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Review

The Potential Roles of BCG Vaccine in the Prevention or Treatment of COVID-19

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Abstract

Coronavirus disease 2019 (COVID-19), which broke out at the end of 2019, is a global pandemic and seriously threatens human health. Vaccination is the most effective way to prevent and control COVID-19. At present, more than 13 COVID-19 vaccines have been urgently authorized for use, but the emergence of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants has brought unprecedented challenges to the protective efficiency of these COVID-19 vaccines. In particular, the recent emergence of Delta and Omicron variants, which are rapidly spreading worldwide, may bring many challenges to the medical systems. Interestingly, previous studies have shown that the Bacillus Calmette-Guerin (BCG) vaccine used to prevent tuberculosis can induce non-specific trained immunity, protecting against infectious diseases caused by respiratory viruses. Therefore, there is a hypothesis that BCG plays an essential role in reducing the incidence, severity, hospitalization, and mortality of COVID-19 and enhancing the protection efficiency of the COVID-19 vaccine. To confirm this hypothesis, 56 clinical trials have been conducted globally to assess BCG's protective effectiveness against COVID-19 infection. Herein, this review discussed the trained immunity induced by BCG and its underlying mechanisms and summarised BCG's latest research progress in preventing COVID-19, especially the ongoing clinical trials. We hope this review will provide new strategies for fighting against COVID-19.

Keywords: Bacillus Calmette-Guerin (BCG); trained immunity; COVID-19; SARS-CoV-2; variants; vaccines

1. Introduction

Coronavirus disease 2019 (COVID-19) is a respiratory infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Since 2019, COVID-19 has had an significant impact on the global economy, politics, and health system. As of April 21, 2022, the number of confirmed cases globally has reached 505,035,1854, including 6,210,719 deaths (https://covid19.who.int). Vaccination is the best way to prevent and control COVID-19. At present, at least 13 COVID-19 vaccines have been urgently authorized, including (1) inactivated vaccines, BBV152 (Covaxin, India) [1], BBIBP-CorV (Sinopharm, China) [1,2], and Sinovac (CoronaVac, China) [1,2]; (2) Viral vector vaccine, ChAdOx1 nCoV-19 (AstraZeneca, UK) [2,3], Sputnik V (Gamaleya, Russia) [2], CanSino'Ad5-nCoV (China) [3], Ad26.COV2.S (Janssen, USA) [2]; (3) Nucleic acid vaccine, BNT162b1/BNT162b2 (Pfizer-BioNtech, USA) [2], mRNA1273 (Modern, USA) [2], CureVac (Germany) [4]; (4) Subunit vaccine, NVX-CoV2373 (Novavax, USA) [5], EpiVacCorona (Russia) [2], and ZF2001 (Anhui Zhifei Longcom, China) [6,7]. As of April 17, 2022, a total of 11,324,805,837 doses of COVID-19 vaccine have been vaccinated worldwide (https://covid19.who.int).

Although the above vaccines approved for emergency use in COVID-19 prevention have sound protective effects, they also face many challenges [3,8]. First, the ongoing emergence of SARS-CoV-2 variants poses a continuing threat to the efficacy of COVID-19 vaccines, such as immune escape, reduced protection efficiency and antibody neutralization ability [9]. Second, the accessibility of COVID-19 vaccines varies among different countries, which affects the balanced global supply of COVID-19 vaccines. Third, the acceptance of COVID-19 vaccines has been decreased by the shortened process of COVID-19 vaccine development and production. Finally, most COVID-19 vaccines require high transportation conditions, especially the mRNA vaccines should be stored at –80 °C. The substandard transportation conditions may increase the risk of vaccine failure and affect the popularization of these vaccines.

Previous studies have found that the trained immunity induced by the Bacillus Calmette-Guerin (BCG) vaccine might have a protective effect on infectious diseases caused by respiratory viruses, including respiratory syncytial virus (RSV), influenza viruses, and SARS-CoV-2 [3,10–12]. To further explore the impact of BCG vaccination on COVID-19 morbidity, severity, hospitalization, mortality, and the

protection efficiency of the COVID-19 vaccines, this review focuses on the trained immunity induced by the BCG vaccine and its underlying mechanisms, highlights the latest research progress on BCG effect on the prevention of COVID-19, and provides potential solutions for many challenges faced by COVID-19 vaccines.

2. Challenges of COVID-19 Vaccines Fighting against SARS-CoV-2 Variants

SARS-CoV-2 is an RNA virus belonging to coronaviridae, having a high mutation rate [13]. During the COVID-19 pandemic, a growing number of SARS-CoV-2 variants emerged and spread rapidly worldwide (https://www.who.int/activities/tracking-SARS-CoV-2-variants/tracking-SARS-CoV-2-variants, Table 1 [5,6,14–51]). As of March 24, 2022, the World Health Organization (WHO) defined five SARS-CoV-2 variants as Variants of Concern (VOCs) (Fig. 1), including two currently circulating VOCs (Table 1), Delta (B.1.617.2) and Omicron (B.1.1.529), and three previously circulating VOCs such as Alpha (B.1.1.7), Beta (B.1.351), and Gamma (P.1). Furthermore, eight SARS-CoV-2 variants are also listed as previously circulating Variants of Interest (VOIs), including Lambda (C.37) and Mμ (B.1.621). According to the data reported on the Nextstrain website (Fig. 2A), Alpha (first discovered in the UK), Beta (South Africa), and Gamma (Brazil) variants have gradually begun to spread in the world by the first half of 2021. The infection rates of these three variants were 42% (Alpha), 9% (Beta) and 10% (Gamma), respectively. With the first emergency of the Delta variant in India, the proportion of the above three variants declined rapidly. By October 16, 2021, the Delta variant has accounted for 96% of new cases worldwide (Fig. 2A). Subsequently, the dominance of the Delta variant in the population was broken by the Omicron variant observed in South Africa in November 2021. The transmission speed of the Omicron variant was much faster than that of the Delta variant. As of April 16, 2022, the global proportion of Omicron variant infection has reached 99% (https://nextstrain.org/ncov/gisaid/global).

The primary mutation sites of these SARS-CoV-2 variants occurred on the Spike (S) protein, and most of the COVID-19 vaccines were designed based on the S protein of the original strain [14] (Fig. 2B). Many studies have shown that the transmission speed of these SARS-CoV-2 variants is enhanced, and the neutralizing ability and protection efficiency of neutralizing antibodies induced by the COVID-19 vaccine are reduced.

2.1 Challenges of COVID-19 Vaccines Fighting against SARS-CoV-2 VOCs

2.1.1 Alpha (B.1.1.7) Variant

The Alpha variant was firstly discovered in the UK in September 2020. It spread in Europe in the early stage and then became the dominant strain worldwide. As of April 20, 2021, the global proportion of Alpha variant reached 40%. The S protein of Alpha variant has nine characteristic mutations (characteristic mutations for a lineage are defined as nonsynonymous substitutions or deletions that occur in >75% of sequences within that lineage, https://outbreak.info/), including del69/70, del144/144, N501Y, A570D, D614G, P681H, T716I, S982A, and D1118H (Fig. 1). Studies have shown that N501Y mutation can enhance the affinity between the S protein of Alpha variant and angiotensin-converting enzyme 2 (ACE2) receptor of the human [52], have relative resistance to neutralizing antibody, and enhance virus transmission [14,15]. The del144/144 mutation makes it difficult for the Alpha variant to be neutralized by most monoclonal antibodies against the N-terminal domain of S protein [16]. Based on the above mutations, the transmission of the Alpha variant increased [53], the effect of neutralizing antibodies decreased, and the mortality and disease severity of COVID-19 patients increased [54].

Previous studies have evaluated the efficacy of COVID-19 vaccines against the Alpha variant infection. (1) NVX-CoV2373 vaccine. A previous study indicated that the NVX-CoV2373 vaccine was 95.6% effective against the original SARS-CoV-2 strain and 85.6% effective against the Alpha strain [17]. Similarly, in a phase III clinical trial conducted in the UK, 15,187 adults aged 18–84 were randomly divided into two groups vaccinated with 5μg NVX-CoV2373 vaccine or placebo at day 0 and 21, respectively. The results showed that the protection of the NVX-CoV2373 vaccine against SARS-CoV-2 infection was 89.7% (95% confidence interval (CI), 80.2 to 94.6), and the effectiveness against the Alpha variant was 86.3% (95% CI, 71.3 to 93.5) [18]. These results suggest that the NVX-CoV2373 vaccine has slightly reduced efficacy against Alpha variant infection but still provides adequate protection. (2) mRNA-1273 vaccine. It has been shown that the effectiveness of the mRNA-1273 vaccine against SARS-CoV-2 original strain infection is 94.1% (95% CI, 89.3 to 96.8) [25]. With the emergence of the Alpha variant, a controlled study on adults in Qatar showed that the effectiveness of mRNA-1273 vaccine on Alpha variant infection was 81.6% and 94.4%, respectively in the third and fourth weeks after the first dose, and 99.2% in the second week after the double dose [19]. It is suggested that the first dose of the mRNA-1273 vaccine can obtain most of the protection, and the second dose can fight Alpha variant infection more effectively. (3) BNT162b2 vaccine. A study was performed to detect the neutralization antibodies induced by the BNT162b2 vaccine against the SARS-CoV-2 variant. The sera collected from 37 healthy volunteers vaccinated with two doses of the BNT162b2 were inoculated with the Alpha variant. The results showed that compared with SARS-CoV-2 wild type (B.1 lineage), the neutralization effect of neutralizing antibodies induced by two doses of BNT162b2 vaccine against Alpha variant was significantly increased, and the neutralization titers were higher

Fig. 1. The prevalence of mutations in the S protein of the SARS-CoV-2 variants. Mutations in the S protein of five VOCs with >75% prevalence were shown with purple boxes. The darker the colour, the higher the prevalence. The data were obtained from the outbreak.info website (https://outbreak.info/) and accessed on April 16, 2022.

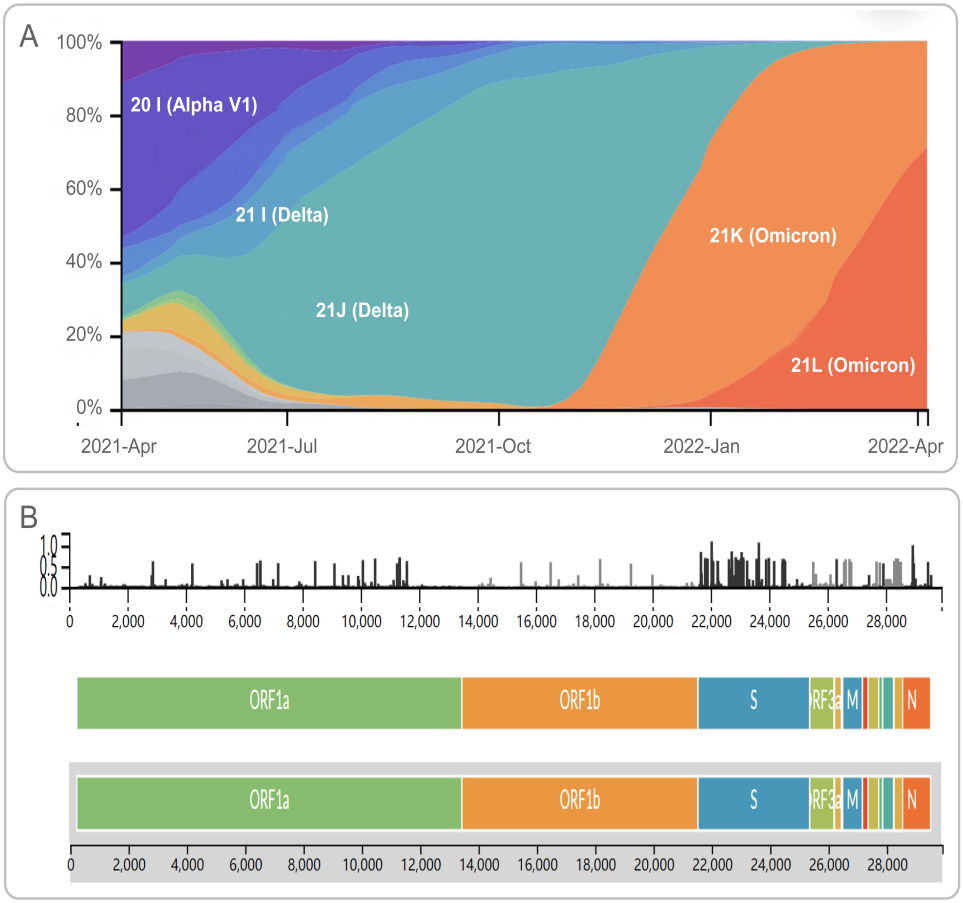

Fig. 2. Frequencies and diversity of SARS-CoV-2 variants. (A) The dynamic change diagram of the frequencies of SARS-CoV-2 variants from February 2021 to April 2022. (B) The diversity of amino acid mutations in SARS-CoV-2 were shown by entropy. The data were obtained from https://nextstrain.org/ncov/gisaid/global, and the data acquisition time was April 16, 2022.

