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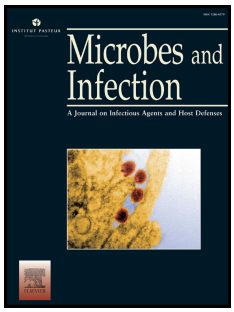

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Highlights of the 3rd international BCG symposium: 100th anniversary of the first administration of BCG

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Abstract

2021 was the year of the 100th anniversary of the first administration of the Bacillus Calmette-Guérin (BCG) to a human being. It was the start of a long journey of the world's most widely used vaccine and the oldest vaccine still in use. More than 4 billion children have been vaccinated with BCG for protection against tuberculosis. However, over the years it became apparent that BCG also has beneficial non-specific effects. As such, it provides protection against various heterologous infectious and non-infectious diseases and is used to treat non-muscle-invasive bladder cancer. As BCG was developed at the Institut Pasteur de Lille by Albert Calmette and Camille Guérin, the Institute has celebrated this important anniversary with an international scientific symposium on all aspects of BCG, held from November 17 to 19, 2021 at the Institut Pasteur de Lille. It covered BCG against tuberculosis and described novel vaccine approaches, the effect of BCG against heterologous infections, including BCG and COVID-19, the effect of BCG against cancer, and BCG against auto-immune and inflammatory diseases. To discuss these areas, the symposium gathered close to 200 participants from all five continents, 2/3 on-line. This article presents the highlights of this 3rd International Symposium on BCG.

Keywords

Tuberculosis; cancer; allergy; trained innate immunity; diabetes; COVID-19

1. Introduction

On July 3rd 1921 the Bacillus Calmette-Guérin (BCG) was administered for the first time as a preventive anti-tuberculosis vaccine to a human being. A baby born to a mother who had died of tuberculosis a few hours after delivery and was subsequently under the care of his grandmother, also suffering from tuberculosis, was given 2 mg BCG orally on days 3, 5 and 7 after birth. The boy stayed perfectly healthy and did not show any sign of tuberculosis during the following years, despite constantly being exposed to the tubercle bacillus *Mycobacterium tuberculosis* [1]. This was the beginning of a more than a century-long history of the most widely used vaccine. Today, 154 countries have a BCG vaccination policy for the entire population, and 53 of them have reached at least 95% vaccination coverage [2]. In addition to its protective effect against tuberculosis, particularly against severe disease in children [3], BCG vaccination has also been associated with a decrease in overall childhood mortality, already observed in the 1920s [4], and has successfully been used in the treatment of non muscle-invasive bladder cancer for several decades now [5]. These observations indicate that the field of BCG applications extends far beyond that of a vaccine to prevent tuberculosis, and that the non-specific effects associated with BCG warrant further investigation and exploration for a variety of different diseases. In addition, since despite wide BCG vaccination coverage tuberculosis remains one of the first causes of death due to a single infectious agent, second only to COVID-19 in 2020 and 2021 [2], improvements of anti-tuberculosis vaccination strategies are urgently needed.

BCG was generated at the Institut Pasteur de Lille by its first Director General Albert Calmette, together with his co-worker Camille Guérin, through 231 serial passages of the *Mycobacterium bovis* “Lait de Nocard” strain on a potato-based medium containing

glycerinated ox-bile. It was therefore appropriate that the Institut Pasteur de Lille decided to celebrate the 100th anniversary of the first administration of the BCG vaccine by organizing an international symposium, which was held from November 17 to 19, 2021 at the institute. This symposium gathered close to 200 participants from all five continents. Two thirds of them participated on-line. Various applications of BCG in the field of tuberculosis, non-tuberculosis infections, including SARS-CoV2 infections, cancer, diabetes, auto-immune and inflammatory diseases were discussed, as well as the BCG adjuvant effects and strategies to improve BCG efficacy against tuberculosis and other diseases. This paper provides a brief summary of the presented data and discussions.

