PERSPECTIVES

100 years of Bacillus Calmette–Guérin immunotherapy: from cattle to COVID-19

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Abstract | Bacillus Calmette–Guérin (BCG) is the most widely used vaccine worldwide and has been used to prevent tuberculosis for a century. BCG also stimulates an anti-tumour immune response, which urologists have harnessed for the treatment of non-muscle-invasive bladder cancer. A growing body of evidence indicates that BCG offers protection against various non-mycobacterial and viral infections. The non-specific effects of BCG occur via the induction of trained immunity and form the basis for the hypothesis that BCG vaccination could be used to protect against the severity of coronavirus disease 2019 (COVID-19). This Perspective article highlights key milestones in the 100-year history of BCG and projects its potential role in the COVID-19 pandemic.

The Bacillus Calmette–Guérin (BCG) vaccine has been used since 1921 to prevent tuberculosis (TB) and is considered to be the world’s most widely used vaccine. In 2002, an estimated 75% of the 130 million children born worldwide were vaccinated with BCG. BCG is also an established anti-cancer immunotherapy and is used intravesically for intermediate-risk and high-risk non-muscle-invasive bladder cancer (NMIBC) to prevent recurrence and progression.

The beginning of 2020 saw the outbreak of the coronavirus disease 2019 (COVID-19) pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). With a global death toll of more than 3 million at the time of writing (7 May 2021), the pandemic has placed extraordinary demands on health-care systems. A growing body of evidence suggests that the benefits of BCG extend beyond uses in TB and bladder cancer, with vaccination offering additional protection against various unrelated pathogens, including those that cause non-mycobacterial and viral infections. Indeed, emerging data suggest that these non-specific effects might be protective against COVID-19, and several randomized controlled trials are underway to investigate this hypothesis. Thus, the COVID-19 pandemic provides a timely opportunity for urologists to reflect on the past 100 years of BCG in clinical practice.

In this timeline Perspective, we review the origins of BCG, its mechanisms of action and contemporary evidence for its use in TB, non-mycobacterial diseases and bladder cancer. We also discuss the role and use of BCG in the COVID-19 era and implications of this use for the urological community.

Origins of BCG
Following the discovery of Mycobacterium tuberculosis as the causative organism in TB by Robert Koch in 1882, scientists raced to develop a vaccine as the TB epidemic peaked across industrialized European cities. In 1908, Albert Calmette and Camille Guérin began working to develop an effective vaccination against TB at the Pasteur Institute in Lille, France. Using ‘lait Nocard’, a virulent strain of Mycobacterium bovis originating from a cow with tuberculous mastitis, the pair subcultured the organism every 3 weeks on a glycerinated beef-bile-potato medium. After 13 years and more than 200 passages, the organism was finally attenuated to a weakened non-virulent form. Calmette and Guérin gave their names to this strain of M. bovis and the resulting vaccine became known as BCG.

In 1921, Benjamin Weill-Halle and Raymond Turpin orally administered the first BCG vaccine to a neonate whose mother had died from TB at the Charité Hospital in Paris. The baby experienced no side effects from the vaccine and did not develop TB, despite maternal exposure. Over the next 3 years, 217 Parisian children were successfully vaccinated against TB, and mass production of the BCG vaccine commenced at the Pasteur Institute. The League of Nations declared the vaccine safe for use in 1928 and vaccination programmes continued uneventfully until 1930, when what would later be known as the Lübeck disaster occurred, in which 72 neonates in Lübeck, Germany, died after receiving the BCG vaccination. Although their deaths were later shown to be due to accidental contamination of vaccine preparations with a virulent strain of M. tuberculosis, BCG vaccination suffered a major setback and enthusiasm for its use rapidly waned. However, a resurgence of TB during World War II led to a return of widespread BCG vaccination and public trust in its safety was restored.