Table 1. The characteristics of the SARS-CoV-2 VOCs and VOIs.

Type	WHO label Variant/lineage	Origin	Mutations in spike protein*	Influence	Reference
VOC*	Alpha(α) B.1.1.7	Sep-2020 United Kingdom	del69/70, del144/144, N501Y, A570D, D614G, P681H, T716I, S982A, D1118H	(1) N501Y mutation can enhance the affinity between Alpha variant S protein and human host cell ACE2 receptor, have relative resistance to neutralising antibodies and enhance virus transmission [16,17]. (2) The del144/144 mutation makes it difficult for the Alpha variant to be neutralised by most monoclonal antibodies against the N-terminal domain of S protein [18]. (3)The effectiveness of the NVX-CoV2373 vaccine on the original variant of SARS-CoV-2 is 95.6%, and the efficacy on the Alpha variant is 85.6% [17]. (4) The protection rate of the NVX-CoV2373 vaccine against SARS-CoV-2 infection was 89.7%, and the effectiveness against the Alpha variant was 86.3% [22]. (5) The effectiveness of the mRNA-1273 vaccine on Alpha variant infection was 81.6% and 94.4%, respectively, in the third and fourth weeks after the first dose and 99.2% in the second week after the double dose [24]. (6) The neutralisation effect of neutralising antibodies induced by two doses of the BNT162b2 vaccine on the Alpha variant was significantly increased, and the neutralisation titers were higher than 1:40 [25]. (7) The effectiveness of one dose and two doses of BNT162b2 vaccine against the Alpha variant was 47.5% and 93.7%; The efficacy of one dose and two doses of ChAdOx1 nCoV-19 vaccine against the Alpha variant was 48.7% and 74.5%, respectively [26].	[14,21]
	Beta(β) B.1.351	May-2020 South Africa	D80A, D215G, K417N, E484K, N501Y, D614G, A701V	(1) E484k mutation can reduce the sensitivity of the SARS-CoV-2 virus to convalescent serum and vaccine-induced neutralising antibodies [27,28]. (2) The infectivity of the Beta variant in Calu-3 cells was enhanced; the three mutations on S protein (E484k, K417N/T, N501Y) significantly increased the ability of infected mice to overexpress ACE2 cells [28]. These mutations increased the propagation rate of the Beta variant by 50% [30]. (3) The effectiveness of the mRNA-1273 vaccine against SARS-CoV-2 original strain infection was 94.1% [23]. (4) The effectiveness of the mRNA-1273 vaccine against Beta variant infection was 47.9% and 73.7%, respectively, in the third and fourth week after the first dose and 96.4% in the second week after the double dose [24]. (5) The effectiveness of NXV-CoV2373 vaccine for Beta variant was 51.0% (95% CI, 0.6 to 76.2) [5]. (6) The efficacy of two doses of ChAdOx1 nCoV-19 vaccine against mild to moderate COVID-19 caused by Beta variant was 10.4% [32]. (7) Compared with SARS-CoV-2 wild-type, the neutralisation titers of convalescent plasma and antiserum inoculated with mRNA vaccine to Beta variant decreased by about 10 and 6 times, respectively [6,33,34]. (8) Compared with SARS-CoV-2 wild-type, the neutralisation titers of antibodies induced by ZF2001 and BBIBP-CorV vaccines to Beta variant decreased by 1.59 times and 1.56 times [6].	[5,6,19,22–28]

Table 1. Continued.

Type	WHO label Variant/lineage	Origin	Mutations in spike protein*	Influence	Reference
	Gamma(γ) P.1	Nov-2020 Brazil	L18F, T20N, P26S, D138Y, R190S, K417T, E484K, N501Y, D614G, H655Y, T1027I, V1176F	(1) Compared with the SARS-CoV-2 wild type, the neutralisation of the Gamma variant by various monoclonal antibodies, convalescent plasma, and inoculated serum decreased or even lost [35]. (2) The neutralisation titer of antibody-induced by BNT162b2 vaccine to Gamma variant was 3.3 times lower than that of Alpha variant [14]. (3) The neutralisation effect of the BNT162b2 vaccine on the Gamma variant was significantly lower than that of the SARS-CoV-2 original strain [25].	[20,29,30]
	Delta(δ) B.1.617.2	Oct-2020 India	T19R, E156G, del157/158, L452R, T478K, D614G, P681R, D950N	(1) L452R and P681R mutations may enhance the infection and proliferation of the virus [30]. L452R can make the Delta variant escape the attack of CD8+ T cells. Delta variant is an immune escape variant with more robust host cell entry and fusion ability [31]. (2) COVID-19 patients infected with the Delta variant had a higher risk of emergency admission or death and a more significant burden on the health system than the Alpha variant [32]. (3) Early data from the UK showed that BNT162b2 and ChAdOx1 nCoV-19 vaccines had only 33% protection efficiency against the Delta variant after the first dose [33,34]. (4) The effectiveness of two doses of BNT162b2 vaccine on Alpha variant and Delta variant was 93.7% and 88%, respectively; The efficacy of two doses of ChAdOx1 nCoV-19 vaccine against Alpha variant and Delta variant was 74.5% and 67%, respectively [21]. (5) Compared with the SARS-CoV-2 wild type, the neutralising antibody titer of the Delta variant decreased by 5.8 times, which was significantly higher than that of the Alpha variant (reduced by 2.6 times) and the Beta variant (reduced by 4.9 times [35]. (6) Compared with G614 variant, the neutralisation titer of serum neutralising antibody induced by BNT162b2, mRNA-1273 and Ad26 COV2.S vaccine to Delta variant decreased by 3 times, 2 times and 12 times, respectively [36].	[21,30–36]
	Omicron(o) B.1.1.529	Nov-2021 South Africa	A67V, del69/70, T95I, G142D, del143/145, N211I, del212/212, G339D, S371L, S373P, S375F, Q493R, G496S, Q498R, N501Y, Y505H, T547K, D614G, H655Y, N679K, P681H, N764K, D796Y, N856K, Q954H, N969K, L981F	(1) D614G can increase the viral load of hathe master's upper respiratory tract, which may be conducive to virus transmission [37]. (2) N501Y mutation can enhance the affinity between virus S protein and ACE2 and prevent neutralising antibodies [14,15]. (3) The Omicron variant has strong transmissibility and immune evasion, which can cause re-infection of COVID-19 patients infected with other variants of SARS-CoV-2 [38,39]. (4) Compared with the Delta variant, the disease severity and risk of death of patients infected with the Omicron variant were significantly reduced [40,41]. The Omicron variant has a 6-fold higher risk of reinfection than the Delta variant [41]. (5) Compared with the Delta variant, the Omicron variant grows 70 times faster in the human bronchus and 10 times slower in the lung [42]. (6) The neutralizing effect of neutralising antibody induced by BNT162b1/BNT162b2 vaccine on Omicron variant decreased 41 times compared with SARS-CoV-2 wild type [43]. Compared with the Delta variant, the neutralising effect of the antibody induced by the BNT162b2 vaccine on the Omicron variant was reduced by 12–44 times [44]. (7) Compared with the SARS-CoV-2 wild type, The neutralisation effect of BNT162b2, mRNA-1273 and Ad26 COV2.S vaccine on the Omicron variant were reduced by 7–45 times [45]. (8) The efficacy of 2 and 3 doses of mRNA vaccine on the Omicron variant was 65% and 86%, respectively [46].	[14,15,37–46]

Table 1. Continued.

Type	WHO label Variant/lineage	Origin	Mutations in spike protein*	Influence	Reference
VOI*	Lambda(λ) C37	Dec-2020 Peru	G75V, T76I, R246N, del247/253, L452Q, F490S, D614G, T859N	(1) L452Q enhances the infectivity of Lambda variant, and its affinity for ACE2, F490S can prevent virus neutralisation by vaccine-induced neutralising antibody and reduce the efficacy of the vaccine and monoclonal antibody [45]. (2) Compared with the D614G strain, the Lambda variant increased the infection ability of LLC-MK2 and Calu-3 cells by 1.6 and 3.3 times, respectively; the neutralisation effect of convalescent serum on the Lambda variant is reduced by 1.3 times [48]. (3) Compared with D614G strain, the neutralisation effect of neutralising antibodies induced by the CanSino'Ad5-nCoV vaccine, BNT162b2 vaccine and mRNA-1273 vaccine on Lambda variant decreased by 2.5 times [48], 3.0 times and 2.3 times [49], respectively. (4) The neutralisation effect of antibody-induced by CoronaVac vaccine on Lambda variant was 3.05 times lower than that of SARS-CoV-2 wild type [50].	[47–50]
	Mμ(μ) B.1.621	Jan-2021 Columbia	T95I, Y144S, Y145N, R346K, E484K, N501Y, D614G, P681H, D950N	(1) D614G mutation can increase the viral load of upper respiratory tract infected hamsters [37]. (2) N501Y mutation on Mμ variant S protein enhances the affinity between SARS-CoV-2 virus S protein and ACE2 and prevents neutralineutralisingy binding [14,15]. (3) E484k mutation can reduce the neutralisation titer of vaccine-induced neutralising antibody to SARS-CoV-2 virus [22]. (4) Mμ variant has surface immune escape characteristics, increases the hospitalisation rate and mortality of COVID-19 [51]. (5) Compared with the wild-type SARS-CoV-2, the neutralisation titer of neutralising antibody induced by BNT162b2 vaccine on Mμ variant decreased 7.6 times [14].	[14,15,22,37,51]

*: VOC: Variant of Concern, Variants with increased transmissibility, virulence, and/or decreased diagnostic, therapeutic, or vaccine efficacy. VOI: Variant of Interest, Variants with mutations suspected or confirmed to cause a change in transmissibility, virulence, or diagnostic/therapeutic/vaccine efficacy, plus community transmission, a cluster of cases, or detection in multiple countries.

**: The data were obtained from the outbreak.info website (https://outbreak.info/). The inclusion threshold is 75%, and the data acquisition time is February 10, 2022.

than 1:40 [20]. These data show that the neutralizing antibodies induced by the BNT162b2 vaccine can effectively neutralize the Alpha variant. Another case-control analysis conducted in the UK (people over 16 years old) showed that the effectiveness of one dose of BNT162b2 vaccine against Alpha variant was 47.5%, and two doses of BNT162b2 vaccine against Alpha variant was 93.7%, respectively [21]. Similarly, the efficacy of one dose of ChAdOx1 nCoV-19 vaccine against Alpha variant was 48.7%, and two doses of ChAdOx1 nCoV-19 vaccine against Alpha variant was 74.5%, respectively [21]. It is suggested that the efficacy of two doses of ChAdOx1 nCoV-19 vaccine on Alpha variant was lower than that of BNT162b2 vaccine, but the second dose of both vaccines provide higher protection.

2.1.2 Beta (B.1.351) Variant

The Beta variant was first developed in South Africa in May 2020. Its S proteins have eight characteristic mutations: D80A, D215G, del241/243, K417N, E484K, N501Y, D614G, and A701V (https://outbreak.info/). It was found that E484k mutation can reduce the sensitivity of the SARS-CoV-2 virus to convalescent serum and vaccine-induced neutralizing antibodies [22,23], resulting in a relative resistance. In the early recovery period of COVID-19 patients, the neutralization activity of plasma to Beta variant decreased significantly. Still, there was no significant change in the neutralization activity of the Alpha variant [16]. The infectivity of the Beta variant in Calu-3 cells (human lung adenocarcinoma cell line) was enhanced. Three mutations on S protein (E484k, K417N/T, and N501Y) significantly increased the ability of infected mice to overexpress ACE2 cells [23] and enhanced the affinity of virus S protein for ACE2 [55]. These mutations increased the propagation rate of the Beta variant by 50% [24].

In addition, studies have analyzed the impact of the Beta variant on the protective efficacy of COVID-19 vaccines. Preliminary data showed that the effectiveness of the mRNA-1273 vaccine against SARS-CoV-2 original strain infection was 94.1% [25]. With the emergence of the Beta variant, studies have shown that the neutralizing effect of neutralizing antibodies induced by the mRNA-1273 vaccine against the Beta variant is reduced [28]. A controlled study in adults conducted in Qatar showed that the effectiveness of the mRNA-1273 vaccine against Beta variant infection was 47.9% and 73.7%, respectively, in the third and fourth week after the first dose, and 96.4% in the second week after the double dose [19]. Therefore, it is suggested that two doses of the mRNA-1273 vaccine can effectively fight the Beta variant infection, and the second injection's optimal vaccination time may vary according to different populations and regions, which needs to be studied and determined. A study on serum neutralization showed that compared with the original strain of SARS-CoV-2, the sera of people who received two doses of the BNT162b2 vaccine had a lower neutralization effect on the Beta variant, but the BNT162b2 vaccine still had a protective effect on the Beta variant [23]. According to previous reports, the NXV-CoV2373 vaccine was 95.6% effective against the primary strain of the SARS-CoV-2 strain, and the vaccine was 60.0% effective against the Beta variant [17]. Recently, a Phase II clinical trial in South Africa yielded similar results, and the analysis data showed that the effectiveness of the NXV-CoV2373 vaccine against the Beta variant was 51.0% (95% CI, 0.6 to 76.2) [5]. Therefore, it is suggested that the protective effect of the NXV-CoV2373 vaccine against the Beta variant is lower than its protective effect on SARS-CoV-2 original strain.

