

Expanded Scope of Bacillus Calmette-Guerin (BCG) Vaccine Applicability in Disease Prophylaxis, Diagnostics, and Immunotherapeutics

Oluwafolajimi A. Adesanya^{1,✉}, Christabel I. Uche-Orji², Yeshua A. Adedeji², John I. Joshua², Adeniyi A. Adesola², Chibuike J. Chukwudike²

Abstract

Following the discovery of the Bacillus Calmette-Guerin (BCG) vaccine, its efficacy against *Mycobacterium tuberculosis* was soon established, with several countries adopting universal BCG vaccination schemes for their populations. Soon, however, studies aimed to further establish the efficacy of the vaccine in different populations discovered that the vaccine has a larger effect in reducing mortality rate than could be explained by its effect on tuberculosis alone, which sparked suggestions that the BCG vaccine could have effects on other unrelated or non-mycobacterial pathogens causing diseases in humans. These effects were termed heterologous, non-specific or off-target effects and have been shown to be due to both innate and adaptive immune system responses. Experiments carried out in a bid to further understand these effects led to many more discoveries about the applicability of the BCG vaccine for the prevention, diagnosis, and treatment of certain disease conditions. As we approach the second century since the discovery of the vaccine, we believe it is timely to review these interesting applications of the BCG vaccine, such as in the prevention of diabetes, atherosclerosis, and leukemia; the diagnosis of Kawasaki disease; and the treatment of multiple sclerosis, non-muscle invading bladder cancer, and stage III melanoma. Furthermore, complications associated with the administration of the BCG vaccine to certain groups of patients, including those with severe combined immunodeficiency and HIV, have been well described in literature, and we conclude by describing the mechanisms behind these complications and discuss their implications on vaccination strategies, especially in low-resource settings.

Keywords: BCG; autoimmunity; immunotherapeutics; complications; non-specific effects

Introduction

In 1908, a bacteriologist – Albert Calmette and veterinarian – Camille Guerin, began working on an experiment to develop a possible vaccine against tuberculosis (TB) at the Pasteur Institute. In 1921, about 13 years later, they announced the discovery of their vaccine, which was obtained by attenuating the bovine strain of tuberculosis *Mycobacterium bovis*, by serial subculturing

(231 passages) on glycerinated bile potato medium.¹ The Bacillus Calmette-Guerin (BCG) vaccine has been shown to be effective in the prevention of disseminated and meningeal tuberculosis in infants, however, its efficacy in preventing pulmonary tuberculosis in adults, ranges from 0% to 80%.² The current World Health Organization (WHO) recommended route of administration is an intradermal injection of the vaccine in the deltoid. With over 4 billion people vaccinated, and about 100 million newly-vaccinated infants every year, BCG is currently the most widely used vaccine globally.² However, beyond its effects against tuberculosis, the BCG vaccine has been shown to have certain heterologous or non-specific effects against other unrelated pathogens and on certain disease states, which could be protective, diagnostic or immunotherapeutic.³ The vaccine has also been shown to be harmful under some cases, such as in immuno-deficient patients.³ As we approach the 100 year mark since the discovery of the BCG vaccine, we believe it is timely to review the applications of BCG beyond its use as a protective agent against TB, such as for protection against diabetes, atherosclerosis and leukemia; as a diagnostic agent against Kawasaki disease; and as an immunotherapeutic agent against noninvasive bladder cancer, stage III melanoma, allergic asthma, and multiple sclerosis. We also discuss the genealogy of the BCG vaccine, its complications, and make recommendations about the implications of these adverse effects on vaccination strategies.

Methodology

We conducted a search on the PubMed, Google Scholar, HINARI, and EMBASE databases with a combination of terms including: BCG, Bacillus Calmette-Guerin, strains, non-specific

Editor: Stijn van der Veen

Author affiliations: ¹ Institute for Advanced Medical Research and Training (IAMRAT), College of Medicine, University of Ibadan, Ibadan, Nigeria;

² Department of Medicine, College of Medicine, University of Ibadan, Ibadan, Nigeria.

✉ Corresponding author: Oluwafolajimi A. Adesanya, Institute for Advanced Medical Research and Training (IAMRAT), College of Medicine, University of Ibadan, Ibadan, Nigeria. E-mail: folajimiadesanya@gmail.com

Author contributions: All authors conceived and designed the study, performed the literature search and prepared the manuscript; OAA reviewed and edited the manuscript; all authors have approved the final manuscript for publication.

Funding: No funding was received for this work.

Conflicts of interest: The authors reported no conflict of interest.

Copyright © 2020 the Author(s). Published by Wolters Kluwer Health, Inc. This is an open access article distributed under the terms of the Creative Commons Attribution-Non Commercial-No Derivatives License 4.0 (CCBY-NC-ND), where it is permissible to download and share the work provided it is properly cited. The work cannot be changed in any way or used commercially without permission from the journal.

Infectious Microbes & Diseases (2020) 2:4

Received: 19 August 2020 / Received in final form: 2 October 2020 / Accepted: 6 October 2020

<https://doi.org/10.1097/IM9.000000000000040>

effects, heterologous effects, protection, diagnosis, immunotherapeutic, cancer, Kawasaki disease, Alzheimer disease, complications, autoimmunity, immunodeficiency, and multiple sclerosis. Following a review of the titles and abstracts to eliminate irrelevant articles and duplicates, we proceeded to report our findings as a narrative review of the literature.

