REVIEW

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COVID-19 pandemic: SARS-CoV-2 specific vaccines and challenges, protection via BCG trained immunity, and clinical trials

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ABSTRACT

Introduction: The coronavirus disease 2019 (COVID-19) pandemic continues to spread worldwide and vaccination remains the most effective approach to control COVID-19. Currently, at least ten COVID-19 vaccines have been authorized under emergency authorization. However, these vaccines still face many challenges.

Areas covered: This study reviews the concept and mechanisms of trained immunity induced by the Bacille Calmette Guérin (BCG) vaccine and identifies questions that should be answered before the BCG vaccine could be used to combat COVID-19 pandemic. Moreover, we present for the first time the details of current BCG vaccine clinical trials, which are underway in various countries, to assess its effectiveness in combating the COVID-19 pandemic. Finally, we discuss the challenges of COVID-19 vaccines and opportunities for the BCG vaccine. The literature was found by searching the PubMed (https://pubmed.ncbi.nlm.nih.gov/), Web of Science (www.webofknowledge.com), Embase (https:// www.embase.com), and CNKI (https://www.cnki.net/) databases. The date was set as the default parameter for each database.

Expert opinion: The advantages of the BCG vaccine can compensate for the shortcomings of other COVID-19 vaccines. If the efficacy of the BCG vaccine against COVID-19 is confirmed by these clinical trials, the BCG vaccine may be essential to resolve the challenges faced by COVID-19 vaccines.

1. Introduction

The coronavirus disease 2019 (COVID-19) pandemic caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV -2) has had serious consequences on global public health, as well as the global economy. Thus, there is an urgent need to control the pandemic, which can only be achieved through the generation of an immune defense shield to protect the population from COVID-19. Presently, although many vaccine manufacturers are producing COVID-19 vaccines at full capacity, they are far from meeting the global demand for COVID-19 vaccines. Therefore, achieving herd immunity remains an issue, and thus, SARS-CoV-2 continues to spread worldwide. More notably, the emergence of SARS-CoV-2 variants may destroy the current achievements of COVID-19 vaccine research and development. Furthermore, the race to develop safe and effective treatment methods has seen vaccine candidates against COVID-19 being developed at a larger scale and a rapid pace. Currently, there are more than 200 potential vaccines in various developmental stages [1-3]. These vaccine candidates use various strategies that include novel approaches to prove that these vaccines are safe and immunogenic in humans. However, in order to be effective in controlling the COVID-19 pandemic, they must be able to generate specific neutralizing antibodies, as well as reasonably longlasting immunity.

1.1. Concerns regarding COVID-19 vaccines

A previous study has shown that specific antibodies induced by vaccination may aggravate clinical symptoms, which is known as antibody-dependent enhancement (ADE) [4]. It has been reported that ADE is involved in clinical observations of increased severity of symptoms associated with early high levels of SARS-CoV-2 antibodies in patients [5]. In the past, in order to develop effective vaccines against coronavirus diseases, some vaccine candidates, although useful in producing protective antibodies, led to more severe disease after subsequent inoculation with the virus [6]. Interestingly, vaccines never generate immunity in all vaccinated people due to several complicated factors, ranging from genetic and immunological factors to the quality of the vaccines themselves and how they are administered [7]. Moreover, even if it is assumed that the vaccine will effectively induce immunity in a vaccinated population, the time frame of vaccine-induced

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Article highlights

- At least ten COVID-19 vaccines have been authorized under an emergency use authorization (EUA) to prevent COVID-19 in more than 40 countries.
- These vaccines still face many challenges, such as the emergence of variant strains, public hesitation, poor cold chain logistics, and low production capacity.
- It is hypothesized that the BCG vaccine may stimulate a non-specific protective effect against COVID-19.
- The BCG vaccine has several advantages, including safety, accessibility, affordability, production capacity, transportation, storage, and distribution.
- To confirm this hypothesis, more than 51 clinical trials investigating the effectiveness of the BCG vaccine against COVID-19 have been conducted in various countries.
- Once this hypothesis is confirmed, the BCG vaccine may help solve the problems faced by the approved COVID-19 vaccines.

protection is questionable [8]. Thus, in the current scenario, it is difficult to assess the long-term effectiveness of COVID-19 vaccines. As such, the persistence of anti-COVID-19 antibodies post-vaccination needs to be closely monitored, which is a time-consuming process. More importantly, safety and efficacy are significantly dependent on the vaccine type, technology or platform used, and immunization strategies. Therefore, although the virulence and pathogenicity of live attenuated vaccines have significantly reduced [9], there is a possibility that they may become pathogenic over time due to mutations [10].

Currently, at least 10 vaccines have been authorized under an emergency use authorization (EUA) for the prevention of COVID-19 in over 40 countries, including the Moderna mRNA-1273 vaccine (United States) [11,12], Pfizer-BioNTech's BNT162b2 vaccine (United States & Germany) [13], Sinovac vaccine (China) [14], Sinopharm's two COVID-19 vaccines (China) [15,16], CanSino' Ad5-nCoV vaccine (China) [17], Sputnik V vaccine (Russia) [18], EpiVacCorona vaccine (Russia) [19], AstraZeneca's ChAdOx1 nCoV-19 (England) [20], and Janssen's Ad26.COV2.S (United States) [21]. Although COVID-19 specific vaccines have been approved for use in priority populations, it is still too early to confidently say that humans have won this battle as we still face the following questions: 1) The supply of these vaccines cannot meet the needs of the global population and many low- and middle-income countries (LMICs), especially the least developed countries (LDCs), cannot obtain enough vaccines [22]; 2) How long does the vaccine-induced protection last? This question needs more time in order to provide accurate answers; 3) The variants found in the United Kingdom (B.1.1.7 variant) and South Africa (501Y.V2 variant) have the potential to escape the immune response induced by the vaccines [23,24], as such, will they weaken the effectiveness of the vaccine? 4) Due to the unprecedented acceleration of clinical trials, the possible long-term side effects of these vaccines have not been fully elucidated.

1.2. The BCG vaccine: a magic bullet against COVID-19?

Thus far, multiple drugs and drug combinations have been used for the treatment of COVID-19 but none have been approved for large-scale clinical practice [25,26]. Due to the lack of approved treatment methods, prevention of further spreading of COVID-19 is of utmost importance. Interestingly, currently available vaccines, such as the Bacille Calmette-Guérin (BCG) vaccine, oral polio vaccine (OPV), and measles or MMR vaccines have been suggested to be effective against COVID-19 until effective COVID-19 specific vaccines are widely administered [27–29]. Several epidemiological studies and a recent BCG vaccination study in healthcare workers (HCWs) have shown that the BCG vaccine can indeed reduce the severity of the disease [30].

The BCG vaccine is a live attenuated vaccine that has been used for more than 100 years as a prophylactic agent against tuberculosis (TB). The BCG vaccine is in the essential list of the World Health Organization (WHO) and is used in childhood immunization programs in many countries. Currently, there is no direct evidence to support the use of the BCG vaccine for the prevention of coronavirus infections [31]. However, the available data show that the BCG vaccine provides nonspecific protection against lethal infections that are not related to the vaccine's target pathogen by inducing trained nonspecific innate immune cells to improve host responses against subsequent infections [28,32,33]. Although induction of trained immunity (TI) by BCG vaccination leads to beneficial heterologous effects, the mechanisms underlying its persistence and magnitude remain unknown. Indeed, there is a need for clinical studies investigating the efficacy of the BCG vaccine in vulnerable populations who have a higher risk of being infected by COVID-19, such as HCWs, to gain insights into the underlying mechanism of heterologous effects of the BCG vaccine and confirm this hypothesis.

According to the WHO report, many LMICs, especially LDCs, recommend BCG vaccination nationally [31]. However, the international health regulation (IHR) scores of these countries are generally low (Figure 1 and Table S1), putting these countries at risk of the COVID-19 pandemic. If the effectiveness of the BCG vaccine is proven through clinical trials, immunization with BCG can not only protect individuals from COVID-19 but also induce heterologous herd immunity, if provided to enough people promptly to provide even partial protection [34]. Thus, BCG vaccination may be an excellent supplementary way to curtail the spread of SARS-CoV-2 and reduce global morbidity and mortality, as proposed in our previous study [22].

Thus, this study will review the concept and mechanisms of TI and present detailed information regarding the ongoing BCG vaccine clinical trials against COVID-19. Moreover, the results of this study will provide evidence for evaluating the role of the BCG vaccine in reducing the impact of the COVID-19 pandemic. Finally, we will discuss the challenges of currently available COVID-19 vaccines and the opportunities and advantages of the BCG vaccine.

Total Average	44	71	61	75	66	69	64		80
Legislation and Financing	43	74	67	80	64	74	66		
Coordination and Focal Point	51	78	69	82	74	70	70		
Zoonotic event	50	72	58	81	77	61	68		70
Food safety	43	78	53	77	64	73	65		
Laboratory	56	79	73	81	69	78	72		
Surveillance	61	78	78	79	72	81	73		60
Human Resources	49	70	60	71	64	65	63		
Health Emergency Framework	40	73	58	74	65	74	63		50
Health Service Provision	41	64	61	79	68	67	63		00
Risk Communication	43	68	71	66	60	65	60		
Points of Entry	36	70	53	60	64	67	56		40
Chemical Events	32	60	45	68	56	67	54		
Radiation Emergencies	32	59	45	78	57	52	55		
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Figure 1. International health regulation scores.

The data were obtained from State Parties Self-Assessment Annual Reporting on implementing the International Health Regulations released by WHO. The detailed information on IHR scores among countries can be found in Table S1. Available online: https://extranet.who.int/e-spar (updated on 9 March 2020).

2. Trained immunity induced by BCG vaccination

2.1. The concept of trained immunity

The concept of TI, which was developed in recent decades, describes the nonspecific and long-term immune memory acquired by innate immune cells after encountering a primary stimulus (pathogen or vaccination), which could mount a heightened inflammatory response, including upregulated production of pro-inflammatory cytokines or enhanced myelopoiesis to a second infection with the same pathogens or different ones [35]. A variety of stimuli, including β -glucan (a fungal cell wall component), lipopolysaccharide (LPS), and BCG, can trigger TI, the most studied of which is the BCG vaccine. Several epidemiological studies and randomized controlled trials have reported that BCG vaccination can protect against pathogens unrelated to Mycobacterium tuberculosis [36,37]. More importantly, controlled experimental studies in humans have provided direct evidence for the protective efficacy of BCG vaccination against clinically relevant pathogens, such as those causing yellow fever and malaria [37-39]. Thus, it has been hypothesized that these

BCG-induced nonspecific beneficial effects are partly mediated by TI induction.