Furthermore, the previous studies also evaluated the protective efficacy of other COVID-19 vaccines against Beta variant infection. The effectiveness of two doses of ChAdOx1 nCoV-19 vaccine against mild to moderate COVID-19 caused by Beta variant was 10.4% [26], and the neutralization titers of antibodies induced by ZF2001 and BBIBP-CorV vaccines to Beta variant decreased by 1.59 times and 1.56 times [6]. These results suggest that the protective efficacy of COVID-19 vaccines against the Beta variant decreased [6,27,28].

2.1.3 Gamma (P.1) Variant

The Gamma variant firstly reported in Brazil in November 2020 includes 12 characteristic mutations: L18F, T20N, P26S, D138Y, R190S, K417T, E484K, N501Y, D614G, H655Y, T1027I, V1176F (https://outbreak.info/). Interestingly, both Gamma and Beta variants share four mutations, including K417T, E484K, N501Y, and D614G. These mutations may also affect the efficacy of the COVID-19 vaccines against the Gamma variant infection and the effectiveness of monoclonal antibodies. Wang Pengfei *et al*. [29] investigated the neutralizing effect of 18 monoclonal antibodies, convalescent plasma, and serum after vaccination with the Moderna or Pfizer vaccine against Gamma variant infection. The results showed that compared with SARS-CoV-2 wild type, the neutralization of various monoclonal antibodies, convalescent plasma, and vaccinated serum against Gamma variant decreased or even lost [29]. Similarly, a study in Italy collected the sera of 90 health care workers (HCWs) who received two doses of the BNT162b2 vaccine and tested their neutralizing antibodies titers against the SARS-CoV-2 variant. The results showed that the neutralization titer of neutralizing antibody induced by BNT162b2 vaccine to Gamma variant was 3.3 times lower than that of Alpha variant [30]. In addition, another study on the neutralization effect of serum antibody after two doses of BNT162b2 vaccine showed that the neutralization effect of neutralizing antibody against Gamma variant was significantly lower than that of neutralizing antibody against original strain [20]. Furthermore, it was found that there was a significant difference in the neutralization of serum antibodies collected on days 10–14 and 15–20 after the second dose of vaccine [20]. These data show that the

neutralization effect of the BNT162b2 vaccine against the Gamma variant is reduced, and the antibody level induced by the vaccine varies at different times after vaccination.

2.1.4 Delta (B.1.617.2) Variant

Delta variant was first discovered in India in October 2020. About six months later, the variant became the most common SARS-CoV-2 variant in India (https://nextstrain.org/ncov/gisaid/global?f_country=IndiaFZ). Delta variant's S protein has eight characteristic mutations: T19R, E156G, del157/158, Ll452R, T478K, D614G, P681R and D950N (https://outbreak.info/). Studies have shown that L452R and P681R mutations may enhance the infection and proliferation of the virus [30]. Delta variant is an immune escape variant with a more robust host cell entry and fusion ability, and its L452R mutation can make itself escape the attack of $CD8^+$ T cells [31]. A cohort study conducted in the UK from March to May 2021 found that COVID-19 patients infected with the Delta variant had a higher risk of emergency admission or death and a more significant burden on the health system than the Alpha variant [32]. Previous studies on the effect of Delta variant against COVID-19 vaccine found that BNT162b2 and ChAdOx1 nCoV-19 vaccines had only 33% protection efficiency against Delta variant after the first dose [33,34]. A case-control analysis conducted in the UK (people over the age of 16) showed that the effectiveness of two doses of BNT162b2 vaccine on Alpha variant and Delta variant was 93.7% and 88%, respectively, and the efficacy of two doses of ChAdOx1 nCoV-19 vaccine against Alpha variant and Delta variant was 74.5% and 67%, respectively [21]. Both studies suggest that booster immunization significantly improved the protective efficiency of BNT162b2 and ChAdOx1 nCoV-19 vaccines. Therefore, British experts called on people to vaccinate the second dose of vaccine in time [34].

Previous studies have also assessed the neutralization of COVID-19 vaccine-induced neutralizing antibodies against the Delta variant. A study conducted in the UK evaluated the neutralizing antibodies induced by the BNT162b2 vaccine against the SARS-CoV-2 variant. Compared with the SARS-CoV-2 wild type, the titer of neutralizing antibody against Delta variant decreased by 5.8 times (95% CI, 5.0 to 6.9), which was significantly higher than that of the Alpha variant (decreased by 2.6 times, (95% CI, 2.2 to 3.1)) and Beta variant (reduced by 4.9 times, (95% CI, 4.2 to 5.7)) [35]. These data suggest that the protective ability of the BNT162b2 vaccine against the Delta variant was lower than that of the Alpha and Beta variants. Furthermore, studies have shown that compared with the G614 variant, the titer of neutralizing antibody induced by BNT162b2, mRNA-1273, and Ad26 COV2.S vaccines against Delta variant decreased by three times, two times, and 12 times, respectively [36]. It can be found that the Delta variant reduces the efficacy of the COVID-19 vaccines, especially the Ad26 COV2.S vaccine.

2.1.5 Omicron (B.1.1.529) Variant

The Omicron variant was first detected in South Africa in November 2021 and was defined as a VOC by WHO in the early stages of its spread. Subsequently, it was found that the most significant difference from the Delta variant is the faster transmission speed [56]. The S protein of the Omicron variant has 30 characteristic mutations (Fig. 1): A67V, del69/70, T95I, G142D, del143/145, N211I, del212/212, G339D, S371L, S373P, S375F, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H, T547K, D614G, H655Y, N679K, P681H, N764K, D796Y, N856K, Q954H, N969K, and L981F (https://outbreak.info/). The number of mutations in the S protein of the Omicron variant is about twice that of other variants. Among them, D614G is an essential mutation. It can also be found in Alpha, Beta, Gamma, Delta, Lambda, Mμ, and other variants that can increase the viral load hamster upper respiratory tract, which may be conducive to virus transmission [37]. Similar results have also been verified in other studies. For example, data from Hong Kong showed that the Omicron variant grows 70 times faster in the human bronchus and ten times slower in the lung than the Delta variant [42]. Additionally, the N501Y mutation can enhance the affinity between S protein and ACE2 and prevent neutralizing antibody binding [14,15].

The Omicron variant has strong transmissibility and immune evasion [56] and can lead to reinfection with the Omicron variant in COVID-19 patients already infected with other variants [38,39]. It was reported that the Omicron variant has a 6-fold higher risk of reinfection than the Delta variant [41]. In addition, studies from the UK and South Africa found that compared with the Delta variant, the disease severity and risk of death of patients infected with the Omicron variant were significantly reduced [40,41], which may be due to the reduced ability of Omicron variant to resist the interferon response of host cells [57].

In addition, neutralizing effect of antibodies induced by the COVID-19 vaccine against the Omicron variant and the effectiveness of COVID-19 vaccines against the Omicron variant were also evaluated. A previous study found that the neutralizing effect of antibodies induced by the BNT162b1/BNT162b2 vaccine against the Omicron variant decreased 41 times compared with SARS-CoV-2 wild type [43]. Compared with the Delta variant, the neutralizing effect of antibodies induced by the BNT162b2 vaccine against the Omicron variant was reduced by 12–44 times [44]. Compared with the SARS-CoV-2 wild type, the neutralization effect of BNT162b2, mRNA-1273, and Ad26 COV2.S vaccines against the Omicron variant was reduced by 7–45 times [45]. In addition, the efficacy of 2 and 3 doses of mRNA vaccines (BNT162b2 and mRNA-1273) against Delta variant were 85% (95% CI, 83 to 87%) and 94% (95% CI, 92 to 95%), and against Omicron variant were 65% (95% CI, 51 to 75%) and 86% (95% CI, 77

to 91%), respectively [46]. From these data, it is not difficult to find that the protection efficiency of the mRNA vaccines against the Omicron variant is lower than that of the Delta variant, and the protection efficiency of the three-dose vaccination is significantly higher than that of the two-dose vaccination. In short, the Omicron variant is a variant with more mutation sites and high transmission power, which leads to the decline of the neutralization effect of vaccine-induced neutralizing antibodies and patient serum antibodies and another wave of COVID-19 pandemic.

2.2 Representative VOIs

2.2.1 Lambda (C.37) Variant

Lambda variant was first discovered in Peru [58], and its S protein has eight characteristic mutations, including G75V, T76I, R246N, del247/253, L452Q, F490S, D614G, and T859N (https://outbreak.info/). The two mutations, F490S and L452Q in the receptor domain, are antigenic site mutations recognized by the antibody. Studies have shown that L452Q mutation enhances the infectivity of the Lambda variant and its affinity for ACE2. F490S can prevent the neutralization of the virus by vaccine-induced neutralizing antibodies and reduce the efficacy of the vaccine and monoclonal antibody [47,48]. Compared with the D614G strain, the Lambda variant increased the infection ability of LLC-MK2 (rhesus monkey kidney cell line) and Calu-3 cells by 1.6 and 3.3 times, and the neutralization effect of convalescent serum against the Lambda variant is reduced by 1.3 times, respectively [48]. The possible reason may be that the concentration of serum antibodies in the convalescent phase is low.

Moreover, another two studies confirmed the impact of the Lambda variant on COVID-19 vaccines. The results indicated that compared with D614G strain, the neutralisation effect of neutralizing antibodies induced by the CanSino'Ad5-nCoV vaccine, BNT162b2 vaccine, and mRNA-1273 vaccine against the Lambda variant decreased by 2.5 times, 3.0 times, and 2.3 times [48,49], respectively. Similarly, another study showed that the neutralization effect of antibody-induced by CoronaVac vaccine against the Lambda variant was 3.05 times lower than that of SARS-CoV-2 wild type [50]. In conclusion, the Lambda variant reduces the efficacy of vaccines and monoclonal antibodies, which threatens the immune protection induced by vaccines and the effectiveness of monoclonal antibody therapy.

2.2.2 Mμ (B.1.621) Variant

Mμ variant was first discovered in Columbia in January 2021. Its S protein has nine characteristic mutations, including T95I, Y144S, Y145N, R346K, E484K, N501Y, D614G, P681H, and D950N (https://outbreak.info/, Fig. 1). Among these mutations, three ones have attracted attention. D614G mutation can increase the viral load in upper respiratory tract of infected hamsters, which may be conducive to virus transmission [37], but do not lead to changes in antibody neutralization characteristics [59,60]. The N501Y mutation on the S protein of Mμ variant enhances the affinity between S protein and ACE2 and prevents neutralizing antibody binding [14,15]. Furthermore, the E484k mutation can reduce the neutralization titer of vaccine-induced neutralizing antibody against SARS-CoV-2 virus [22]. These mutations lead to Mμ variant with enhanced surface immune evasion, increasing hospitalization and mortality in COVID-19 patients [51]. It has been found that compared with the SARS-CoV-2 wild-type, the neutralization titer of neutralizing antibodies induced by BNT162b2 vaccine against Mμ variant decreased 7.6 times [14]. In addition, some studies reported that although mutation of the S protein resulted in attenuated neutralization of vaccine-induced neutralizing antibodies against the Mμ variant, BNT162b2 vaccine-triggered neutralizing antibodies could still neutralize the Mμ mutant [61]. Based on these data, the Mμ variant is characterized by enhanced transmissibility, increased COVID-19 disease severity, and depressed neutralization of serum antibodies, which not only affects the immune protection and monoclonal antibody treatment of the COVID-19 vaccines, but also leads to the immune escape of the Mμ variant against the COVID-19 vaccines.

3. BCG-Induced Trained Immunity and its Preventive and Protective Effect on COVID-19

3.1 BCG and Trained Immunity

BCG is a live attenuated TB vaccine developed and bred by Albert Calmette and Camille Guerin about 100 years ago [62], and is the only vaccine approved for TB prevention. BCG has a certain protective effect on TB pleurisy and miliary TB in children [63]. At present, 154 countries and territories have introduced neonatal BCG vaccination policies, of which 53 countries report vaccination coverage of at least 95% [64]. In addition to its application in preventing TB, BCG has also been proven to prevent and treat infections caused by other bacteria and viruses and even some tumors. Many studies have found that the prevention and treatment effect of BCG on bacteria other than *M. tuberculosis* or tumors is related to the non-specific immune response induced by the BCG, which is called "trained immunity" [65–67].