Genealogy of the BCG vaccine

Following introduction of BCG in 1921, several countries took different approaches to vaccinating their populations. While the United Kingdom, and countries in Africa and Asia adopted a universal vaccination policy, other countries such as Italy, the Netherlands, Canada, Belgium, and the United States, either reserved the vaccination for high-risk groups within their populations, or did not adopt any form of BCG vaccination policy.⁴ In a similar manner, the BCG vaccine strain used by different countries for immunizing their populations differs widely. This is because after the initial strain was discovered by Calmette and Guerin, the seeds were distributed to different countries who then sought to culture their own strains of the vaccine. The successive cycles of serial passaging ensued the initial BCG strain accumulated genomic variations, including single nucleotide polymorphisms, deletions, and duplications, leading to the emergence of multiple sub-strains.⁵ The BCG vaccine can be classified into four groups based on the duplication variants (DU2). The DU2-I and DU2-II groups are often referred to as the early BCG vaccine strains, and include the BCG Russia, BCG Japan, and BCG Moreau/Brazil sub-strains, while the DU2-III and DU2-IV groups are referred to as the late strains and include the BCG Pasteur, BCG Denmark, and BCG Connaught sub-strains.⁵ According to Behr et al.,⁶ these various vaccine sub-strains differ in their biochemical compositions, immunogenicity, virulence, and growth properties, with the later sub-strains possessing alphamycolic and ketomycolic acids in their cell wall, while being defective in cell wall

methoxymycolic acids, which the early sub-strains produce in abundance. They went on to explain that a point mutation in the *mma3* gene is responsible for the impairment in methoxymycolic acid production by the later BCG sub-strains.⁶ These mycolic acids stimulate macrophages to produce increased levels of interferon (IFN)- γ , tumor necrosis factor (TNF)- α and myeloperoxidase upon exposure to innate trigger factors,⁷ however, according to Vander Beken et al.,⁸ while methoxymycolic acid induces activated macrophages to produce pro-inflammatory cytokines, ketomycolic acids promote anti-inflammatory, alternatively activated macrophages. The translational effect of this molecular structure determined inflammatory pattern is that methoxymycolic acid-producing early BCG sub-strains are more potent immunogenic agents than the ketomycolic acid producing late sub-strains.⁹ Figure 1 illustrates the genealogy of BCG vaccine strains from 1921.

BCG for prophylaxis

The non-specific effects of BCG are numerous and have been reviewed by Moorlag et al.² Heterologous lymphocytic responses, including antigen cross-reactivity due to molecular mimicry, bystander activation of unrelated lymphocytes and lymphocyte dependent activation of innate immunity, as well as activation of innate immune memory through epigenetic and metabolic changes in circulating monocytes leading to the production of pro-inflammatory cytokines have been described as possible mechanisms behind these non-specific or heterologous effects.¹⁰ Studies from Guinea Bissau,¹¹ Uganda,¹² and Denmark¹³ have linked BCG administration to a reduction in all-cause infant mortality rates, an effect that has been explained to be a direct consequence of the heterologous effects of the BCG vaccine in preventing unrelated viral infections during childhood. This finding was confirmed in a WHO Special Advisory Committee systematic review by Zimmermann et al.,¹⁴ which reported a 30% reduction in all-cause mortality in infants

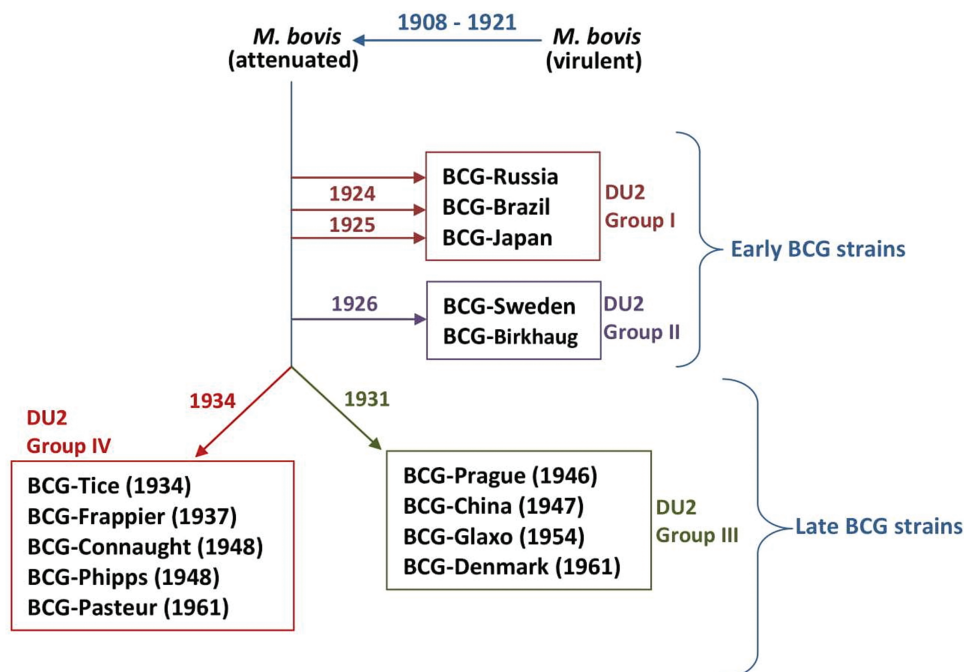


Figure 1. Genealogy of the BCG vaccine. BCG: Bacillus Calmette-Guerin.

following BCG vaccination (RR 0.70; confidence interval 95%, 0.49–1.01). A study by Post et al.¹⁵ identified BCG vaccination as an important factor which contributes to the prevention of childhood pneumonia, stating that its effects translate into the reduction of the risk of pneumonia related deaths by up to 50%. Furthermore, BCG vaccination was associated with a decrease in infection-related illnesses in infants during the first three months of life,¹⁶ while studies in animal models have suggested conflicting results on the efficacy of BCG vaccination in protecting against atherosclerosis. Ovchinnikova et al.¹⁷ demonstrated that the BCG strain inactivated by subjection to extended freeze drying was able to reduce the size of atherosclerotic lesions by increasing interleukin (IL)-10 production and reducing the production of pro-inflammatory cytokines like IL-6, IL-13, and TNF- α . Similarly, van Dam et al.¹⁸ showed that in hyperlipidemic mice models BCG vaccination significantly reduced the plasma total cholesterol by 34% ($P=0.03$), reduced non-HDL cholesterol by 36% ($P=0.002$), and reduced macrophage foam cell formation by 18% ($P=0.02$). These effects were attributed to accelerated plasma clearance of cholesterol through increased hepatic uptake.¹⁸ Conversely, Lamb et al.¹⁹ reported in their own experiment that BCG vaccination increased aortic atherosclerosis in cholesterol-fed rabbits.