2.2. Mechanisms responsible for the induction and sustained memory of trained immunity

Thus far, the mechanisms responsible for BCG-induced TI are only partially understood and multiple regulatory layers are thought to be involved in this process (Figure 2). Myeloid cell populations were identified as the primary cellular basis mediating BCG-induced TI [40]. Mature myeloid cells, including monocytes and macrophages, have been shown to be the key players involved in BCG-induced TI that leads to protection against unrelated pathogens [38,41,42]. An initial challenge of monocytes by BCG during infection leads to more open chromatin architecture, along with an increase in transcriptionally active histone modifications, such as histone H3 acetylation at lysine 27 (H3K27ac) and histone H3 trimethylation at lysine 4 (H3K4m3), and a decrease in transcriptionally repressive histone modifications, such as histone H3 trimethyllation at lysine 9 (H3K9m3) and at promoters or enhancers of



Figure 2. Mechanisms responsible for BCG – induced trained immunity.

BCG or its triggered cytokines reprogram the epigenetics and metabolism of monocytes/macrophages, and some of these changes are partially retained after cessation of the stimulus, inducing a trained state. The trained monocytes/macrophages heightened innate immune responses to a second stimulation unrelated to *M. tuberculosis*, mediated by faster and enhanced metabolic and epigenetic rewiring. In addition, BCG-vaccination induces the hematopoietic stem/progenitor cells to undergo epigenetic changes or increased DNA accessibility of certain genes, leading to enhanced myelopoiesis. Some of these epigenetic changes with HSPCs are conserved in their differentiated progeny, i.e. monocytes. These modulations of HSPCs by BCG ultimately mediate the long-term innate immune memory.

IL-1 β , TNF α , and IL-6 [38,41–43]. These dynamic epigenetic changes are also observed in genes that regulate metabolism-related signaling pathways, such as PI3K/AKT/mTOR, which promote cellular metabolism toward glycolysis, leading to high glucose consumption and lactate production [38,42,44].

During glycolysis, some of the generated metabolic intermediates could act as epigenetic modifiers, contributing to the induction of H3K4m3, H3K9m3, and H3K27ac at the promoters or enhancers of stimulated genes, thereby suggesting the presence of complex interactions between epigenetic and metabolic reprogramming in trained monocytes [42]. Some of these modifications, especially histone 3 lysine 4 methylation, may be preserved as a latent enhancer after cessation of BCG exposure, thereby establishing the epigenetic memory in monocytes and permitting the cells to enter a more responsive state [45]. When encountering subsequent stimuli, this preserved modification allows the trained monocytes to reacguire the increases in H3K27ac and H3K4m3 and decreases in H3K9m3 in a faster and stronger manner, which is followed by enhanced production of pro-inflammatory cytokines, such as IL-1β, TNFα, IL-6, and metabolic reprogramming toward aerobic glycolysis to heighten the TI phenotype [40].

Mature myeloid cells, such as monocytes and macrophages, play a central role in BCG-induced TI. These cells have a short lifespan of just several days, which is inconsistent with the long-term innate immune memory of TI shown by trained monocytes found in circulation, which was shown to last from three months to one year post-BCG vaccination [41]. This paradox suggests that BCG-induced TI also occurs at the level of myeloid progenitors and immature myeloid cells. Indeed, a recent elaborate study in mice demonstrated that intravenous BCG vaccination reprograms hematopoietic stem cells (HSCs) in the bone marrow via IFN- γ signaling, thereby promoting their differentiation into progenitors with restricted

myeloid-lineage potential, which is referred to as enhanced myelopoiesis [43]. This priming process of myelopoiesis enables the generation of trained monocytes/macrophages with enhanced protection against M. tuberculosis infection [43]. Similarly, another study reported that β-glucan could also modulate hematopoietic stem and progenitor cells (HSPCs), induce myelopoiesis via IL-1ß signaling, and confer long-term innate memory to TI [46]. Most recently, in people vaccinated with intradermal BCG, a similar myeloid differentiation bias of HSPCs has been observed, which functions by regulating the DNA accessibility of inflammation-related sites [47]. Notably, some epigenetic changes within HSPCs are preserved in differentiated peripheral monocytes. Thus, these studies indicate that both mature myeloid cells and HSPCs as the cellular basis are responsible for BCG-induced TI and their sustained memory.

2.3. Unresolved questions remain to be addressed

Although myeloid cells are the cellular basis and epigenetic and metabolic reprogramming have been identified as key molecular factors that drive TI, the regulatory mechanisms which mediate this process remain largely unexplored. First, mature myeloid cell populations (monocytes and macrophages) and myeloid progenitors (HSPCs) are heterogeneous populations and both comprise several subsets. When encountering BCG or other pathogens, it is possible for a defined subpopulation or all subpopulations to develop TI. Recently developed single-cell RNA sequencing has been used to investigate this process [48]. Second, the BCG vaccine is typically administered as an injection into dermal tissue. However, intravenous administration in mice and non-human primates was shown to induce stronger protection against M. tuberculosis infection [43,49]. Thus, can different BCG vaccination strategies induce innate immune cells to produce the same or completely different TI in humans? Moreover, which vaccination route can induce the human host to produce the best protection against M. tuberculosis-related pathogens and what is the mechanism underlying this difference? Third, intradermal BCG vaccination in humans induces the transcriptional programs of HSPCs toward myelopoiesis [47]. However, no significant alterations in HSPCs were observed in mice vaccinated with BCG intradermally [43]. This may help explain these differences. Fourth, BCG or other stimuli, such as β -glucan and LPS, can generate different types of TI [40] but the factors that mediate the development of stimulus-specific innate immune memory remain unknown. Finally, to date, most studies on BCG-induced TI have focused on the analysis of acquired innate memory in circulating monocytes in the peripheral blood or myeloid progenitors in the bone marrow [50]. Thus, knowledge regarding the induction process and mechanisms underlying BCG-induced TI in local tissues, especially in the lungs, is still lacking. The respiratory mucosa of the lung is the primary site of defense against a broad range of pathogens, including coronaviruses, adenoviruses, influenza, M. tuberculosis, and Streptococcus pneumonia. Thus, further studies are required to clarify these issues and improve our view of host defenses and innate immune memory, as well as provide a novel approach for targeting TI in order to develop better vaccines against TB or other infectious diseases.

3. BCG vaccine and COVID-19

As an old vaccine, BCG has been widely used to fight against TB for decades [51]. Interestingly, a growing number of studies have demonstrated that BCG vaccine induced a significantly higher levels of IFN-γ and IL-10, giving it the prophylactic effect against bacterial and viral infections in humans, such as experimental yellow fever, human papillomavirus (HPV), respiratory syncytial virus (RSV), influenza A virus (H1N1), herpes simplex virus (HSV), hepatitis B virus (HBV), melanoma, bladder cancer, severe oral aphthosis, *S. pneumoniae* or virus infection, and *Salmonella typhi* [37,52]. Furthermore, BCG vaccination shows nonspecific immunotherapy effects on clinical symptoms caused by viruses, such as skin warts and genital warts caused by HPV [53].

Based on these evidences, it is hypothesized that BCG vaccination has the potential to protect against COVID-19. Preliminary and unprecise epidemiological data suggest a negative association between national BCG vaccination policies and the prevalence and mortality of COVID-19 [54,55]. Before COVID-19 vaccines can be widely administered, BCG vaccination may be an effective tool to prevent COVID-19 [22,27]. As the mechanisms of BCG-induced TI are not fully understood and our understanding of the immunological responses to COVID-19 remains lacking, more mechanistic studies on the effects of BCG vaccination on COVID-19 are urgently needed. In particular, which phase of the disease is appropriate for intervention? How can the proper strain of the BCG vaccine be selected? What is the BCG dose and delivery method that can generate the best protection against COVID-19? In addition, a clinical evaluation involving a large cohort regarding the effectiveness of BCG vaccination against COVID-19 is needed. The following section provides a comprehensive presentation of ongoing clinical trials of the BCG vaccine against COVID-19.

4. Current clinical trials of TB vaccines against COVID-19

Currently, over 51 clinical trials involving TB vaccines (including 43 clinical trials of BCG, 7 clinical trials of VPM1002, and 1 clinical trial of RUTI) against COVID-19 are being conducted worldwide (Table 1). Although individuals of any age can be infected with SARS-CoV-2, high-risk participants (HRPs), such as older patients and HCWs, have the highest risk of SARS-CoV -2 infection. Therefore, 54.90% (28/51) and 19.61% (10/51) of clinical trials were performed to prevent SARS-CoV-2 infection or to reduce its clinical consequences in HCWs and the elderly $(\geq 50 \text{ years old})$, respectively. Of the 51 clinical trials, 9 Phase IV clinical trials are being conducted to evaluate the effectiveness of BCG vaccination in preventing COVID-19 infection. Furthermore, there are 28 phase III clinical trials, 3 phase II clinical trials, and 11 phase unknown clinical trials also underway. Among the clinical trials of TB vaccines against COVID-19, 28 clinical trials of the BCG vaccine and 5 clinical trials of VPM1002 are recruiting or authorized, while one clinical trial of RUTI has been active but not yet recruiting. Here, we review the progress of the afore-mentioned clinical trials of these three types of TB vaccines against COVID-19.

4.1. Clinical trials of BCG vaccines against COVID-19

Although several reports have claimed that BCG is not effective in fighting COVID-19 [56-59], a growing number of studies have shown that countries with routine BCG vaccination programs have significantly fewer reported cases and deaths caused by COVID-19 [27,28,30,60-62]. However, these observational studies need to be further confirmed by clinical trials. In the absence of direct evidence, the WHO does not recommend BCG vaccination to prevent COVID-19 [31]. Therefore, clinical trials regarding the effectiveness of BCG vaccination for COVID-19 prevention are urgently needed. Currently, 43 clinical trials on BCG vaccine prevention of COVID-19 have been registered, including 9 Phase IV clinical trials, 20 Phase III clinical trials, 3 phase II clinical trials, and 11 clinical trials without a definite trial stage. Herein, we will focus on Phase IV and Phase III clinical trials that have already started recruiting volunteers (Table 1). Detailed information regarding the other clinical trials is shown in Table S2.