In 1974, the WHO created the Expanded Programme on Immunization to ensure universal access of mothers and children to routinely recommended infant vaccines. Intradermal (ID) BCG vaccination at birth has been included in this programme since inception, resulting in more than 4 billion vaccines administered to date.
| Study                                                                 | Location     | Primary outcome                                                                 | Estimated enrolment | Status                  | Projected end date |
|----------------------------------------------------------------------|--------------|----------------------------------------------------------------------------------|----------------------|-------------------------|--------------------|
| Application of BCG vaccine for immune-prophylaxis among Egyptian health-care workers during the pandemic of COVID-19  | Egypt        | Incidence of COVID-19 infection                                                  | 900                  | Not yet recruiting      | December 2020      |
| Efficacy of BCG vaccination in the prevention of COVID-19 via the strengthening of innate immunity in health-care workers (COVID-BCG) | France       | Incidence of documented COVID-19 among health-care workers exposed to SARS-CoV-2 and vaccinated with BCG compared with placebo | 1,120                | Recruiting              | February 2021      |
| Reducing health-care workers absenteeism in the COVID-19 pandemic through the BCG vaccine (BCG-CORONA) | Netherlands  | Number of days of health-care workers' unplanned absenteeism for any reason      | 1,500                | Active, not recruiting  | April 2021         |
| BCG vaccination for health-care workers in COVID-19 pandemic          | South Africa | Incidence of health-care workers hospitalized owing to COVID-19                  | 500                  | Recruiting              | April 2021         |
| Prevention of respiratory tract infection and COVID-19 through BCG vaccination in vulnerable older adults (BCG-PRIME) | Netherlands  | Incidence of clinically relevant respiratory tract infection or COVID-19 infection | 5,200                | Recruiting              | April 2021         |
| Clinical trial evaluating the effect of BCG vaccination on the incidence and severity of SARS-CoV-2 infections among health-care professionals during the COVID-19 pandemic in Poland | Poland       | Incidence of COVID-19 infections                                                 | 1,000                | Recruiting              | April 2021         |
| BCG vaccination to prevent COVID-19 (ACTIVATEII)                      | Greece       | Positive for the respiratory questionnaire consisting of questions concerning the appearance of symptoms possibly, probably and/or definitively related to COVID-19 on visit 3 | 900                  | Recruiting              | May 2021           |
| Study to assess VPM1002 in reducing health-care professionals' absenteeism in the COVID-19 pandemic | Germany      | Number of days absent from work due to respiratory disease (with or without documented SARS-CoV-2) | 59                   | Active, not recruiting  | May 2021           |
| Reducing COVID-19-related hospital admission in the elderly by BCG vaccination | Netherlands  | SARS-CoV-2 hospital admission                                                    | 2,014*               | Active, not recruiting  | May 2021           |
| Study to assess VPM1002 in reducing hospital admissions and/or severe respiratory infectious diseases in the elderly in the COVID-19 pandemic | Germany      | Number of days with severe respiratory disease at hospital and/or at home        | 2,038                | Active, not recruiting  | September 2021     |
| Efficacy and safety of VPM1002 in reducing SARS-CoV-2 (COVID-19) infection rate and severity (COBRA) | Canada       | Incidence of COVID-19 infection                                                  | 3,626                | Active, not recruiting  | December 2021      |
| Using BCG vaccine to protect health-care workers in the COVID-19 pandemic | Denmark      | Days of unplanned absenteeism for any reason                                     | 1,500                | Active, not recruiting  | December 2021      |
| BCG to reduce absenteeism among health-care workers during the COVID-19 pandemic (EDCTP) | Denmark      | Days of unplanned absenteeism due to illness                                    | 1,050                | Recruiting              | March 2022         |
| Using BCG to protect senior citizens during the COVID-19 pandemic     | Denmark      | Incidence of acute infection                                                     | 1,900                | Recruiting              | March 2022         |
deletion of RD2 led to the late strains, including BCG Pasteur, Danish, Glaxo, Tice, Montreal (also known as Frappier) and Connaught. Although 20 genetically distinct BCG strains exist, 5 strains currently account for >90% of the vaccines used worldwide: Pasteur 1173 P2, Danish 1331, Glaxo 1077 (derived from the Danish strain), Tokyo 172-1 and Russian BCG-I. Other commonly used strains include the Montreal and Tice strains, the latter of which is the only strain available for intravesical use in patients with NMIBC in the USA following suspension of BCG-Connaught production by the FDA in 2011 after mould was discovered at the manufacturing facility. As the Connaught strain supplied a significant proportion of the global market, the end of its production has led to a worldwide BCG shortage.

Observational studies suggest that various BCG vaccine strains might have different immunogenicity and adverse reaction profiles, with the Russian strain potentially associated with reduced scarification. A 2019 randomized controlled trial comparing vaccination with the BCG-Denmark (Copenhagen 1331), BCG-Japan (Tokyo 172-1) and BCG-Russia (Russia BCG-I) strains in neonates in Guinea-Bissau found no difference in all-cause morbidity (BCG-Denmark versus BCG-Russia, hospitalization incidence rate ratio (IRR) 1.08 (95% CI 0.84–1.37); BCG-Japan versus BCG-Russia, IRR 1.15 (95% CI 0.93–1.43)) or mortality (BCG-Denmark versus BCG-Russia, mortality rate ratio 1.15 (95% CI 0.74–1.80); BCG-Japan versus BCG-Russia, mortality rate ratio 0.71 (95% CI 0.43–1.19)) among strains. However, in keeping with other studies, BCG-Russia produced fewer scars than other strains (BCG-Denmark versus BCG-Russia, prevalence ratio (PR) 1.07 (95% CI 1.04–1.10); BCG-Japan versus BCG-Russia, PR 1.06 (95% CI 1.02–1.09)). Although the presence of a BCG vaccine scar does not predict susceptibility to TB, these findings suggest that differences could exist in the immune response to different BCG strains. Further evidence supporting this hypothesis comes from a large randomized trial in 303,092 neonates in Hong Kong that showed that the risk of TB after vaccination with BCG-Pasteur was 45% (95% CI 22%–61%) less than the risk after vaccination with BCG-Glaxo.

Additionally, a cohort study in Kazakhstan showed differences between the efficacy of different strains: neonatal vaccination with BCG-Tokyo, BCG-Serbia and BCG-Russia was shown to reduce the risk of clinically diagnosed TB by 69% (95% CI 61–75%), 43% (95% CI 31–53%) and 22% (95% CI 7–35%), respectively.

Whether strain selection influences the efficacy of intravesical BCG in treating NMIBC is unclear. Several different strains including BCG Tice, BCG Moreau, BCG Connaught and BCG-Tokyo-172 with different quantities of colony-forming units are currently in use for intravesical instillations. In a prospective randomized controlled trial comparing BCG Connaught with BCG Tice in 132 patients with high-risk NMIBC, 5-year recurrence-free survival was significantly higher in the BCG Connaught group (74% versus 48%, P = 0.0108), but progression-free survival (P = 0.3442) and overall survival (P = 0.2652) did not differ between the groups. However, this study had several limitations, including a lack of maintenance treatment. A larger retrospective study comparing the same strains in 2,099 patients showed that BCG Connaught was more effective than BCG Tice for time-to-first recurrence (HR 1.48; 95% CI 1.20–1.82; P < 0.001).