BCG vaccination has been linked to protection against hyperoxic lung injury in neonatal mouse models as a result of an increase in expression of FGF-BP1, VEGF, IL-13, and NFkB1 genes in the lungs, resulting in a decrease in alveolar septal fibrosis and smooth muscle actin production.²⁰ BCG has also been demonstrated to have anti-neoplastic properties later in life, protecting against isologous tumors in mice,²¹ reducing leukaemia mortality^{22,23} and lung cancer²⁴ in humans, and increasing survival of patients with malignant melanoma.²⁵ Repeated BCG vaccination was associated with the prevention of diabetes in non-obese mouse models,²⁶ however, evidence from some human studies have failed to establish such an effect.^{27,28} Gofrit et al.²⁹ also hypothesized that BCG vaccination may protect against Alzheimer disease, following a study by Zou et al.³⁰ that showed BCG vaccination alleviates cognitive defects and neuroinflammation in mouse models through the recruitment of inflammation-resolving monocytes to the brain.

BCG for diagnosis

BCG vaccination has been proposed as an indirect tool for the diagnosis of Kawasaki disease (KD), which is an acute febrile illness of childhood, often diagnosed before the age of 5. In 1970, Kawasaki described redness and swelling that appears over the site of BCG vaccination to be an important diagnostic marker of the disease,³¹ which is still used today. However, Kakisaka et al.³² reported a case of BCG site erythema in an 11 month old with human herpes virus type 6 infection and not KD. Similarly Muthuvelu et al.³³ reported a case of BCG site redness and swelling in a 7 month old with measles. To test the validity of the association between BCG site redness and KD, Uehara et al.³⁴ conducted a survey of 15 524 patients with KD in Japan, and found that 7745 (49.9%) had the BCG site induration and redness characteristic of the condition. A similar study in Mexico involving 399 KD patients found that 97 patients (24.3%) had the BCG vaccination site reaction.³⁵ This difference in results could be attributed to ethnic differences and different BCG vaccine strains used.³⁶ To further test this association, Tseng et al.³⁷ studied the correlation between the lesion patterns and the severity of KD, and found that the intensity of the cutaneous

inflammation is proportional to the severity of the associated coronary abnormalities and aspartate aminotransferase elevation. Histologically, the vaccination site erythema and induration is characterized by small vessel dilation in the dermis, with CD4⁺ T lymphocyte and CD13⁺ macrophage infiltration as well as an increased secretion of IL-1 α , TNF- α , IL-2, and IFN- γ .³⁸ The proposed mechanism behind this BCG reactivation effect seen in KD involves cross-reactivity between the mycobacterial heat shock protein (hsp) 65 and the human homolog HSP63,³⁹ and it has been hypothesized that in patients without a BCG scar, a tuberculin skin test could be of value for the diagnosis of KD. This theory was first tested by Bertotto et al.⁴⁰ in a clinical trial involving 11 children with KD. They reported a strongly positive tuberculin skin reaction in all patients in the test group and in none in the control group. However, when Kollmann et al.⁴¹ tried to reproduce this result in another trial involving nine patients with KD, they reported that none of the patients had a positive tuberculin skin reaction, a contradiction which was attributed to the use of a different tuberculin product. BCG vaccination site reaction still remains an important marker for KD diagnosis.

BCG for therapy

BCG immunotherapy is the gold standard for the adjuvant treatment of non-muscle invasive bladder cancer. It was first reported by Morales et al.⁴² in 1972, and there are over 3 million treatment courses administered annually.^{43,44} The mechanism behind this action involves recruitment of immunocompetent cells to the bladder by BCG, followed by the induction of the tumor cells in the bladder to secrete cytokines, which alerts the immune cells to their presence and location, eventually leading the tumor cells to be killed by the immune cells.⁴⁵ Some cytokines implicated in this mechanism include IL-8,⁴⁶ IL-6, and TNF- α .⁴⁷ Recently, Ibarra et al.⁴⁸ reported that the BCG-induced cytokine production by the bladder tumor cells is regulated through a Ca²⁺ signaling pathway. BCG is also recognized as an intravesical treatment strategy for inoperable stage III in-transit melanoma. Although the mechanism behind this effect is still unclear, some studies have reported a 50% regression of injected lesions and 17% regression of uninjected lesion in immunocompetent patients following direct injection of BCG into the cutaneous metastatic melanoma lesions.⁴⁹ It was hypothesized by Yang et al.⁴⁹ that this effect may be secondary to the stimulation of γ 9 δ 2 T cells by the BCG ligands.

A 1997 study had suggested a strong inverse relationship between BCG vaccination and atopic disorders such as allergic asthma.⁵⁰ Five years later, Choi and Koh reported the results of a randomized controlled clinical trial investigating the effect BCG vaccination on adult asthma. They found that BCG vaccination significantly increased pulmonary function and reduced the use of rescue medication in the study participants.⁵¹ Furthermore, BCG has been linked to neuroprotective functions by alleviating lipopolysaccharides-induced neuroinflammation in mouse models.⁵² Lacan et al.⁵³ showed that BCG protects against Parkinson disease in mice, through the induction of a regulatory T-cell immune response, while Lee et al.⁵⁴ reported that BCG vaccination suppresses autoimmune encephalomyelitis by independently inducing a Th17 and IFN- γ immune response in mouse models. BCG has also been linked to the treatment of multiple sclerosis, with a study recording a significant reduction in magnetic resonance imaging activity in patients with relapsing-remitting multiple sclerosis following BCG treatment.⁵⁵ In a clinical trial on

the therapeutic effect of BCG on the demyelination event characteristic of multiple sclerosis, Ristori et al.⁵⁶ reported BCG vaccination decreased the conversion rate of clinically isolated syndrome into multiple sclerosis. They found that the mean number of gadolinium-enhancing lesions were lower in the BCG group at 6 months, 12 months, and 18 months, compared with the placebo group. More recently, Gofrit et al.⁵⁷ reported findings from a prospective cohort study which showed that BCG administration was associated with a four-fold reduction in the incidence of Alzheimer diseases in bladder cancer patients receiving Intravesical BCG treatment. It is important to note, however, that BCG is currently not recognized as a treatment procedure for either encephalomyelitis, Alzheimer disease, clinically isolated syndrome or multiple sclerosis.

