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

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Running title: Current status of clinical trials of BCG against COVID-19

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Abstract

The pandemic COVID-19 continues to spread worldwide, and the specific COVID-19 vaccine is the most effective approach to control the COVID-19. An extraordinary research effort has resulted in the rapid development of several vaccine candidates, clinical trials initiation, and the acquisition of registration certificates. Currently, at least four COVID-19 vaccines have been authorized under an emergency use authorization. However, these vaccines still face many challenges. Interestingly, boosting innate immunity with Bacille Calmette Guérin (BCG) has been shown to play a role in controlling the COVID-19 pandemic. BCG is well known for its nonspecific beneficial effects due to epigenetic and metabolic reprogramming of innate immune cells, termed trained immunity (TI). Throughout the literature, it has been shown that the induction of TI using the BCG vaccine can provide protection through altered immune responses against a range of viral infections. Additionally, recent studies showed that BCG vaccination is beneficial against COVID-19. In this review, we discuss the challenges of SARS-CoV-2 specific vaccines, concepts and mechanisms of TI to BCG and COVID-19, and for the first time detailed the current clinical trials of BCG against COVID-19 underway in different countries.

Keywords: Bacille Calmette-Guérin vaccine (BCG), SARS-CoV-2, COVID-19 pandemic, Trained Immunity, Clinical Trials.

1. Introduction

Coronavirus disease 2019 (COVID-19) pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has a serious consequence on the health of the populations and the world economy. It is an urgent need for controlling the existing situation that can only be met through the generation of an immune defence shield to protect the populations from the COVID-19 pandemic. There is no known effective treatment available for the SARS-CoV-2 virus. Currently, no enough COVID-19 specific vaccine is widely vaccinated, with the result is that SARS-CoV-2 continues to spread throughout the world. Many efforts for the development and production of vaccines are underway. The race to develop safe and effective treatment methods has seen vaccine candidates against COVID-19 develop at a larger scale and a rapid pace. There are currently about 200 potential vaccines at various development stages [1-3]. These vaccine candidates use various strategies that include novel approaches to prove that the vaccines are safe and immunogenic in humans. However, in addition to the effectiveness and safety of vaccines, they must be able to generate a specific type of the antibodies, at a particular concentration (titer), and to protect for a reasonable time.

1.1 Concerns about COVID-19 vaccines

It has been shown that specific antibodies generated by vaccination may aggravate the disease, also known as antibody-dependent enhancement (ADE) [4]. In the past, to develop effective vaccines against coronavirus diseases, some of the vaccine candidates, although useful in producing antibodies, led to more severe disease with the subsequent inoculation of the virus [5]. Interestingly, vaccines never generate immunity to all the vaccinated people due to complicated reasons that range from genetic and

immunological factors to the quality of the vaccines themselves and how they are administered [6]. Even if it is assumed that the vaccine will induce effective immunity to a sufficient number of vaccinated people, the time frame of vaccine protection is questionable [7]. In the current scenario, it is not easy to assess the effectiveness of COVID-19 vaccines. There is a need to monitor the persistence of anti-COVID-19 antibodies post-vaccination, and it is a time-consuming process. More importantly, the safety and efficacy are significantly dependent on the type of vaccine, i.e., the technology or platform used. The attenuated SARS-CoV-2 made the least pathogenic by genetic manipulation that are most immunogenic. However, there is a possibility that they will become pathogenic over a period of time due to mutations [8].

At present, at least four vaccines have been authorized under an emergency use authorization (EUA) for the prevention of COVID-19 among more than 40 countries, including Moderna COVID-19 (mRNA-1273) vaccine (ModernaTX, Inc; Cambridge, Massachusetts) [9, 10], Pfizer-BioNTech COVID-19 (BNT162b2) vaccine (Pfizer, Inc; Philadelphia, Pennsylvania) [11], CoronaVac™ vaccine (Sinovac Life Sciences, Beijing, China, https://baijiahao.baidu.com/s?id=1681132239745336390&wfr=spider&for=pc), and Sinopharm's COVID-19 vaccine (Sinopharm China National Biotec Group, Beijing, China, http://english.cctv.com/2020/12/31/ARTIZ7wK4ax0xENYwgnKGZ5E201231.shtml). Although COVID-19 specific vaccines have been approved for vaccination among priority groups, it is still too early to say that humans have won in this battle. Because we still face the following questions: 1) The supply of these vaccines for EUA can not meet the needs of all human beings, and many developing countries, especially the least developed countries (LDCs), cannot obtain enough vaccines [12]; 2) How long can the protection of these vaccines last? It still needs time to verify; 3) The variants found in the

United Kingdom (B.1.1.7 variant) and South Africa (501Y.V2 variant) have the potential to escape an effective immune response [13, 14], will they weaken the effectiveness of the vaccine? 4) Due to the unprecedented acceleration of clinical trials, the possible side effects of these vaccines have not been fully exposed.

1.2 BCG vaccine: A magic bullet against COVID-19?

For the treatment of COVID-19 disease, multiple drugs and their combinations have been tried, but none approved [15, 16]. Due to the lack of approved treatment methods, prevention of COVID-19 from further spread must be of utmost importance. Interestingly, the available vaccines such as Bacille Calmette-Guérin (BCG), oral polio vaccine (OPV), and the measles or MMR vaccines have been suggested to be used for protecting against COVID-19 until enough COVID-19 specific vaccines are widely vaccinated [17-19]. Several epidemiological studies and a latest BCG vaccination study in health care workers have shown that the BCG vaccine can indeed reduce the severity of the disease [20, 21].

BCG vaccine is a live attenuated vaccine and has been used for more than 100 years as a prophylactic agent used for tuberculosis (TB). The BCG is in the essential list of the World Health Organization and used for childhood immunization programs in many countries. At present, there is no direct evidence to support the use of the BCG vaccine for the prevention of coronavirus infection [22]. However, the available data shows that the BCG vaccine provides nonspecific protection against lethal infections that are not related to the target pathogen of the vaccine by inducing trained nonspecific innate immune cells for improved host responses against subsequent infections [18, 23, 24]. Though induction of trained immunity (TI) by BCG vaccination mediates beneficial heterologous effects, the mechanisms underlying its persistence and magnitude is not known. Indeed, there is a need for clinical studies of the

BCG vaccine in vulnerable populations, such as healthcare workers (HCWs), to get insights into the underlying mechanism of BCG's heterologous effects to confirm this hypothesis.

According to the WHO report [22], most countries nationally recommend BCG vaccination in developing countries. However, the international health regulation scores (IHR) of these countries are generally low (Figure 1 and Table S1), putting these countries at risk of the COVID-19 pandemic. If the BCG vaccine's effectiveness is proven through clinical trials, immunization with BCG can not only protect individuals but also, if provided to enough people promptly that provides even partial protection, it is possible to induce sufficient herd immunity. Thus, BCG vaccination may be an excellent supplementary way to curtail the spread of the SARS-CoV-2 and reduce morbidity and mortality across the globe, as proposed in our previous study [20].