However, when maintenance BCG was used, the opposite was true (HR 0.66; 95% CI 0.47–0.93, P = 0.019). The authors hypothesize that this might be due to BCG Connaught reaching an earlier cumulative optimized dose than BCG Tice. Subsequently, a 2020 retrospective comparison of BCG Tice (n = 321) with BCG Moreau (n = 339) showed no difference in recurrence-free (HR 0.88; 95% CI 0.56–1.38, P = 0.58) or progression-free survival (HR 0.55; 95% CI 0.25–1.21, P = 0.14). Likewise, a meta-analysis comparing nine strains across 65 trials was unable to demonstrate the superiority of one BCG strain over another at preventing recurrence. Ultimately, comparative trials are needed to determine the effect of strain selection on BCG efficacy and to identify whether an optimal strain exists for treatment of NMIBC.

### Table 1 (cont.) | Summary of randomized controlled trials of BCG vaccination and COVID-19

| Study | Location | Primary outcome | Estimated enrolment | Status | Projected end date |
|-------|----------|----------------|---------------------|--------|-------------------|
| Use of BCG vaccine as a preventive measure for COVID-19 in health-care workers (ProBCG) (NCT04659941) | Brazil | Incidence of SARS-CoV-2 infection; incidence of severe forms of COVID-19 | 1,000 | Recruiting | October 2022 |
| BCG against COVID-19 for prevention and amelioration of severity trial (BAC to the PAST) (NCT04534803) | USA | Incidence of severe COVID-19 disease | 2,100 | Not yet recruiting | November 2022 |
| COVID-19: BCG as therapeutic vaccine, transmission limitation and immunoglobulin enhancement (BATTLE) (NCT04369794) | Brazil | Clinical evolution of COVID-19; SARS-CoV-2 elimination; seroconversion rate and titration (IgA, IgM, IgG) | 1,000 | Recruiting | August 2023 |

*Actually enrolled. BCG, Bacillus Calmette–Guérin.

**BCG and tuberculosis**

TB is the leading infectious cause of death globally, responsible for an estimated 1.4 million deaths in 2019 (REF. ). In countries with a high incidence of TB (>40 per 100,000), BCG vaccination reduces the risk of childhood meningeal and miliary TB by 85% and pulmonary TB by 50%. These protective effects last ~10–15 years, although a small number of studies have demonstrated protection of up to 50 years after vaccination.

However, adults and adolescents, not children, are thought to be responsible for the majority of community transmission of TB. The protective effect of BCG vaccination is far more variable in adolescents and adults than children, with protection rates ranging from 0–80%. Reasons for this high variability are not clear, but could include differences in BCG strain, host genetics and previous exposure to environmental mycobacteria that can...
A vaccine that prevents pulmonary TB in adolescents and adults is, therefore, urgently needed for TB control. Fortunately, novel insights into the basic immunology of *M. tuberculosis* infection and clinical vaccine development have led to the development of potential vaccine candidates, some of which are now in advanced clinical trials. In particular, two phase IIb trials are worth highlighting. In a 2018 clinical trial of re-vaccination of adolescents in South Africa, a reduction in the rate of TB infection was observed in the BCG re-vaccinated groups (efficacy 45.4%, *P* = 0.03), as measured by the rate of sustained Quantiferon-TB Gold In-tube assay conversion, compared with participants who received a placebo vaccination (30.5%, *P* = 0.16)\(^{41}\). In a second 2018 trial, protection against TB could be achieved by vaccination of *M. tuberculosis*-infected adults with an adjuvanted subunit vaccine containing two *M. tuberculosis* proteins (efficacy 54%, 95% CI 13.9–75.4%; *P* = 0.04)\(^{42}\).

Interestingly, although intravenous (IV) BCG injections had been consigned to the history books, this approach was revisited in a 2020 study in adult rhesus macaques\(^{51}\). The study compared ID and IV injection of BCG and observed that IV BCG was highly effective in protecting against *M. tuberculosis* challenge, with six out of ten vaccinated animals showing no signs of infection as defined by lack of granuloma formation. Furthermore, nine out of ten IV BCG-immunized macaques had no signs of tuberculous disease, as determined by a lack of lung fluorodeoxyglucose activity at the time of necropsy. By contrast, no evidence of protection against infection or disease was seen in macaques receiving ID injection of the standard human dose. Although these results are encouraging, meticulous safety testing is required to prove that IV vaccination is safe in humans. As seen in the Lübeck disaster, direct IV injection of a contaminated vaccine could lead to fatal infection. Nevertheless, these studies represent milestones in the efforts to develop a novel TB vaccine that protects against both neonatal and adult TB.

**Non-specific effects of BCG**

A growing body of evidence indicates that vaccines have non-specific effects on the immune system\(^{54}\). Also known as heterologous or off-target effects, these afford additional protection beyond that offered against their target disease.