The BCG vaccine has been linked to the treatment of diabetes. In a proof-of-concept randomized placebo controlled clinical trial by Faustman et al.⁵⁸ on the effect of BCG on the treatment of long-term type I diabetes, they reported a significant rise in insulin production (determined by measuring the serum C-peptide levels) in the BCG vaccinated test group, an effect which was strongly associated with the death of insulin-autoreactive T cells and rise in the levels regulatory T-cells.⁵⁹ In addition, BCG causes an increase in glucose consumption in immune cells due to increased glycolysis and reduced oxidative phosphorylation,^{60,61} thus helping to achieve glycemic control in type I diabetes patients. Kuhlreiber et al.⁶² recently reported for the first time, the effect of BCG vaccination in up-regulating the expression of Myc, an important transcription factor utilized by four metabolic pathways: glutaminolysis, HIF-1 α /mTOR, glycolysis, and polyamine synthesis, all of which accelerate the utilization of glucose. Through this mechanism, BCG vaccination is able to trigger the correction of elevated blood glucose in type I diabetes patients.⁶² In a recently published paper, Klein⁶³ described the findings of a retrospective analysis of two studies investigating the effect of BCG vaccination on the development of type I diabetes mellitus, and revealed that the administration of a booster dose of the BCG vaccine reinforcing postnatal BCG vaccination had a protective effect against type I diabetes, compared with post-natal BCG administration without booster dose administration.⁶³ In addition, the study noted that post-natal BCG administration without a booster was in turn better at protecting against type I diabetes than no BCG vaccination at all.

A number of epidemiological studies have presented evidence to suggest that BCG vaccination is associated with reduced transmission, as well as morbidity and mortality rates of COVID-19.^{64–66} This is because countries with long-standing BCG vaccination policies such as Japan, India, and other countries in Africa and South East Asia, reported much lower incidence and mortality figures in the early days of the pandemic, compared with nations without universal BCG vaccination policies.⁶⁷ This correlation was met with a considerable amount of scepticism from the broader scientific community, with Kirov⁶⁸ suggesting that many of these studies did not accommodate for confounding factors such as race, income levels, population age and time from community spread, establishing that population age, was indeed a more significant factor influencing the COVID-19 epidemiological profile than BCG vaccination policy.⁶⁸ More recently, Aksu et al.⁶⁹ published the results of their retrospective study, which revealed that BCG vaccination was in fact not associated with severity of disease in COVID-19 patients in Turkey. Finally, as the pandemic progressed, many of the nations whose epidemiological profile initially supported this correlation, have also begun to witness a sharp rise in their incidence and mortality

figures, suggesting at the very least that protective effects BCG vaccination on COVID-19, if any at all, is not long-lasting. While the mechanism behind the proposed effect of BCG on COVID-19 is attributed to its non-specific/heterologous effects² and the concept of “trained immunity,”⁷⁰ there is a need for randomized controlled trials to provide some concrete evidence of any such effects.

Complications of BCG administration

In the years immediately following the introduction of the BCG vaccine, between 1929 and 1933, a tragic accident called “the Lübeck disaster” happened, in which 251 new-borns were vaccinated with BCG contaminated with *Mycobacterium tuberculosis*, after which 173 of them developed TB and 72 died.⁷¹ The live, attenuated nature of the BCG vaccine makes it particularly risky as it relies on asymptomatic bacteremia, which triggers an immune response eventually leading to immune resistance. While this bacteremia is largely expected to be asymptomatic, in particular groups of people, especially the immunocompromised, this bacteremia could lead to symptomatic disease of devastating proportions. Such symptomatic disease could either be localized (known as BCGitis) or disseminated (known as BCGosis) symptoms.^{72,73} The prevalence of BCGitis is estimated to be around 1 in 2500, while that of BCGosis is estimated to be around 1 in 100 000. Some studies have shown that BCG may increase susceptibility to HIV through induction of trained immunity and the activation of CD4⁺ T-cells.⁷⁴ These activated CD4⁺ cells would then act as loci for accelerated HIV viral replication.⁷⁵

Another well characterized complication of BCG vaccination in HIV patients is the immune reconstitution inflammatory syndrome, which follows antiretroviral therapy.⁷⁶ In a multi-center cross-sectional study by Marciano et al.,⁷⁷ where they assessed 349 BCG-vaccinated patients with severe combined immunodeficiency, they found that 51% of patients presented with BCG vaccination associated complications, resulting from impaired cellular immunity, phagocytic function, and IFN- γ mediated immunity, while 34% had disseminated disease.⁷⁷ The most common complications recorded was extra-regional lymphadenopathy in the skin and lungs, and they noted that the age at BCG vaccination was a strong predictor of the resulting BCG complications, as patients who were vaccinated within the first month of life bore the highest risk burden.⁷⁷

Localized or disseminated BCG related complications have also been reported in patients with chronic granulomatous disease^{78,79} and studies investigating the cause of idiopathic disseminated BCG complications have revealed that certain inborn errors of immunity against mycobacterial diseases could be responsible for such presentation.⁸⁰ Patients with this condition have intact immunity against other microbes, except for mycobacterial pathogens including the BCG vaccine, and mutations in 14 genes have been described to be the cause of this rare phenotype known as Mendelian susceptibility to mycobacterial disease.⁸¹ BCG complications have also been reported while using the vaccine as a therapeutic agent, such as in the treatment of superficial bladder cancer, in which 5% of patients treated report complications. These complications may arise as early as a few months after treatment, to as late as several years after treatment, and they could involve autoimmune conditions like reactive arthritis,⁸² which would be asymmetric and localized to large joints. Another autoimmune complication of BCG treatment of superficial bladder cancer is Reiter syndrome (triad of conjunctivitis, urethritis, and arthritis),⁸³ however, this seems

to only occur 60% of the time in certain susceptible patients with HLA-B27. The autoimmune complications of BCG instillation therapy for superficial bladder cancer are a result of molecular mimicry, leading to cross-reactivity between the mycobacterial heat shock protein 65 (hsp65) and the mammalian cartilage proteoglycan link protein.⁸⁴ Intravesical BCG instillation treatment has also been associated with Guillain Barre syndrome⁸⁵ and Henoch-Schoenlein purpura,^{86–88} both of which are also autoimmune conditions.