4.1.1. NCT04348370 (BADAS) and NCT04632537 (NUEVA): BCG vaccine for HCWs as defense against COVID-19 (United States)

From 3 January 2020, to 29 April 2021, the United States of America have reported 31,835,314 confirmed cases of COVID-19 and 567,971 deaths, therefore ranking first in the world regarding COVID-19 incidence and mortality. Currently, the Pfizer-BioNTech and Moderna COVID-19 vaccines have been authorized and recommended to prevent COVID-19 in the United States (https://www.cdc.gov/coronavirus/2019-ncov

Intervention Primary outcome	Experimental group: BCGHealth Care Workers vaccine concentrate absenteeism and solvent for solution for injection, i.d.,Placebo: Concentrate and solvent for solution for iniarrion i d	Experimental group: Days of unplanned absenteeisr Experimental group: Days of unplanned absenteeisr 0.1 ml BCG vaccine due to illness with 2–8 × 10e5 CFU, with 2–8 × 10e5 CFU, id.Placebo: 0.1 ml	 Compare the second secon	Experimental group: BCGSARS-CoV-2 related hospital vaccine (Danish strain admission 1331, SSI, Denmark), i. d.Placebo: 0.9% NaCl, i.d.	Experimental group: Clinical evolution of COVID-19, 0.1 ml BCG vaccine classified as mild, moderate with 2–8 × 10e6 CFU, and severe, SARS-CoV-2 i.d.Placebo: 0.9% elimination, virus detection 1 saline solution, i.d. PCR, seroconversion rate and titration, titration of anti SAR	Experimental group: Incidence of COVID 19 Infection 0.1 ml vaccine with [Time Frame: 6 months] 2 × 10e5 CFU, i.d. Placebo: 0.1 ml saline, i.d.	Experimental group: To investigate the effect of M-M-RVAXPRO® bisphosphonates and the powder and solvent MMR vaccine on BCG-induce for suspension for trained immunity as injection, i.d.Placebo: a preventive approach again Solution for injection, COVID-19 i.d.	Experimental group: Susceptibility for COVID-19 0.1 ml BCG vaccine, i. d.Placebo: 0.1 ml of
Countries	Netherlands	Cape Verde; Guinea- Bissau; Mozambique	Netherlands	Netherlands	Brazil	United States	Netherlands	Greece
Population Age	18 Years and older (HCWs)	18 Years and older (HCWs)	60 Years and older	60 Years and older	18 Years and older	18–75 Years old (HCWs)	18–50 Years old	50 Years and older
Phase	Phase 4	Phase 4	Phase 4	Phase 4	Phase 4	Phase 4	Phase 4	Phase 4
Target size	1000	1050	5200	2014	1000	1800	100	006
Recruitment Status	Authorized	Recruiting	Recruiting	Not recruiting	Recruiting	Recruiting	Authorized	Authorized
Primary sponsor	University Medical Center	University of Southern Denmark	UMC Utrecht	Radboud University	University of Campinas, Brazil	Texas A&M University	Radboudumc	Hellenic Institute for the Study of Sepsis
Acronym	BCG-CORONA	EDCTP	BCG-PRIME		BATTLE	BADAS	BCG-PLUS	
Vaccine (strain)	BCG vaccine (Danish strain 1331)	BCG vaccine (Danish strain 1331)	BCG vaccine (Danish strain 1331)	BCG vaccine (Danish strain 1331)	BCG (NA)	BCG (Tice strain)	BCG vaccine (Danish strain 1331)	BCG (Moscow strain 361–1)
Trial ID	EUCTR2020-000919- 69-NL	NCT04641858	NCT04537663	NCT04417335	NCT04369794	NCT04348370	EUCTR2020-002456- 21-NL	EUCTR2020-002448- 21- GRNCT04414267

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Trial ID	Vaccine (strain)	Acronym	Primary sponsor	Recruitment T Status	arget size P	hase	Population Age	Countries	Intervention	Primary outcome
CTRI/2020/06/025798	BCG (NA)		None	Not Recruiting	d 02	hase 1 4	8-80 Years old	India	Experimental group: T Reinitiation of intravesical BCG from the beginning of the regimer, 120 mg intravesical BCG (50 ml Normal Saline) Placebo: Resumption of Intravesical BCG from where it was interrupted; 120 mg intravesical BCG dissolved (50 ml Normal Saline)	he recurrence rates, grade and stage progression in NMIBC patients with restarting the intravesical BCG regimen versus continuing the regimen, stratified by different phases of interruption.
NCT04648800	BCG (Moreau strain)		Hanna Czajka	Recruiting	1000 P	hase 2 3	(HCWs) (HCWs)	Poland	Experimental group 1: D with positive RT23 test reading, not randomized and not vaccinated against tuberculosis; Experimental group 2: 0.10 ml BCG with 1.5- 6 × 10e5 CFU, i.d. Placebo: 0.1 ml normal saline (0.10% NaCU i.d.	eath and life- or health- threatening condition
NCT04632537	BCG (Tice strain)	NUEVA	Henry M. Jackson Foundation for the Advancement of Military Medicine	Recruiting	550 P	hase 1 3	8-64 Years old	United States	Experimental group: Ir 0.10 ml BCG with 2 × 10e6 CFU, i.d. Placebo: 0.1 ml normal saline (0.9% NaCl) i.d.	cidence of symptomatic rt-PCR- confirmed SARS-CoV-2 infection
NCT04542330	BCG vaccine (Danish strain 1331)		Bandim Health Project	Recruiting	1900 P	hase 6 3	55–110 Years old	Denmark	Experimental group: '' 0.10 ml BCG with 2- 8 × 10e5 CFU, i.d. Placebo: 0.1 ml normal saline (0.9% NaCl).i.d.	cute infection' identified either by a doctor, antibiotics use, hospitalization, or death due to infection.
NCT04534803	BCG (NA)	BAC to the PAST	Harvard Medical School	Not recruiting	2100 P	hase 7 3	0 Years and older	United States	Experimental group: N 0.1 ml of reconstituted BCG vaccine given intradermally at baseline. Placebo: 0.1 ml of diluent (saline) given intradermally at baseline	umber of people diagnosed with severe Covid-19 disease
										(Continued)

Table 1. (Continued).										
Trial ID	Vaccine (strain)	Acronym	Primary sponsor	Recruitment Status	Target size	Phase	Population Age	Countries	Intervention	Primary outcome
NCT04475302	BCG (NA)		Tuberculosis Research Center, India	Recruiting	2175	Phase 3	60-80 Years old	India	Experimental group: Each 1 ml contains between 2 × 10e6 anc 8 × 10e6 CFU, i.p. Placebo: No intervention	Mortality due to COVID-19 disease
NCT04461379	BCG (Tokio 172 strain)		Hospital Universitario Dr. Jose E. Gonzalez	Not recruiting	806	Phase 3	18 Years and older (HCWs)	Mexico	Experimental group: 0.1 ml BCG vaccine with 0.075 mg, i.d. Placebo: 0.1 ml NaCl 0.9% solution, i.d.	Cumulative incidence of infection in 6 months, cumulative incidence of hospitalization for COVID-19, incidence of specific
NCT04384549	BCG vaccine (Danish strain 1331)	COVID-BCG	Assistance Publique – Hôpitaux de Paris	Not recruiting	1120	Phase 3	18 Years and older (HCWs)	France	Experimental group: 0.1 ml BCG vaccine (A. Vaccine) with 2– 8 × 10e5 CFUS. Placebo: One i.d. of 0.1 ml Nacl	Incidence of al. Incidence of documented COVID- J 19 among HCWs exposed to SARS CoV2 and vaccinated with BCG compared to placebo
NCT04379336	BCG vaccine (Danish strain 1331)		TASK Applied Science	Recruiting	500	Phase 3	18 Years and older (HCWs)	South Africa	Experimental group: 0.1 ml BCG vaccine with 0.075 mg of attenuated <i>Mycobacterium bovis</i> , i.d.Placebo: 0.1 ml 0.9% NaCl. i.d.	Incidence of HCWs hospitalized due to COVID-19 per arm
NCT04373291	BCG vaccine (Danish strain 1331)		Bandim Health Project	Not recruiting	1500	Phase 3	18–100 Years old (HCWs)	Denmark	Experimental group: 0.1 ml BCG-Denmark vaccine, i.d.Placebo: 0.1 ml sterile 0.9% NaCL i.d.	Number of days of unplanned absenteeism for any reason, number of days of unplanned absenteeism for any reason
NCT04362124	BCG (NA)		Universidad de Antioquia	Not recruiting	1000	Phase 3	18–65 Years old (HCWs)	Colombia	Experimental group: 0.1 ml BCG with 1 × 10e5 to 33 × 10e5 CFU, i.d.Placebo: 0.1 ml normal saline solurion i.d	Incidence of COVID-19 cases confirmed or probable in the study population
NCT04350931	BCG vaccine (Danish strain 1331)		Ain Shams University	Withdrawn	006	Phase 3	18–65 Years old	Egypt	Experimental group: Experimental group: 0.1 ml BCG with 2– 8 × 10e5 CFU, i.d. Placebo: 0.1 ml normal saline (0.9%	incidence of confirmed COVID-19 [Time Frame: 9 months] Effectiveness of BCG vaccine [Time Frame: 9 months]
NCT04328441	BCG vaccine (Danish strain 1331)	BCG-CORONA	UMC Utrecht	Active, not recruiting	1500	Phase 3	18 Years and older (HCWs)	Netherlands	Experimental group: 0.1 ml BCG vaccine with 0.075 mg of attenuated Mycobacterium bovis, i.d.Placebo: 0.1 ml NaCl 0.9%, i.d.	Health Care Workers absenteeism
										(Continued)