Over the past 50 years, numerous studies have proved that BCG vaccination is able to induce non-specific protective effects. Carl Naslund in Sweden first described this phenomenon in 1932, noting that BCG-vaccinated neonates (*n* = 1,323) had a mortality rate that was almost threefold lower than those who were unvaccinated (*n* = 3,350; 6.6% versus 22.2%)\(^{55}\). Subsequent epidemiological studies have corroborated this finding, showing a correlation between BCG vaccination and reduced childhood mortality\(^{46–48}\), and hospitalization rates attributable to other respiratory infections\(^{59}\), independent of its effect on TB.

Strong evidence for the non-specific effects of BCG vaccination comes from a series of randomized controlled trials in Guinea-Bissau, a country with a high childhood mortality rate\(^{56–58}\). The main study found that low-birth-weight neonates vaccinated at birth had a >40% reduction in neonatal mortality rate compared with low-birth-weight neonates who had delayed vaccination at 6 weeks\(^{62}\). This effect seems to be mainly due to protection against neonatal sepsis and respiratory infection\(^{1}\). Subsequently, the phase III ACTIVATE trial assessed whether BCG confers similar protective effects against respiratory infections in the elderly\(^{61}\). In this double-blind, randomized trial, 198 hospitalized elderly patients received either BCG or placebo vaccine at discharge and were followed up for 12 months for the occurrence of new infections. At interim analysis, BCG vaccination significantly increased the time to first infection compared with placebo (median 16 weeks versus 11 weeks; *P* = 0.039).

The incidence of new infections was 42.3% (95% CI 31.9%–53.4%) after placebo vaccination compared with 25% (95% CI 16.4%–36.1%) after BCG vaccination; most of the protection in the BCG group was determined to be against respiratory tract infection of probable viral origin (HR 0.21, *P* = 0.013).

Evidence from murine models and human trials has shown that BCG vaccination protects against secondary infections with *Candida albicans*\(^{65}\), *Schistosoma mansoni*\(^{9–12}\), and *Plasmodium*\(^{9–12}\). In a randomized study, participants received either BCG or placebo vaccination followed by a trivalent seasonal influenza vaccine 14 days later\(^{13}\). The data showed that BCG-vaccinated subjects had markedly enhanced antibody responses against the...
A(H1N1)pdm09 vaccine strain compared with patients who had received placebo (0.041), with a trend towards more rapid seroconversion (P = 0.08). In another randomized placebo-controlled study, yellow fever vaccine recipients who had received the BCG vaccine 1 month previously had significantly lower yellow fever vaccine viraemia than subjects who had received previous placebo vaccination (P < 0.05)14. The lower vaccine viraemia was also accompanied by lower circulating inflammatory cytokines in BCG-vaccinated individuals — lower levels of circulating inflammatory cytokines were seen because the high cytokine production capacity following BCG vaccination leads to a rapid local antimicrobial response and subsequent elimination of the pathogen, thereby preventing a systemic reaction and high levels of circulating cytokines64,65. However, yellow fever antibody titres did not differ between placebo and BCG-vaccinated groups 90 days after receiving the vaccine, indicating that the trained immunity induced by BCG conferred protection via an antigen-unspecific process66.

BCG vaccination has also been shown to have a protective effect against atopy measured by skin-test reactivity to three allergens, *Dermatophagoides pteronyssinus*, *Dermatophagoides farinae* and cockroach70. This finding has been validated in randomized controlled trials; one Dutch study of 121 newborns showed that BCG-vaccinated infants used significantly less medication for eczema than unvaccinated infants (P = 0.04)71. In the separate Dutch Calmette Study, neonatal BCG vaccination reduced the cumulative incidence of atopic dermatitis in children with atopic predisposition compared with BCG-unvaccinated predisposed infants (22.7% versus 25.4%; RR 0.90 (95% CI 0.66–1.02). The lower vaccine viraemia was also accompanied by lower circulating inflammatory cytokines in BCG-vaccinated infants — lower levels of circulating inflammatory cytokines were seen because the high cytokine production capacity following BCG vaccination leads to a rapid local antimicrobial response and subsequent elimination of the pathogen, thereby preventing a systemic reaction and high levels of circulating cytokines64,65. However, yellow fever antibody titres did not differ between placebo and BCG-vaccinated groups 90 days after receiving the vaccine, indicating that the trained immunity induced by BCG conferred protection via an antigen-unspecific process66.

BCG and cancer

The concept of using microbial products as immunotherapy for cancer was pioneered by William B. Coley at the turn of the 20th Century. After learning about a patient with an inoperable malignant neck tumour that disappeared after developing erysipelas, Coley started treating his own patients with cancer with intratumoural injections of live *Streptococcus pyogenes*72 and, later, mixtures of *S. pyogenes* and *Serratia marcescens*73. Although he observed tumour regression in several patients, the injections had unpredictable efficacy and lethal adverse effects sometimes occurred.

In 1929, Raymond Pearl noted that patients with lesions of active TB had a lower frequency of cancer on post-mortem examination74, although he was unable to provide an explanation for this finding. In 1959, landmark studies by Lloyd Old at the Memorial Sloan-Kettering Institute in New York demonstrated the inhibition of cancer by BCG: mice infected with BCG showed increased resistance to the transplantation of the murine tumour cell lines S-180, carcinoma 755 and Ehrlich ascites75.