In this review, we discuss the challenges of SARS-CoV-2 specific vaccines, concepts and mechanisms of TI concerning BCG and COVID-19, and the question to be answered before using BCG in combating the COVID-19. For the first time, we also present the details of the current clinical trials of the BCG vaccine underway in different countries to assess its role in reducing the effect of the COVID-19 pandemic.

2. The trained immunity induced by BCG vaccination

2.1 The concept of trained immunity

The conception of TI developed in recent decades describes the non-specific and long-term immune memory acquired by innate immune cells after encountering a primary stimulus (pathogen or vaccination), which could mount a heightened inflammatory response including up-regulated production of pro-inflammatory cytokines or enhanced myelopoiesis to a second infection of the same pathogens or

different ones [25]. A variety of stimuli, including β-glucan (a fungal cell wall component), LPS (Lipopolysaccharide), and BCG can trigger TI, and the most studied of which are BCG vaccine. Several epidemiological studies and randomized controlled trials have reported that BCG vaccination could protect against *Mycobacteria tuberculosis*-unrelated pathogens [26]. More importantly, controlled experimental studies in humans have provided direct evidence for the protective efficacy of BCG vaccination against clinically relevant pathogens, such as the yellow fever vaccine and malaria [27, 28]. It has been hypothesized that these BCG-induced non-specific beneficial effects are at least partly mediated by the induction of TI.

2.2 Mechanisms responsible for induction and sustained memory of trained immunity

So far, the mechanisms responsible for BCG-induced TI were only partially understood, and multiple regulatory layers have been supposed to be involved in this process (Figure 2). The myeloid cell populations were identified as the primary cellular basis that mediated BCG-induced TI [29]. The mature myeloid cells, including monocytes and macrophages, have been shown to be the leading performers of BCG-induced TI to protect against unrelated pathogens [27, 30, 31]. An initial challenge of monocytes by BCG during infection leads to more open chromatin architecture with increases of transcriptionally active histone modifications, such as histone H3 acetylation at lysine 27 (H3K27ac) and histone H3 trimethylation at lysine 4 (H3K4m3) and a decrease of transcriptionally repressive histone modifications, such as histone H3 trimethylationat lysine 9 (H3K9m3), at promoters or enhancers of IL-1β, TNFα, IL-6 [27, 30-32]. These dynamic epigenetic changes are also observed in genes that regulate metabolism-

121 related signalling pathways, such as PI3K/AKT/mTOR, which enable the promotion of cellular
122 metabolism towards glycolysis with high glucose consumption and high lactate production [27, 31, 33].
123 During the process of glycolysis, some of the generated metabolic intermediates could act as the
124 epigenetic modifiers, in turn, contribute to the induction of H3K4m3, H3K9m3 and H3K27ac at the
125 promoters or enhancers of stimulated genes, hence showing a complex interaction between epigenetic
126 reprograming and metabolic reprogramming in trained monocytes [31]. Some of these modifications,
127 especially the histone 3 lysine 4 methylation, may preserve as a latent enhancer after cessation of BCG
128 exposure, thereby establishing the epigenetic memory in monocytes and permitting the cells to a more
129 responsive state [34]. When encountering subsequent stimuli, this preserved modification allows the
130 trained monocytes to reacquire the increases of H3K27ac and H3K4m3, and decrease of H3K9m3 in a
131 faster and stronger fashion, followed by enhanced production of pro-inflammatory cytokines, such as IL-
132 1β, TNFα, IL-6 and metabolic reprogramming towards aerobic glycolysis to heighten the TI phenotype
133 [29].
134 While mature myeloid cells, such as monocytes and macrophages, play central roles in BCG-induced
135 TI. These cells have a short life-span within several days, which is inconsistent with the long-term innate
136 immune memory of TI shown by trained monocytes in the circulation lasting for 3 months to 1 year after
137 BCG vaccination [30]. This paradox suggests that BCG-induced TI should also occur at the level of
138 myeloid progenitors and the level of mature myeloid cells. Indeed, a recent elaborate study in the mouse
139 has demonstrated that intravenous BCG vaccination reprograms hematopoietic stem cells (HSCs) in the
140 bone marrow via IFN-γ signaling, promoting their differentiation into progenitors with restricted
141 myeloid-lineage potential, referred to as enhanced myelopoiesis [32]. This primed process of
142 myelopoiesis enables to generate trained monocytes/macrophages with enhanced protection against *M.*

143 *tuberculosis* infection [32]. Similarly, another study has reported that β-glucan could also modulate
144 hematopoietic stem and progenitor cells (HSPCs), and induce myelopoiesis via IL-1β signalling,
145 conferring long-term innate memory to TI [35]. Most recently, similar myeloid differentiation bias of
146 HSPCs through regulating the DNA accessibility of particular inflammation-associated loci in humans
147 of intradermal BCG vaccination has been observed [36]. Some of the epigenetic changes within HSPCs
148 are identified to be preserved in its differentiated peripheral monocytes. These studies indicate that both
149 mature myeloid cells and HSPCs as the cellular basis are responsible for BCG-induced TI and their
150 sustained memory.

151 ### *2.3 Unresolved questions remain to be addressed*

152 Although the myeloid cells as the cellular basis, and epigenetic and metabolic reprogramming as
153 the molecular basis have been identified as the key factors that drive TI, more regulatory mechanisms
154 remain mostly unexplored but are of significant regulatory potential. First, the mature myeloid cell
155 populations (monocytes and macrophages) and myeloid progenitors (HSPCs) are heterogeneous
156 populations, and both of them comprise several subsets. Whether a defined subpopulation or all
157 subpopulations have the potential to develop TI when encountering BCG or other pathogens? Recently
158 developed single-cell RNA sequencing could be used to figure this out [37]. Second, BCG is typically
159 given as an injection into the dermal tissue. However, intravenous administration in the mouse and non-
160 human primates demonstrate stronger protection against *M. tuberculosis* infection [32, 38]. Do the
161 different BCG vaccination strategies elicit the innate immune cells to develop the same or entirely
162 different TI in humans? What is the mechanism underlying this difference? Is the inoculate route that
163 confers the human host's best protective effects against *M. tuberculosis*-related pathogens? Third,

intradermal BCG vaccination in humans induces transcriptional programs of HSPCs toward myelopoiesis [36]. However, no significant alteration of HSPCs was observed in the mouse vaccinated by intradermal BCG [32]. What might help explain these differences except species variation? Fourth, BCG or other stimuli, such as β-glucan and LPS, can generate different TI [29], but what factors mediate the development of stimulus-specific innate immune memory? Finally, to date, most of the studies on BCG-induced TI focus on the analysis of acquired innate memory in circulating monocytes in the peripheral blood or in myeloid progenitors in the bone marrow [39]. The induction process and BCG-induced TI mechanisms in local tissues, especially in the lung, are still lacking. After all, the lung's respiratory mucosa is the primary site to defence for a broad range of pathogens, including coronavirus, adenoviruses, influenza, *M. tuberculosis*, and *Streptococcus pneumonia*. Thus, further studies are required to decipher these outstanding issues to improve our view of host defence and innate immune memory and provide a novel approach for targeting trained immunity to develop better vaccines against tuberculosis or other infectious diseases.

