study of GRAd-COV2 for the Prevention of COVID-19 in Adults (COVITAR)	GRAd	18	IM / Single or two dose	Phase 2, 3 (NCT04791423) Actual enrollment : 10300	ReiThera Srl. / Italy
Modified Vaccina Virus						
COH04S1	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
Parainfluenza virus						
CVXGA1	Phase 1 Study of Intranasal PIV5-vectored COVID-19 Vaccine Expressing SARS-CoV-2 Spike Protein in Healthy Adults (CVXGA1-001)β	PIV5	18 to 75	IN / Single dose	Phase 1 (NCT04954287) Estimated enrollment : 90	CyanVac LLC / US

could be the first line of defense against the virus (Gallo *et al.*, 2021). Probiotics could be a potent delivery system for the COVID-19 vaccine because they can interact with antigen-presenting cells to induce robust mucosal innate immune responses in the gut and respiratory tract (Moradi-Kalbolandi *et al.*, 2021). *Lactiplantibacillus plantarum* has been proposed as a potent COVID-19 vaccine. Previously, *L. plantarum* has been studied as a modulator of mucosal antiviral immunity in the context of oral vaccines to prevent influenza and Newcastle disease (Yang *et al.*, 2017; Ho *et al.*, 2020). Although further *in vivo* studies are needed, a recent *in vitro* study indicated that recombinant *L. plantarum* with the optimized spike gene of SARS-CoV-2 may induce mucosal immune responses against SARS-CoV-2 (Wang *et al.*, 2020) (Table 5).

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

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	Phase 2b/3 Trial of VSV-ΔG 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	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 (NCT04497298) Actual enrollment : 90	Institute Pasteur / France
Influenza Virus						
DelNS1-nCoV-RBD LAIV	Study to Evaluate Safety and Immunogenicity of DelNS1-nCoV-RBD LAIV for COVID-19	MVA	18 to 55	IN / Two dose	Phase 1 (NCT04809389) Estimated enrollment : 115	The University of Hong Kong / Hong Kong
Newcastle Disease Virus						
rNDV	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

Table 5. Vaccine candidates of preclinical stages based on bacterial or viral vectors to evaluate the protective effects against COVID-19

Vector	Inserted genes	Study / Injection route	Host	Reference
Bacterial vector				
F. tularensis (LVS ΔcapB)	SARS-CoV2 spike (Various regions)	Protective effect in the lung Intradermally, intranasally vaccination	Hamster	Jia *et al.* (2021)
S. typhimurium	SARS-CoV2 spike	Immunogenicity, Oral vaccination	Rat	Zhu *et al.* (2020b)
M. paragordonae	RBD	Immunogenicity, Efficacy Subcutaneously vaccination	Mouse	Kim *et al.* (2021)
L. plantarum	SARS-CoV2 spike	Stability, Antigenicity	*In vitro* (LP18)	Wang *et al.* (2020)
Replication incompetent vector				
Parainfluenza Virus 5 (PIV5)	SARS-CoV2 spike	Protective effect Mucosal immunization	Mouse, Ferret	An *et al.* (2021)
Lentivirus (LV)	SARS-CoV2 spike	Protective effect Intranasal vaccination	Mouse, Hamster	Ku *et al.* (2021b)
Modified Vaccinia Virus Ankara (MVA)	SARS-CoV2 spike	Immunogenicity, efficacy Intramuscular vaccination	Mouse	Liu *et al.* (2021)
	SARS-CoV2 spike	Vaccine efficacy Adaptive immunity	Mouse	Tscherne *et al.* (2021)
Replication competent vector				
Vesicular Stomatitis Virus (VSV)	SARS-CoV2 spike	Protective effect Intramuscular vaccination	Hamster	Yahalom-Ronen *et al.* (2020)
	SARS-CoV2 spike	Protective effect Intranasal, Intraperitoneal vaccination	Mouse	Case *et al.* (2020)
Measles Virus (MeV)	SARS-CoV2 spike & Different length of RBD	Safety and efficacy, Protective effect Subcutaneous, Intranasal vaccination	Mouse, Hamster	Lu *et al.* (2021)
	SARS-CoV2 spike	Immunogenicity, Th1 immune responses Intraperitoneal vaccination	Mouse, Hamster	Hörner *et al.* (2020)
Influenza Virus	RBD	Protective effect Intranasal vaccination	Mouse	Loes *et al.* (2020)
	RBD	Immunogenicity, Protective effect Intranasal, Intramuscular vaccination	Mouse	Koonpaew *et al.* (2021)
Newcastle Disease Virus (NDV)	SARS-CoV2 spike	Protective effect Intramuscular vaccination	Mouse	Sun *et al.* (2020a)
	SARS-CoV2 spike (Live or Inactivated)	Protective effect Intramuscular vaccination	Mouse, Hamster	Sun *et al.* (2020b)