Further work published in 1971 by Zbar and Tanaka at the National Cancer Institute was instrumental in confirming the anti-neoplastic properties of BCG76. Zbar found that intranasal injection of BCG into guinea pigs induced regression of ID tumours, prevented the development of lymph node metastases and eradicated nodal micrometastases77. Zbar later went on to describe the four criteria for optimal BCG therapy: proximity between BCG and tumour cells, a small tumour burden, a host capable of mounting an immunological reaction to mycobacterial antigens, and sufficient numbers of viable mycobacteria78. This work led to the clinical use of BCG as cancer therapy, first in acute lymphoblastic leukaemia, in which adjuvant BCG reduced relapse rates79, and later in malignant melanoma in which skin lesions regressed following treatment with intralesional BCG injection79. These reports created a great deal of interest in BCG and prompted a number of clinical trials using BCG against kidney80, colon81 and lung82 cancer. Unfortunately, these trails failed to show a significant benefit of BCG in these tumour types, although, notably, most of the studies were in patients with advanced disease who, based on Zbar’s criteria, would not be expected to respond to BCG82.
BCG and bladder cancer

The most successful application of BCG for the treatment of malignancy was in NMIBC. Preliminary work by Coe and Feldman showed that BCG elicited a delayed hypersensitivity response in guinea pig bladders83, and when injected into a malignant melanoma metastasis in the bladder, complete eradication was observed44.

In the early 1970s, Alvaro Morales started investigating the use of BCG in bladder cancer and in 1972 devised the BCG protocol that remains in use today83. Interestingly, Morales chose the schedule of six weekly instillations simply because the Armand Frappier strain of BCG he was using was supplied in six separate vials with adverse events lasting less than 1 week85. In a landmark study in 1976, Morales treated nine patients with recurrent NMIBC with weekly intravesical and ID BCG for 6 weeks, observing a 12-fold reduction in bladder cancer recurrence ($P < 0.01$)85. These promising data prompted two randomized controlled trials testing the effectiveness of transurethral resection (TUR) followed by intravesical BCG for NMIBC versus surgical resection alone. In a 1980 study in 37 patients, BCG significantly reduced tumour recurrence rate compared with resection alone (17% versus 42%, $P = 0.029$)86. Similarly, a 1985 randomized trial of 86 patients with 43 in each treatment arm demonstrated a significant reduction in the number of recurrent tumours in patients receiving BCG compared with a control group receiving standard care (cystoscopy and fulguration) (43 versus 27 patients, respectively; $P < 0.001$)87. Furthermore, patients treated with BCG also demonstrated reduced tumour progression requiring cystectomy compared with patients in the control group (3 versus 15 patients, respectively; $P < 0.001$)87.

Subsequent meta-analyses have confirmed that BCG is associated with a decreased risk of recurrence compared with TUR alone88,89. Han et al. analysed 4,767 patients undergoing TUR from 25 randomized controlled trials and found a significant difference in the odds ratio for tumour recurrence between groups treated with and without BCG (OR 0.61, 95% CI 0.46–0.80, $P < 0.0001$)89. BCG has also been shown to be superior to chemotherapy for recurrence prevention90–92. A meta-analysis of 2,749 patients from 11 randomized controlled trials reported that BCG was superior to mitomycin C in reducing tumour recurrence (OR 0.56, 95% CI 0.38–0.84, $P = 0.005$)90. Furthermore, in the subgroup treated with BCG maintenance, all six studies showed significant superiority of BCG over mitomycin C (OR 0.43, 95% CI 0.35–0.53, $P < 0.001$)90. Similarly, in a meta-analysis using individual patient data from nine randomized controlled trials ($n = 2,820$), Malmstrom and colleagues observed a 32% reduction in risk of recurrence in patients treated with maintenance BCG compared with those treated with mitomycin C ($P < 0.0001$)90. Patients treated without maintenance BCG demonstrated a 28% increase in recurrence risk ($P = 0.006$)90.

With regard to tumour progression, several meta-analyses have concluded that intravesical BCG prevents — or at least delays — progression to invasive disease93,94. The largest of these studies analysed 24 trials ($n = 4,863$ patients) and showed a 27% reduction in the odds of progression (9.8% versus 13.8%; OR 0.73, $P = 0.001$) in patients treated with maintenance BCG compared with either TUR alone or TUR with chemotherapeutic agents other than mitomycin C95. Notably, however, the Malmstrom meta-analysis was unable to confirm a significant difference between BCG and mitomycin C for either progression ($P = 0.6164$) or survival ($P = 0.2783$)90. These conflicting results could perhaps be explained by differences in patient characteristics, maintenance schedules, follow-up period and statistical power93.

Another area of controversy related to BCG therapy is the optimal duration of maintenance regimens. Although different regimens have been used, a 2002 meta-analysis was unable to determine which regimen was most effective for NMIBC treatment96. However, a 3-year maintenance schedule is supported by data from the SWOG 8507 (REF.97) and EORTC 30962 (REF.98) studies. SWOG 8507 showed that maintenance BCG given as a weekly instillation for 3 weeks over a 3-year period reduced the recurrence rate compared with induction BCG alone99. Accordingly, EORTC 30962 used a similar regimen and showed that 3 years of maintenance treatment reduced the recurrence rate compared with 1 year of BCG maintenance in patients with high-risk disease (95% CI 1.13–2.30; $P = 0.009$)100. As such, current European Association of Urology (EAU) and American Association of Urology (AUA) guidelines recommend a risk-stratified schedule for maintenance therapy, such that patients with intermediate-risk disease should receive maintenance therapy for 1 year whereas patients with high-risk disease receive treatment for up to 3 years100.