3. BCG vaccine and COVID-19

The evidence that BCG vaccination induced-TI can result in significant heterologous effects against various viral infections, especially respiratory viruses, implicates that BCG vaccination has the potential to afford protection to COVID-19. Though preliminary and unprecise, epidemiological data suggest a negative association between national BCG vaccination policy and the prevalence and mortality of COVID-19 [40, 41]. It has been hypothesized that BCG vaccination could be a potent preventive tool against COVID-19 ahead of a specific vaccine vaccinated [17, 42]. As the mechanisms of BCG-induced TI are not fully understood, and the understanding of immunological responses of COVID-19 is in its

fancy, more mechanistic studies underlying the effect of BCG vaccination on COVID-19 are urgently needed. Especially, which phase of the disease is proper to be intervened? How to select the proper strain of the BCG vaccine? What is the dose and delivery method of BCG that could generate the best protection against COVID-19? In addition, a large cohort of clinical evaluation of the effectiveness of BCG vaccination on COVID-19 is needed.

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

As of 5 January 2020, there are at least 51 clinical trials of TB vaccines (including 43 clinical trials of BCG, 7 clinical trials of VPM1002, and 1 clinical trial of RUTI®) against COVID-19 worldwide (Table 1). Although individuals of any age can be infected with SARS-CoV-2, older patients and HCWs have the highest risk of SARS-CoV-2. Therefore, most of these clinical trials are performed to prevent SARS-CoV-2 infection or to reduce its clinical consequences in these populations. Of the 51 clinical trials, 9 Phase IV clinical trials are being conducted to evaluate the effectiveness of BCG vaccination to prevent COVID-19 infection. Furthermore, there are 27 Phase III clinical trials, 3 Phase II clinical trials, and 12 Phase unknown clinical trials also underway. Among these 51 clinical trials of TB vaccines against COVID-19, 28 clinical trials of BCG vaccine, and 5 clinical trials of VPM1002 are recruiting or authorized, one clinical trial of RUTI® has been active but not yet recruiting. Here, we will review the progress of clinical trials of these three types of TB vaccines against COVID-19 disease in order to provide new ideas for COVID-19 vaccine development.

4.1 Clinical trials of BCG vaccines against COVID-19

As an old vaccine that can induce a long-term boosting of innate immune responses termed as

205 “trained immunity”, BCG has been paid more and more attention to its role in combating a wide variety
206 of pathogens, including SARS-CoV-2 [17, 43]. Although several reports claimed that BCG is not
207 effective in fighting COVID-19 [44-47], a growing number of studies showed that countries with routine
208 BCG vaccination programs have significantly fewer reported cases and deaths of COVID-19 [17, 21, 43,
209 48-50]. However, these observational studies should be confirmed by clinical trials. For the absence of
210 direct evidence, WHO does not recommend BCG to prevent COVID-19 [22]. At present, 43 clinical
211 trials on BCG prevention of COVID-19 have been registered, including 9 Phase IV clinical trials, 20
212 Phase III clinical trials, 3 phase II clinical trials, and 11 clinical trials without a definite trial stage. Herein,
213 we will focus on Phase IV and Phase III clinical trials of BCG vaccines against COVID-19 that have
214 already started recruiting volunteers (Table 1). Detailed information on other clinical trials can be found
215 in Table S2.

216 **4.1.1 NCT04348370 (BADAS) and NCT04632537 (NUEVA): BCG vaccine for health care**
217 **workers as defense against COVID-19 (United States)**

218 In the United States of America, from January 3, 2020, to January 8, 2021, there have been
219 21,447,670 confirmed cases of COVID-19 with 362,287 deaths. Both data rank first in the world.
220 Currently, Pfizer-BioNTech COVID-19 vaccine and Moderna’s COVID-19 vaccine have been
221 authorized and recommended to prevent COVID-19 in the United States
222 (https://www.cdc.gov/coronavirus/2019-ncov/vaccines/different-vaccines.html), but the supply of the
223 vaccines is far from enough. In addition to the research on the COVID-19 specific vaccines
224 (AstraZeneca’s COVID-19 vaccine, Janssen’s COVID-19 vaccine, and Novavax’s COVID-19 vaccine),
225 the United States is also conducting three clinical trials on BCG vaccine against COVID-19, including
226 NCT04348370 (Phase IV), NCT04632537 (Phase III), and NCT04534803 (Phase III). Two clinical trials

(NCT04348370 and NCT04632537) have begun to recruit volunteers. Both of them were conducted to evaluate BCG's efficacy to reduce the risk of infection by SARS-CoV-2 and mitigate COVID-19 disease severity in at-risk health care providers. NCT04348370 is a double-blind, randomized, and placebo-controlled Phase IV clinical trial. This clinical trial plans to recruit 1,800 participants (18-75 years old) who will be randomized to the BCG group and placebo group in a 1:1 ratio. The BCG group will receive a single dose of 0.1 ml FDA-approved BCG vaccine (Tice strain, 2×10^5 CFUs) by slow intradermal injection in the deltoid area. The placebo group will receive a single dose of 0.1 ml saline with the same injection site and route. NCT04632537 is a multicenter, prospective, double-blind, randomized placebo-controlled trial with 550 participants. The immunization strategies and strains of clinical trial NCT04632537 are the same as those of clinical trial NCT04348370, but the difference lies in the immunization dose and sample size. The two clinical trials have a ten-fold difference in vaccination doses, which provides an opportunity to observe and evaluate the immunoprotective effects of different doses. The primary outcome measure is the hazard ratios for the development of COVID-19 calculated with the Cox proportional-hazards model. The results will be reported as the proportion of HCWs with PCR-positive or seroconvert in six months. The secondary outcome measure is the severity of COVID-19 that will be determined with the COVID-19 Severity Scale Scoring based on the level of care required for volunteers who test positive for COVID-19. Both clinical trials are currently recruiting volunteers and are expected to end in November 2021 and November 2022, respectively.

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

As a large country with a population second only to China, India's public health system and the number of HCWs are far behind China. However, from January 3, 2020, to January 8, 2021,

India's cumulative number of confirmed cases and deaths of COVID-19 have reached 10,413,417 and 150,570, respectively. The international community currently believes that the COVID-19 epidemic in India is like a gunpowder keg. Once it is ignited, it will bring a devastating blow to India [51, 52]. Fortunately, India has recommended nationwide BCG vaccination, which may bring a ray of light to India. Furthermore, elderly individuals and HCWs are at high risk of COVID-19. Therefore, strategies to protect these individuals are desperately needed to safeguard continuous patient care. Currently, seven clinical trials have been conducting in India, including NCT04475302 (Recruiting, Phase III), CTRI/2020/09/027684 (Recruiting, Phase unknown), CTRI/2020/06/025798 (Not recruiting, Phase IV), CTRI/2020/07/026668 (Not recruiting, Phase III), CTRI/2020/05/025013 (Not recruiting, Phase II), CTRI/2020/04/024833 (Not recruiting, Phase unknown), and CTRI/2020/06/025854 (Not recruiting, Phase unknown). On July 17, 2020, India conducted a Phase III clinical trial (NCT04475302) to evaluate the effectiveness of the BCG vaccine in reducing morbidity and mortality in 1,450 elderly individuals (60-80 years of age) in COVID-19. According to the experimental plan, individuals in the BCG group will receive a single dose of Freeze-dried BCG vaccine (2-8×10^6 CFUs/ml) manufactured by Serum Institute of India. Individuals in the placebo group will not receive any intervention. For CFUs, the investigator only gave a range rather than a precise value, which may directly impact the immunogenicity of the vaccine and the side effects on the volunteers. This clinical trial is currently recruiting volunteers and is expected to end in May 2021.