Trained immunity refers to the non-specific immune response of the self-immune system to the secondary stimulation of the same pathogen or different pathogens after the initial challenge caused by some pathogen infection or vaccination. The trained immune response is usually faster and more active (Fig. 3) [68,69]. Previous studies have shown that when the ligand of some pathogenic microorganisms binds to specific receptors on innate immune cells, it induces long-lasting enhanced metabolic activity and epigenetic changes in innate immune cells [70]. The increased

metabolic activity provides faster energy support and necessary metabolites for the immune response. Epigenetic changes increased the susceptibility of transcription factors to promoter and enhancer regions of inflammatory genes. When immune cells are repeatedly stimulated by the same pathogen or infected with other pathogens, they accelerate the process of gene expression. The combination of these metabolic and epigenetic effects up-regulates the production of pro-inflammatory cytokines (IL-1β, TNFα, and IL-6), promotes bone marrow proliferation, and promotes a faster and more robust immune response to secondary stimuli from the same or different pathogens, and may even prevent subsequent secondary infections (Fig. 4) [68,70].

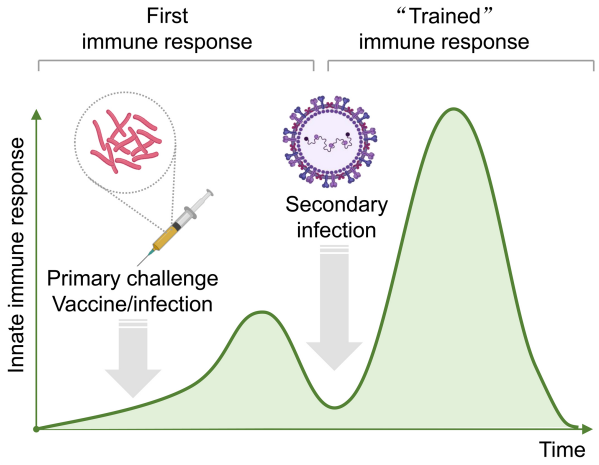

Fig. 3. The curve of trained immunity over time. After the initial immunization, the host's innate immune response was gradually enhanced and reached the first immune peak followed by a steadily decreased trend. Subsequently, the second infection with another pathogen will rapidly induce a more intense immune response and then reach a second immune peak followed by a gradually decreased trend.

Interestingly, there is growing evidence that BCG vaccination induces trained immunity against multiple respiratory viruses, such as RSV and influenza viruses [71]. Therefore, in the face of the COVID-19 pandemic, some researchers have proposed a hypothesis that BCG vaccination may be one of the ways to prevent or control the spread of COVID-19 and reduce the morbidity and mortality of COVID-19 [2,72].

3.2 Effect of BCG-Mediated Trained Immunity on the Morbidity and Mortality of COVID-19

To explore the impact of BCG vaccination on the incidence, mortality and severity of COVID-19, many studies have been conducted in many countries and organizations around the world. Statistics show a correlation between the morbidity and mortality of COVID-19 and BCG vaccination rates in different countries and regions at the beginning of the global pandemic (Table 2, Ref. [12,73–89]). For example, the number of confirmed COVID-19 cases in developed countries such as North America and Western Europe is significantly higher than that in developing countries such as South America, Asia, and Africa, where BCG vaccination policies for newborns are widely adopted. Observing this phenomenon, Madan *et al.* [73] published a cross-sectional study that included data collected from 174 countries. The 174 countries were divided into four groups based on TB incidence and BCG vaccination coverage, and the incidence and mortality of COVID-19 in each group were statistically analyzed. The results showed that the incidence and mortality of COVID-19 in countries with a high incidence of TB were lower than those in countries with low TB incidence. Similarly, the incidence and mortality of COVID-19 were lower in countries with high BCG vaccination coverage [73]. Furthermore, a large-scale observational study involved in 175 countries found similar results, the results show that countries with a universal BCG vaccination program (n = 138) have significantly lower incidence of COVID-19 than countries without a universal BCG vaccination program (n = 37) [0.0147 $\pm$ 0.027 vs 0.1892 $\pm$ 0.244, respectively, $p < 0.0001$] [74]. Countries with BCG neonatal vaccination programmes also had significantly lower per capita mortality than countries without BCG neonatal vaccination programmes [0.0004 $\pm$ 0.001 vs 0.0113 $\pm$ 0.020, respectively, $p < 0.0001$] [74]. The findings of both studies indicate specific protective mechanisms against COVID-19 in TB endemic areas. These findings also promote research on the pathogenesis and immune response process of COVID-19 in various countries.

To further explore the relationship between BCG vaccination coverage and COVID-19, Klinger D *et al.* [75] conducted a meta-regression analysis based on the data from 160 countries. They divided countries into three groups based on BCG vaccination coverage $\leq$70% group, $>$70% group, and the unvaccinated group. The study found that countries with BCG coverage $\leq$70% had 6.5 fewer COVID-19 cases per 10,000 people than unvaccinated countries [75]. Therefore, it suggests that countries with higher BCG vaccination coverage have lower morbidity and mortality of COVID-19. Klinger D 's study data took BCG vaccination coverage rate of 70% as the grouping limit. In China, Gong *et al.* [12] conducted a larger analysis of COVID-19 data obtained from 225 countries released by WHO on 12 August 2020. According to BCG vaccination coverage, Gong *et al.* [12] divided these countries into three groups: countries with BCG vaccination coverage $\geq$90%, countries that recommended BCG vaccination but BCG vaccination coverage $<$90%, and countries that have never introduced BCG vaccination. Results showed that the incidence of COVID-19 was significantly lower in countries where BCG was recommended than in countries where BCG was not recommended, and the mortality of COVID-19 was considerably lower in countries with

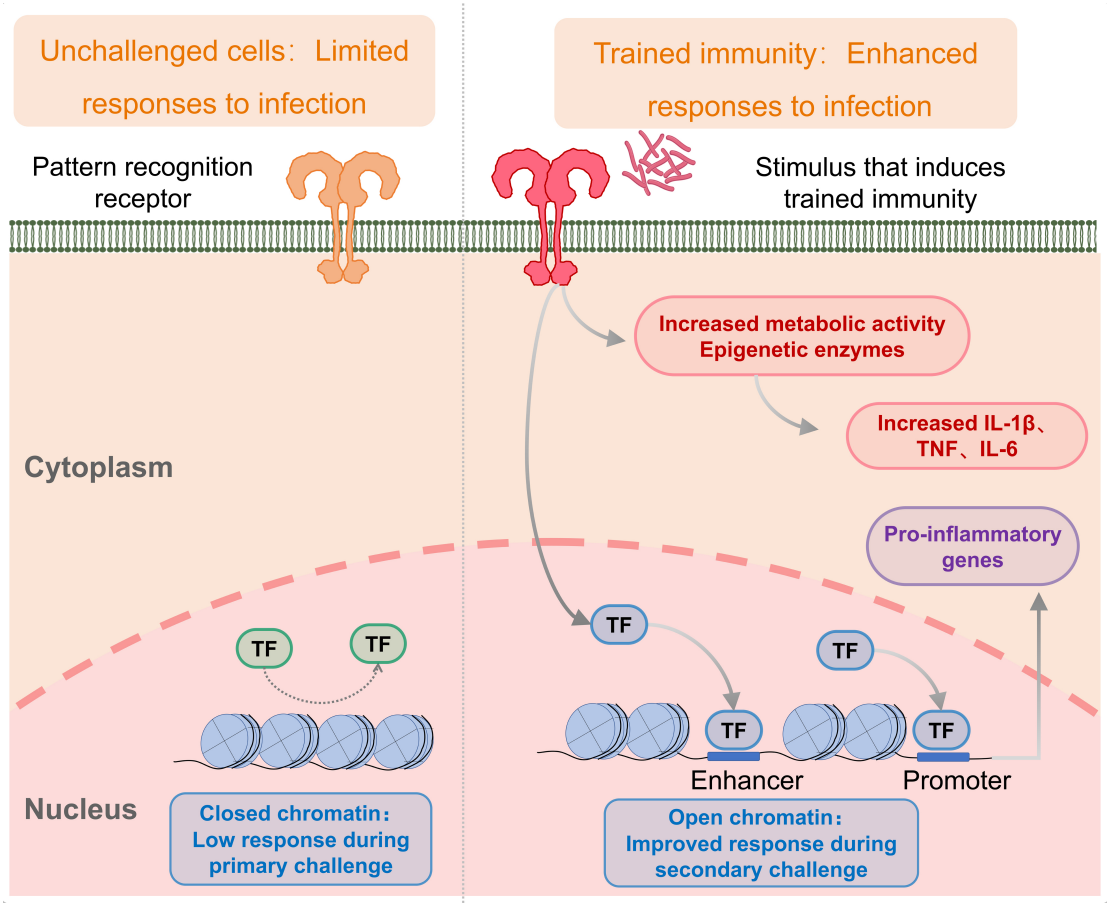

Fig. 4. The mechanism of trained immunity induced by the BCG vaccine. Trained immunity is an enhanced innate immune response to different pathogens after an initial challenge, such as vaccination or infection. When the ligand of some pathogenic microorganisms binds to specific receptors on innate immune cells, it induces durable enhancement of metabolic activity and epigenetic changes in innate immune cells. Epigenetic changes increase the susceptibility of transcription factors to promoter and enhancer regions of inflammatory genes when immune cells undergo repeated stimulation or other pathogens, accelerating the gene expression process. In addition, the increased metabolic activity provides faster energy support and necessary metabolites for the immune response. This combination of metabolism and epigenetics prompts the immune system to respond faster and more vigorously to secondary stimuli caused by the same or different pathogens.

BCG coverage ≥90% [12]. Data from these two studies suggest that increased BCG coverage may have a role in reducing morbidity and mortality of COVID-19. However, when Gong conducted statistics, it was found that countries with high BCG coverage were mainly developing countries with low human development index (HDI). Therefore, Gong *et al*. [12] further analyzed the relationship between HDI and COVID-19 in these 225 countries. They divided these countries into four levels based on the 2019 Human Development Report and found that countries with low HDI had lower incidence and mortality rates of COVID-19 than countries with high HDI. In contrast, countries with low HDI are mostly developing countries, and BCG vaccination policy is widely adopted. Once again, expanding BCG vaccination coverage may reduce the incidence and mortality of COVID-19.

Those studies indicate a negative correlation between BCG vaccination and the incidence and mortality of COVID-19. However, many studies have shown that in the early stage of epidemiological studies, there are often a large number of confounding factors, which have a significant impact on epidemiological data and often lead to distortion of research results. Confounding factors such as differences in health systems, levels of economic development, income, education, population, rural and urban environments, time lag between COVID-19 outbreaks, number and standard of diagnostic tests, and national control strategies are also present in the above studies [90]. To minimize the impact of these confounding factors on epidemiological results, some researchers analyzed the relationship between the BCG index (the level of universal BCG vaccination cov-

Table 2. Evidence supporting the association between BCG vaccination and COVID-19 morbidity and mortality.

Studies	Years	Subjects	Results	Reference
A cross-sectional study	30 May 2020	174 countries	Countries with high TB incidence have lower levels of COVID-19 than countries with low TB incidence. Similarly, countries with high BCG coverage have lower rates of COVID-19, suggesting that some protective mechanisms exist in TB-endemic areas.	[73]
An observational study	24 April 2020	175 countries	Countries with neonatal BCG vaccination policies (n = 138) had lower prevalence and COVID 19 related deaths than countries without neonatal BCG vaccination policies (n = 37).	[74]
Meta regression analysis	5 Sep 2020	160 countries	Compared with unvaccinated countries, countries with BCG coverage $\leq$70% had 6.5 fewer cases of COVID-19 per 10,000 people, with BCG coverage; 70% of countries saw a decrease of 10.1 cases.	[75]
Linear regression analysis	4 Nov 2020	213 countries	The incidence of COVID-19 in countries where BCG is recommended is significantly lower than in countries where BCG is not recommended, and the mortality rate of COVID-19 in countries where BCG vaccination coverage is $\geq$90% is significantly lower than in countries where BCG is not recommended. Countries with low HDI have lower incidence and mortality of COVID-19 than countries with high HDI. Most countries with low HDI generally implement BCG vaccination policy. Increased BCG vaccination coverage may reduce morbidity and mortality from COVID-19. BCG coverage is negatively correlated with COVID-19 morbidity and mortality.	[12]
Epidemiological analysis	9 Jul 2020	Worldwide	There is a strong correlation between national BCG mandatory vaccination policies and COVID-19 mortality. A 10% increase in the BCG index was associated with a 10.4% decrease in COVID-19 deaths. These results indicate that BCG vaccination has a protective effect on COVID-19.	[76]
Multiple regression analysis	31 Jul 2020	171 countries	Countries with universal BCG vaccination had a 30-fold reduction in COVID-19 deaths (95% CI, 17 to 52) compared with countries without universal BCG vaccination; BCG vaccination helps reduce mortality from COVID-19.	[77]
Longitudinal, retrospective observational study	19 Jan 2021	6201 HCW	The serum positive rate of SARS-CoV-2 and clinical symptoms associated with COVID-19 were significantly lower in the BCG vaccination group than in the non-BCG vaccination group, suggesting that BCG may have certain value in reducing the severity of COVID-19 disease.	[78]
A cohort study	9 Jul 2020	120 adult patients,	Individuals vaccinated with BCG were less likely to require hospitalization during the course of disease (3.7% vs 15.8%, $p = 0.019$). Suggesting that BCG has the potential to reduce the severity of COVID-19.	[79]
An observational study	5 June 2020	In Rhode Island, US	BCG vaccine was associated with reduced mortality rates (while adult pneumococcal and adult seasonal influenza vaccines were not)	[80]

Table 2. Continued.