Other autoimmune complications have also been reported in the central nervous system, including central nervous system vasculitis,⁸⁹ mycotic aneurysms, and meningitis; in the cardiovascular system, such as aortitis⁹⁰; in the eyes, including endophthalmitis and uveitis⁹¹; and in the skin, such as vitiligo.⁹²

Recommendations and conclusion

The current WHO recommended vaccination scheme dictates that all new-borns receive a dose of the BCG vaccine at birth, however, for immunodeficient infants, such as those with HIV or severe combined immunodeficiency, who are usually asymptomatic at birth and often times cannot be diagnosed until several months after birth, this puts them at risk of developing disseminated BCG complications. In order to prevent this, it may be beneficial to either institute robust immunodeficiency screening programs to prevent administration of BCG under unsafe conditions or to delay BCG vaccination for 2–6 months after delivery, when the immune status of infants can be confirmed.³ The latter would be particularly applicable to developing countries and low-resource healthcare settings, which usually bear the highest burden of HIV and TB, and where the facilities for immunodeficiency screening may be insufficient, unavailable, or unaffordable. Looking holistically at the entire body of evidence presented, it would seem that the BCG vaccine may indeed be grossly underutilized as a pharmacological agent, with immense potential in clinical applicability that we are just beginning to unearth. As we approach the second century post-discovery of BCG, it is important that larger, methodological pre-clinical as well as clinical studies be carried out to establish a strong scientific basis for many of BCG's potential applications, especially in the treatment of neurodegenerative disorders and diabetes - conditions which we expect to become more of an epidemiological burden due to rapid globalization and the accompanying lifestyle changes. Consequently, should BCG become a recognized modality of treatment for more disease conditions with significant epidemiological profile, it is important that production and distribution of the vaccine be increased commensurate with the increased demand for the vaccine, so as to maintain the supply chain and avoid vaccine shortages, which could be devastating to nations in Africa and South-East Asia, where tuberculosis remains endemic, and which rely heavily on vaccine supplies to suppress the disease. While we focus on expanding the frontiers and exploring uncharted territories, it is important we do not lose ground to our familiar infectious foes.

In conclusion, we have explored the genealogy of the BCG vaccine, discussing the molecular variation responsible for the different efficacies recorded with different sub-strains, discussed the several expanded applications of the BCG vaccine beyond its use as a protective agent against TB, which include its use in the prevention of atherosclerosis, hyperoxic lung injury, Alzheimer disease, lung cancer, leukemia, and malignant melanoma; in the diagnosis of Kawasaki disease and the treatment of superficial bladder cancer, inoperable melanoma, allergic asthma, multiple

sclerosis and type I diabetes. We highlighted the autoimmune complications associated with BCG vaccination in immunodeficient patients as well as during its use as a treatment option for non-muscle invading bladder cancer, and we presented recommendations to prevent these complications in low-resource settings. While some of these BCG applications have been confirmed by several studies, others have produced conflicting results by different studies. As we approach the second century since the discovery of BCG, more studies would be required to unearth new effects of the vaccine and establish already familiar ones.

References

- Luca S, Mihaescu T. History of BCG vaccine. *Maedica* 2013;8(1):53–58.
- Moorlag SJCFM, Arts RJW, van Crevel R, Netea MG. Non-specific effects of BCG vaccine on viral infections. *Clin Microbiol Infect* 2019;25(12):1473–1478.
- Yamazaki-nakashimada MA, Unzueta A, Berenise L, González-saldaña N, Sorensen RU. BCG: a vaccine with multiple faces. *Hum Vaccin Immunother* 2020;16(8):1841–1850.
- Boman G. The ongoing story of the Bacille Calmette-Guérin (BCG) vaccination. *Acta Paediatr* 2016;105(12):1417–1420.
- Brosch R, Gordon SV, Garnier T, et al. Genome plasticity of BCG and impact on vaccine efficacy. *Proc Natl Acad Sci U S A* 2007;104(13):5596–5601.
- Behr MA, Schroeder BG, Brinkman JN, Slayden RA, Barry CE. A point mutation in the *mma3* gene is responsible for impaired methoxymycolic acid production in *Mycobacterium bovis* BCG strains obtained after 1927. *J Bacteriol* 2000;182(12):3394–3399.
- Korf J, Stoltz A, Verschoor J, De Baetselier P, Grooten J. The *Mycobacterium tuberculosis* cell wall component mycolic acid elicits pathogen-associated host innate immune responses. *Eur J Immunol* 2005;35(3):890–900.
- Vander Beken S, Al Dulayymi JR, Naessens T, et al. Molecular structure of the *Mycobacterium tuberculosis* virulence factor, mycolic acid, determines the elicited inflammatory pattern. *Eur J Immunol* 2011;41(2):450–460.
- Hayashi D, Takii T, Fujiwara N, et al. Comparable studies of immunostimulating activities in vitro among *Mycobacterium bovis* bacillus Calmette-Guérin (BCG) substrains. *FEMS Immunol Med Microbiol* 2009;56(2):116–128.
- Goodridge HS, Ahmed SS, Curtis N, et al. Harnessing the beneficial heterologous effects of vaccination. *Nat Rev Immunol* 2016;16(6):392–400.
- Kristensen I, Aaby P, Jensen H. Routine vaccinations and child survival: follow up study in Guinea-Bissau, West Africa. *Br Med J* 2000;321(7274):1435–1439.
- Nankabirwa V, Tumwine JK, Mugaba PM, et al. Child survival and BCG vaccination: a community based prospective cohort study in Uganda. *BMC Public Health* 2015;15(1):175.
- Biering-Sorensen S, Aaby P, Napirna BM, et al. Small randomized trial among low-birth-weight children receiving bacillus Calmette-Guérin vaccination at first health center contact. *Pediatr Infect Dis J* 2012;31(3):306–308.
- Zimmermann P, Finn A, Curtis N. Does BCG vaccination protect against nontuberculous mycobacterial infection? A systematic review and meta-analysis. *J Infect Dis* 2018;218(5):679–687.
- Post CL, Victora CG, Valente JG, Leal MC, Niobey FM, Sabroza PC. Prognostic factors for hospital case-fatality due to diarrhea or pneumonia: a case-control study. *Rev Saude Publica* 1992;26(6):369–378.
- Mawa PA, Webb EL, Filali-Mouhim A, et al. Maternal BCG scar is associated with increased infant proinflammatory immune responses. *Vaccine* 2017;35(2):273–282.
- Ovchinnikova OA, Berge N, Kang C, et al. *Mycobacterium bovis* BCG killed by extended freeze-drying induces an immunoregulatory profile and protects against atherosclerosis. *J Intern Med* 2014;275(1):49–58.
- van Dam AD, Bekkering S, Crasborn M, et al. BCG lowers plasma cholesterol levels and delays atherosclerotic lesion progression in mice. *Atherosclerosis* 2016;251:6–14.
- Lamb DJ, Eales LJ, Ferns GA. Immunization with bacillus Calmette-Guérin vaccine increases aortic atherosclerosis in the cholesterol-fed rabbit. *Atherosclerosis* 1999;143(1):105–113.
- Kumral A, Işcan B, Tuzun F, et al. Bacillus Calmette-Guérin vaccination: a novel therapeutic approach to preventing hyperoxic lung injury. *J Matern Neonatal Med* 2015;28(16):1950–1956.