After an introductory lecture on the history of BCG, Ann Ginsberg (Bill and Melinda Gates Foundation) and Soumya Swaminathan (World Health Organization) introduced the audience to the situation of tuberculosis in the world today and the challenges in BCG world-wide supply, respectively. These presentations reminded us of the importance of tuberculosis in the world, with global estimates that decline only very slowly, especially in resource-poor countries, and described the impact of COVID-19 on the drop of tuberculosis notifications, contrasting with an increase in the numbers of deaths due to tuberculosis, for the first time in more than a decade. They presented the challenges with the current BCG vaccination, including manufacturing issues, global supply problems, despite a total number of 22 suppliers, variable efficacies of BCG, waning of BCG-mediated immunity and the absence of immunological correlates of protection. They also discussed the potential of BCG re-vaccination showing first phase 2b trial results on the protective effect of BCG re-vaccination against *M. tuberculosis* infection in adults. At the same time, they stressed the need for improved tuberculosis vaccines and provided examples of vaccine candidates currently in clinical development. Finally, the WHO roadmap for tuberculosis vaccine development was

presented, including the WHO preferred product profile for new tuberculosis vaccines and strategic guidance spanning the spectrum from pipeline diversification to accelerated clinical development and implementation. This opening session was then followed by 5 sessions, covering respectively novel vaccine approaches against tuberculosis, off-target effects of BCG, BCG and COVID-19, BCG and cancer, and BCG and inflammatory and auto-immune diseases.

2. Session 1: Novel vaccine approaches against tuberculosis

The first session started with the lecture of Olubukola Idoko (London School of Hygiene and Tropical Medicine) on the human immune responses to BCG and approaches to identify correlates of protection against tuberculosis. Numerous studies in mice and humans have documented the role of cellular immune responses in protection against tuberculosis, and BCG is known to induce Th1-type immune responses in children and adults. However, B cells and antibodies have also recently been suggested as important players in protection, and systems serology studies have identified features of antibodies that differ between active tuberculosis and latent *M. tuberculosis* infection. O. Idoko discussed potential mechanisms of antibody-mediated protection. In addition to adaptive immune responses, innate immune cells, such as myeloid cells and NK cells, trained by BCG, may also be involved in protection. These issues should be taken in to account for the development of next-generation vaccines. A large clinical study by the Expanded Program on Immunization Consortium [6] using systems biology to explore BCG immunity in newborns is currently underway.

The next contribution was from Mark Hatherill (South African Tuberculosis Vaccine Initiative) who lined out the BCG revaccination project in South Africa. After reviewing the evidence for and against protection against tuberculosis in various BCG revaccination trials, this project was initiated based on observations in a randomized controlled trial (RCT) that

BCG re-vaccination may reduce sustained QuantiFERON conversion as a measure of *M. tuberculosis* infection by 45% during the 30 first months after vaccination [7]. In the new study 1,800 QuantiFERON-negative children and young adolescents are enrolled, as neonatal BCG vaccination-induced immunity decreases after ten years and there is a rapid increase in tuberculosis rates after 15 years of age in South Africa. They will be followed up for 48 months to evaluate prevention of protection and potentially prevention of disease. Study subjects were fully enrolled in 2021. Therefore, results are expected by 2026.

The third presentation in this session was by Robert A. Seder (National Institutes of Health) who explored alternative BCG administration routes, especially intravenous administration, using a non-human primate model [8]. A standard intradermal dose was compared to a high intradermal dose, a high intravenous dose, a high aerosol dose and a mixed high aerosol and low intradermal dose. Except for the intravenous route, all routes provided comparable protection against *M. tuberculosis* challenge. Most of the intravenously vaccinated animals had no measurable *M. tuberculosis* infection upon challenge. The striking immunological differences between these animals and the others were a strong antigen-specific $CD8^+$ T cell response in the blood, but most of all the strong and sustained induction of resident memory T cells in the lungs after intravenous vaccination. Depletion of the $CD4^+$ T cells with antibodies resulted in complete loss of protection. In order to use this model to identify immunological correlates of protection, the animals were intravenously vaccinated with various doses of BCG, leading to a dose - T cell response correlation and a dose-response for protection. However, even at the low BCG doses and with low local T cell responses, some animals were protected, which may be useful to identify correlates of protection by multi-dimensional systems approaches.