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 lentivirus (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/AstraZeneca), 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-

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	JNJ-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
	CIGB-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
	COVOVAX	1	2 trials in 1 country	Serum Institute of India
	COVAX-19	1	4 trials in 2 countries	Vaxine / CinnagGne 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
	QazVac	2	3 trials in 1 country	Kazahstan 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

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, NCT-04530396, 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 vaccina 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

(EUA) for COVID-19 in blood cancer patients (NCT04639466, NCT04977024) (Table 3). Furthermore, it has been reported that mice injected with a single or two-dose of MVA vector-based vaccine incorporating the SARS-CoV-2 spike gene are all survived after challenging with SARS-CoV-2 virus (García-Arriaza *et al.*, 2021). Another preclinical study showed that MVA-SARS-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 (Barría *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

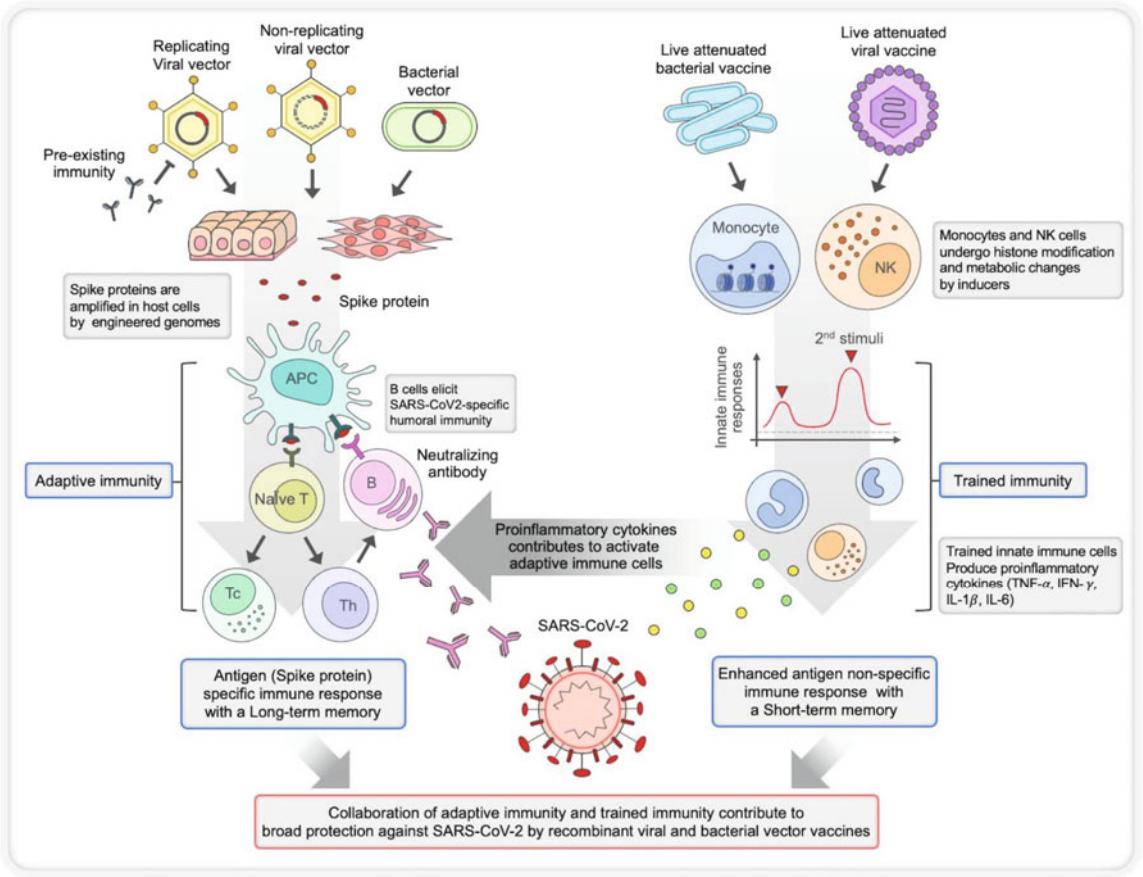

Fig. 1. COVID-19 vaccines based on recombinant viral and bacterial delivery systems can provide a better protection via combinatorial effect of adaptive and trained immunity. First, they possess various pattern-associated molecular patterns (PAMPs) leading to adjuvant free vaccination. Second, they can induce adaptive T cell-mediated and humoral immune responses specific to SARS-CoV-2 infection. Third, they can induce broad-spectrum off-target vaccine effects via trained immunity, providing a better protective effect even against vaccine escape variants.

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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