- [21] Weiss DW, Bonhag RS, Deome KB. Protective activity of fractions of tubercle bacilli against isologous tumours in mice. *Nature* 1961;190(4779):889–891.
- [22] Davignon L, Robillard P, Lemonde P, Frappier ABCG. Vaccination and leukemia mortality. *Lancet* 1970;296(7674):638.
- [23] Morra ME, Kien ND, Elmaraezy A, et al. Early vaccination protects against childhood leukemia: a systematic review and meta-analysis. *Sci Rep* 2017;7(1):1–9.
- [24] Usher NT, Chang S, Howard RS, et al. Association of BCG vaccination in childhood with subsequent cancer diagnoses: a 60-year follow-up of a clinical trial. *JAMA Netw Open* 2019;2(9):e1912014.
- [25] Kölmel KF, Grange JM, Krone B, et al. Prior immunisation of patients with malignant melanoma with vaccinia or BCG is associated with better survival. An European Organization for Research and Treatment of Cancer cohort study on 542 patients. *Eur J Cancer* 2005;41(1):118–125.
- [26] Shehadeh N, Etzioni A, Cahana A. Repeated BCG vaccination is more effective than a single dose in preventing diabetes in non-obese diabetic (NOD) mice. *Isr J Med Sci* 1997;33(11):711–715.
- [27] Parent MÉ, Siemiatycki J, Menzies R, Fritschi L, Colle E. Bacille Calmette-Guérin vaccination and incidence of IDDM in Montreal, Canada. *Diabetes Care* 1997;20(5):767–772.
- [28] Huppmann M, Baumgarten A, Ziegler A-G, Bonifacio E. Neonatal Bacille Calmette-Guérin vaccination and type 1 diabetes. *Diabetes Care* 2005;28(5):1204–1206.
- [29] Gofrit ON, Bercovier H, Klein BY, Cohen IR, Ben-Hur T, Greenblatt CL. Can immunization with Bacillus Calmette-Guérin (BCG) protect against Alzheimer's disease? *Med Hypotheses* 2019;123:95–97.
- [30] Zuo Z, Qi F, Yang J, et al. Immunization with Bacillus Calmette-Guérin (BCG) alleviates neuroinflammation and cognitive deficits in APP/PS1 mice via the recruitment of inflammation-resolving monocytes to the brain. *Neurobiol Dis* 2017;101:27–39.
- [31] Kawasaki T. Two cases with acute febrile mucocutaneous lymph node syndrome possibly induced by vaccinia or measles vaccination. *Jpn J Pediatr* 1970;23:657–662.
- [32] Kakisaka Y, Ohara T, Katayama S, et al. Human herpes virus type 6 can cause skin lesions at the BCG inoculation site similar to Kawasaki disease. *Tohoku J Exp Med* 2012;228(4):351–353.
- [33] Muthuvelu S, Lim KSC, Huang LY, Chin ST, Mohan A. Measles infection causing Bacillus Calmette-Guérin reactivation: a case report. *BMC Pediatr* 2019;19(1):251.
- [34] Uehara R, Igarashi H, Yashiro M, Nakamura Y, Yanagawa H. Kawasaki disease patients with redness or crust formation at the bacille calmette-guérin inoculation site. *Pediatr Infect Dis J* 2010;29(5):430–433.
- [35] Garrido-García LM, Castillo-Moguel A, Vázquez-Rivera M, Cravioto P, Fernando G. Reaction of the BCG scar in the acute phase of Kawasaki disease in Mexican children. *Pediatr Infect Dis J* 2017;36(10):e237–241.
- [36] Araki T, Kodera A, Kitada K, et al. Analysis of factors associated with development of Bacille Calmette-Guérin inoculation site change in patients with Kawasaki disease. *J Int Med Res* 2018;46(4):1640–1648.
- [37] Tseng HC, Ho JC, Guo MMH, et al. Bull's eye dermatoscopy pattern at bacillus Calmette-Guérin inoculation site correlates with systemic involvements in patients with Kawasaki disease. *J Dermatol* 2016;43(9):1044–1050.
- [38] Sato N, Sagawa K, Sasaguri Y, Inoue O, Kato H. Immunopathology and cytokine detection in the skin lesions of patients with kawasaki disease. *J Pediatr* 1993;122(2):198–203.
- [39] Sireci G, Dieli F, Salerno A. T cells recognize an immunodominant epitope of heat shock protein 65 in Kawasaki disease. *Mol Med* 2000;6(7):581–590.
- [40] Bertotto A, Spinozzi F, Vagliasindi C, Radicioni M, De Rosa O, Vaccaro R. Tuberculin skin test reactivity in Kawasaki disease. *Pediatr Res* 1997;41(4 I):560–562.
- [41] Kollmann TR, Klein EJ, Stefanelli CB, Marcuse EK. Purified protein derivative anergy in Kawasaki disease. *Pediatr Infect Dis J* 2001;20(1):81–82.
- [42] Morales A, Eiding D, Bruce AW. Intracavitary Bacillus Calmette Guerin in the treatment of superficial bladder tumors. *J Urol* 1976;116(2):180–182.
- [43] Meyer JP, Persad R, Gillatt DA. Use of bacille Calmette-Guérin in superficial bladder cancer. *Postgr Med J* 2002;78(922):449–454.
- [44] Alexandroff AB, Jackson AM, O'Donnell MA, James K. BCG immunotherapy of bladder cancer: 20 years on. *Lancet* 1999;353(9165):1689–1694.
- [45] Redelman-Sidi G, Glickman MS, Bochner BH. The mechanism of action of BCG therapy for bladder cancer—a current perspective. *Nat Rev Urol* 2014;11(3):153–162.