Studies	Years	Subjects	Results	Reference
An Ecological study	16 Mar 2021	25 level 4 European countries	Countries with high BCG coverage in children have a reduced risk of COVID-19, while there is no significant correlation between MCV coverage and COVID-19. But there are significant differences in Healthcare Access and Quality Index (HAQI) across the 140 countries. The correlation between BCG coverage and COVID-19 becomes uncertain when combined with the HAQI function system analysis.	[81]
A Multivariable Analysis	5 Aug 2020	140 countries	Countries with mandatory BCG vaccination policies have lower incidences of COVID-19 and disease-related deaths than countries without BCG vaccination policies. Countries that eliminated BCG vaccination policies before 2000 showed no significant difference in COVID-19 incidence and disease-related mortality compared with countries that had never implemented BCG vaccination policies.	[82]
An Ecological study	3 Aug 2020	135 countries	BCG coverage (%) was inversely associated with COVID-19-related mortality, but not morbidity.	[83]
Correlation analysis	25 Jan 2021	173 countries	Conclusions support the view that BCG vaccination may help prevent deaths from COVID-19.	[84]
A Multivariable Analysis	11 Jul 2020	Worldwide	BCG coverage was negatively correlated with the number of deaths per million (DPM), especially among young people 0-24 years of age. These results suggest that BCG vaccination can help control the spread of COVID-19 and reduce the severity of the disease.	[85]
Linear regression analysis	28 Aug 2020	55 countries	A significant negative correlation was observed between COVID-19 morbidity and mortality and BCG coverage.	[86]
A Cohort Study	5 Aug 2020	225 countries	BCG vaccination is safe and reduces the incidence of COVID-19	[87]
Investigative study	12 Aug 2020	430 people	In Japan, BCG coverage among young people has a significant impact on preventing local transmission of COVID-19.	[88]
Exploratory study	22 Oct 2020	Japan	Countries that have implemented neonatal BCG vaccination policies have reduced COVID-19-related deaths by 58%. However, the results of this exploratory study are based on secondary cross-sectional data.	[89]

erage in a country) and COVID-19 mortality after controlling confounding factors. For example, Escobar and his colleagues performed a major epidemiological study among European countries [76]. A negative correlation was found between the BCG index and COVID-19 mortality in European countries. On the other hand, a 10% increase in the BCG index was associated with a 10.4% decrease in COVID-19 mortality, suggesting that BCG may have a protective effect during the COVID-19 epidemic. With the outbreak of COVID-19 in the Americas, this study analyzed COVID-19-related mortality rates in STATES without BCG vaccination policies and states with BCG vaccination policies located at major entry points in Mexico and Brazil and found that COVID-19 mortality rates in states without BCG vaccine were significantly higher than in countries with BCG vaccination [76]. However, this study did not consider the significantly higher population density in South America than in North America, so the results are controversial. Another study performed multiple regression analyses on data from 171 countries, adjusting for socioeconomic and climatic covariates to avoid potential confounding factors [77]. The results showed a 30-fold reduction in COVID-19 deaths in countries with universal BCG vaccination policies compared with countries without universal BCG vaccination policies, further supporting the role of BCG vaccination in reducing COVID-19 deaths [77].

Although the above ecological or epidemiological data to some extent support the negative association between BCG vaccination rates and COVID-19 morbidity and mortality, other studies have questioned this. Some epidemiological studies have shown no clear association between BCG vaccination policies and morbidity and mortality of COVID-19 (Table 3, Ref. [24,29,91–97]). For example, Szigeti *et al*. [91] conducted a comprehensive epidemiological study using data from public databases in 68 countries. The relationship between BCG prevalence and mortality in COVID-19 cases found no significant correlation [91]. In addition, several previous epidemiological analyses suggested that although some ecological studies showed that BCG vaccination had a protective effect on COVID-19, the results of these studies were significantly affected by confounding factors. Their literature review found that, after adjusting for confounding variables, there was no longer a clear association between BCG vaccination policy and COVID-19 morbidity and mortality, especially after adjusting for detection rates [24].

The above studies indicate that confounding factors significantly impact the analysis and evaluation of BCG vaccination's protective effect against COVID-19, leading to the opposite conclusion of the study. Therefore, several cohort studies or longitudinal retrospective observational studies have further analyzed the relationship between BCG vaccination rates and COVID-19 morbidity and mortality. The advantage of these studies over previous epidemiological and ecological studies is that they eliminate confounding factors as much as possible. In a longitudinal, retrospective observational study performed in 6201 HCWs, 29.6% of the participants in the experimental group reported a history of BCG vaccination, and 68.9% of the participants in the control group had never received BCG vaccination [78]. Clinical symptoms associated with COVID-19 and serum anti-SARS-CoV-2 IgG concentrations were compared. The results showed that the sero-positive rate of SARS-CoV-2 and clinical symptoms related to COVID-19 were significantly lower in the BCG vaccinated group than those in the non-BCG vaccinated group [78].

Similarly, a Rhode Island study analyzed the hospitalization rates of 120 patients with COVID-19 and found that those vaccinated with BCG had lower hospitalization rates than those who had not been vaccinated with BCG [79]. These findings suggest that BCG may have some value in reducing the severity of COVID-19 disease. Though the above two studies in order to reduce the influence of confounding factors, choose the same country of the people in the same area as the research object, the application of unified testing means to control confounding variables as much as possible. However, there are still unavoidable potential confounding factors, such as the research object of the age, BCG vaccination time, growing environment and condition. To further reduce the influence of these variables on the results, a cohort study in Israel compared 3064 patients with BCG vaccination and 2869 patients without BCG vaccination. The positive rate of the COVID-19 test in the two groups was analyzed, and no statistically significant difference was found between the two groups [29]. Since Israel abolished its mandatory BCG neonatal vaccination program in 1982, the study looked at individuals born three years before and three years after the BCG vaccination program stopped. This study minimised the impacts of confounding factors such as health systems, national levels of economic development, education, rural and urban environments, time lag between COVID-19 outbreaks, number and standard of diagnostic tests, and national control strategies. In addition, Aksu K *et al*. [92] conducted a natural experiment in Sweden to evaluate the effect of BCG vaccination on COVID-19 related outcomes by using the regression discontinuities method. Two groups of volunteers were recruited, including 1026,304 people born before 1975 and 1018,544 people born after 1975. The impact of BCG vaccination on COVID-19 related outcomes was evaluated between two groups. Analysis results showed that although BCG vaccination at birth was not protective against COVID-19 in middle-aged individuals, universal BCG vaccination reduced the number of COVID-19 cases by 19% and the number of hospitalizations by 25% [92]. The rigorous experimental design and sufficiently large sample size of this study allow its reliability and precision to exceed that of a randomized controlled trial.

Table 3. Evidence that does not support the association between BCG vaccination and COVID-19 morbidity and mortality.

Studies	Years	Subjects	Results	Reference
Validation study	7 Oct 2020	68 countries	Global COVID-19 epidemiological data are unreliable, and the results do not definitively indicate whether BCG has a protective effect against COVID-19 infection.	[91]
Analyses of the epidemiological studies	27 Oct 2020	Review of literature	After adjusting for confounding variables (particularly detection rates), there was no association between BCG vaccination policy and morbidity or mortality from COVID-19.	[24]
Cohort study	13 May 2020	3064 + 2869 people (Israelis)	There was no statistical difference in the positive rate of COVID-19 detection between the BCG vaccination group and the non-BCG vaccination group.	[29]
Regression discontinuity study	23 Aug 2020	2,044,848 people in Sweden	BCG vaccination at birth does not protect against COVID-19 in middle-aged individuals	[92]
Analyses of the epidemiological studies	9 May 2020	Review of literature	There is insufficient evidence to support or deny the hypothesis that COVID-19 reduces morbidity and mortality in countries where compulsory BCG vaccination.	[93]
Comparative analyses of the data	14 Aug 2020	Review of literature (18 countries)	There is no evidence that BCG vaccination has a beneficial effect on reported COVID-19 cases or deaths.	[94]
A systematic review and meta-analysis	11 May 2020	Review of literature	Studies have rejected a true correlation between COVID-19 incidence and BCG vaccination rates and/or national vaccination policies.	[95]
Retrospective study	21 Sep 2020	334 people	Intravesical BCG injection did not reduce the incidence of Novel coronavirus infection.	[96]
A retrospective cross-sectional study	17 Sep 2020	123 adults	BCG vaccination is not associated with the severity of COVID-19 pneumonia. Instead, age and low income are significant determinants of severe COVID-19 pneumonia.	[97]

In summary, there is pronounced heterogeneity in the results of these studies. Therefore, it is impossible to answer whether BCG vaccination is meaningful to control the spread of COVID-19, decrease the morbidity and mortality of COVID-19, and reduce the severity of COVID-19 disease. Furthermore, although the above studies have minimized the interference of confounding factors, there are still many potential confounding factors that may affect the study results. Therefore, many clinical trials are needed to assess the significance of BCG vaccination in controlling the COVID-19 pandemic.

4. Clinical Trials Currently Underway to Evaluate the Efficacy of BCG in Preventing COVID-19

At present, as many as 56 clinical trials are conducted to evaluate the protective efficacy of BCG in preventing COVID-19 worldwide, including 13 in phase IV, 27 in phase III, 4 in phase II, and 13 in the unknown phase (Table 4). These 56 clinical trials will be categorized and discussed by country, and we will focus on analyzing the clinical trial status, results, and the potential impact on fighting against COVID-19.

4.1 Eleven Clinical Trials in Netherlands

As of March 24, 2022, there have been 7,626,640 confirmed cases of COVID-19 with 21,836 deaths, and 33,943,469 doses of the COVID-19 vaccine have been administered in the Netherlands (https://covid19.who.int). As a developed country, the incidence and mortality of tuberculosis are very low in the Netherlands, and the BCG vaccination program has not been widely promoted [3]. Therefore, it is admirable that Switzerlands is at the forefront of research in evaluating the preventive effect of BCG against COVID-19. Currently, 11 clinical trials have been conducted in the Netherlands to assess the protective effect of BCG against COVID-19, including seven clinical trials in phase IV (EUCTR2021-000182-33-NL, EUCTR2020-003470-47-NL, NCT04537663, EUCTR2020-002456-21-NL, NCT04417335, EUCTR2020-000919-69-NL and EUCTR2020-001591-15-NL), one clinical trial in phase III (NCT04328441), and three clinical trials in unknown phase (NL8609, NL8547, and NL8477). Interestingly, the BCG strains used in these clinical trials are all BCG Danish strand 1331 strain. The objectives of these clinical trials are to assess the effect of BCG immunization in reducing the hospitalization rate and COVID-19 disease severity in the elderly ($\geq$60 years old) and the work absenteeism rate as well as clinical symptoms of healthcare workers (HCWs). Furthermore, several clinical trials have analyzed the prime-booster strategy of the BCG and COVID-19 vaccines.

The elderly have become a vulnerable group in the COVID-19 epidemic due to a weakened immune system and primary diseases like hypertension and diabetes. Three clinical trials (NCT04537663, NCT04417335, and NL8547) were performed to evaluate the effect of BCG vaccination in reducing the hospitalization rate and the severity of COVID-19 in 5200, 2014, and 1600 older adults ($\geq$60 years old) in the Netherlands, respectively. The results of these three trials have not been reported, and they will provide data support for assessing the impact of BCG on COVID-19 in the elderly. Additionally, HCWs are a vital force in the fight against COVID-19, and reducing HCWs absenteeism is a critical factor in defeating the pandemic. Two clinical trials (EUCTR2020-000919-69-NL and NL8477) were conducted by the University Medical Center and the University Medical Center Utrecht in 1000 and 1500 HCWs, respectively. Both clinical trials focused on assessing the impact of BCG vaccination on reducing the absenteeism of HCWs. Previous studies have shown that the prime-booster strategy of BCG and specific vaccines has a good effect on improving their protective efficiency. Still, this strategy was rarely used by COVID-19 vaccines under emergency use authorization (EUA). Surprisingly, we found that the clinical trial EUCTR2021-000182-33-NL carried out by Radboudumc made a bold attempt to this immune strategy. This clinical trial aimed to analyze whether BCG vaccination before mRNA1273 vaccination could improve the immune effect of the COVID-19 vaccine. The results of this trial may provide data support for exploring new strategies to fight COVID-19. No experimental results have been announced yet, and follow-up continuous attention is required. Nevertheless, this is another way to explore the potential impact of the joint application of the BCG vaccine and COVID-19 vaccine in COVID-19 prevention and control.