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

From January 3, 2020, to January 8, 2021, the cumulative number of confirmed cases and deaths of

COVID-19 in Brazil has reached 7,961,673 and 200,498, ranking second only to India and the United States in the world. The health care system and HCWs in Brazil are facing unprecedented challenges. At present, four clinical trials of BCG vaccine against COVID-19 are performing in Brazil, including a Phase IV clinical trial (NCT04369794, recruiting), 2 Phase II clinical trials (NCT04659941 and RBR-4kjqtg, recruiting), and a Phase unknown clinical trial (RBR-5ysj54, not recruiting). Generally speaking, the sample size of the four clinical trials is relatively large, but many details such as the type of BCG strain and the exact vaccination dose are not disclosed. Here, we call on all researchers conducting clinical trials to provide as much information as possible when registering, especially those related to vaccine doses. Because disclosure of this critical information will facilitate the evaluation of the vaccine by international colleagues. On April 30, 2020, a prospective, randomized, double-blind, and multicenter Phase IV clinical trial (NCT04369794) was conducted by the University of Campinas (Brazil). This clinical trial will evaluate the impact of previous or current BCG exposure on the clinical evolution of COVID-19, elimination of SARS-CoV-2 at different times and disease phenotypes, and seroconversion rate and titration (anti-SARS-CoV-2 IgA, IgM, and IgG). Volunteer in BCG group (n = 500) will receive a single dose of 0.1 ml of lyophilized, live, and attenuated BCG intradermal vaccine (2-8×10^6 CFUs). Volunteer in the placebo group (n = 500) will receive a single dose of 0.9% saline solution in the same volume and route as BCG vaccine. The primary outcomes include clinical evolution of COVID-19, SARS-CoV-2 elimination, seroconversion rate, and titration. This clinical trial is currently recruiting volunteers and is expected to end in August 2023.

4.1.4 NCT04648800: Clinical trial evaluating the effect of BCG vaccination on the incidence and severity of SARS-CoV-2 infections among healthcare professionals during the COVID-19 pandemic in Poland

In Poland, the number of confirmed cases and deaths of COVID-19 began to rise rapidly in the autumn and winter of 2020. According to the WHO report, from January 3, 2020, to January 8, 2021, there have been 1,365,645 confirmed cases of COVID-19 with 30,574 deaths. As of January 2021, two clinical trials (NCT04648800 and EUCTR2020-002111-22-PL) of BCG vaccination against COVID-19 have been conducted in Poland. Both are phase III clinical trials, but only the clinical trial NCT04648800 has started to recruit volunteers. NCT04648800, a multicenter, randomized, partially blinded, placebo-controlled Phase III clinical trial of BCG vaccination against COVID-19, was conducted in Rzeszow/Krakow/ Katowice/Warsaw to determine whether the BCG vaccination affects the course of COVID-19. This clinical trial has started to recruit 1000 HCWs (25 years and older) in Rzeszow, Krakow, Katowice, and Warsaw. The volunteers will be divided into three groups: Group I with positive RT23 test reading, not randomized and not vaccinated against tuberculosis; Group II (with negative RT23 test reading) will receive a dose (0.1 ml) contains 50 mg of BCG vaccine with 150,000 - 600,000 of live Brazilian Moreau strain; Group III with negative RT23 test reading, receiving placebo (0.1 ml normal saline). The primary outcome measures are death and life- or health-threatening condition. The second outcome measures include the onset of clinical symptoms of COVID-19, asymptomatic SARS-CovV-2 infection, hospitalization, and ICU hospitalization, and dyspnoea. This clinical trial is currently recruiting volunteers and is expected to end in April 2021.

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

As of January 8, 2021, Iran's confirmed cases had reached 1,268,263, ranking 15th globally. To assess the BCG vaccine's effect on preventing COVID-19 infection in HCWs exposed to SARS-CoV-2, a double-blind, placebo-controlled, randomized controlled, and multicenter Phase III clinical trial was

conducted by Professor Alborzi Clinical Microbiology Research Center in cooperation with Shiraz University of Medical Sciences in Iran. This study will recruit 500 HCWs (over 18 years of age) and groups will be divided into a 1: 1 ratio. The BCG group will be given a single dose of 0.1 ml intradermal injection of BCG vaccine in the deltoid muscle. The placebo group will be given a single dose of 0.1 ml 0.9% NaCl solution intradermal injection in the same muscle. Although the BCG vaccine's volume and injection site has been opened, the specific number of CFUs was not disclosed in this clinical trial. Participants will be follow-up for 12 months via text messages to obtain information on whether they are infected with COVID-19 and related issues. Furthermore, blood samples will be collected before randomization and at 12 months to determine exposure to SARS-CoV-2. This study includes two primary outcomes: COVID-19 infection and severe COVID-19 disease. This clinical trial is currently recruiting volunteers and is expected to end in June 2021.

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

As of January 8, 2021, the cumulative number of confirmed COVID-19 cases and deaths in South Africa have reached 1,149,591 and 31,368, which puts South Africa at risk of COVID-19 infection. On April 5, 2020, a placebo-controlled and randomized controlled Phase III clinical trial was conducted by TASK Applied Science to investigate whether and why BCG-revaccination can reduce incidence rate and/or disease severity in HCWs during the COVID-19 outbreak in Cape Town, South Africa. This study involves 500 participants who will receive an intradermal injection of 0.1ml of the suspended BCG vaccine (0.075mg of attenuated *Mycobacterium bovis* Danish strain 1331) or a placebo injection of 0.1ml 0.9% NaCl. This project aims to compare the incidence of HCWs hospitalized due to COVID-19 and the incidence of SARS-CoV-2 infection in HCWs, et al. This clinical trial is currently recruiting volunteers. It is expected to end in April 2021.