- [46] De Boer EC, Somogyi L, De Ruiter GJW, De Reijke TM, Kurth KH, Schamhart DHJ. Role of interleukin-8 in onset of the immune response in intravesical BCG therapy for superficial bladder cancer. *Urol Res* 1997;25(1):31–34.
- [47] de Reijke TM, Vos PCN, de Boer EC, et al. Cytokine production by the human bladder carcinoma cell line T24 in the presence of bacillus Calmette-Guérin (BCG). *Urol Res* 1993;21(5):349–352.
- [48] Ibarra C, Karlsson M, Codeluppi S, et al. BCG-induced cytokine release in bladder cancer cells is regulated by CA²⁺ signaling. *Mol Oncol* 2019;13(2):202–211.
- [49] Yang J, Jones MS, Irene Ramos R, et al. Insights into local tumor microenvironment immune factors associated with regression of cutaneous melanoma metastases by *Mycobacterium bovis* Bacille Calmette-Guérin. *Front Oncol* 2017;7:61.
- [50] Shirakawa T, Enomoto T, Shimazu SI, Hopkin JM. The inverse association between tuberculin responses and atopic disorder. *Science* 1997;275(5296):77–79.
- [51] Choi IS, Koh YI. Therapeutic effects of BCG vaccination in adult asthmatic patients: a randomized, controlled trial. *Ann Allergy Asthma Immunol* 2002;88(6):584–591.
- [52] Yang J, Qi F, Yao Z. Neonatal Bacillus Calmette-Guérin vaccination alleviates lipopolysaccharide-induced neurobehavioral impairments and neuroinflammation in adult mice. *Mol Med Rep* 2016;14(2):1574–1586.
- [53] Lačan G, Dang H, Middleton B, et al. Bacillus Calmette-Guérin vaccine-mediated neuroprotection is associated with regulatory T-cell induction in the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine mouse model of Parkinson's disease. *J Neurosci Res* 2013;91(10):1292–1302.
- [54] Lee J, Reinke EK, Zozulya AL, Sandor M, Fabry Z. *Mycobacterium bovis* Bacille Calmette-Guérin infection in the CNS suppresses experimental autoimmune encephalomyelitis and Th17 responses in an IFN- γ -independent manner. *J Immunol* 2008;181(9):6201–6212.
- [55] Ristori G, Buzzi MG, Sabatini U, et al. Use of Bacille Calmette-Guérin (BCG) in multiple sclerosis. *Neurology* 1999;53(7):1588–1589.
- [56] Paolillo A, Buzzi MG, Giugni E, et al. The effect of Bacille Calmette-Guérin on the evolution of new enhancing lesions to hypointense T1 lesions in relapsing remitting MS. *J Neurol* 2003;250(2):247–248.
- [57] Gofrit ON, Klein BY, Cohen IR, Ben-Hur T, Greenblatt CL, Bercovier H. Bacillus Calmette-Guérin (BCG) therapy lowers the incidence of Alzheimer's disease in bladder cancer patients. *PLoS One* 2019;14(11):e0224433.
- [58] Faustman DL, Wang L, Okubo Y, et al. Proof-of-concept, randomized, controlled clinical trial of Bacillus-Calmette-Guérin for treatment of long-term type 1 diabetes. *PLoS One* 2012;7(8):e41756.
- [59] Fenner A. BCG enriches Treg cells. *Nat Rev Urol* 2018;15(10):591.
- [60] Stienstra R, Netea MG. Firing up glycolysis: BCG vaccination effects on type 1 diabetes mellitus. *Trends Endocrinol Metab* 2018;29(12):813–814.
- [61] Kühtreiber WM, Tran L, Kim T, et al. Long-term reduction in hyperglycemia in advanced type 1 diabetes: the value of induced aerobic glycolysis with BCG vaccinations. *NPJ Vaccines* 2018;3(1):23.
- [62] 2020;Kühtreiber WM, Takahashi H, Keefe RC, et al. BCG vaccinations upregulate Myc, a central switch for improved glucose metabolism in diabetes. *iScience*. 23(5):101085.
- [63] Klein BY. Newborn BCG vaccination complemented by boosting correlates better with reduced juvenile diabetes in females, than vaccination alone. *Vaccine* 2020;38(41):6427–6234.
- [64] Miller A, Reandelar MJ, Fasciglione K, Roumenova V, Li Y, Otazu GH. Correlation between universal BCG vaccination policy and reduced morbidity and mortality for COVID-19: an epidemiological study. Preprint. Posted online September 14, 2020. *medRxiv*. doi:10.1101/2020.03.24.20042937.
- [65] Hegarty PK, Kamat AM, Zafirakis H, DiNardo A. BCG vaccination may be protective against COVID-19. Preprint. Posted online March 27, 2020. *ResearchGate*. doi:10.13140/RG.2.2.35948.10880.
- [66] Dolgikh S. Further evidence of a possible correlation between the severity of COVID-19 and BCG immunization. *J Infect Dis Epidemiol* 2020;6(120). doi:10.23937/2474-3658/1510120.
- [67] Dayal D, Gupta S. Connecting BCG vaccination and COVID-19: Additional data. Preprint. Posted online April 19, 2020. *medRxiv*. doi:10.1101/2020.04.07.20053272.
- [68] Kirov S. Association between BCG policy is significantly confounded by age and is unlikely to alter infection or mortality rates. Preprint. Posted online April 11, 2020. *medRxiv*. doi:10.1101/2020.04.06.20055616.
- [69] Aksu K, Naziroğlu T, Özkan P. Factors determining COVID-19 pneumonia severity in a country with routine BCG vaccination. *Clin Exp Immunol* 2020;doi:10.1111/cei.13507.