Trial ID	Vaccine (strain)	Acronym	Primary sponsor	Recruitment . Status	Target size	Phase	Population Age	Countries	Intervention	Primary outcome
NCT04327206	BCG vaccine (Danish strain 1331)	BRACE	Murdoch Childrens Research Institute	Recruiting	10,078	Phase 3	18 Years and older (HCWs)	Australia	Experimental C group:0.1 ml vaccine with 2–8 × 10e5 CFUs. Placebo: Intracutaneously 0.1 ml NaCl 0,9%.	OVID-19 disease incidence; Severe COVID-19 disease incidence
IRCT202004 11047019N1	BCG (NA)		Professor Alborzi Clinical Microbiology Research Center, Shiraz University of Mdical Sciences	Recruiting	200	Phase 3	18 Years and older (HCWs)	Iran	Experimental group: C 0.10 ml BCG Vaccine, i. d.Placebo: 0.1 ml of 0.9% NaCl solution, i. d.	OVID-19 infection
EUCTR2020-002503- 19-NL	BCG vaccine (Danish strain 1331)		UMC Utrecht	Authorized	7244	Phase 3 3	l8 Years and older (HCWs)	Spain;Australia; Netherlands; United Kingdom	Experimental group: BCGH Vaccin SSI concentrate and solvent for solution for injection, i.d.Placebo: Concentrate and solvent for solution for injection. i.d.	lealth Care Workers incidence of COVID-19 disease
EUCTR2020-002111- 22-PL	BCG (NA)		University of Rzeszów	Authorized	1000	Phase 3 3	(HCWs) (HCWs)	Poland	Experimental group: C Anti-Tuberculosis Vaccine BCG 10, powder and solvent for suspension for injection, i.d.Placebo: Solution for injection, i.d.	OVID-19 cases and deaths
EUCTR2020-001888- 90-DK	BCG vaccine (Danish strain 1331)	BCG-DENMARK- COVID	University of Southern Denmark	Authorized	1500	Phase 3	18–64 Years old (HCWs)	Denmark	Experimental group: T- 2 × 10e5 ~ 8 × 10e5 CFUs/ml, i.d.Placebo: Solution for injection, i.d.	o reduce absenteeism among health care workers with direct patient contacts during the COVID-19 pandemic.
EUCTR2020-001783- 28-HU	BCG vaccine (Danish strain 1331)	BACH	National Korányi Institute of Pulmonology	Authorized	1000	Phase 3	18–64 Years old (HCWs)	Hungary	Experimental group: BCGH vaccine, suspension for injection, i.d. Placebo: Solution for injection. i.d.	lealth Care Workers absenteeism
EUCTR2020-001678- 31-FR	BCG vaccine (Danish strain 1331)	COVID BCG	Assistance Publique Hopitaux de Paris	Authorized	1120	Phase 3	18 Years and older (HCWs)	France	Experimental group: 2–8Tl CFU/ml, i.d.Placebo: Solution for injection, i.d.	he protection of BCG for health care workers exposed to COVID-19
CTRI/2020/07/026668	BCG (NA)	BRIC	Indian Council of Medical Research	Not Recruiting	800	Phase 3 3	18–60 Years old	India	Experimental group: Ir 0.1 ml BCG vaccine i. d.; Placebo: 0.1 ml Normal saline	rcidence of COVID-19 by 9 months of follow-up.
RBR-4kjqtg	BCG (NA)		Universidade Federal de Goias	Recruiting	400	Phase 2	18 Years and older (HCWs)	Brazil	Experimental group: R receive a dose of BCG vaccine; The control group will not be vaccinated.	eduction of positivity for COVID-19;Reduction of health problems of health care workers related to COVID-19

(Continued)

Table 1. (Continued).										
Trial ID	Vaccine (strain)	Acronym	Primary sponsor	Recruitment Status	Target size	Phase	Population Age	Countries	Intervention	Primary outcome
NCT04659941	BCG (NA)	ProBCG	Universidade Federal do Rio de Janeiro	Recruiting	1000	Phase 2	18 Years and older (HCWs)	Brazil	Experimental group: 0.1 ml of the reconstituted vaccine to be administered intradermally.Placebo 1:0.1 ml 0.09% NaCl saline solution to be administered intradermally	Compare the cumulative incidence of SARS-CoV-2 infection
CTRI/2020/05/025013	BCG (NA)		Medical Education and Drugs Department	Not Recruiting	60	Phase 2 2	20-40 Years old	India	Experimental group 1: 0.1 ml BCG with 2 × 10e6 and 4. Experimental group 2: BCG plus STANDARD of CARE as suggested by DCG; Placebo 1: Tamiflu, Hydroxychloroquine, Azithromycin: None; Placebo 2: SALINE plus STANDARD of CARE as suggested by DCG;	Total duration of Hospitalization with COVID-19 symptoms such as febrile respiratory distress, decrease in Viral Titer, duration of COVID-19 symptoms
RBR-5ysj54	BCG (NA)		Universidade Federal do Rio de Janeiro	Not Recruiting	1000	N/A	18 Years and older	Brazil	Experimental group: Experimental group: vaccineted with BCG vaccine; Control group: normal saline	It is expected to find a smaller number of coronavirus infections in the BCG vaccinated group when compared to those vaccinated with Dlacebo
NL8609	BCG (NA)	BCG-PLUS	Radboudumc	Not Recruiting	100	V/N	1850 years old	Netherlands	 Placebo treatment; 2. BCG vaccination; 3. BCG vaccination + oral bisphosphonate supplementation (alendronic acid); 4. BCG vaccination + MMR vaccine; 5. MMR varcine alone 	The fold-increase in production of pro-inflammatory cytokines by PBMCs/monocytes following vaccination.
NL8547	BCG (NA)	BCG-CORONA- ELDERLY	Radboudumc	Recruiting	1600	A/A	60 Years and older	Netherlands	Participants will be randomized between intracutaneous administration of BCG vaccine or placebo in a 1.1 rario.	SARS-CoV-2 related hospital admission
NL8477	BCG (NA)	BCGcorona	University Medical Center Utrecht	Not Recruiting	1500	N/A	18 Years and older (HCWs)	Netherlands	Participants will be randomized between intracutaneous administration of BCG vaccine or placebo in a 1:1 ratio	Number of days of unplanned absenteeism for any reason
										(Continued)

3904-	Vaccine (strain) BCG (NA) BCG vaccine (Danish strain 1331)	Acronym	Primary sponsor Assiut University University of Southern Denmark	Recruitment 1 Status Recruiting Authorized	larget size 100	Phase N/A	Population Age 12–80 years old 65 Years and older	Countries Egypt Denmark	Intervention Group A: COVID-19 P positive with positive tuberculin test;Group B, COVID-19 positive with negative tuberculin test.Test the delayed hypersensitivity reaction and immunity to previous BCG vaccination (both group). Experimental group: 0.1 ml BCG vaccine (AJ Vaccine) with 2– 8 × 10e5 CFUs.	Primary outcome Dneumonia severity index; Need for ICU admission The primary outcome is acute infection identified either by a doctor, antibiotics use, hospitalization or death due to infection.
BCG vaccii (Danish 1331)	strain		UMCU Utrecht	Authorized	5200	A/N	18 Years and older	Netherlands	0.1 ml NaCl Experimental group: BCGl Vaccin SSI concentrate and solvent for solution for injection, i.d.Placebo: Concentrate and solvent for solution for injection, i.d.	Based on accrual of the two endpoints, the primary endpoint will be either (a) COVID-19 or (b) clinically relevant respiratory tract infection (RTI) requiring medical intervention, potentially including COVID-1
BCG vacci (Danish 1331)	ne strain		Murdoch Children's Research Institute (MCRI)	Authorized	7244	N/A	18–64 Years old (HCWs)	Spain;Australia; Netherlands; United Kingdom	Experimental group: BCGI Vaccin SSI concentrate and solvent for solution for injection, i.d.Placebo: Concentrate and solvent for solution for iniaction i.d	Vumber of participants with COVID-19 disease defined as fever or at least one sign or symptom of respiratory disease
BCG (NA)			SGT University	Recruiting	400	N/A	18–50 Years old (HCWs)	India	Experimental group: 0.1 ml BCG vaccine i. d.; Placebo: Unknown	Aate of infection of COVID-19 in healthcare workers re- vaccinated with BCG as
BCG (NA)			Indian Council of Medical Research	Not Recruiting	1450	N/A	60–95 Years old	India	Experimental group: 1 0.1 ml BCG vaccine i. d.; Placebo: No intervention	Proportion of patients with Severe COVID disease based on COVID Severity Scale and proportion of death due to
BCG vaccin (Danish 1331)	ie strain		Dr Narayanan Parameswaran	Not Recruiting	1826	N/A	18–65 Years old (HCWs)	India	Experimental group: 1 0.1 ml, i.d.;Placebo: 0.1 ml Normal saline, i. d.	Proportion of HCW with symptomatic COVID 19 disease 6 months after randomization.

(Continued)

Table 1. (Continued).