Different findings were reported by Frank Verreck (Biomedical Primate Research Centre), who showed that a single pulmonary administration of BCG conferred protection signals upon a high-dose challenge against which standard intradermal BCG vaccination did not protect. He refined the model by challenging with repeated low doses and found that endobronchial vaccination provided better protection against infection by *M. tuberculosis* than vaccination by the intradermal route. This also resulted in significantly lessened disease scores. While in the blood the T cell responses were similar between the two routes of immunization, the endobronchial route induced significantly higher T cell responses in the lungs than the intradermal route. Moreover, endobronchial vaccination after intradermal priming induced a stronger initial CD4+ T cell response than the endobronchial administration alone, but the response converged several weeks later and did not further improve protection.

An improved BCG vaccine candidate was described by Stefan Kaufmann (Max Planck Institut für Infectionsbiologie). In order to augment the $CD8^+$ T cell stimulation by BCG, his group developed VPM1002, a recombinant BCG derivative that produces listeriolysin and contains a deletion in the urease operon to decrease the pH within the phagosome containing the BCG [9], which increased the $CD8^+$ T cell responses and enhanced protection in mice, including in post-exposure mouse models; yet the vaccine was safer in SCID mice than the original BCG. It also induced stronger central memory responses, showed better antigen presentation, cross-priming, activation of the inflammasome and autophagy/xenophagy. Two phase 1 trials with this vaccine have been completed, in young adults, respectively in Germany and South Africa, as well as 2 phase 2 trials in South African newborns with or without HIV exposure, using BCG as a comparator. Several Phase 3 trials in neonates have now started in sub-Saharan Africa and India to evaluate VPM1002 for safety and efficacy in prevention of infection, disease and recurrence.

A different approach was taken by Carlos Martin (Universidad Zaragoza) who attenuated *M. tuberculosis* by the genetic deletion of the *phoP* and *fadD26* genes, which removes the lipids that interfere with *M. tuberculosis* immunogenicity, increases the secretion of Antigen 85 and CFP-10, but prevents ESAT-6 secretion [10]. This vaccine called MTBVAC protected mice against all *M. tuberculosis* lineages better than BCG and induced good trained innate immunity. However, when ESAT-6 was removed from MTBVAC, improved protection was lost [11]. The vaccine was also tested in guinea pigs and rhesus macaques, where it showed improved protection over BCG. Two phase 1 clinical trials have been completed, respectively in adults in Switzerland and in neonates in South Africa, and have shown acceptable safety and stronger T cell immunogenicity that BCG, up to one year after vaccination. Two phase 2 trials are currently being conducted in South Africa in both QuantiFERON^{+} and QuantiFERON^{-} adolescents and adults primed in their childhood with BCG, as well as in newborns, using BCG as the comparator. Efficacy trials are planned in three different Africa sites to start soon.

3. Session 2: Off-target effects of BCG

Victoria Nankabirwa (Makerere University School of Public Health) presented a study that compared early versus late BCG vaccination in HIV-1-exposed infants in Uganda to evaluate the effect of deferred BCG vaccination on the risk of severe illness in the first 14 weeks of life [12]. This study is currently running, and 4,500 infants will be enrolled. Mortality and severe illness with hospitalization, including frequency, duration and illness outcome, will be monitored during the first 14 weeks of life. Potential confounders, such as socioeconomic status, delivery history, breastfeeding, nutrition, other vaccinations, weight and length, are

also taken into account. Currently, 3,809 infants have been randomized, and blood samples have been collected from 1,200 infants for cytokine measurements.

Results on the non-specific effects of BCG on heterologous infections in Uganda were shown by Sarah Prentice (London School fog Hygiene and Tropical Medicine). While since several decades various animal studies have provided strong evidence for heterologous protection offered by BCG against different bacterial, viral, parasite and fungal infections, observational studies in humans provided less unambiguous results, and human challenges studies sometimes came to conflicting conclusions. Therefore, S. Prentice and colleagues conducted a RCT to measure the occurrence of non-tuberculous infections in infants with early BCG vaccination compared to delayed BCG vaccination [13], and observed a significant reduction in non-tuberculosis infections after at-birth vaccination. Especially severe illness frequencies were reduced in boys vaccinated at birth with BCG. However, these data contrast with previous RCTs, showing no or minimal effect of at-birth BCG vaccination. These differences may be due to differences in maternal priming, in other routine immunizations, exposure to non-tuberculosis mycobacteria or other confounding factors. Interestingly, in a BCG re-vaccination RCT designed to evaluate the effect of BCG revaccination on *M. tuberculosis* infection [7], a significant decrease in upper respiratory tract infections was observed in the BCG-revaccinated participants compared to the placebo group, which has also been seen in other studies.