Despite high success rates, BCG fails in approximately 25–45% of patients, with a further 40% of patients eventually relapsing despite an initial response95,99–101. Furthermore, intravesical BCG can cause adverse effects, which — although mild in the majority of cases — can be poorly tolerated and curtail treatment schedules102. Haematuria and cystitis are the most common local side effects occurring in approximately 20% and 35%, respectively, and tend to arise in the first 48 h after BCG instillation103. Systemic adverse effects include pyrexia, fatigue and general malaise. Severe systemic effects are rare (<1%) and are the result of disseminated infection (also known as BCG-osis), which can present as sepsis, reactive arthritis, pneumonitis, rash, or — rarely — mycotic aneurysm104–106.

Mechanisms of BCG in bladder cancer

The precise mechanism of action by which BCG immunotherapy exerts its effect remains incompletely elucidated. However, it is clear that BCG induces a robust immune response that leads to adaptive immunity and anti-tumour activity. Following intravesical instillation, BCG is believed to attach to and invade the urothelium. Although murine studies indicate that BCG binds to the urothelium via fibronectin receptors107, this mechanism of attachment is not supported by studies in humans108,109. Regardless, once BCG attaches to the urothelium, it is internalized by urothelial cells, bladder cancer cells and immune cells110. Internalization stimulates a local and systemic innate immune response leading to the release of several chemokines and cytokines including IL-6, IL-8, GM-CSF and TNF110–113. In fact, the release of these pro-inflammatory cytokines might be used to predict response to BCG therapy for bladder cancer. For example, the CyPRIT assay, a nomogram constructed using urinary levels of cytokines induced by BCG (IL-2, IL-6, IL-8, IL-18, IL-1RA, TRAIL, IFNγ, IL-12p70 and TNF), was able to predict the likelihood of recurrence with 85.5% accuracy (95% CI 77.9–93.1%)114. Following this cytokine and chemokine burst, cytotoxic immune cells, including neutrophils, monocytes, CD4+ and CD8+ T cells, B cells, macrophages and natural killer (NK) cells, are recruited. Immune-mediated cytotoxicity of bladder cancer cells subsequently occurs via activation of NK cells, T cells and secretion of soluble factors such as TRAIL by polymorphonuclear cells110.

The success of BCG therapy also depends on the induction of adaptive
immunity. BCG antigens are presented on the cell surface of antigen-presenting cells and urothelial cells via MHC class II. These molecules interact with CD4+ T cell receptors, leading to activation and differentiation of a primarily T helper 1 cell immune response. Together, both innate and adaptive immune system responses lead to lasting protection against tumour recurrence and progression.

**BCG and trained immunity**

The non-specific effects of BCG might be explained in part by trained immunity — the concept that innate immune cells (such as monocytes and macrophages) encountering a vaccine or microbial component can be trained by a primary stimulus to improve responsiveness to a similar or unrelated secondary stimulus.

Host immune responses are divided into innate and adaptive responses and, until relatively recently, it was believed that only adaptive immunity possessed immunological memory. However, studies over the past decade have shown that innate immune cells also possess intrinsic memory characteristics and can undergo functional reprogramming to enable an enhanced response to secondary stimulation. This reprogramming is achieved through histone modifications, which are epigenetic alterations of the chromatin structure in the nucleus that result in enhanced chromatin accessibility, and facilitate transcription of genes important for antimicrobial responses and improved cell function. Additionally, metabolic changes such as increased glycolysis, oxidative phosphorylation and glucose consumption lead to selective accumulation or depletion of certain metabolites, such as glutamate and malate, that regulate this process.

BCG vaccination is able to endow circulating monocytes with characteristics of trained immunity through epigenetic and metabolic rewiring of myeloid progenitors in the bone marrow. This process begins with autophagy, the process of intracellular degradation of cell and microbial components. Phagosomal digestion of BCG causes the release of muramyl dipeptide (MDP) as well as metabolic changes through activation of the Akt-mTOR-HIFα pathway. MDP binds to nucleotide-binding oligomerization domain-containing protein 2 (NOD2) receptor to induce epigenetic histone alterations. Epigenetic modifications result in increased access to the promoter regions of genes related to inflammatory pathways, such as cytokines and pattern recognition receptors. As a result, these BCG-trained innate immune cells express higher levels of pattern recognition receptors and produce increased levels of pro-inflammatory cytokines than non-BCG-trained cells when challenged by a secondary and potentially unrelated stimulus, thus increasing the capacity of the innate immune cells to mount a faster and augmented response to such a secondary stimulation.

Emerging data have identified a key role for the stimulator of interferon genes (STING) pathway in mediating innate immune responses. STING is a cytosolic receptor, and activation of STING induces potent cytokine and cellular immune responses against microbial infection. STING-dependent signalling is activated by binding cyclic dinucleotides either secreted by intracellular bacteria or distinct host cyclic dinucleotides generated by host cell receptors after recognition of cytosolic double-stranded DNA. STING agonists might, therefore, be a novel class of vaccine adjuvants capable of inducing cellular immune responses and protective efficacy against intracellular pathogens. Studies of a recombinant BCG that released high levels of STING agonist demonstrated a significantly augmented pro-inflammatory cytokine response (TNF, IL-6 (P < 0.05); IL-1β (P < 0.001)) in vitro in murine macrophages challenged with M. tuberculosis. Attenuation of M. bovis for clinical use as BCG results in the loss of the early secretory antigenic target system (ESX-1), which is critical for activation of the STING pathway. This loss potentially limits subsequent BCG responsiveness owing to reduced induction of immune cell-recruiting chemokines. To compensate, several recombinant versions of the BCG vaccine have been produced. One example is the VPM1002 vaccine, which activates the STING pathway and has been shown to be potentially more immunogenic than conventional BCG: in a phase II trial with a 6-month follow-up, 48 HIV-unexposed newborn infants were vaccinated with either VPM1002 (n = 36) or BCG Danish strain (n = 12). Although both vaccines induced IL-17 responses, VPM1002 vaccination led to a significant increase in CD8+IL-17+ T cells at day 14 (P = 0.0156) and month 6 (P = 0.0002) compared with BCG. Trials are currently investigating whether VPM1002 can protect health-care workers and the elderly against severe COVID-19.