Trial ID	Vaccine (strain)	Acronym	Primary sponsor	Recruitment Status	Target size	Phase	Population Age	Countries	Intervention	Primary outcome
NCT04439045	VPM1002 (rBCGAureC::hly)	COBRA	University Health Network, Toronto	Not recruiting	3626	Phase 3	18 Years and older	Canada	Experimental group: 0.1 ml VPM1002 with 2–8 × 10e5 CFU, i.d.Placebo: 0.1 ml 0.9% sodium chloride, i.d.	Incidence of SARS-CoV-2 infection (confirmed by positive test) following vaccination with either VPM1002 or placebo
NCT04435379	VPM1002 (rBCGAureC::hly)		Vakzine Projekt Management GmbH	Recruiting	2038	Phase 3	60 Years and older	Germany	Experimental group: 0.1 ml VPM1002 with 2-8 × 10e5 CFU.Placebo: Physiological saline 0.1 ml	Number of days with severe respiratory disease at hospital and/or at home
NCT04387409	VPM1002 (rBCG∆ureC::hly)		Vakzine Projekt Management GmbH	Recruiting	1200	Phase 3	18 Years and older (HCWs)	Germany	Experimental group: 0.1 ml VPM1002 with 2–8 × 10e5 CFU, i.d.Placebo: Physiological saline 0.1 ml, i.d.	Number of days absent from work due to respiratory disease (with or without documented SARS-CoV-2 infection)
EUCTR2020-001675- 33-DE	VPM1002 (rBCGAureC::hly)		Vakzine Projekt Management GmbH	Authorized	2038	Phase 3	18 Years and older	Germany	Experimental group: VPM1002, 2– 8 × 10e6 CFU/ml, i. d.Placebo: Solution for injection, i.d.	Reduction of days with severe respiratory infectious diseases at hospital and/or at home in elderly subjects
EUCTR2020-001376- 15-DE	VPM1002 (rBCGAureC::hly)		Vakzine Projekt Management GmbH	Authorized	1200	Phase 3	18 Years and older (HCWs)	Germany	Experimental group: VPM1002, 2– 8 × 10e6 CFU/ml, i. d.Placebo: Solution for iniection. i.d.	Health Care Workers absenteeism
CTRI/2020/04/024749	VPM1002 (NA)		Serum Institute of India Pvt Ltd	Recruiting	5946	Phase 3	18–99 Years old (HCWs)	India	Experimental group: 0.1 ml reconstituted vaccine, i.d.Placebo: 0.1 ml 0.9% sodium chloride, i.d.	Number of subjects with laboratory confirmed COVID- 19 infection among HCWs
VPM1002 (NA)		Accelagen Pty Ltd	Not Recruiting	3468	ohase 3		18 Years and older (HRPs)	Australia	Experimental group: 0.1 ml VPM1002 live vaccine with 2– 8 × 1065 FGU, i.d. Placebo: 0.1 ml 0.9% sodium chloride i d	ACTRN12620000707955 The incidence SARS CoV-27 COVID-19 infection associated with acute respiratory symptoms
NCT04453488	e ۲л		Fundació Institut Germans Trias i Pujol	Not recruiting	315	Phase 3	18 Years and older (HCWs)	Spain	Experimental group: 25 µg of fragmented, purified and liposomed heat-inactivated Mycobacterium tuberculosis bacilli in 0.3 ml, s.c. Placebo: Physiological serum, 0.9% NaCl, s.c.	% positive serology at the end of the study or positive PCR test in the course of routine clinical practice
 The data were obta clinicaltrialsregistereu The list of abbreviatio injection; SAR5-CoV-2 The more informatio 	ined from Internation u/ctt-search/search), <i>A</i> ons: BCG, Bacillus Calr 2, severe acute respira in can be found in Ta	ral Clinical Trials Regi: Australian New Zealan mette-Guérin; CFUs, cc atory syndrome coron. ible S2.	stry Platform (https://ww dd Clinical Trials Registry Jony-forming units, COVI avirus 2.	w.who.int/ictrp (https://anzctr.c ID-19, corona vi	/en/), Cli org.au/De rus disea	nicalTrial efault.asp ise 2019;	ls.gov (https://clinical xx), and Iranian Regis HCWs, healthcare wc	trials.gov/ct2/resu try of Clinical Trial irkers; HRPs: High-	ts?cond=COVID-19), EU Cl s (https://en.irct.ir/trial/47; Risk Participants; i.d., intra	inical Trials Register (https://www. 279) on 8 January 2021. dermal injection; s.c., subcutaneous

/vaccines/different-vaccines.html). However, the vaccine supply is insufficient to ensure widespread vaccination. In addition to research on COVID-19 specific vaccines (AstraZeneca's ChAdOx1 nCoV-19 vaccine, Janssen's Ad26.COV2.S vaccine, and Novavax's NVX-CoV2373 vaccine), the United States has also conducted 3 clinical trials on BCG vaccine against COVID-19, including NCT04348370 (Phase IV), NCT04632537 (Phase III), and NCT04534803 (Phase III). Two clinical trials (NCT04348370 and NCT04632537) have begun recruiting volunteers. Both were conducted to evaluate the efficacy of BCG vaccination in reducing the risk of infection by SARS-CoV -2 and mitigate COVID-19 disease severity in at-risk health care providers.

4.1.2. NCT04475302: BCG vaccine in reducing morbidity and mortality in elderly individuals in COVID-19 hotspots (India)

As a large country with a population size second only to China, India's public health system and the number of HCWs are far behind those found in China. Although India has received a total of 147,727,054 doses of vaccine as of 26 April 2021, double mutated virus, B.1.617, is pushing India's public health system to the brink of collapse. From 3 January 2020, to 29 April 2021, India's cumulative number of confirmed COVID-19 cases and deaths has reached 18,376,524 and 204,832, respectively. The international community currently believes that the COVID-19 epidemic in India strongly resembles a gunpowder keg, once it is ignited, it will bring a devastating blow to India [63,64]. Fortunately, India has employed a nationwide BCG vaccination, which may bring a ray of hope. Furthermore, as elderly individuals and HCWs are at a high risk of COVID-19, strategies to protect these individuals are urgently needed to safeguard continuous patient care. Currently, 7 clinical trials have been conducted in India, including NCT04475302 (Recruiting, Phase III), CTRI/ 2020/09/027684 (Recruiting, Phase unknown), CTRI/2020/06/ 025798 (not recruiting, Phase IV), CTRI/2020/07/026668 (Not recruiting, Phase III), CTRI/2020/05/025013 (not recruiting, Phase II), CTRI/2020/04/024833 (Not recruiting, Phase unknown), and CTRI/2020/06/025854 (not recruiting, phase unknown). On 17 July 2020, India conducted a Phase III clinical trial (NCT04475302) to evaluate the effectiveness of the BCG vaccine in reducing COVID-19-related morbidity and mortality in 1,450 elderly individuals (60-80 years of age). However, one major issue is that volunteers in the placebo group did not receive any intervention, even with normal saline. Moreover, the investigator reported a range of inoculation doses without giving a precise value, which may affect later analyses of the clinical trial data. Thus, we call on all vaccine researchers to submit data that are as detailed as possible when registering clinical trials, especially those key data, such as vaccination dose, population grouping, and inclusion and exclusion criteria.

4.1.3. NCT04369794: COVID-19: BCG as therapeutic vaccine, transmission limitation, and immunoglobulin enhancement (BATTLE, Brazil)

From 3 January 2020, to 29 April 2021, the cumulative number of confirmed COVID-19 cases and deaths in Brazil has reached

14,441,563 and 395,022, respectively, therefore ranking second only to India and the United States. According to the WHO statistics, the number of confirmed COVID-19 cases and deaths in Brazil has increased significantly since December 2020, which may be associated with the emergence of the SARS-CoV-2 variant [65]. Thus, the healthcare system and HCWs in Brazil face unprecedented challenges. Currently, 4 clinical trials of the BCG vaccine against COVID-19 have been conducted in Brazil, including a Phase IV clinical trial (NCT04369794, recruiting), 2 Phase II clinical trials (NCT04659941 and RBR-4kjqtg, recruiting), and a phase unknown clinical trial (RBR-5ysj54, not recruiting). The sample size of the four clinical trials was relatively large. However, many details, such as the type of BCG strain and the exact vaccination dose, have not been disclosed.

4.1.4. NCT04648800: 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

In Poland, the number of confirmed COVID-19 cases and deaths began to rise rapidly in the autumn and winter of 2020. According to the WHO report, from 3 January 2020, to 29 April 2021, there have been 2,785,353 confirmed COVID-19 cases and 67,073 deaths. As of January 2021, 2 clinical trials (NCT04648800 and EUCTR2020-002111-22-PL) of BCG vaccination against COVID-19 were conducted in Poland. Both are phase III clinical trials but only the NCT04648800 clinical trial has started recruiting volunteers. NCT04648800 is a multicenter, randomized, partially blinded, placebocontrolled phase III clinical trial. The volunteers were divided into three groups: group I with positive TB skin test (TST) + observation, group II with negative TST + BCG vaccine, and group III with negative TST + placebo. Unlike other clinical trials on BCG vaccination to prevent COVID-19, this clinical trial used the TST to distinguish those participants who had been vaccinated with BCG at birth. On this basis, volunteers are divided into three groups instead of two groups and the following three guestions can be perfectly answered: (1) Does BCG vaccination affect the disease course of COVID-19? (2) Will COVID-19 symptoms be less severe among volunteers with a negative TST after additional BCG vaccination than in the case of non-vaccinated subjects? (3) Do people with a positive TST have less severe COVID-19 symptoms than those with negative test results? This clinical trial is currently recruiting volunteers and is expected to end in April 2021. We look forward to the publication of the results of this clinical trial because it will provide strong direct evidence regarding whether BCG vaccination can effectively prevent COVID-19.

4.1.5. IRCT20200411047019N1: Investigating the effect of BCG vaccine on preventing COVID-19 infection in healthcare staff exposed to SARS-CoV-2 (Iran)

As of 29 April 2021, Iran's confirmed number of COVID-19 cases had reached 2,459,906, ranking 14th globally. To assess the effect of the BCG vaccine on preventing COVID-19 infection in HCWs exposed to SARS-CoV-2, a double-blind,

placebo-controlled, randomized controlled, multicenter phase III clinical trial was conducted by the Professor Alborzi Clinical Microbiology Research Center in cooperation with the Shiraz University of Medical Sciences in Iran. This study will recruit 500 HCWs (over 18 years of age) and groups will be divided into a 1:1 ratio. Although the volume of the BCG vaccine and injection site have been revealed, the specific number of colony forming units (CFUs) was not disclosed in this clinical trial. Participants will be followed up for 12 months via text messages and blood samples will be collected before randomization and at 12 months to determine exposure to SARS-CoV-2.

4.1.6. NCT04379336: BCG vaccination for HCWs in COVID-19 pandemic (South Africa)

As of 29 April 2021, the cumulative number of confirmed COVID-19 cases and deaths in South Africa reached 1,578,450 and 54,285, respectively. As of 27 April 2021, a total of 182,983 vaccine doses have been administered. On 5 April 2020, a placebo-controlled, randomized controlled phase III clinical trial was conducted by TASK Applied Science to investigate whether and why BCG-revaccination could reduce the incidence rate and/or disease severity in HCWs during the COVID-19 outbreak in Cape Town, South Africa. This project aimed to compare the number of HCWs hospitalized due to COVID-19 and the incidence of SARS-CoV -2 infection in HCWs. This clinical trial is currently recruiting volunteers and was expected to end in April 2021.