Eva Kaufmann (McGill University) focused on the mechanism of trained innate immunity by BCG in mice. In contrast to subcutaneous BCG vaccination intravenous vaccination results in high amounts of bacilli in the bone marrow and enhanced innate immune training [14]. While BCG does not infect hematopoietic stem cells, it modifies their transcriptomic profile, which is then transmitted to the daughter cells. These trained

monocytes provide protection against tuberculosis in the absence of adaptive immunity. Innate immune protection requires Interferon signaling and is sustainable. On the other hand, *M. tuberculosis* infection impairs the generation of protective trained innate immunity. Intravenous vaccination with BCG also provided significant protection against influenza A virus in mice, but not against SARS-CoV2.

Nelly Amenyogbe (Telethon Kids Institute) talked about the effect of BCG vaccination on neonatal sepsis. She showed that subcutaneous BCG vaccination of neonatal mice protected them from sepsis after challenge with cecal slurry, with a significant reduction in inflammatory response and a faster bacterial clearance. Three days after vaccination the neonatal mice expanded the neutrophil pools in the spleen, and transfer of neutrophils from the spleen of BCG vaccinated mice passed on the protection to naïve mice. Neutrophil expansion and protection depended on G-CSF. The effect of BCG vaccination on neutrophils was also observed in human neonates in the Gambian EPIC cohort [6, 15].

The effect of BCG vaccination on immunogenicity of heterologous vaccines was covered by Peta Zimmermann (Universität Freiburg) [16], which could occur by epigenetic reprogramming of monocytes to increase antigen presentation and/or by increasing the expression of co-stimulatory molecules and cytokines. She reviewed studies in adults showing increased immunogenicity to inactivated influenza vaccine, but not to live influenza vaccine by BCG co-administration. However, a later study showed that this enhancement was only observed for H1N1, but not for H3N1, nor for the Influenza B virus component of the vaccine. Furthermore, BCG vaccination did not appear to have an adjuvant effect to a polysaccharide typhoid fever vaccine. However, BCG appeared to prevent the inhibition of innate immune responses by the typhoid vaccine. In infants, BCG did not appear to enhance immunogenicity of the hepatitis B vaccine or the pertussis vaccine. In contrast, in different studies at-birth BCG

vaccination enhanced immunogenicity to hepatitis vaccine, both at the antibody and the T cell levels. Other studies showed a slight enhancement of antibody responses to tetanus, diphtheria, measles, mumps and pneumococcal antigens, but lower responses to hepatitis B vaccine after BCG vaccination. It is difficult to compare these results because different vaccine schedules and timepoints of analysis were used the various studies. Other confounders, such age, BCG strain, maternal BCG status and sex may also play a role.

To end this session, Christine Stabell Benn (Bandim Health Project, University of Southern, Denmark) shared her findings on the effect of parental BCG priming on childhood mortality in Guinea-Bissau. She reminded us that vaccination with the measles vaccine in the presence of maternal antibodies amplifies its beneficial non-target effects. The positive effect of maternal antibodies to measles vaccine, but not to other antigens, on reduced childhood mortality through measles vaccination has been observed in several clinical studies. She also showed that at-birth BCG vaccination in Denmark reduced all-cause hospitalization in a RCT, but only for babies from mothers who had been BCG vaccinated. In a study carried out in Guinea-Bissau, children with recorded BCG vaccination born to mothers with a BCG scar had much lower all-cause mortality rates than those born to mothers without a scar. This effect was further increased when both parents had a BCG scar. The mechanism underlying these observations are not identified yet, but may be related to intergenerational inheritance of trained innate immunity [17].

4. Session 3: BCG and COVID-19

One of the most recent interrogations about the non-specific effects of BCG concerns COVID-19, and a special session was devoted to this issue, including three invited speakers and several additional short communications. Nigel Curtis (University of Melbourne) started this

session by describing the BRACE project designed to evaluate the impact of BCG vaccination on COVID-19 in health care workers [18]. In this study, the effect of BCG vaccination on severe disease, including deaths and hospitalization, as well as milder symptomatic and asymptomatic disease is examined. The BRACE trial is a multicenter study involving 34 trial sites in 5 countries (Australia, The Netherlands, Spain, the United Kingdom and Brazil) and will enroll 2,828 health-care workers. At the same time the study will also provide large-scale information on whether BCG has an impact on other respiratory diseases and allergies. The effect of BCG on immunogenicity of other vaccines, including the anti-COVID vaccines, will also be studied. Biologicals samples are taken at several time points over one year to study the underlying mechanisms in a system approach.