**Trained immunity and bladder cancer**

Trained immunity might also have a role in the effect of intravesical BCG for NMIBC as an anti-tumour mechanism. This role is not well defined; data on epigenetic modifications in innate immune cells during intravesical BCG instillations are lacking. However, increased cytokine and chemokine production by innate immune cells during intravesical BCG therapy is well described and might indirectly support a role for trained immunity in the mechanisms of BCG immunotherapy for NMIBC.

Further support for the role of trained immunity comes from studies evaluating the role of recombinant BCG and STING agonists in bladder cancer. The STING signalling pathway not only mediates innate immune responses against microbial infection but is also important for the anti-cancer immune response. Activation of the STING signalling pathway by tumour-derived DNA results in
production of type I interferon and other pro-inflammatory cytokines that stimulate cross-presentation of tumour antigens and mobilization of tumour-specific CD8+ T cells. Thus, STING agonists have the potential to induce an anti-tumour immune response. Studies have evaluated the effect of BCG in combination with a STING agonist in cell-line models representing human monocytes, the bladder cancer cell line RT112 and primary bladder epithelial cells. They showed a >20-fold increase in immune cell recruiting chemokines including CXCL10 as a result of synergistic effect of BCG and the STING agonist.

In another study, use of a recombinant BCG strain overexpressing a STING agonist stimulated epigenetic changes that promoted enhanced anti-tumour immunity compared with wild-type BCG in rat NMIBC tumour models. A significantly reduced number of immunosuppressive M2 macrophages and increased pro-inflammatory M1 macrophages (P < 0.01) was observed with the recombinant BCG strain compared with wild-type BCG. Additionally, myeloid cells treated with the recombinant BCG strain exhibited enhanced phagocytosis and autophagy to a greater degree than wild-type BCG. These findings highlight the improved ability of BCG to elicit trained immunity when combined with a STING agonist.

**BCG in the COVID-19 era**

The COVID-19 pandemic is a public health crisis caused by infection with SARS-CoV-2. COVID-19 has reached at least 212 countries and territories, with a global death toll of more than 3 million at the time of writing (7 May 2021). The outbreak of COVID-19 has placed extraordinary demands on health-care systems whilst also triggering a global recession that is set to extend to protection against COVID-19 through the induction of trained immunity.

**Ecological studies.** Several ecological studies have proposed a correlation between BCG vaccination policies and COVID-19-related mortality and morbidity. For instance, countries with a BCG vaccination programme for TB had a lower daily incidence of COVID-19 (0.8 per million) than countries without such a programme (34.8 per million). Furthermore, both mortality and crude case fatality rate were lower in countries with a BCG vaccination programme compared with those without (0.08 per million and 4.1% versus 34.8 per million and 5.1% respectively). Similarly, the presence of mandatory national polices for universal BCG vaccination was associated with flattened growth curves for confirmed cases of COVID-19 and resulting deaths in the first 30-day period of country-wise outbreaks.

Overall, countries including BCG in their national vaccination programmes had a significantly lower incidence of COVID-19 cases (mean cases per population ratio: 0.0147 ± 0.027 versus 0.1892 ± 0.244, P < 0.0001) and mortality (mean of deaths per population ratio: 0.0004 ± 0.001 versus 0.0113 ± 0.020, P < 0.0001) than countries that excluded it. Subsequently, an assessment of the global linkage between BCG vaccination and COVID-19 mortality showed a strong correlation between the BCG index (an estimation of the degree of universal BCG vaccination deployment in a country) and COVID-19 mortality (r² = 0.99, P = 8 × 10⁻10), indicating that every 10% increase in the BCG index was associated with a 10.4% reduction in COVID-19 mortality.

Although intriguing, these findings must be interpreted with caution, as ecological studies carry inherent limitations. First, any risk reduction seen as a result of BCG vaccination applies to a population rather than an individual. Second, these studies are susceptible to bias from confounders such as differences in national demographics, reporting biases, testing rates for COVID-19 and the stage of the pandemic in each country. For example, an analysis of the COVID-19 spread rate in 74 countries stratified according to BCG vaccination policy (no policy, past universal vaccination policy or current universal policy) reported that, although a lower COVID-19 spread rate and mortality was observed in countries with a current universal vaccination policy compared with countries with no or prior universal vaccination policy, adjusting for COVID-19...
testing rates meant that those countries with high testing rates (defined as 10 or more tests per thousand inhabitants) no longer showed a significant association between BCG policy and COVID-19 spread rate. Accordingly, in a study of Israeli adults aged 35–41 years with COVID-19–compatible symptoms, individuals born during a period of universal BCG vaccination had similar rates of positive COVID-19 test results to those who were born after cessation of universal vaccination (11.7% versus 10.4%, 95% CI −0.3–2.9%; P = 0.09)\(^{151}\). Likewise, a preliminary study was unable to demonstrate an association between COVID-19 case fatality and BCG vaccination before 1980 (P = 0.258) or with year of establishment of universal vaccination (P = 0.852)\(^{151}\). Several other studies have also failed to demonstrate a correlation between vaccination policy and SARS-CoV-2 infection\(^{152–154}\).