4.1.7. NCT04537663 (BCG-PRIME) and NCT04328441 (BCG-CORONA): prevention of Covid-19 in vulnerable older adults and reducing health care worker's absenteeism in Covid-19 pandemic through BCG vaccine (Netherlands)

In the Netherlands, from 3 January 2020, to 29 April 2021, there have been a total of 1,481,323 confirmed COVID-19 cases and 17,104 deaths. As of 24 April 2021, a total of 5,207,554 vaccine doses have been administered. As a country that does not officially recommend BCG vaccination, the Netherlands is conducting the most clinical trials of BCG vaccination against COVID-19 globally, including 4 Phase IV clinical trials (EUCTR2020-000919-69-NL, EUCTR2020-002456-21-NL, NCT04537663, and NCT04417335), 1 Phase III clinical trial (NCT04328441), and 4 clinical trials with unknown phases (NL8609, NL8547, NL8477, and EUCTR2020-003470-47-NL). Currently, 3 clinical trials have begun to recruit volunteers, namely NCT04537663 (Phase IV), NCT04328441 (Phase III), and NL8547 (Phase unknown). Based on the criteria mentioned above, we will provide a detailed summary of the clinical trials NCT04537663 and NCT04328441. Both clinical trials were subsidized by UMC Utrecht and conducted using the same BCG Danish strain 1331. However, there are also significant differences between the two trials, such as objectives, population, sample size, and outcome measures. NCT04537663 is an adaptive multicenter, double-blind, randomized, placebo-controlled trial that includes 5,200 to 7,000 vulnerable elderly individuals. This trial aimed to determine the impact of BCG vaccination on the incidence of clinically relevant respiratory infections or COVID-19 in vulnerable elderly patients. The NCT04328441 trial is a placebocontrolled, adaptive, multicenter, randomized, controlled phase III clinical trial. Based on the hypothesis that BCG vaccination can reduce HCWs absenteeism during the epidemic phase of COVID-19, this clinical trial aims to recruit 1,500 HCWs. Both studies began recruiting volunteers and were expected to end in April 2021. The results of both clinical trials will provide sufficient evidence to evaluate the effectiveness of BCG in protecting the HRPs, such as HCWs and the elderly, from COVID-19 infection.

4.1.8. NCT04542330: Using BCG to protect senior citizens during the COVID-19 pandemic (Denmark)

In Denmark, from 3 January 2020, to 2 March 2021, there were 249,785 confirmed COVID-19 cases and 2,481 deaths. It is well known that seniors and HCWs are at high risk of SARS-CoV-2 infection. Thus, to investigate the hypothesis that BCG vaccination can reduce the risk of COVID-19 and other infections among senior citizens and HCWs during the COVID-19 pandemic, 4 clinical trials (NCT04542330, NCT04373291, EUCTR2020-001888-90-DK, and EUCTR2020-003904-15-DK) have been conducted in Denmark. Interestingly, the Bandim Health Project (NCT04542330 and NCT04373291) and the University of Southern Denmark (EUCTR2020-001888-90-DK and EUCTR2020-003904-15-DK) have each conducted two clinical trials, both of which included HCWs and elderly patients. Both designs reduce errors in the research process and provide the possibility for future data analysis and comparison.

4.1.9. NCT04347876: outcome of COVID-19 cases based on tuberculin test: can previous BCG alter the prognosis? (Eqypt)

According to the WHO report (https://covid19.who.int/region/ emro/country/eg), from 14 February 2020, to 8 January 2020, COVID-19-related morbidity and mortality in Egypt was 1,362.89 and 75.12 per 100,000 people, respectively. In Egypt, the BCG vaccine coverage is as high as 96%. On 15 April 2020, the Assiut University conducted a Phase III clinical trial to determine whether BCG vaccination can significantly reduce the pneumonia severity index, need for ICU admission, number of days necessary to cure COVID-19, and mortality. This study recruited 100 participants (12-80 years old) admitted with confirmed COVID-19. All volunteers were divided into two groups according to the results of the tuberculin test (positive or negative, indicating a history of previous BCG vaccination or not). The study has two design flaws: (1) A positive tuberculin test is not only caused by BCG vaccination but may also be caused by non-tuberculous mycobacteria or environmental mycobacteria. However, the researchers did not exclude this factor, which may have led to a lack of accuracy in the research results. (2) Phase III clinical trials generally require approximately 20,000 participants but this study only recruited 100 participants, which will affect the reliability of the results. Nevertheless, the clinical trial also has a major advantage, being the only clinical trial that includes children aged 12-17 years. These data will fill the gap in our knowledge regarding the effectiveness of BCG vaccination for COVID-19 prevention in children. This clinical

trial is currently recruiting volunteers and was expected to end on 30 June 2020, but the results have not yet been published.

4.1.10. NCT04327206: BCG vaccination to protect health care workers against COVID-19 (BRACE, Australia)

Although the number of confirmed cases and deaths in Australia is much lower than that in the United States, Australia has not yet carried out a national BCG vaccination program, which may increase the risk of COVID-19 infection in HCWs and the elderly when compared to the countries that recommend BCG vaccination [30]. Currently, only one clinical trial of BCG against COVID-19 was conducted in Australia (NCT04327206). This Phase III clinical trial will recruit 10,078 HCWs to determine whether BCG vaccination reduces the incidence and severity of COVID-19 during the 2020 pandemic. Compared with other clinical trials of BCG against COVID-19, the biggest advantage of this clinical trial is its large sample size (10,078). Therefore, the data provided by this clinical trial will be able to convincingly demonstrate the protective efficiency of BCG against COVID-19.

4.2. Clinical trials of VPM1002 vaccines against COVID-19

VPM1002 is a live recombinant BCG *DureC::hly* vaccine derived from the parental strain of Danish BCG and was originally developed to improve the efficacy and safety of the BCG vaccine [9]. Preclinical studies have shown that VPM1002 can induce an enhanced safety profile and Th1-/Th17-type immune responses in mice, guinea pigs, rabbits, and nonhuman primate animal models [66,67]. Several clinical trials conducted in South Africa and Germany have also indicated that VPM1002 could stimulate multifunctional T cells which secrete high levels of IFN-y or B cells that produce antibodies [68,69]. These data suggest that VPM1002 may be significantly more effective than BCG in reducing the severity of SARS-CoV -2 infection. However, as a live attenuated vaccine, VPM1002 is modified by replacing the BCG urease C gene with the membrane perforating listeriolysin O (LLO) encoding gene (hly) of Listeria monocytogenes [70]. Thus, whether its defensive efficiency against COVID-19 infection is significantly better than that of the classic BCG vaccine still needs to be verified via clinical trials.

Currently, 7 VPM1002 clinical trials have been registered to investigate its ability to prevent COVID-19 in adults and the elderly. Four of them were carried out in Germany (NCT04387409, NCT04435379, EUCTR2020-001376-15-DE, and EUCTR2020-001675-33-DE), while the other three were conducted in India (CTRI/2020/04/024749), Canada (NCT04439045), and Australia (ACTRN12620000707965), respectively (Table 1). Here, we will give more attention to the clinical trials that have begun recruiting volunteers.

4.2.1. NCT04387409 and NCT04435379: studies to assess VPM1002 in reducing HCWs' absenteeism and hospital admissions and/or severe respiratory infectious diseases in the elderly during COVID-19 pandemic (Germany)

Germany has been at the forefront of clinical trials regarding the effectiveness of the VPM1002 vaccine against COVID-19.

Currently, Germany has registered 4 VPM1002 clinical trials globally, including 2 clinical trials registered on Clinicaltrials. gov (NCT04387409 and NCT04435379) and 2 registered in the EU Clinical Trials Register (EUCTR2020-001376-15-DE and EUCTR2020-001675-33-DE). The four clinical trials were all carried out by Vakzine Projekt Management GmbH, three of which will recruit volunteers over 18 years old and one which will recruit seniors over 60 years old. Furthermore, two clinical trials (NCT04435379 and NCT04387409) had a vaccination dose of 0.1 mL VPM1002 with $2-8 \times 10^5$ CFUs, while the other two clinical trials (EUCTR2020-001675-33-DE and EUCTR2020-001376-15-DE) had a vaccination dose of 0.1 mL VPM1002 with 2-8 \times 10⁶ CFUs. Notably, the data from these clinical trials will reveal the preventive effect of the BCG vaccine on COVID-19 in people of different ages and evaluate the protective efficiency of different BCG doses against COVID-19.

4.2.2. CTRI/2020/04/024749: study to evaluate the efficacy of recombinant BCG VPM1002 in reducing infection incidence and disease severity of SARS-COV-2/COVID-19 among high-risk subjects (India)

In recent years, India has conducted several clinical research studies regarding the VPM1002 vaccine for TB prevention. Recently, the Serum Institute of India Pvt Ltd (SIIPL), the world's largest vaccine manufacturer, was supported by the Department of Biotechnology's National Biopharma Mission to conduct a double-blind, randomized, placebo-controlled, multicenter phase III clinical trial of the VPM1002 vaccine. This study recruited 5946 HCWs in close contact with COVID-19 patients to determine whether the VPM1002 vaccine can induce an enhanced immunity that helps protect patients from SARS-CoV-2. Participants in the VPM1002 vaccine group or placebo group will receive a single dose of 0.1 mL VPM1002 or 0.9% sodium chloride via intradermal injection. However, the number of CFUs in the 0.1 mL VPM1002 vaccine was not disclosed, which is extremely important for evaluating vaccine efficacy and should not be ignored. The current trial will assess the efficacy and safety of VPM1002 by counting and analyzing the number of subjects with laboratory-confirmed COVID-19 infections among HCWs or other high-risk subjects. Moreover, they will also evaluate the number of laboratory-confirmed COVID-19 infections presenting with severe, critical, or lifethreatening disease, as assessed by investigators, among HCWs or other high-risk subjects.