4.2 Eight Clinical Trials in India

India is a populous country second only to China, but the level of medical security is relatively backward. According to the population data released by the WHO, India has a population of 1.4 billion (https://population.un.org/wpp/Download/Standard/Population/). By March 14, 2022, India had 1.8 billion vaccinations, which means that only one dose of COVID-19 vaccine per person is average. Based on these disadvantages, India has 43,016,372 confirmed COVID-19 cases, ranking second only to the United States, and the cumulative number of deaths is as high as 516,755, ranking third after the United States and Brazil (https://covid19.who.int/table). Unlike Switzerland, India has universal BCG vaccination, and BCG coverage has reached 92% in 2019 (http://www.bcgatlas.org/). As far as India's challenges are concerned, if BCG proves to be an excellent preventative against COVID-19, then India has one more powerful weapon against COVID-19. To provide sufficient evidence for this hypothesis, eight clinical trials have been conducted in India to evaluate the preventive and protective effects of BCG against COVID-19, including one Phase IV

clinical trial (CTRI/2020/06/06/025798), three Phase III clinical trials (NCT04475302, CTRI/2020/07/026668 and CTRI/2020/04/024749), one Phase II clinical trial (CTRI/2020/05/05/0205013), and three clinical trials with unknown phase (CTRI/2020/06/025854, CTRI/2020/09/027684 and CTRI/2020/04/04/024833). Seven of these clinical trials were used to evaluate the preventive effect of BCG on COVID-19, but one was used to assess the therapeutic effect of BCG immunization in patients with COVID-19. Clinical trial CTRI/2020/05/025013 was carried out to evaluate the therapeutic effect of BCG as a therapeutic agent on 60 hospitalized patients aged 20–40 years with COVID-19 and explore the relationship between BCG and COVID-19. These patients were divided into two groups, BCG group with 0.1 mL BCG (2–8 $\times$ 10^6 CFUs) and placebo control group with 0.9% sodium chloride via intradermal injection. The clinical trial is ongoing, and experimental data have not yet been released. The strength of this clinical trial is that it evaluated the therapeutic rather than preventive effect of BCG immunization in COVID-19 patients. At the same time, its disadvantage is that the number of cases recruited is relatively small, which may reduce the reliability and stability of data obtained from this clinical trial.

Overall, the eight clinical trials conducted in India cover a wide range of age groups (20–40, 18–50, 18–60, 18–65, 18–80, 18–99, 60–80, and 60–95 years old, respectively) and high-risk groups such as HCWs, the elderly, and other high-risk workers. Therefore, if the final data from these clinical trials support the hypothesis that the BCG vaccine can prevent and treat COVID-19, widespread BCG vaccination in a populous and relatively underdeveloped country like India may be an effective and inexpensive way to combat the COVID-19 pandemic.

4.3 Five Clinical Trials in Brazil

COVID-19 pandemic quickly disrupted health services, including Brazil's Unified Health System (SUS). As of March 25, 2022, Brazil has confirmed 29,729,991 COVID-19 cases, ranking third only to the United States and India; The total number of deaths is 657,998, second to the United States. As of March 10, 2022, 383,413,147 doses of the COVID-19 vaccine had been administered, and total vaccine doses administered per 100 population and persons fully vaccinated per 100 population are 180.38 and 71.35, respectively (https://covid19.who.int/table). From these data, it can be seen that despite the high COVID-19 vaccination rate in Brazil, the COVID-19 morbidity and mortality remain high. To reverse this unfavourable situation, some scientists in Brazil turned their attention to the BCG vaccine. At present, five clinical trials have been conducted in Brazil, including one Phase IV clinical trial (NCT04369794), two Phase II clinical trials (NCT04659941 and RBR-4kjqtg), and two clinical trials with unknown phase (ISRCTN47802196 and RBR-5ysj54). NCT04369794 is a multicenter, randomized, and double-blind clinical trial conducted in Brazil on April 30, 2020, enrolling 1000 adults over 18 years. These participants will be divided into the BCG vaccine group with 0.1 mL BCG (2–8 $\times$ 10^6 CFUs) and the placebo control group with 0.1 mL 0.9% sodium chloride. This study explores the effects of BCG on promoting the production of cytokines, inducing trained immunity, slowing disease progression, and decreasing the severity of COVID-19 patients. Furthermore, another clinical trial, ISRCTN47802196, was launched on May 19, 2021, which randomly recruited 200,805 COVID-19 patients with or without BCG vaccination from 1996 to 1998. The purpose was to evaluate the impact of BCG vaccination and re-vaccination on the occurrence and severity of COVID-19 in Brazil. The significant advantage of this clinical trial is that the sample size exceeds 200,000, which lays a solid foundation for proving the effectiveness of BCG in preventing COVID-19.

4.4 Four Clinical Trials in Denmark

As of March 25, 2022, Denmark had a total of 3,029,228 confirmed cases and 5518 deaths, ranking 36th and 85th in the world, respectively. As of March 5, 2022, as many as 13,178,651 vaccine doses have been administered in Denmark. Total vaccine doses administered per 100 population and persons fully vaccinated per 100 population were 226.3 and 82.26, respectively, higher than the global average. Fortunately, although the number of confirmed cases is high in Denmark, the mortality rate is relatively low, which may be inseparable from the COVID-19 vaccination strategy actively pursued by the Danish government and the strong support of the health system. In addition, Denmark is actively exploring new ways to fight COVID-19, such as the BCG vaccine. At present, four clinical trials were performed in Denmark to evaluate the potential effect of BCG vaccination on preventing and controlling the COVID-19 pandemic. Like other countries, these clinical trials in Denmark have focused on groups at high risk for COVID-19, such as the elderly and HCWs. The clinical trial EUCTR2020-003904-15-DK conducted by the University of Southern Denmark and the clinical trial NCT04542330 conducted by Bandim Health Project intend to recruit 1900 and 1300 participants, respectively. All participants will be randomly divided into two groups, the intervention group will receive BCG Danish strain 1331, and the control group will receive normal saline. The objective of both clinical trials is to evaluate the protective effect of BCG immunization against COVID-19 infection in the elderly over 65 years. In addition to the elderly, HCWs in Denmark are also a high-risk group, accounting for more than 20% of the cumulative number of confirmed cases. A growing number of HCWs with COVID-19 will cause large-scale absenteeism, further leading to a shortage of medical resources. Therefore, there is an urgent need to develop strategies to prevent HCWs absenteeism. Two

clinical trials, EUCTR2020-001888-90-DK (Estimated enrollment 1500) and NCT04373291 (Estimated enrollment 1293), have been conducted to evaluate the potential beneficial effects of BCG vaccination by reducing the work absenteeism rate of HCWs or alleviating the clinical process of COVID-19 infection. Both studies use the BCG vaccine to strengthen the non-specific protection of HCWs during the COVID-19 pandemic.

By analysing the available public data, we found an interesting thing: four clinical trials in Denmark were conducted independently by two different institutions, and each institution conducted a clinical trial in the elderly and HCWs. The rational layout of these clinical trials provides a model for a country to systematically evaluate BCG vaccines on the prevention and treatment of COVID-19. Furthermore, by comprehensively analyzing these experimental data, researchers can eliminate some confounding factors and improve the reliability and authenticity of the data.

4.5 Four Clinical Trials in Germany

As a developed country, the incidence of TB in Germany in 2020 is estimated to be 5.5 per 100,000 population, and the mortality rate is calculated to be 0.35 per 100,000 population (https://www.who.int/teams/global-tuberculosis-programme/data). Germany's BCG vaccination policy has changed several times in its history, and the BCG policy was different between former East Germany and former West Germany. Former West Germany stopped recommending the BCG vaccine (BCG Denmark strain) in 1974 except for infants at risk, but former East Germany continued a mandatory BCG vaccine (Boehring-Hoechst/Jenapharm) until the German reunification in 1990 (http://www.bcgatlas.org/). Therefore, there is a significant difference in BCG coverage between former East Germany and former West Germany, but confounding factors such as geography, genetic background, and current epidemic prevention policy are similar in Germany. These unique advantages provide natural conditions for assessing the protective effect of BCG against COVID-19.

Currently, four clinical trials of the VPM1002 vaccine (a BCG replacement vaccine) have been performed in Germany. Another feature of the four clinical trials conducted in Germany is that they all used the VPM1002 vaccine instead of the BCG vaccine. In addition, the Max Planck Institute genetically modified the VPM1002 vaccine to improve its immune activity. Previous studies have demonstrated that the VPM1002 vaccine showed superior efficacy and safety than BCG in fighting against *Mycobacterium tuberculosis* infection in mice, guinea pigs, rabbits, nonhuman primates, HIV-unexposed newborn infants, and young adults [35,38,87,88]. Thus, based on the experience of trained immunity induced by the BCG vaccine, some scientists speculate that the VPM1002 vaccine may also (partially) have potential preventive and therapeutic effects on COVID-19.

In Germany, Vakzine Projekt Management GmbH conducted four Phase III clinical trials to evaluate the potential of the VPM1002 vaccine to prevent COVID-19. Briefly, two clinical trials (NCT04435379 and EUCTR2020-001675-33-DE) were performed to assess the efficacy and safety of the VPM1002 vaccine in reducing the hospitalization rate of COVID-19 and severe respiratory infectious diseases in adults. The two clinical trials have the same sample size of 2038, and the difference is the vaccination dose and the age of the population (Table 4). The former used a dose of 2–8 $\times$ 10^5 CFUs to vaccinate adults over 18 years, while the latter used a dose of 2–8 $\times$ 10^6 CFUs to vaccinate the elderly over 60 years, which will lay the foundation for subsequent data analysis and enhance the credibility of the research results. Interestingly, two additional clinical trials (NCT04387409 and EUCTR2020-001376-15-DE) were conducted to evaluate the effectiveness and safety of the VPM1002 vaccine in reducing the absenteeism of HCWs in the SARS-CoV-2 pandemic. The clinical trial EUCTR2020-001376-15-DE was conducted to evaluate the effectiveness and safety of VPM1002 in reducing the absence of HCWs. Regrettably, due to the premature termination of the study, only 59 subjects were recruited, of which 29 received VPM1002 and 30 received placebo. The primary assessment objective, a reduction in HCWs' absenteeism, was not statistically analyzed due to the early termination of the trial. Nonetheless, preliminary data suggest that the serious adverse events (0.00% vs 3.33%), vaccine compatibility (10.34% vs 23.33%), and COVID-19 infection rate (6.90% vs 10.00%) in the VPM1002 vaccine group were lower than these in the placebo control group. These data preliminarily show that the VPM1002 vaccine may have a specific protective effect on the prevention and control of COVID-19. However, this clinical trial was terminated prematurely due to poor recruitment, and only the result data of 59 participants were analyzed. We expect that the results of the other three clinical trials will provide more substantial evidence of whether the VPM1002 vaccine can prevent COVID-19 infection.

4.6 Four Clinical Trials in the United States of America

As a low TB incidence and mortality, BCG is not generally recommended for use in the United States. Currently, the Centers for Disease Control (CDC) of the United States recommends BCG vaccination only for HCWs and children who have a negative tuberculin skin test and who are continually exposed and cannot be separated from adults with a high risk of TB. However, as of March 25, 2022, the United States has confirmed 79,139,385 cases of COVID-19, with cumulative death of 967,905, ranking first in the world. Therefore, in the face of the world's worst COVID-19 pandemic and widespread vaccination of specific COVID-19 vaccines, the United States has sought other anti-epidemic strategies, including BCG.

Table 4. Current clinical trials of BCG vaccines for COVID-19.