4.1.7 NCT04537663 (BCG-PRIME) and NCT04328441 (BCG-CORONA): Prevention of Covid-19 in vulnerable older adults and reducing health care workers absenteeism in Covid-19 pandemic through BCG vaccine (Netherlands)

In the Netherlands, from January 3, 2020, to January 9, 2021, there have been 850,790 confirmed cases of COVID-19 with 12,084 deaths. As a country that does not officially recommend BCG vaccination, the Netherlands has the most clinical trials of BCG vaccination against COVID-19 globally. At present, there are nine clinical trials of BCG vaccination against COVID-19 (Table 1), of which three clinical trials have begun to recruit volunteers such as NCT04537663 (Phase IV), NCT04328441 (Phase III), and NL8547 (Phase unknown). Based on the criterion mentioned above, we will give a detailed summary on clinical trials NCT04537663 and NCT04328441. Both clinical trials were subsidized by UMC Utrecht and shared with the same BCG Danish strain 1331. However, there are also significant differences between them, such as the objective, population, sample size, interventions, and outcome measures. NCT04537663 is an adaptive multicenter, double-blind, randomized, placebo-controlled trial including 5,200 to 7,000 vulnerable elderly. This trial aims to determine the impact of BCG vaccination on the incidence of clinically relevant respiratory infections or COVID-19 in vulnerable elderly. Participants will receive an intradermal injection of BCG-Vaccine SSI or placebo (0.1ml 0.9% NaCl) in a 1:1 ratio. It is frustrating that the exact dose of BCG vaccine was not described. The lack of this crucial information may impact the transparency of the experiment. The primary endpoint of this clinical trial will be either (a) COVID-19 or (b) clinically relevant respiratory tract infection requiring medical intervention, potentially including COVID-19 episodes. NCT04328441 is a placebo-controlled adaptive multicenter randomized controlled Phase III clinical trial. Base on the hypothesis that BCG vaccination can reduce HCWs absenteeism during the epidemic phase of COVID-19, this clinical trial will recruit

1,500 participants (over 18 years old) and randomize between intracutaneous administration of BCG vaccine or placebo in a 1:1 ratio. The BCG group or placebo group will be given a single dose of 0.1ml BCG vaccine with 0.075mg of attenuated *Mycobacterium bovis* (Danish strain 1331) or 0.1ml 0.9% saline solution by intradermal injection, respectively. This study will evaluate BCG's efficacy on 1) reducing absenteeism among HCWs with direct patient contacts during the COVID-19 pandemic; 2) reducing hospital admission, ICU admission, or death in HCWs with direct patient contacts during the COVID-19 pandemic. Both studies have begun recruiting volunteers and are expected to end in April 2021.

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

In Denmark, from January 3, 2020, to January 8, 2021, there have been 176,837 confirmed cases of COVID-19 with 1,487 deaths. It is well known that seniors and HCWs are at high risk of SARS-CoV-2 infection in COVID-19. To investigate the hypothesize that BCG vaccination can reduce the risk of COVID-19 and other infections among senior citizens and HCWs during the COVID-19 pandemic, four clinical trials (NCT04542330, NCT04373291, EUCTR2020-001888-90-DK, and EUCTR2020-003904-15-DK) have been conducted in Denmark. Clinical trials NCT04542330 (Phase III, recruiting) and NCT04373291 (Phase III), sponsored by Bandim Health Project, will be performed in 1900 seniors and 1500 HCWs, respectively. Clinical trials EUCTR2020-001888-90-DK (Phase III) and EUCTR2020-003904-15-DK (Phase unknown), sponsored by the University of Southern Denmark, will be performed in 1500 HCWs and 1900 seniors, respectively. As early as September 9, 2020, a placebo-controlled randomized Phase III clinical trial (NCT04542330) was conducted on the population of 1900 seniors (65 years of age or above). This trial's primary objective is to determine whether BCG vaccination reduces

the risk of acute infection in the elderly during the COVID-19 pandemic. Participants randomized to the experimental group or placebo control group will receive an adult 0.1 ml dose of BCG-Denmark vaccine with 2-8×10^5 CFUs or 0.1 ml normal saline (0.9% NaCl) in the skin covering the left upper deltoid muscle, respectively. The primary outcome measure is acute infection identified either by a doctor, antibiotics use, hospitalization, or death due to infection. This clinical trial is currently recruiting volunteers and is expected to end in March 2022.

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

According to the WHO report (https://covid19.who.int/region/emro/country/eg), from February 14 to January 8, 2020, Egypt's morbidity and mortality are 1,362.89 per 100,000 and 75.12 per 100,000, respectively. In Egypt, the BCG coverage is as high as 96%. On April 15, 2020, Assiut University conducted a Phase III clinical trial to determine whether BCG vaccination can significantly reduce the pneumonia severity index, need for ICU admission, number of days to cure COVID-19 disease, and mortality. This study will recruit 100 participants (12 - 80 years old) admitted with proven COVID-19 positive disease. All volunteers will be divided into two groups according to the tuberculin test's positive or negative result (history of previous BCG or not). The study has two design flaws: (1) A positive tuberculin test is not only caused by BCG vaccination but may also be caused by non-tuberculous mycobacteria or environmental mycobacteria. However, the researchers did not exclude this factor, which may lead to a lack of accuracy in the research results. (2) Participants in Phase III clinical trials generally require about 20,000, but this study only recruits 100 participants, which will affect the reliability of the results. This clinical trial is currently recruiting volunteers and is expected to end in June 30, 2020, but the results have not been published.

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

Although the number of confirmed cases and deaths in Australia is much lower than that in the United States, Australia has not yet carried out a national BCG vaccination, which may increase the risk of COVID-19 infection in HCWs and the aged compared with the countries that recommend BCG vaccination. This two-group multicenter, placebo-controlled, and randomized controlled Phase III clinical trial was originally conducted by Murdoch Children's Research Institute in cooperation with Royal Children's Hospital. This study will recruit 10,078 HCWs to determine if BCG vaccination reduces the incidence and severity of COVID-19 during the 2020 pandemic. Participants will be randomly divided into a BCG group and a placebo control group. They will receive a single dose of 0.1 ml BCG vaccine (Danish strain 1331, 2-8 × 10^5 CFUs) or 0.1 ml of 0.9% Saline by intradermal injection in the deltoid muscle, respectively. Each volunteer will receive a 12-month follow-up and feedback on their daily health status and detail COVID-19 infection through a smartphone application. Furthermore, each volunteer's severe disease information will be obtained from hospital medical records and/or government databases. Volunteers' blood samples will be collected before randomization, the third month, and the 12th month after vaccination to assess SARS-CoV-2 infection. The outcome measures of the study will consist of two primary outcome measures (COVID-19 disease incidence and severe COVID-19 disease incidence) and 30 secondary outcome measures. This clinical trial is currently recruiting volunteers and is expected to end in March 2022.

4.2 Clinical trials of VPM1002 vaccines against COVID-19

VPM1002 is a live recombinant BCG *ΔureC*::*hly* vaccine derived from the parental strain of BCG Danish [53]. Originally, VPM1002 was developed to improve the efficacy and safety of the BCG vaccine. Preclinical studies have shown that VPM1002 can induce an enhanced safety profile and Th1- / Th17-type immune responses in mouse, guinea pig, rabbit, and non-human primate animal models [54, 55]. Several clinical trials conducted in South Africa and Germany indicated that VPM1002 could stimulate multifunctional T cells secreting a high level of IFN-γ or B cells producing antibodies [56, 57]. These data suggest that VPM1002 may have more significant potential than BCG in reducing the severity of SARS-CoV-2 infection. However, as a live attenuated vaccine, VPM1002 is modified by replacing the urease C gene of BCG with membrane perforating listeriolysin O (LLO) encoding gene (hly) of *Listeria monocytogenes* [58]. Whether its defensive efficiency against COVID-19 infection is significantly better than BCG still needs more clinical trials to verify.