4.3. Clinical trial of the RUTI vaccine against COVID-19

RUTI is a therapeutic vaccine based on fragmented and detoxified *M. tuberculosis* which is used to prevent active TB in subjects with latent TB infection (LTBI) [71]. This vaccine was initially developed by Archivel Farma S.L. (Spain) in collaboration with Parexel (USA). Previous studies have demonstrated that the RUTI vaccine provides a strong humoral and cellular immune response against *M. tuberculosis* antigens [72], induces a balanced immune response, and reflects its impact on TI [73]. Moreover, a pre-clinical study has indicated that the RUTI vaccine has good efficacy in controlling LTBI in mice, guinea pigs, goats, and mini-pigs [74]. Clinical trials have also



Figure 3. The global situation of confirmed COVID-19 cases (A) and deaths and the regional situation of confirmed COVID-19 cases (B). The data was obtained from WHO COVID-19 dashboard (https://covid19.who.int/) and showed as cases per weekly. Each chart's blue date represents the date when the number of confirmed cases or deaths per week shows a downward trend. The data collection time is 3 March 2021.

suggested that the RUTI vaccine has acceptable tolerability, immunogenicity, and safety in healthy volunteers (NCT00546273) and patients with LTBI [74,75] or multidrug-resistant TB (NCT02711735) [9,76].

Based on these encouraging results, Fundació Institut Germans Trias i Pujol in Spain performed a double-blind, randomized, placebo-controlled phase III clinical trial to evaluate the efficacy of the RUTI vaccine in preventing SARS-CoV-2 infection (NCT04453488). Three hundred and fifteen HCWs over the age of 18 will receive two doses of 0.3 mL RUTI vaccine (25 μ g) or 0.3% NaCl (0.9%) via subcutaneous injection in the deltoid region at the baseline visit and after 2 weeks \pm 3 days, respectively. The primary outcome of this study was the rate of positive serology at the end of the study or a positive PCR test during routine clinical practice. It should also be noted that the immunogenicity of the vaccine remains uncertain. A phase II clinical trial in patients with LTBI demonstrated that the safety profile of the RUTI vaccine was considered acceptable. However, its immunogenicity profile varied among groups [74]. Therefore, these issues should be considered in clinical trials of RUTI vaccines against COVID-19.

5. Challenges of COVID-19 vaccines

Vaccination has been the best way to fight against SARS-CoV-2 infection [77]. Currently, there are 76 and 182 COVID-19 vaccines in clinical and preclinical development, respectively (https://www.who.int/publications/m/item/draft-landscape-of-

covid-19-candidate-vaccines). What is exciting is that more than ten COVID-19 specific vaccines have been approved under EUA by over 40 countries [11–13,20,21,78]. By analyzing the data regarding weekly confirmed cases and deaths released by the WHO, we found that after vaccination with the COVID-19 vaccines, the number of weekly confirmed cases and deaths worldwide began to show a downward trend from early January 2021, but an upward trend from February and March 2021 (Figure 3(a)), especially in the Americas, Europe, Southeast Asia, Eastern Mediterranean, and Western Pacific (Figure 3(b)). This demonstrates that COVID-19 vaccination effectively reduces morbidity and mortality but this trend has been changed to upward due to the combined effects of other confounding factors, such as the lifting of lockdowns, the absence of masks, the restoration of social activities, uncertain duration of immunity after vaccination, and the emergence of mutant strains.

Although the COVID-19 vaccines approved under EUA have brought hope to people under the threat of the COVID-19 pandemic, they still face several challenges.

5.1. Challenge I: The emergence of SARS-CoV-2 variants

As the most crucial SARS-CoV-2 surface protein, the spike (S) protein plays an essential role in viral infection [79]. Therefore, 44 of the 76 COVID-19 vaccines in clinical trials were based on the S protein, according to the WHO report (https://www.who.int/publications/m/item/draft-landscapeof-covid-19-candidate-vaccines). Currently, the COVID-19 vaccines approved under EUA have been proven to have adequate protection efficiency and safety against the original SARS-CoV-2 strain. However, SARS-CoV-2 variants have emerged in the United Kingdom (20I/501Y.V1, lineage B.1.1.7), South Africa (20 H/501Y.V2, lineage B.1.351), Brazil (20 J/501Y.V3, lineage P.1), and India (double mutated virus, B.1.617), which has raised concerns regarding the effectiveness of these vaccines [80-83]. It has been reported that these SARS-CoV-2 variants may have a higher transmission rate and are associated with escape from neutralizing antibodies induced by COVID-19 vaccines [82,84]. Therefore, there is an urgent need to evaluate the protective efficiency of COVID-19 vaccines against SARS-CoV-2 variants. Recently, a study has shown that the Covaxin vaccine effectively neutralizes the UK SARS-CoV-2 variant and reduces the possibility of mutant virus escape [85]. Similarly, it was also reported that the protective efficiency of NVX-CoV2373 against the original strain and the UK variant strain was 95.6% and 85.6%, respectively [86]. However, studies have also shown that the neutralizing effect of the Moderna mRNA-1273 vaccine and Pfizer-BioNTech BNT162b2 vaccine on SARS-CoV-2 variants (E484K and N501Y) was slightly reduced [87], while the NVX-CoV2373 vaccine was only 49.4% effective in preventing the South African SARS-CoV -2 variant strain [86]. These data indicate that evaluating the protective efficiency of currently approved COVID-19 vaccines against SARS-CoV-2 variants will be an urgent but time-consuming project.

5.2. Challenge II: Some HRPs were not included in the clinical trials of COVID-19 vaccines

Currently, most subjects in clinical trials of COVID-19 vaccines are HCWs, with only a small number of older adults included in the study cohort. However, other HRPs, such as infants, children, and pregnant women, were excluded. Due to their physical condition, these HRPs have lower immunity than normal adults and are more likely to be infected by SARS-CoV-2. Although several COVID-19 vaccines, such as BNT162b2, mRNA-1273, and ChAdOx1 nCoV-19 (AZD1222), can also be used in older adults and have been shown to be somewhat effective, the vaccine dose requires clarification through further studies [88,89]. An age-to-age infection matrix suggested that transmission of SARS-CoV-2 was more common from adults to children than from children to adults, which means that children are at a disadvantage in the COVID-19 pandemic [90]. For pregnant women, the safety of the vaccines is the primary factor determining their acceptance of the COVID-19 vaccination. A recent study evaluated COVID-19 vaccine acceptance among pregnant women in 16 countries and the results showed that only 52.0% of pregnant women indicated an intention to receive the COVID-19 vaccine [91]. Unfortunately, children, pregnant women, and the elderly are often excluded from COVID-19 vaccines clinical trials, resulting in a lack of first-hand data regarding the safety and effectiveness of the COVID-19 vaccines in these populations. In the future, we propose to include children, pregnant women, and the elderly in COVID-19 vaccines clinical trials.

5.3. Challenge III: The cold chain logistics for the transportation of COVID-19 vaccines

Mass vaccination is a very effective public health intervention during the COVID-19 pandemic. However, after COVID-19 vaccines have become available, transporting these vaccines quickly and efficiently from manufacturers to hospitals or communities is the next crucial step in the whole process of vaccine circulation [92]. The most significant difference between the vaccine supply chain and transportation of other goods lies in the cold chain logistics requirements [93]. The transportation and storage of inactivated vaccines, such as the Sinovac vaccine and Sinopharm's COVID-19 vaccine need to be carried out at 2°C to 8°C, with a complete industrial chain and mature technology [94]. However, Pfizer/BioNTech's BNT162b2 and Moderna mRNA-1273 require temperatures of -80°C and -20°C, respectively. Thus, the delivery of these mRNA vaccines requires specialized freezers that most doctors' offices and pharmacies are unlikely to have on-site. As such, the easiest solutions are aircraft transportation and dry ice storage. However, the global air cargo throughput is insufficient to distribute these COVID-19 vaccines internationally and the instability of dry ice may render the vaccine ineffective [95]. The WHO

estimates a huge risk of losing COVID-19 vaccines due to cold chain failures and nonfunctional freezers [96]. Therefore, the best solution may be to use the ultra-low temperature freezer as the transport carrier but this equipment is expensive and difficult to obtain, so solving the challenges in the cold chain logistics of COVID-19 vaccines in a short time remains difficult.

5.4. Challenge IV: Global imbalance of supply of COVID-19 vaccines

The development of effective COVID-19 vaccines is a global marathon competition from basic research and clinical trials to the current vaccine supply and distribution. Developed countries have always been in a leading position in this competition. COVAX, which is co-led by the WHO, Coalition for Epidemic Preparedness Innovations (CEPI), and the Global Alliance for Vaccines and Immunization (GAVI), is committed to accelerating the development, production, and global fair distribution of COVID-19 vaccines (https://www.who.int/initia tives/act-accelerator/covax). Worryingly, WHO and its partners' efforts may not meet the needs of the global population, especially those in LMICs (http://infojustice.org/archives/ 42255), as it was reported that 80% of the population in LMICs could not benefit from COVID-19 vaccines provided by COVAX [92]. Global cooperation is urgently needed in response to an imbalance in the supply and distribution of COVID-19 vaccines. As such, developed countries should actively support LMICs to expand their testing capabilities and obtain COVID-19 vaccines reasonably and affordably [97].

6. Opportunities of BCG vaccine

As mentioned in the previous section, the currently available COVID-19 vaccines face many challenges. Interestingly, the above-mentioned shortcomings of COVID-19 vaccines represent advantages of the BCG vaccine. BCG is the only vaccine approved for TB prevention [9]. Moreover, due to its ability to induce TI, the BCG vaccine has been used to treat other viral infections in humans [9,38]. Recently, it was proposed that the BCG vaccine may have a protective effect against COVID-19 [22]. As previously mentioned, 51 clinical trials were conducted to verify this hypothesis. Once this hypothesis is demonstrated, the BCG vaccine may help overcome the challenges associated with COVID-19 vaccines.