Trial ID	Vaccine (strain)	Countries	Recruitment Status	Target size	Phase	Population Age	Intervention	Primary outcome
ACTRN12620-000707965	VPM1002 (NA)	Australia	Not Recruiting	3468	Phase 3	18 Years and older (HRPs)	Experimental group: 0.1 mL VPM1002 with 2–8 × 10e^5 CFU, i.d.; Placebo: 0.1 mL 0.9% sodium chloride, i.d.	The incidence of SARS CoV-2/COVID-19 infection is associated with acute respiratory symptoms.
NCT04327206	BCG vaccine (Danish strain 1331)	Australia Brazil Netherlands Spain United Kingdom	Not recruiting	10,078	Phase 3	18 Years and older (HCWs)	Experimental group: 0.1 mL vaccine with 2–8 × 10e^5 CFUs i.d.; Placebo: Intracutaneously 0.1 mL NaCl 0.9% i.d.	COVID-19 disease incidence; Severe COVID-19 disease incidence.
ISRCTN4780-2196	BCG (Moreau strain)	Brazil	Recruiting	200,805	N/A	N/A	Experimental group: BCG vaccine (Moreau) strain, no placebo.	COVID-19 incidence; COVID-19 severity
NCT04659941	BCG (NA)	Brazil	Recruiting	1000	Phase 2	18 Years and older (HCWs)	Experimental group: 0.1 mL of the reconstituted vaccine to be administered intradermally; Placebo 1: 0.1 mL of 0.9% NaCl saline solution to be administered intradermally	Compare the cumulative incidence of SARS-CoV-2 infection and severe forms of COVID-19; Assess the BCG vaccine-mediated immune response in HCWs.
RBR-4kjqtg	BCG (NA)	Brazil	Recruiting	400	Phase 2	18 Years and older (HCWs)	Experimental group: receive a dose of BCG vaccine; The control group will not be vaccinated.	Reduction of positivity for COVID-19; Reduction of HCWs related to COVID-19
RBR-5ysj54	BCG (NA)	Brazil	Not Recruiting	1000	N/A	18 years and older	Experimental group: vaccinated with BCG vaccine; Control group: normal saline.	Expected to find a smaller number of coronavirus infections and severe coronavirus infections in the BCG vaccinated group compared to those inoculated with placebo.
NCT04369794	BCG (NA)	Brazil	Recruiting	1000	Phase 4	18 years and older	Experimental group: 0.1 mL BCG vaccine with 2–8 × 10e^6 CFU, i.d.; Placebo: 0.9% saline solution, i.d.	Clinical evolution of COVID-19; SARS-CoV-2 elimination; Seroconversion rate and titration
NCT04439045	VPM1002 (rBCGΔureC::hly)	Canada	Not recruiting	3626	Phase 3	18 years and older	Experimental group: 0.1 mL VPM1002 with 2–8 × 10e^5 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
NCT04826718	BCG (NA)	Cape Verde	Recruiting	400	N/A	18 years and older	Other: Questionnaire	Total number of days absent from work due to COVID-19; Unplanned Absenteeism; Symptomatology after infection by SARS-CoV-2; Presence or absence of anti-SARS-CoV-2 Acs; Duration of anti-SARS-CoV-2 Acs

Table 4. Continued.

Trial ID	Vaccine (strain)	Countries	Recruitment Status	Target size	Phase	Population Age	Intervention	Primary outcome
NCT04641858	BCG vaccine (Danish strain 1331)	Cape Verde; Guinea-Bissau; Mozambique	Recruiting	1050	Phase 4	18 Years and older (HCWs)	Experimental group: 0.1 mL BCG vaccine with 2–8 × $10e^5$ CFU, i.d.; Placebo: 0.1 mL 0.9% NaCl, i.d.	Days of unplanned absenteeism due to illness
NCT04362124	BCG (NA)	Colombia	Not recruiting	1000	Phase 3	18–65 Years old (HCWs)	Experimental group: 0.1 mL BCG with 1 × $10e^5$ to 33 × $10e^5$ CFU, i.d.; Placebo: 0.1 mL normal saline solution, i.d.	Incidence of COVID-19 cases confirmed or probable in the study population.
NCT04542330	BCG vaccine (Danish strain 1331)	Denmark	Recruiting	1900	Phase 3	65–110 Years old	Experimental group: 0.10 mL BCG with 2–8 × $10e^5$ CFU, i.d.; Placebo: 0.1 mL normal saline (0.9% NaCl), i.d.	"Acute infection" identified either by a doctor, antibiotics use, hospitalisation, or death due to infection.
EUCTR2020-003904-15-DK	BCG vaccine (Danish strain 1331)	Denmark	Authorised	1900	Phase 3	65 years and older	Experimental group: 0.1 mL BCG vaccine with 2–8 × $10e^5$ CFUs; Placebo: 0.1 mL NaCl.	The primary outcome is acute infection identified by a doctor, antibiotics use, hospitalisation or death due to infection.
NCT04373291	BCG vaccine (Danish strain 1331)	Denmark	Completed	1293	Phase 3	18–100 Years old (HCWs)	Biological: BCG-Denmark; Biological: Saline.	Number of days of unplanned absenteeism for any reason
EUCTR2020-001888-90-DK	BCG vaccine (Danish strain 1331)	Denmark	Authorised	1500	Phase 3	18–64 Years old (HCWs)	Experimental group: 2–8 × $10e^5$ CFUs/mL, i.d.; Placebo: Solution for injection, i.d.	To reduce absenteeism among HCWs with direct patient contacts during the COVID-19 pandemic.
NCT04347876	BCG (NA)	Egypt	Recruiting	100	N/A	12–80 Years old	Group A: COVID-19 positive with positive tuberculin test; Group B: COVID-19 positive with a negative tuberculin test.	Pneumonia severity index; Need for ICU admission
NCT04350931	BCG vaccine (Danish strain 1331)	Egypt	Not recruiting	900	Phase 3	18 years and older	Experimental group: 0.1 mL BCG with 2–8 × $10e^5$ CFU, i.d.; Placebo: 0.1 mL normal saline (0.9% NaCl), i.d.	Incidence of confirmed COVID-19; Effectiveness of BCG vaccine
NCT04384549	BCG vaccine (Danish strain 1331)	France	Recruiting	1120	Phase 3	18 Years and older (HCWs)	Experimental group: 0.1 mL BCG vaccine (AJ Vaccine) with 2–8 × $10e^5$ CFUs; Placebo: One i.d. of 0.1 mL NaCl	Incidence of documented COVID-19 among health care workers exposed to SARS-CoV-2 and vaccinated with BCG compared to placebo.
EUCTR2020-001678-31-FR	BCG vaccine (Danish strain 1331)	France	Authorised	1120	Phase 3	18 Years and older (HCWs)	Experimental group: 2–8 CFU/mL, i.d.; Placebo: Solution for injection, i.d.	The protection of BCG for HCWs exposed to COVID-19
NCT04435379	VPM1002 (rBCGΔureC::hly)	Germany	Recruiting	2038	Phase 3	60 years and older	Experimental group: 0.1 mL VPM1002 with 2–8 × $10e^5$ CFU; Placebo: Physiological saline 0.1 mL.	Number of days with severe respiratory disease at the hospital and at home

Table 4. Continued.

Trial ID	Vaccine (strain)	Countries	Recruitment Status	Target size	Phase	Population Age	Intervention	Primary outcome
NCT04387409	VPM1002 (rBCGΔureC::hly)	Germany	Active, not recruiting	59	Phase 3	18 Years and older (HCWs)	Experimental group: 0.1 mL VPM1002 with 2–8 × $10e^5$ CFUs, 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 (rBCGΔureC::hly)	Germany	Authorised	2038	Phase 3	18 years and older	Experimental group: VPM1002, 2–8 × $10e^6$ CFU/mL, i.d.; Placebo: Solution for injection, i.d.	Reduction of days with severe respiratory infectious diseases at the hospital and/or at home in elderly subjects
EUCTR2020-001376-15-DE	VPM1002 (rBCGΔureC::hly)	Germany	Prematurely Ended	59	Phase 3	18 Years and older (HCWs)	Experimental group: VPM1002, 2–8 × $10e^6$ CFU/mL, i.d.; Placebo: Solution for injection, i.d.	Health Care Workers absenteeism
NCT04414267 (EUCTR2020-002448-21-GR)	BCG (Moscow strain 361-1)	Greece	Completed	301	Phase 4	50 years and older	Experimental group: 0.1 mL BCG vaccine, i.d.; Placebo: 0.1 mL of sodium chloride 0.9%, i.d.	Susceptibility for COVID-19
NCT03296423	(BCG vaccine Bulgaria strain 1331)	Greece	Completed	200	Phase 4	65 years and older	Experimental group: 0.1 mL BCG Vaccine i.d.; Placebo: 0.1 mL of sodium chloride 0.9%, i.d.	Time to the first infection
EUCTR2020-001783-28-HU	BCG vaccine (Danish strain 1331)	Hungary	Authorised	1000	Phase 3	18–64 Years old (HCWs)	Experimental group: BCG vaccine, suspension for injection, i.d.; Placebo: Solution for injection, i.d.	Health Care Workers absenteeism
CTRI/2020/09/027684	BCG (NA)	India	Recruiting	400	N/A	18–50 Years old (HCWs)	Experimental group: 0.1 mL BCG vaccine i.d.; Placebo: unknown	Rate of infection of COVID-19 in healthcare workers re-vaccinated with BCG as compared to controls
CTRI/2020/07/026668	BCG (NA)	India	Not Recruiting	800	Phase 3	18–60 Years old	Experimental group: 0.1 mL BCG vaccine i.d.; Placebo: 0.1 mL Normal saline	Incidence of COVID-19 by 9 months of follow-up.
NCT04475302	BCG (NA)	India	Not recruiting	2175	Phase 3	60–80 Years old	Experimental group: Each 1 mL contains between 2 × $10e^6$ and 8 × $10e^6$ CFUs, i.p.; Placebo: No intervention	Mortality due to COVID-19 disease
CTRI/2020/06/025854	BCG (NA)	India	Not Recruiting	1450	N/A	60–95 Years old	Experimental group: Single dose of 0.1 mL of BCG vaccine; Placebo: No BCG vaccine.	Proportion of patients with Severe COVID-19 disease and balance of death due to COVID-19 disease
CTRI/2020/06/025798	BCG (NA)	India	Not Recruiting	70	Phase 4	18–80 Years old	Experimental group: 120 mg intravesical BCG (50 mL Normal Saline)	The rates of infectivity of SARS-CoV-2 virus in patients of NMIBC on BCG therapy

Table 4. Continued.

Trial ID	Vaccine (strain)	Countries	Recruitment Status	Target size	Phase	Population Age	Intervention	Primary outcome
CTRI/2020/05/025013	BCG (Moreau strain)	India	Not Recruiting	60	Phase 2	20–40 Years old	Experimental group 1: 0.1 mL BCG with $2 \times 10e^6$ and $8 \times 10e^6$ CFUs/1 mL, i.d.; Experimental group 2: BCG plus standard of care as suggested by DCGI; Placebo 1: Tamiflu, Hydroxychloroquine, Azithromycin; Placebo 2: saline plus standard of care as indicated by DCGI	The total duration of Hospitalization with COVID-19 symptoms such as febrile respiratory distress, decrease in Viral Titer, duration of COVID-19 symptoms
CTRI/2020/04/024833	BCG vaccine (Danish strain 1331)	India	Not Recruiting	1826	N/A	18–65 Years old (HCWs)	Experimental group: 0.1 mL, i.d.; Placebo: 0.1 mL Normal saline, i.d.	The proportion of HCWs with symptomatic COVID 19 disease 6 months after randomisation.
CTRI/2020/04/024749	VPM1002 (NA)	India	Not Recruiting	5946	Phase 3	18–99 Years old (HCWs)	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.
IRCT2020041-1047019N1	BCG (NA)	Iran (the Islamic Republic of)	Recruiting	500	Phase 3	18 Years and older (HCWs)	Experimental group: 0.10 mL BCG Vaccine, i.d.; Placebo: 0.1 mL of 0.9% NaCl solution, i.d.	COVID-19 infection
NCT04461379	BCG (Tokio 172 strain)	Mexico	Not recruiting	908	Phase 3	18 years and older	Biological: BCG vaccine; Other: Placebo	Cumulative incidence of infection in 6 months, the cumulative incidence of hospitalisation for COVID-19, incidence of specific Antibodies
EUCTR2021-000182-33-NL	BCG (NA)	Netherlands	Authorised	40	Phase 4	18–64 Years old	Biological: BCG vaccine	Analyse whether BCG-vaccination before COVID-19 vaccination can enhance the immunogenicity of the COVID-19 mRNA vaccine developed by BioNTech and Pfizer.
EUCTR2020-003470-47-NL	BCG vaccine (Danish strain 1331)	Netherlands	Authorised	5200	Phase 4	18 years and older	Experimental group: BCG Vaccine SSI concentrate; Placebo: Concentrate and solvent for solution for injection, i.d.	Based on the accrual of the two endpoints, the primary endpoint will be either COVID-19.
NCT04537663	BCG vaccine (Danish strain 1331)	Netherlands	Recruiting	5200	Phase 4	60 years and older	Drug: BCG Vaccine; Other: Placebo	A clinically relevant respiratory tract infection, or COVID-19.
EUCTR2020-002456-21-NL	BCG vaccine (Danish strain 1331)	Netherlands	Not Recruiting	100	Phase 4	18–50 Years old	Experimental group: M-M-RVAXPRO® powder and solvent for suspension for injection; Placebo: Solution for injection.	To investigate the effect of bisphosphonates and the MMR vaccine on BCG-induced trained immunity as a preventive approach against COVID-19.