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

4.2.1 NCT04387409: Study to assess VPM1002 in reducing healthcare professionals' absenteeism in COVID-19 pandemic (German)

Germany has been at the forefront of clinical trials related to the effectiveness of the VPM1002 vaccine against COVID-19. Currently, Germany has registered four clinical trials of VPM1002 globally,

including two clinical trials registered on the Clinicaltrials.gov (NCT04387409 and NCT04435379) and two clinical trials registered on the EU Clinical Trials Register (EUCTR2020-001376-15-DE and EUCTR2020-001675-33-DE). On May 13, 2020, a double-blind, randomized, placebo-controlled, and multicenter Phase III clinical trial was conducted by Vakzine Projekt Management GmbH in cooperation with FGK Clinical Research GmbH to investigate whether vaccination of HCWs with VPM1002 could reduce the absenteeism during the COVID-19 pandemic. A total of 1200 HCWs (over 18 years old) with high expected exposure to SARS-CoV-2 infected patients will be centrally randomized in a 1:1 ratio to receive a single dose of 0.1ml VPM1002 with 2-8×10^5 CFUs or 0.1 ml physiological saline by intradermal injection, respectively. All volunteers' information on absenteeism, hospitalizations, adverse events or serious adverse events, and intensive care unit admissions will be collected through an online questionnaire. This clinical trial is currently recruiting volunteers and is expected to end on June 30, 2021.

4.2.2 NCT04435379: Study to assess VPM1002 in reducing hospital admissions and/or severe respiratory infectious diseases in the elderly in COVID-19 pandemic (German)

In addition to testing the VPM1002 vaccine in adults over the age of 18, Vakzine Projekt Management GmbH and FGK Clinical Research GmbH subsequently evaluated the VPM1002 vaccine's preventive effect on COVID-19 infection in the elderly population in German. This Phase III, randomized, double-blind, placebo-controlled, and multicenter clinical trial will recruit 2038 elderly participants to assess the efficacy and safety of VPM1002 in reducing hospital admissions and/or severe respiratory infectious diseases in the COVID-19 pandemic. Subjects who fulfill the inclusion/exclusion criteria will be centrally randomized in a 1:1 ratio to receive a single dose of 0.1 ml VPM1002 (freeze-dried, 2-8×10^5 CFUs) or 0.1 ml physiological saline by intradermal injection, respectively. All volunteers will be asked to provide their 240-day follow-up data through an online questionnaire, including

hospitalization, adverse events/serious adverse events, ICU hospitalizations, and other secondary endpoints. Furthermore, subjects (with or without symptoms) diagnosed with SARS-CoV-2 infection will be followed up for at least six weeks (from the date of test results), regardless of the total test time. This clinical trial is currently recruiting volunteers and is expected to end in May 31, 2021.

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

India has been working hard for many years in the clinical research of the VPM1002 vaccine for TB prevention. Recently, Serum Institute of India Pvt Ltd (SIIPL), the world's largest vaccine manufacturer, has been supported under the Department of Biotechnology's National Biopharma Mission for conducting a double-blind, randomized, placebo-controlled, and multicenter Phase III clinical trial of the VPM1002 vaccine. This study will recruit 5946 HCWs and high-risk individuals in close contact with COVID-19 patients to determine if the VPM1002 vaccine can induce an enhanced immunity to fight against SARS-CoV-2. Participants in the VPM1002 vaccine group or placebo group will receive a single dose of 0.1 ml VPM1002 or 0.9% sodium chloride to be administered as an intradermal injection, respectively. Nevertheless, the number of CFUs in the 0.1 ml VPM1002 vaccine was not disclosed, which is very important for evaluating a vaccine and should not be ignored. The current trial will assess the efficacy and safety of VPM1002 by counting and analyzing the number of subjects with laboratory-confirmed COVID-19 infection among HCWs or other high-risk subjects. The number of laboratories confirmed COVID-19 infection with the severe, critical, or life-threatening disease assessed by investigator among HCWs or other high-risk subjects.

4.3 Clinical trial of RUTI® vaccine against COVID-19

RUTI® is an therapeutic vaccine based on fragmented and detoxified *M. tuberculosis* to prevent active TB in subjects with latent TB infection (LTBI) [59]. This vaccine is initially developed by Archivel Farma S.L. (Spain) in collaboration with Parexel (USA). Previous studies have demonstrated that the RUTI® vaccine provides a strong humoral and cellular immune response against antigens from *M. tuberculosis* [60], induces a balanced immune response and reflects its impact on trained immunity [61]. A preclinical study has indicated that the RUTI® vaccine has good efficacy in controlling LTBI in mice, guinea pigs, goats, and mini-pigs [62]. Clinical trials also suggested that the RUTI® vaccine has acceptable tolerability, immunogenicity, and safety in healthy volunteers (NCT00546273) and patients with LTBI [62, 63] or multidrug-resistant TB (NCT02711735) [53, 63].

Based on these encouraging results, Fundació Institut Germans Trias i Pujol in Spain performed a double-blind, randomized, placebo-controlled Phase III clinical trial to evaluate the efficacy of the RUTI® vaccine to prevent SARS-CoV-2 infection (NCT04453488). Three hundred and fifteen HCWs over the age of 18 will be given two doses of 0.3 ml RUTI® vaccine (25 μg) or 0.3 ml 0.9% NaCl by subcutaneous injection in the deltoid region at the baseline visit and after 2 weeks +/- 3 days, respectively. The primary outcome of this study is a rate of positive serology at the end of the study or a positive PCR test in the course of routine clinical practice. It should also be noted that the stability of the vaccine's immunogenicity is still uncertain. A Phase II clinical trial in patients with latent tuberculosis infection demonstrated that the safety profile of the RUTI® vaccine was considered acceptable. However, the immunogenicity profile of the RUTI® vaccine was variable among groups [62]. Therefore, these issues should be considered in clinical trials of the RUTI® vaccine against COVID-19.

5. Challenges of COVID-19 vaccines and opportunities of BCG vaccines

In many countries, the SARS-CoV-2 specific vaccine candidates are already available for vaccinating vulnerable groups such as HCWs and the elderly. However, the war with the COVID-19 pandemic continues as there is already the third wave of COVID-19 pandemic in many western countries. In addition, recent studies reported that the variants of SARS-CoV-2 have already emerged with multiple mutations in spike protein. The mutated SARS-CoV-2 variants with a specific change to the spike protein are more concerning than other harmless changes to the previously observed virus. The new mutations could change the biochemistry of the spike and could affect the transmissibility of the virus. It is also essential to know that the spike protein is based on current COVID-19 vaccines that are expected to generate an immune response against this protein. The variant strains with a spike protein mutation of D614G currently predominate globally. Several mutant variants' analyses revealed that D614G, along with several variants, were significantly more infectious. The study also showed that the variants with changes to A475V, L452R, V483A, and F490L were more resistant to some neutralizing antibodies [64, 65]. In these circumstances, it is difficult to assess the impact of the SARS-CoV-2 specific vaccines in controlling the COVID-19 pandemic effectively.