6.1. Opportunity I: Better safety and larger applicable population

Accumulating evidence has shown that age is a crucial risk factor associated with the severity of symptoms caused by SARS-CoV-2 [98]. Therefore, there is an urgent need to reduce the incidence and mortality of COVID-19 in infants and the elderly. Unfortunately, these two groups of people are often excluded from the scope of COVID-19 vaccination programs. Thus, the BCG vaccine can compensate for this shortcoming. Adverse events following immunization (AEFI) are essential indicators to evaluate vaccine safety [99]. A recent review

conducted in Africa compared the AEFI of nine vaccines and found that the BCG vaccine was generally well-tolerated in neonates [94], suggesting that its safety is reliable [100]. In addition, studies have found that BCG vaccination of pregnant women had no obvious effect on the fetus, indicating that BCG vaccination is also safe for pregnant women [101,102]. A study in the elderly also suggested that TI elicited by BCG vaccination may improve immune responses and serve as a strategy to combat COVID-19 [98]. These results demonstrate that the BCG vaccine has reliable safety and its applicability has achieved full coverage, from neonates to the elderly.

6.2. Opportunity II: Less public hesitation, better affordability and accessibility

Currently, COVID-19 vaccines are being limited by public hesitation, affordability, and accessibility. A cohort study demonstrated that compared to Caucasian participants, COVID-19 vaccine hesitancy was greater among ethnic minorities and COVID-19 vaccine affordability and accessibility were found to be lower in African-American participants in the United States [103]. A similar hesitation was observed in the Philippines [104]. In addition, in a social survey in the United States, approximately 40% of the respondents who had not been vaccinated or had not scheduled vaccinations indicated that they were unwilling to receive the COVID-19 vaccine [105]. These data indicate that the public's acceptance of the COVID-19 vaccine is still a vital factor that hinders the widespread COVID-19 vaccination. In contrast, the BCG vaccine has better acceptance, affordability, and availability. According to the WHO statistics, in 2019, over 153 countries recommend BCG vaccination as a standard part of national childhood immunization programs, of which 87 reported coverage of \geq 90% [31]. Our previous study also found that the BCG vaccination coverage in LMICs, especially those in the LDCs, is very high, which may be associated with the low cost and high benefit of the BCG vaccine [22]. Moreover, the BCG vaccine price is relatively low, and even in some developing countries, governments provide free vaccination services, which dramatically increases the accessibility and affordability of the BCG vaccine. If the clinical trial results support the hypothesis that BCG can effectively prevent COVID-19, the BCG vaccine will solve many challenges faced by currently available COVID-19 vaccines, such as multiple doses imposing financial burdens and patient compliance barriers [106].

6.3. Opportunity III: Simple transportation and storage

As discussed in Section 5.3, cold-chain logistics affect the worldwide distribution of COVID-19 vaccines. It has been reported that even highly developed countries, such as the United States, cannot provide sufficient cold-chain logistics to ensure the national distribution of COVID-19 vaccines, let alone LMICs in the epidemic and economic vortex [96]. Although LMICs, especially the LDCs, still have a gap in their understanding, attitude, and practice of vaccination cold-chain logistics systems, this does not mean that these countries experience obstacles regarding the transportation, storage,

and distribution of routine vaccines, such as the BCG vaccine. A recent study evaluated the knowledge and practice of vaccination logistics management systems among HCWs in Nigeria and the results suggested that 83.9% and 81.1% of HCWs had good knowledge and attitude, respectively, regarding the management systems [107]. In addition, it has been reported that the stability of the dry powder BCG vaccine did not decrease after storage at 25°C for one year [108]. These data indicate that compared to COVID-19 vaccines, the BCG vaccine has absolute advantages in regards to transportation, storage, distribution, and vaccination, especially in countries where COVID-19 vaccines are not available.

6.4. Opportunity IV: Larger production capacity and a shorter wait

COVAX has an ambitious plan to provide at least 2 billion doses of COVID-19 vaccines by the end of 2021, which unfortunately will only account for 20% of the COVID-19 vaccine needs of participating countries [92]. Furthermore, many highincome countries have signed bilateral agreements with COVID-19 vaccine manufacturers, while most LMICs face difficulties in obtaining and providing COVID-19 vaccines to their citizens [109]. In sharp contrast, the production technology of the BCG vaccine is very well established, as it is a vaccine that has been used for decades. Sufficient production capacity, effective demand forecasting, and national-level procurement strategy enable the BCG vaccine to be supplied in large guantities in a short time [31]. Coupled with the low requirements for transportation and storage, mass-produced BCG vaccines can be distributed to hospitals and clinics in time for vaccination.

7. Conclusions

In summary, at least ten COVID-19 specific vaccines have been approved under the EUA for vulnerable populations. However, these approved COVID-19 vaccines still face many challenges, such as the emergence of SARS-CoV-2 variants, short protective duration, low acceptance, inaccessibility, unaffordability, unfair distribution, and inconvenient transportation and storage. Interestingly, as an ancient TB vaccine, the BCG vaccine has attracted attention due to its ability to induce TI. The TI induced by the BCG vaccine seems to have advantages that can compensate for the shortcomings of currently available COVID-19 vaccines, including better safety, long duration of protective efficacy (10 to 15 years), larger applicable population, less public hesitation, better affordability and accessibility, simple transportation and storage, and higher production capacity. Accumulating data indicate that the BCG vaccine may have good potential in preventing COVID-19 via its trained innate immunity, but this hypothesis needs to be confirmed by clinical trials. Currently, 51 clinical trials of BCG vaccines (including 7 of VPM1002 and 1 of RUTI) against COVID-19 have been conducted, including 9 phase IV clinical trials, 28 phase III clinical trials, 3 phase II clinical trials, and 11 phase unknown clinical trials. Thus, the results of these clinical

trials will provide substantial evidence regarding whether the BCG vaccine is effective in preventing COVID-19.

8. Expert opinion

Globally, as of 29 April 2021, there have been 149,216,984 confirmed cases of coronavirus disease 2019 (COVID-19), including 3,144,028 deaths reported by the World Health Organization (WHO). Vaccination is the best way to defend against COVID-19. Currently, there are 76 and 182 COVID-19 vaccines in clinical and preclinical development, respectively. Moreover, at least ten vaccines have been authorized under an emergency use authorization (EUA) to prevent COVID-19 in over 40 countries, including Moderna's mRNA-1273 vaccine (United States), Pfizer-BioNTech's BNT162b2 vaccine (United States & Germany), Sinovac vaccine (China), Sinopharm's two COVID-19 vaccines (China), CanSino' Ad5-nCoV vaccine (China), Sputnik V vaccine (Russia), EpiVacCorona vaccine (Russia), AstraZeneca's ChAdOx1 nCoV-19 (England), and Janssen's Ad26.COV2.S (United States). As of 28 April 2021, a total of 968,452,196 vaccine doses have been administered worldwide.

However, despite their efficacy, COVID-19 vaccines face many challenges: (1) The emergence of SARS-CoV-2 variants in the United Kingdom (20I/501Y.V1, lineage B.1.1.7), South Africa (variant 20 H/501Y.V2, lineage B.1.351), and Brazil (20 J/ 501Y.V3, lineage P.1) has raised concerns regarding the effectiveness of these vaccines. (2) The duration of immunity induced by the COVID-19 vaccines remains uncertain. It has been shown that vaccinated patients could still be infected with SARS-CoV-2 and patients who have been cured are also at risk of being infected again. Notably, protective antibodies in recovered patients are present for only a few months. (3) Some high-risk participants (HRPs) were not included in the COVID-19 vaccines clinical trials, such as infants, children, and pregnant women. (4) Cold chain logistics for the transportation of COVID-19 vaccines. The WHO estimates a massive risk of losing COVID-19 vaccines due to cold chain failures and nonfunctional freezers. (5) Global imbalance in the supply of COVID-19 vaccines. It was reported that 80% of the population in low- and middle-income countries (LMICs) could not benefit from the COVID-19 vaccines provided by COVAX.

The Bacille Calmette-Guérin (BCG) vaccine is a live attenuated vaccine used for more than 100 years as a prophylactic agent against tuberculosis (TB). Several epidemiological studies and randomized controlled trials have reported that BCG vaccination could also protect against other unrelated pathogens. More importantly, controlled experimental studies in humans have provided direct evidence for the protective efficacy of BCG vaccination against clinically relevant pathogens, such as those who cause yellow fever and malaria. Thus, it has been hypothesized that these BCG-induced nonspecific beneficial effects may also help prevent COVID-19. To confirm this hypothesis, more than 51 clinical trials investigating the efficacy of the BCG vaccine against COVID-19 have been conducted worldwide, including 9 phase IV clinical trials, 28 phase III clinical trials, 3 phase II clinical trials, and 11 phase unknown clinical trials.

Although there are currently several COVID-19 vaccines already approved for emergency use, there are still as many

as 51 clinical trials investigating BCG-induced prevention of COVID-19, indicating that the BCG vaccine has several advantages over other COVID-19 vaccines. Interestingly, the shortcomings of the above-mentioned COVID-19 vaccines represent advantages of the BCG vaccine, such as: (1) Better safety and larger applicability in the general population. BCG has reliable safety and its applicability is considerably larger, having full coverage from neonates to the elderly. (2) Less public hesitation, better affordability, and accessibility. According to WHO statistics, in 2019, over 153 countries recommended BCG vaccination as a standard part of national childhood immunization programs. Moreover, the price of the BCG vaccine is relatively low and even in some developing countries, governments provide free vaccination services, therefore dramatically increasing the accessibility and affordability of the BCG vaccine. (3) Simple transportation and storage. The delivery of mRNA COVID-19 vaccines, such as Pfizer/ BioNTech's BNT162b2 and Moderna's mRNA-1273, requires specialized freezers that most offices of doctors and pharmacies are unlikely to have on-site. In contrast, it has been reported that the dry powder BCG vaccine remained stable even after storage at 25°C for one year. These data show that, compared to the COVID-19 vaccines, the BCG vaccine has absolute advantages in regards to transportation, storage, distribution, and applicability, especially in countries where COVID-19 vaccines are not available. (4) Larger production capacity and shorter wait time. As a vaccine used for decades, the production technology for the BCG vaccine is very well established. Sufficient production capacity, effective demand forecasting, and national-level procurement strategies enable the supply of large quantities of the BCG vaccine quickly. Moreover, coupled with the low requirements for transportation and storage, mass-produced BCG vaccines could be distributed to hospitals and clinics in time for vaccination.

Once this hypothesis is confirmed, the BCG vaccine may provide a possible solution to the problems faced by COVID-19 vaccines due to its advantages regarding safety, availability, affordability, production capacity, transportation, storage, and distribution capacity

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