A 2021 study has attempted to reconcile the conflicting findings from different studies regarding protection of BCG against COVID-19 (REF\(^{155}\)). By modelling the effect of BCG vaccination across different times during the pandemic the authors show that the protective effect of BCG vaccination against COVID-19 is strongest during the early stages of the pandemic. This protection gradually dissipates in later stages as governments introduce social distancing and other preventative measures.

Clinical data. Although results from ecological studies are conflicting, clinical data to support the hypothesis that BCG offers protection against COVID-19 are emerging. In a retrospective study, volunteers who received the BCG vaccine within the past 5 years had a lower incidence of self-reported sickness (20.7% versus 31.1%, P < 0.05) and extreme fatigue (8.3% versus 18.9%, P < 0.01) during the COVID-19 pandemic compared with those who had never received the vaccine\(^{156}\). Data from 120 consecutive adult patients with COVID-19 from a predominantly Latino and Hispanic population receiving care at a major federally qualified health centre demonstrated that BCG-vaccinated patients were less likely to require hospital admission during the disease course than non-BCG-vaccinated individuals (3.7% vs 15.8%, P = 0.019). This association remained unchanged after adjusting for demographics and comorbidities (P = 0.017) using multivariate regression analysis\(^{157}\). Accordingly, a study that assessed morbidity due to SARS-CoV-2 infections in a large cohort of health-care professionals from a multi-site Los Angeles health-care organization\(^{158}\) reported that almost 30% of the study participants had a history of BCG vaccination, and that BCG vaccination was associated with a decrease in self-reported COVID-19 diagnoses (1.9% versus 2.9%; P = 0.029), reduced seroprevalence of anti-SARS-CoV-2 IgG (2.7% versus 3.8%; P = 0.044) and a decrease in COVID-19–specific symptoms (75.6% versus 72.7%; P = 0.017).

Ultimately, these findings need confirmation and phase III–IV trials are currently underway to establish whether BCG vaccination of health-care workers and the elderly could protect them from COVID-19 (TABLE 1). If proved effective, BCG vaccines could be repurposed to provide a rapid response to the pandemic\(^{158}\). However, the relevance of these trials has been called into question now that COVID-19–specific vaccinations are available. Published efficacy data from the BioNTech/Pfizer\(^{159}\) and Moderna\(^{160}\) trials indicate vaccine efficacy rates of approximately 95%, with a third vaccine from The University of Oxford/AstraZeneca providing at least 62% protection\(^{161}\).

Following the launch of national COVID-19 vaccination programmes in several countries, the question of whether such BCG trials ought to continue should be considered. Several important arguments have been made in support of continuing these trials\(^{161}\). First, the duration of protection conferred by COVID-19 vaccines and the extent to which the vaccine response is affected by co-morbidities and frailty in the elderly is unknown. One possibility is that a combination of BCG with a COVID-19–specific vaccine might aid the immune response and induce lasting protection. Furthermore, global demand for COVID-19 vaccines is likely to vastly outstrip available supply, with the vaccine remaining inaccessible to almost one-quarter of the world’s population until 2022 (REF\(^{162}\)). By contrast, BCG vaccines are widely available and might be useful to bridge the gap until production of COVID-19 vaccines is able to meet demand. Finally, establishing whether BCG vaccination is able to protect against COVID-19 severity might provide a tool for use against emerging pathogens in future pandemics.

The outlook for BCG vaccination for COVID-19. Until results from these trials are published, BCG vaccination should only be used for COVID-19 in a trial setting, according to the WHO guidance\(^{161}\); the BCG vaccine is already in short supply and use outside this setting risks jeopardizing supplies for TB prevention in endemic areas. If trials demonstrate a positive treatment effect, a major concern for urologists and their patients will be how the supply of BCG for bladder cancer is affected. Urologists have faced global shortages of BCG for a decade and, in the short term, this shortage could be further exacerbated if BCG is repurposed for the COVID-19 effort. However, notably, the dose of BCG used for vaccination is 1/500th the dose used for bladder cancer treatment, meaning that, in theory, at least 500 subjects could be vaccinated from a single vial of BCG\(^{162}\). Furthermore, the renewed interest in BCG might encourage manufacturers who have hitherto been reluctant to increase production (or indeed, have left the market)\(^{163}\) to become newly incentivized to increase BCG production, benefitting patients with bladder cancer and also increasing available vaccine supplies for TB prevention in endemic areas.

Conclusions

A century after the first use of BCG in humans, the vaccine has become established for the prevention of disseminated forms of TB and is also used intravesically as the standard treatment for high-risk NMIBC. Besides its use in TB and bladder cancer, the BCG vaccine might have beneficial non-specific effects and the mechanisms involved in such effects are now becoming better understood. Phase IV trials are currently underway to prospectively measure the role of these non-specific effects on protection against SARS-CoV-2 infection. Nevertheless, many questions regarding the mechanism of action of BCG remain, and it is hoped that these trials might provide new insights to improve our understanding of how to modulate the host response to prevent damage whilst protecting against infections, including COVID-19.

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