This presentation was followed by that of Henri Werkhoven (University Medical Center of Utrecht) who shared first results on a RCT of BCG vaccination against COVID-19 in the elderly. This population was chosen because it is the group with the highest mortality rates. Risk of COVID-19, severity and duration of disease were measured, as well as risk of other respiratory infections. In two cohorts of respectively 2,014 and 6,112 participants, randomized 1:1 for BCG:Placebo and followed for one year, no effect of BCG could be evidenced. This was in contrast to a communication by Denise Faustman (Harvard Medical School), who took advantage of an ongoing RCT to examine the effect of BCG vaccination in type 1 diabetes (see below). In this study participants were vaccinated three times with a different BCG strain or placebo 2 to 3 years prior to the COVID-19 pandemic and followed for COVID-19 until April 2021, at which time 10.4% in the placebo group had evidence for SARS-CoV2 infection by PCR diagnosis, and none in the BCG group. The difference between these two contradictory study outcomes may be due to BCG strain differences, multi-dosing in the second compared to a single dose in the first study and/or timing between BCG vaccination and SARS-CoV2 infection.

Richard White (London School of Hygiene and Tropical Medicine) studied the impact of COVID-19 on BCG vaccination coverage and paediatric tuberculosis mortality. He showed a direct relationship between even small BCG supply shortfalls and increase in tuberculosis-caused deaths [19], and BCG vaccination coverage has declined in many countries, sometimes by more than 50% and up to 96% in Bangladesh, with a weighted global average of 25%. The mathematical modelling study [20] predicted that if the disruption occurred for 3 months or 6 months, percentages in the numbers of deaths would increase by roughly 8 or 16, respectively in the absence of any catch-up intervention. This effect of vaccination disruption is predicted to be strongly reduced by catch-up vaccination, especially if catch-up vaccination coverage approaches 100%, and is only minimally affected by the time of catch-up vaccination. Importantly, only 78% of BCG vaccines were shipped by UNICEF due to air freight restrictions, and a major BCG producer has left the market to concentrate on COVID-19 vaccine production.

5. Session 4: BCG and cancer

Molly Ingersoll (Institut Pasteur) introduced the audience into the mucosal immunity in bladder cancer. She reminded us that BCG treatment together with surgery significantly improved progression-free survival of non muscle-invasive bladder cancer over many years, compared to surgery alone [21]. She showed the complexity of the immune response during BCG immunotherapy and explained that robust innate and Th1 biased immune responses seem to be important for the prevention of recurrence and progression. She used a mouse model to study how these immune responses develop following immunotherapy and examined the differences between the sexes. She showed that pre-existing immunity improves treatment outcome by enhancing innate cell infiltration in both male and female

mice. While there were no differences in immune cell infiltration between the sexes, tumor-specific T cells infiltrated the male tumors better than the female tumors.

An approach to improve the efficacy of BCG against bladder cancer was proposed by William Bishai (Johns Hopkins University). Since approximately 30% of patients do not respond to BCG treatment, W. Bishai asked the question whether this may be related to the anti-inflammatory properties of BCG and set out to augment the pro-inflammatory properties of BCG by enhanced STING-mediated signaling through over-production of cyclic di-AMP in recombinant BCG strains. By over-expressing *disA*, coding for the mycobacterial deadenylate cyclase, 15 times more cyclic di-AMP was produced, which resulted in increased protection against tuberculosis in a guinea pig model [22] and also augmented the anti-tumor efficacy in mouse and rat models of bladder cancer, outperforming small molecule STING agonists. It increased IFN-γ-producing and decreased regulatory T cell frequencies. Furthermore, it synergized with checkpoint inhibitors and increased glucose metabolism and H3K4 methylation on promoters of inflammatory genes, indicative of trained innate immune induction.