As discussed earlier, there are more than 200 SARS-CoV-2 vaccine candidates, and some of them have shown promising results during the clinical trials. However, many factors need to be considered before achieving success with SARS-CoV-2 specific vaccines. The factors such as the number of doses and their frequency need to be determined, particularly if repeated or annual vaccination is required. Scale-up of production to billions of doses and delivery to all the world regions is a tremendous logistic challenge. Information regarding what level of antibodies correlate with protection against the disease

and the development of standardized viral neutralization along with other assays to compare vaccine candidates are needed. There is also concern about the potential disease enhancement and other theoretical safety concerns related to each type of vaccine need to be understood and carefully monitored.

In contrast, the BCG vaccine has been in use for more than a hundred years for the prophylaxis of TB. There is convincing preclinical evidence as well as human studies that show the immunostimulatory antiviral effects of the vaccine. Interestingly, recent epidemiological studies and limited studies in health care workers showed that BCG offers a protective effect by reducing the severity of the COVID-19 and hence mortality. The BCG may not eliminate or prevent the infection. The vaccine might offer some heterological enhanced nonspecific immunity by induction of lymphocyte response and cell mediated and innate immunity against SARS-CoV-2 infection. In addition, the data has shown that BCG vaccination is safe and can protect the elderly against infections. Therefore, the BCG vaccination could bridge the gap until a fully functional vaccine for SARS-CoV-2 is developed and produced. However, to be fully convinced that BCG vaccination can have such protective effects, we need to wait for the results of ongoing controlled trials, which could provide the highest level of proof for the hypothesis that BCG vaccination protects against COVID-19.

6. Discussion

In summary, although some countries have provided COVID-19 specific vaccines for vulnerable groups, it is difficult for people in LDCs to obtain these vaccines in a short time due to the limitations of funds, production technology, and transportation cold chain. It is hypothesized that BCG may stimulate a non-specific protective effect against COVID-19, and a plausible immunological mechanism has been identified as "trained innate immunity". Currently, more than 51 clinical trials of BCG against COVID-

19 have been conducted to determine the hypothesis, including 9 Phase IV clinical trials, 27 Phase III clinical trials, 3 Phase II clinical trials, and 12 Phase unknown clinical trials. These clinical trials' development will provide the most substantial evidence for the BCG vaccine to prevent COVID-19 disease. Once the results of these clinical trials confirm the above hypothesis, we can produce enough BCG in a short time to reduce the severity of the COVID-19 disease and gain time for wide coverage of COVID-19 specific vaccines.

Data availability

All data generated or analysed during this study are included in this published article and its supplementary information files.

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Author contributions

Conceptualization: Wenping Gong and Ashok Aspatwar; Software: Wenping Gong; Data extraction: Wenping Gong; Funding acquisition: Wenping Gong and Ashok Aspatwar; Writing – original draft, Wenping Gong, Ashok Aspatwar, and Shuyong Wang; Writing – review & editing, Xueqiong Wu and Seppo Parkkila. All Authors have read and approved the manuscript.

Competing interests

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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Figure Legends

Figure 1. International health regulation scores. The data were obtained from State Parties Self-Assessment Annual Reporting on the implementation of the International Health Regulations released by WHO. The detailed information of IHR scores among countries can be found in Table S1. Available online: https://extranet.who.int/e-spar (updated on 09 March 2020).

Figure 2. Mechanisms responsible for BCG - induced trained immunity. BCG or its triggered cytokines reprogram the epigenetics and metabolism of monocytes/macrophages and some of these changes are partially retained after cessation of the stimulus, inducing a trained state. The trained monocytes/macrophages allow to heightened innate immune responses to a second stimulation unrelated to Mtb, which is mediated by faster and enhanced metabolic and epigenetic rewiring. In addition, BCG-vaccination induce the hematopoietic stem/progenitor cells to undergo epigenetic changes or increased DNA accessibility of certain genes, leading to enhanced myelopoiesis. Some of these epigenetic changes with HSPCs are conserved in their differentiated progeny, i.e., monocytes. These modulation of HSPCs by BCG ultimately mediate the long-term innate immune memory.

Tables

Table 1. Current clinical trials of BCG vaccines against COVID-19

Supplementary materials

Table S1. SPAR - State Parties Self-Assessment Annual Reporting on the implementation of the International Health Regulations.

Table S2. Detail data of current clinical trials of COVID-19 vaccines.