- [70] Netea MG, Joosten LAB, Latz E, et al. Trained immunity: a program of innate immune memory in health and disease. *Science* 2016;352(6284):427.
- [71] Fox GJ, Orlova M, Schurr E. Tuberculosis in newborns: the lessons of the “Lübeck Disaster” (1929-1933). *PLoS Pathog* 2016;12(1):e1005271.
- [72] Fordham von Reyn C. Routine childhood Bacille Calmette Guerin immunization and HIV infection. *Clin Infect Dis* 2006;42:559–561.
- [73] Hesseling AC, Rabie H, Marais BJ, et al. Bacille Calmette-Guérin vaccine-induced disease in HIV-infected and HIV-uninfected children. *Clin Infect Dis* 2006;42(4):548–558.
- [74] Derrick SC. Trained immunity and susceptibility to HIV. *Clin Vaccine Immunol* 2017;24(1):e00509–e00516.
- [75] Thayil SM, Ho YC, Bollinger RC, et al. *Mycobacterium tuberculosis* complex enhances susceptibility of CD4 T cells to HIV through a TLR2-mediated pathway. *PLoS One* 2012;7(7):e41093.
- [76] Puthanakit T, Oberdorfer P, Punjaisee S, Wannarit P, Sirisanthana T, Sirisanthana V. Immune reconstitution syndrome due to bacillus Calmette-Guérin after initiation of antiretroviral therapy in children with HIV infection. *Clin Infect Dis* 2005;41(7):1049–1052.
- [77] Marciano BE, Huang CY, Joshi G, et al. BCG vaccination in patients with severe combined immunodeficiency: complications, risks, and vaccination policies. *J Allergy Clin Immunol* 2014;133(4):1134–1141.
- [78] Bustamante J, Aksu G, Vogt G, et al. BCG-osis and tuberculosis in a child with chronic granulomatous disease. *J Allergy Clin Immunol* 2007;120(1):32–38.
- [79] Lin CJ, Wang SC, Ku CL, Kao JK, Chen M, Liu CS. Successful unrelated cord blood stem cell transplantation in an X-linked chronic granulomatous disease patient with disseminated BCG-induced infection. *Pediatr Neonatol* 2015;56(5):346–350.
- [80] Casanova J-L, Blanche S, Emile J-F, et al. Idiopathic disseminated Bacillus Calmette-Guérin infection: a French national retrospective study. *Pediatrics* 1996;98(4):774–778.
- [81] Rosain J, Kong XF, Martinez-Barricarte R, et al. Mendelian susceptibility to mycobacterial disease: 2014-2018 update. *Immunol Cell Biol* 2019;97(4):360–367.
- [82] Tinazzi E, Ficarra V, Simeoni S, Artibani W, Lunardi C. Reactive arthritis following BCG immunotherapy for urinary bladder carcinoma: a systematic review. *Rheumatol Int* 2006;26(6):481–488.
- [83] Ng KL, Chua CB. Reiter’s syndrome postintravesical Bacillus Calmette-Guérin instillations. *Asian J Surg* 2017;40(2):163–165.
- [84] van Eden W, Holoshitz J, Nevo Z, Frenkel A, Klajman A, Cohen IR. Arthritis induced by a T-lymphocyte clone that responds to *Mycobacterium tuberculosis* and to cartilage proteoglycans. *Proc Natl Acad Sci U S A* 1985;82(15):5117–5120.
- [85] Webb K, Venkatesan P. Guillain Barré syndrome associated with bladder instillation of Bacille Calmette Guérin (BCG). *JMM Case Rep* 2018;5(8):e005164.
- [86] Hirayama T, Matsumoto K, Tsuboi T, et al. Anaphylactoid purpura after intravesical therapy using bacillus Calmette-Guérin for superficial bladder cancer. Article in Japanese. *Acta Urol Jpn* 2008;54(2):127–129.
- [87] Tsukada H, Miyakawa H. Henoch Schönlein Purpura nephritis associated with intravesical bacillus calmette-guerin (BCG) therapy. *Intern Med* 2017;56(5):541–544.
- [88] Nan DN, Fernández-Ayala M, García-Ibarbia C, Gutiérrez-Santiago M, Hernández JL. Henoch-Schönlein purpura after intravesical administration of bacillus Calmette-Guérin. *Scand J Infect Dis* 2005;37(8):613–615.
- [89] Parent ME, Richer M, Liang P. The first case of bacillus Calmette-Guérin-induced small-vessel central nervous system vasculitis. *Clin Rheumatol* 2018;37(8):2297–2302.
- [90] Shoenfeld Y, Aron-Maor A, Tanai A, Ehrenfeld M. BCG and autoimmunity: another two-edged sword. *J Autoimmun* 2001;16(3):235–240.
- [91] Spratt A, Key T, Vivian AJ. Chronic anterior uveitis following bacille Calmette-Guérin vaccination: molecular mimicry in action? *J Pediatr Ophthalmol Strabismus* 2008;45(4):252–253.
- [92] Donaldson RC, Canaan SA Jr, McLean RB, Ackerman LV. Uveitis and vitiligo associated with BCG treatment for malignant melanoma. *Surgery* 1976;76(5):771–778.

How to cite this article: Adesanya OA, Uche-Orji CI, Adedeji YA, Joshua JI, Adesola AA, Chukwudike CJ. Expanded scope of Bacillus Calmette-Guérin (BCG) vaccine applicability in disease prophylaxis, diagnostics, and immunotherapeutics. *Infect Microb Dis* 2020;2(4):144–150. doi: 10.1097/IM9.0000000000000040