6. Session 5: BCG and inflammatory and auto-immune diseases

Denise Faustman (Harvard Medical School) informed us about the off-target effects of BCG in type 1 diabetes. Previous studies had indicated the importance of TNF-α in reducing auto-immunity and BCG is known as a strong TNF-α inducer. In a group of studies on patients with longstanding type 1 diabetes who have received at least 2 doses of BCG, haemoglobin A1C levels were significantly reduced over placebo, and this reduction was sustained for at least 8 years [23]. BCG restored blood sugar levels by increasing cellular sugar uptake and aerobic glycolysis, which lasted for several decades. It modified genes involved in glucose regulation

through methylation/demethylation and upregulated Myc, a central switch in glucose metabolism. It also increased regulatory T cell signatures in vivo by gradual de-methylation and ensuing increase in the corresponding mRNA levels. In particular, demethylation of *Foxp3* was found to parallel clinical improvement.

Hervé Bercovier (Hebrew University of Jerusalem) explored the potential effect of BCG on Alzheimer's disease. Studies in mice have shown that immunization with BCG alleviates neuroinflammation and cognitive deficits in APP/PS1 mice via the recruitment of inflammation-resolving monocytes in the brain. To investigate the effect of BCG on Alzheimer's disease, H. Bercovier and his colleagues studied bladder cancer patients older than 70 years who have or have not been treated with BCG and followed the occurrence of Alzheimer's disease in these patients until the age of 100 years. They found that BCG treatment resulted in a significantly lower risk to develop Alzheimer's disease [24]. This was confirmed in several studies in variouscountries, and the risk was found to decrease with increasing doses of BCG. Similar observations were made in preliminary studies with Parkinson's disease patients. In contrast, BCG had no effect on non-inflammatory pathologies, such as stroke or type 2 diabetes.

The anti-inflammatory effects of BCG were further assessed by Laure Pittet (University of Melbourne) in allergic diseases. She elaborated on the limitations of clinical studies, where control groups are compared to BCG groups: the BCG status is sometimes inferred by poxy measures, outcome definitions may differ between studies and confounders cannot completely be adjusted for. Systematic reviews and meta-analyses are therefore crucial. Some meta-analyses of epidemiological studies have suggested a beneficial effect of BCG vaccination on childhood asthma, while another meta-analysis of pooled RCT and cohort studies found no evidence of protection against the development of asthma following BCG

vaccination. However, a correction of this latter meta-analysis also suggests a strong beneficial effect of BCG in the prevention of asthma. These studies pointed to the importance of the time of outcome measurements, especially for asthma, and the MIS-BAIR RCT [25] in Australia will follow up the children up to 5 years of age. In a meta-analysis of 3 RCT a beneficial effect was also observed for eczema, but mostly in infants with atopic parents, and it was greater in boys than in girls. In contrast, for the moment there is no evidence of a beneficial effect of BCG vaccination on food allergy and hay fever.

7. Conclusion

In a very inspiring lecture Stanley Plotkin (University of Pennsylvania) concluded by placing the BCG vaccine in the global context of the future of vaccinology. He reviewed epidemics of the past and listed the various strategies for vaccine discovery, as well as the various mechanisms of protective immunity, including the stimulation of innate immunity, particularly relevant for BCG. He then provided examples of existing vaccines, such as influenza vaccines, with their limitations and difficulties to identify immune correlates of protection, and the challenges for the development of new vaccines, such as vaccines against the respiratory syncytial virus and against cytomegalovirus. He also insisted on the success of some very potent vaccines, such as those preventing human papillomavirus infections, and summarized the current status of COVID-19 vaccines, including knowns and unknowns. He concluded by listing unsolved problems in vaccinology.

In conclusion, this third international Symposium specifically dedicated to BCG (the previous symposia were organized in 1948 and 2018), covered many different aspects of BCG as a vaccine against tuberculosis and beyond. Many intriguing protective effects against respiratory infections and cancer, non-infectious inflammatory and auto-immune diseases

were presented, for which the underlying mechanisms are still not fully understood. Furthermore, it is likely that the full potential of therapeutic and prophylactic potential of BCG against a variety of different disease has not yet been discovered, and we witness, 100 years after the first BCG administration to a human being, only the tip of an iceberg that may potentially be enormous and remains to be largely explored. Therefore, we may look forward with confidence that in the 100 years to come many more exciting properties of BCG will be revealed to us.

Declaration of competing interest

The author declares no competing interests.

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