Figure 1.TIF

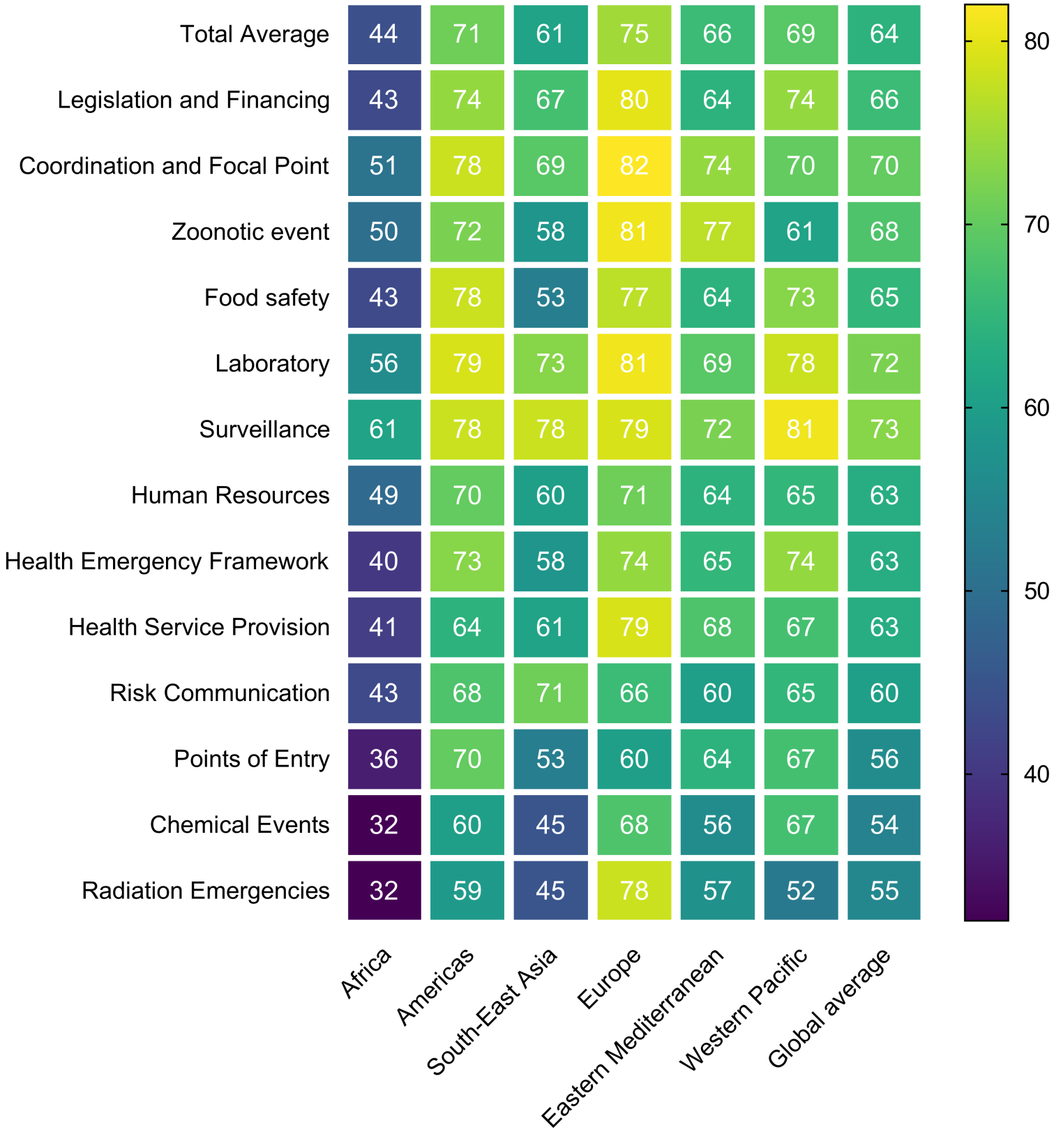
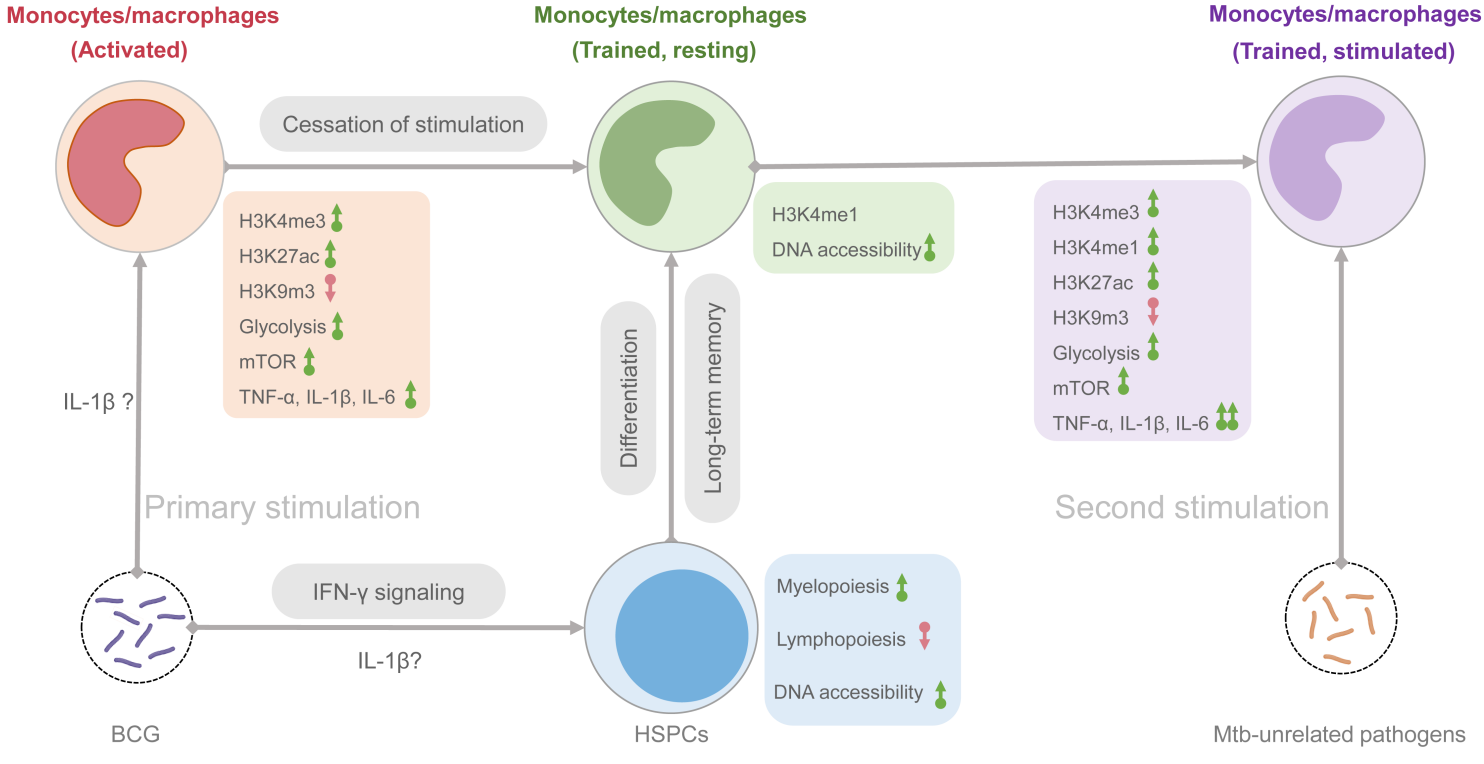

Total Average 44 71 61 75 66 69 64
Legislation and Financing 43 74 67 80 64 74 66
Coordination and Focal Point 51 78 69 82 74 70 70
Zoonotic event 50 72 58 81 77 61 68
Food safety 43 78 53 77 64 73 65
Laboratory 56 79 73 81 69 78 72
Surveillance 61 78 78 79 72 81 73
Human Resources 49 70 60 71 64 65 63
Health Emergency Framework 40 73 58 74 65 74 63
Health Service Provision 41 64 61 79 68 67 63
Risk Communication 43 68 71 66 60 65 60
Points of Entry 36 70 53 60 64 67 56
Chemical Events 32 60 45 68 56 67 54
Radiation Emergencies 32 59 45 78 57 52 55
Africa
Americas
South-East Asia
Europe
Eastern Mediterranean
Western Pacific
Global average
80
70
60
50
40

Figure 2.TIF


Monocytes/macrophages
(Activated)
Monocytes/macrophages
(Trained, resting)
Monocytes/macrophages
(Trained, stimulated)
Cessation of stimulation
H3K4me3
H3K27ac
H3K9m3
Glycolysis
mTOR
TNF-α, IL-1β, IL-6
H3K4me1
DNA accessibility
H3K4me3
H3K4me1
H3K27ac
H3K9m3
Glycolysis
mTOR
TNF-α, IL-1β, IL-6
IL-1β ?
Differentiation
Long-term memory
Primary stimulation
Second stimulation
IFN-γ signaling
IL-1β?
Myelopoiesis
Lymphopoiesis
DNA accessibility
BCG
HSPCs
Mtb-unrelated pathogens