Table 4. Continued.

Trial ID	Vaccine (strain)	Countries	Recruitment Status	Target size	Phase	Population Age	Intervention	Primary outcome
NCT04417335	BCG vaccine (Danish strain 1331)	Netherlands	Not recruiting	2014	Phase 4	60 years and older	Experimental group: BCG vaccine; Placebo: 0.9% NaCl.	SARS-CoV-2 related hospital admission
NCT04328441	BCG vaccine (Danish strain 1331)	Netherlands	Not recruiting	1500	Phase 3	18 years and older	Experimental group: 0.1 mL BCG vaccine with 0.075 mg of attenuated Mycobacterium bovis; Placebo: 0.1 mL NaCl 0.9%.	Health Care Workers absenteeism
EUCTR2020-000919-69-NL	BCG vaccine (Danish strain 1331)	Netherlands	Not Recruiting	1000	Phase 4	18 Years and older (HCWs)	Experimental group: BCG vaccine concentrate and solvent for solution for injection, i.d.; Placebo: Concentrate and solvent for solution for injection, i.d.	Health Care Workers absenteeism
EUCTR2020-001591-15-NL	BCG vaccine (Danish strain 1331)	Netherlands	Authorised	2000	Phase 4	18 years and older	Experimental group: BCG vaccine; Placebo: Pharmaceutical Concentrate and solvent for solution	Primary Objective: To reduce hospital admission of older adults during the SARS-CoV-2 outbreak.
NL8609	BCG (NA)	Netherlands	Not Recruiting	100	N/A	18–50 years old	1 Placebo treatment; 2. BCG vaccination; 3. BCG vaccination + oral bisphosphonate supplementation (alendronic acid); 4. BCG vaccination + MMR vaccine; 5. MMR vaccine alone.	The primary study parameter is the fold-increase in production of pro-inflammatory cytokines by PBMCs/monocytes following immunisation.
NL8547	BCG (NA)	Netherlands	Recruiting	1600	N/A	60 years and older	Participants will be randomised between intracutaneous administration of BCG vaccine or placebo in a 1:1 ratio.	SARS-CoV-2 related hospital admission
NL8477	BCG (NA)	Netherlands	Not Recruiting	1500	N/A	18 Years and older (HCWs)	Participants will be randomised between intracutaneous administration of BCG vaccine or placebo in a 1:1 ratio.	Number of days of unplanned absenteeism for any reason
NCT04648800	BCG (Moreau strain)	Poland	Recruiting	1000	Phase 3	25 Years and older (HCWs)	Experimental group 2: 0.10 mL BCG with 1.5–6 × $10e^5$ CFU, i.d.; Placebo: 0.1 mL normal saline (0.10% NaCl), i.d.	Death and life-or health-threatening condition
EUCTR2020-002111-22-PL	BCG (NA)	Poland	Authorised	1000	Phase 3	18–64 Years old (HCWs)	Experimental group: Anti-Tuberculosis Vaccine BCG 10, powder and solvent for suspension for injection, i.d.; Placebo: Solution for injection. i.d.	COVID-19 cases and deaths

Table 4. Continued.

Trial ID	Vaccine (strain)	Countries	Recruitment Status	Target size	Phase	Population Age	Intervention	Primary outcome
NCT04379336	BCG vaccine (Danish strain 1331)	South Africa	Recruiting	500	Phase 3	18 Years and older (HCWs)	Experimental group: 0.1 mL BCG vaccine with 0.075 mg of attenuated Mycobacterium bovis, i.d.; Placebo: 0.1 mL 0.9% NaCl, i.d.	Incidence of HCWs hospitalised due to COVID-19 per arm.
NCT04453488	RUTI®	Spain	Not recruiting	315	Phase 3	18 Years and older (HCWs)	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
NCT04384614	BCG (NA)	Tunisia	Withdrawn	0	N/A	18–80 Years old	Diagnostic Test: Test PCR; Genetic: TDR; Other: Clinical Examination	Differences related to epidemiological, demographic characteristics
NCT04632537	BCG (Tice strain)	United States	Withdrawn	550	Phase 3	18–64 Years old	Experimental group: 0.10 mL BCG with $2 \times 10e^6$ CFU, i.d.; Placebo: 0.1 mL normal saline (0.9% NaCl), i.d.	Incidence of symptomatic rt-PCR-confirmed SARS-CoV-2 infection
NCT04534803	BCG (NA)	United States	Withdrawn	0	Phase 3	70 years and older	Experimental group: 0.1 mL of reconstituted BCG vaccine given intradermally at baseline. Placebo: 0.1 mL of diluent (saline) given intradermally at baseline	Number of people diagnosed with severe Covid-19 disease
NCT04348370	BCG (Tice strain)	United States	Active, not recruiting	1800	Phase 4	18–75 Years old	Experimental group: 0.1 mL vaccine with $2 \times 10e^5$ CFU, i.d. Placebo: 0.1 mL saline, i.d.	Incidence of COVID 19 Infection
NCT02081326	BCG (N/A)	United States	Active, not recruiting	150	Phase 2	18 Years to 65 Years	Experimental group: 2 BCG vaccinations spaced 4 weeks; Placebo: 2 saline injections spaced 4 weeks	Change in HbA1c values in juvenile-onset type 1 diabetics; COVID-19 and BCG Adaptive Study

(1) The data were obtained from International Clinical Trials Registry Platform (https://www.who.int/ictrp/en/), ClinicalTrials.gov (https://clinicaltrials.gov/ct2/results?cond=COVID-19), EU Clinical Trials Register (https://www.clinicaltrialsregister.eu/ctr-search/search), Australian New Zealand Clinical Trials Registry (https://anzctr.org.au/Default.aspx), Iranian Registry of Clinical Trials (https://en.irct.ir/trial/47279), and Clinical Trials Registry-India (CTRI, http://www.ctri.nic.in/Clinicaltrials/pmaindet2.php?trialid=44460) on March 27, 2022.

(2) The list of abbreviations: BCG, Bacillus Calmette-Guérin; CFUs, colony-forming units; COVID-19, corona virus disease 2019; HCWs, healthcare workers; HRPs: High-Risk Participants; i.d., intradermal injection; s.c., subcutaneous injection; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

Researchers in the United States carried out two Phase III clinical trials (NCT04632537 and NCT04534803) to confirm the prevention and improvement of the BCG vaccine against COVID-19. However, both clinical trials were withdrawn due to capital and other reasons. Furthermore, a randomized and controlled Phase III clinical trial (NCT 04348370) was conducted in 1800 HCWs to assess the BCG vaccine-induced trained immunity to protect HCWs from SARS-CoV-2 infection. The HCWs were divided into the BCG vaccine group with 0.1 ml BCG (2×10^5 CFUs via intradermal injection) and the placebo control group with 0.1 ml sodium chloride. This clinical trial will measure BCG vaccination's impact on the incidence of COVID-19 infection in HCWs and the severity of the COVID-19. The study is expected to be completed in May 2022. In addition, one Phase II clinical trial (NCT02081326) was also conducted to evaluate the effect of BCG in COVID-19 patients with Type 1 diabetes. The participants in the BCG group will receive two doses of BCG vaccine in the first year (with an interval of 4 weeks) and one dose of BCG vaccine each year from the second to the fifth year. Participants in the placebo group will receive the same dose and frequency of 0.9% sodium chloride as the BCG group. This clinical trial was designed to observe whether revaccination with BCG has beneficial immune and metabolic effects in patients with type 1 diabetes and its impact on the incidence and severity of COVID-19 in patients with type 1 diabetes, which makes this clinical trial very distinctive and meaningful.

4.7 Two Clinical Trials in Greece

Like other high-income European countries, Greece has historically implemented universal BCG vaccination, but it is recommended only for high-risk groups, such as infants and children who have contact with an adult infected with TB, immigrants, and refugees (http://www.bcgatlas.org/). In Greece, as of March 25, 2022, as many as 2,884,100 confirmed cases of COVID-19 and 27,125 deaths have been reported to WHO, and a total of 20,181,739 vaccine doses have been administered. Currently, two clinical trials are being conducted in Greece to evaluate the efficacy of BCG in preventing COVID-19. As early as September 21, 2017, a randomized and controlled Phase IV clinical trial was conducted to explore the effect of BCG vaccination against influenza in the elderly. Interestingly, with the outbreak of the COVID-19 pandemic, the primary purpose of this clinical trial shifted from influenza to COVID-19. An interim analysis of this clinical trial was reported on April 29, 2020. The results showed that the first infection time (median 16 weeks vs 11 weeks) and the incidence of new infections (25.0% vs 42.3%) in the BCG group were significantly longer or lower than those in the placebo group [98]. These preliminary data suggest that BCG vaccination is safe, prolongs the first infection time, reduces the incidence of new infections, and has a protective effect on viral respiratory tract infection. This interim analysis provides evidence that "trained immunity induced by BCG vaccination may play a role in combating the COVID-19 epidemic". Furthermore, another double-blind, placebo-controlled Phase IV clinical trial (NCT04414267 and EUCTR2020-002448-21-GR) was also performed to verify whether BCG vaccination can prevent COVID-19 and whether BCG vaccination can regulate the susceptibility of vaccinators to COVID-19 through clinical and immunological standards. The clinical trial is currently recruiting volunteers, and the trial results have not yet been announced. Overall, the two clinical trials in Greece were conducted by the same institution (Hellenic Institute for the Study of Sepsis), but the type of BCG strain, the number of volunteers, and the primary outcome were different between the two clinical trials (Table 4).

5. Conclusions

The COVID-19 pandemic has lasted more than two years since it began in late December 2019, killing more than 6 million people globally. And the continuous emergence of SARS-CoV-2 variants may lead to more pandemic waves. Since there is no effective drug to treat COVID-19, preventive vaccination has become the most promising method for epidemic prevention and control [99]. At present, there are 196 COVID-19 vaccines in preclinical development and 153 COVID-19 vaccines in clinical development. In addition, there are at least 13 COVID-19 vaccines under EUA, including inactivated vaccines, mRNA vaccines, virus vector vaccines, and subunit vaccines. These COVID-19 vaccines are designed based on SARS-CoV-2 S protein. Unfortunately, the S protein has the highest mutation rate, reducing the protection efficiency induced by the COVID-19 vaccines and even causing immune escape [99].

As a live attenuated TB vaccine with a history of 100 years, the BCG vaccine has saved countless lives [100]. However, BCG induced protection can only defence *M. tuberculosis* infection on infants and children rather than adults, and its protective efficacy maintaines 10–15 years. Fortunatelly, previous studies have shown that BCG can induce trained immunity, which has a specific preventive and protective effect against respiratory virus infection, including SARS-CoV-2 [101,102]. Compared with COVID-19 specific vaccines, BCG vaccine has several significant advantages, such as better safety, matured production technologies, low cost, acceptable storage condition, easy to transport, and high accessibility. If this hypothesis can be confirmed by clinical trials in the future, BCG may be a useful tool to fight against COVID-19. Thus, this review focuses on 56 clinical trials conducted to evaluate the preventive effect of BCG against COVID-19, which provides new strategies and ideas for exploring and resolving challenges faced by the COVID-19 vaccines. Furthermore, this review highlighted the prime-booster strategy of the BCG and COVID-19 vaccine and found that prior BCG vaccination significantly increased neutralizing antibody levels induced by the influenza vaccine [103]. In addition, Nether-

lands' clinical trial EUCTR2021-000182-33-NL was also conducted to confirm the prime-booster strategy of the BCG and COVID-19 vaccine. Furthermore, we suggest that the interpretation of the data from these clinical trials should be more cautious and objective. The early termination analysis data of the EUCTR2020-001376-15-DE clinical trial in Germany preliminarily show that the VPM1002 vaccine has a certain protective effect on preventing COVID-19. However, the sample size of this clinical trial is small, the reliability of the results is low, and its effectiveness needs to be further verified.

Abbreviations

COVID-19, Coronavirus disease 2019; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2; BCG, Bacillus Calmette-Guerin; CET, central European time; CFUs, colony-forming units; EUA, emergency use authorization; VOC, Variant of Concern; VOI, Variant of Interest; ACE2, angiotensin converting enzyme 2; HCWs, healthcare workers; HRPs, High-Risk Participants; i.d., intradermal injection; s.c., subcutaneous injection.

Consent for Publication

Not applicable.

Availability of Data and Materials

All data generated or analyzed during this study are included in this published article.

Author Contributions

Conceptualization—WPG and HMW; Data curation—JW and QZ; Formal analysis—JW and QZ; Funding acquisition—WPG; Methodology—JW and QZ; Software—JW, QZ, and WPG; Writing — original draft—JW and QZ; Writing — review & editing—WPG and HMW.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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