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EXPERT REVIEWS

Tuberculosis vaccines in clinical trials

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Effective prophylactic and/or therapeutic vaccination is a key strategy for controlling the global TB epidemic. The partial effectiveness of the existing TB vaccine, bacille Calmette–Guérin (BCG), suggests effective vaccination is possible and highlights the need for an improved vaccination strategy. Clinical trials are evaluating both modifications to the existing BCG immunization methods and also novel TB vaccines, designed to replace or boost BCG. Candidate vaccines in clinical development include live mycobacterial vaccines designed to replace BCG, subunit vaccines designed to boost BCG and therapeutic vaccines designed as an adjunct to chemotherapy. There is a great need for validated animal models, identification of immunological biomarkers of protection and field sites with the capacity for large-scale efficacy testing in order to develop and license a novel TB vaccine or regimen.

KEYWORDS: BCG • clinical trials • mycobacteria • prophylactic • subunit • therapeutic • tuberculosis • vaccine

Tuberculosis (TB) has been one of the major causes of morbidity and mortality worldwide for centuries and control of the spread of *Mycobacterium tuberculosis* infection remains a public health priority. Following documentation of the pathogenesis of TB by anatomists and the identification of the tubercle bacillus by Robert Koch in the 19th Century, various control strategies have been attempted, principally the prompt detection, diagnosis and treatment of TB cases and prophylactic vaccination [1]. Robert Koch produced tuberculin, or purified protein derivative (PPD), which was initially introduced as an (ineffective) treatment for TB disease, before its diagnostic properties were realized, leading to the identification of latent infection with *M. tuberculosis* (LTBI). Early treatment regimens for TB, including bed rest and fresh air in sanitoria, and pulmonary collapse therapies, were poorly effective.

In an attempt to develop a vaccine, Albert Calmette and Camille Guérin attenuated an isolate of *Mycobacterium bovis* through multiple passages. The TB vaccine *M. bovis* bacille Calmette–Guérin (BCG) was first tested in humans in 1921 and the first mass immunization campaign of tuberculin skin test (TST)-negative individuals began in Poland in 1948. BCG has been part of the Expanded Program on Immunization since 1974 and is given soon after birth in countries with a high burden of TB disease and to infants of high-risk groups or children with negative TSTs in countries with a low burden of TB disease [2]. It remains the only licensed TB vaccine and the oldest vaccine currently in use but has variable efficacy, hence case detection and treatment are necessary concurrent TB control strategies. The first chemotherapeutic agents were introduced in the 1940s and 1950s in the form of para-aminosalicylic acid, streptomycin and the oral antibiotics, isoniazid and rifamycin, which remain the principal agents used for treating TB today [1]. Individuals with both LTBI and active TB disease can be effectively treated, but the *M. tuberculosis* bacillus rapidly acquires drug resistance so combination chemotherapy regimens are necessary.

In the early 1990s, TB was identified as one of the top ten causes of disease and mortality worldwide by the Global Burden of Disease Survey and the WHO declared TB a global emergency [3]. This was despite the routine use of BCG as part of the Expanded Program on Immunization schedule throughout the developing world for 20 years, and the World Bank identifying combination drug treatment for TB to be among the most cost effective of all available health interventions. The Global Fund for HIV, TB and malaria was established in 1998 and the WHO developed Directly Observed Treatment and Short Course Drug Therapy (DOTS) in the mid 1990s. However, TB cases and mortality continued to rise, which is largely attributed to the HIV epidemic and the emergence of

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multidrug-resistant (MDR) and extensively drug-resistant strains of *M. tuberculosis* [3,4]. Additional factors disrupting existing control measures are internal migration to high-density urban areas, immigration from high-burden countries and economic crises [5]. It is clear that the existing strategies of mass immunization with BCG, effective case detection and combination chemotherapy are insufficient to control the TB epidemic. The Stop TB Strategy, introduced in 2006, is broader in approach, with the express targets of halting and reversing TB incidence by 2015 and halving TB prevalence and deaths by 2015 compared with 1990 [6].

The current global situation is that there were 9.4 million new cases of TB (139 cases per 100,000) and 1.8 million deaths due to TB in 2008, representing a decline in TB incidence but a rise in the prevalence of TB cases and deaths, as world population growth outweighed the decline in incidence [7]. Most cases occurred in Asia (55%) and Africa (30%) and 80% of cases were in the 22 high-burden countries. The Stop TB targets have all been achieved in Central Europe, high-income countries, Latin America and the Western Pacific region. Eastern Europe and the Eastern Mediterranean have reversed the incidence and halved the prevalence rate but not mortality rate [7]. Southeast Asia regions are on track to achieve the Stop TB Partnership targets of halving prevalence and death rates by 2015, compared with a baseline of 1990. However, the TB incidence, prevalence and death rate continues to rise in African countries even with a low prevalence of HIV, and it is unlikely that the targets of halving prevalence and mortality worldwide will be realized by 2015 [7]. This is despite case detection being 61% and the treatment success rate 86%. The expectation with DOTS was that exceeding greater than 70% case detection and 85% cure rates would reduce incidence *per capita* by 5–10% per year, but after 20 years of DOTS only Estonia has achieved this, underlining the need for the broader Stop TB strategy approach [5].

In addition to addressing the issues of TB–HIV coinfection and MDR-TB; the needs of poor and vulnerable populations, strengthening health infrastructures, engaging care providers and empowering patients and communities, the Stop TB strategy also advocates for, and promotes research into, the development of new diagnostics, drugs and vaccines. Novel interventions and an emphasis on prevention are vital components of the Stop TB strategy if the targets are to be successful in all regions. Indeed, a mathematical model evaluating the potential benefit of novel interventions indicated that novel vaccines, drug regimens and diagnostics would each offer significant benefits in reducing TB incidence and TB mortality, but a combination of the three would augment the benefits [8]. Attributes of an ideal TB vaccine include safety and efficacy in at-risk infants, children and adults (including HIV infection); effectiveness against all forms of TB including pulmonary and MDR-TB, logistically practical (timing of vaccination and noninterference with other childhood immunizations), and a formulation that can be feasibly manufactured on a mass scale and stored and administered under low-technology conditions. Ongoing TB vaccine clinical trials are evaluating hypotheses for the variable efficacy of BCG; alterations to the methodology of BCG administration and novel candidate vaccines designed either to replace or enhance BCG.

The existing TB vaccine: BCG

BCG safety & efficacy

BCG is a well-tolerated vaccine with an 80-year safety record; 100 million doses are administered each year. Complications of BCG vaccination are extremely rare. Significant local reactions (severe ulceration or regional lymphadenitis) occur in less than one per 1000 individuals and fatal disseminated BCG disease in less than two per million vaccinees [2]. The majority of serious complications occur in immunocompromised individuals, due to disseminated infection. The rates of disseminated BCG in HIV-infected infants in South Africa were found to be higher than first expected, leading to revised recommendations from the WHO, whereby HIV infection is now a contraindication to BCG immunization [9,10]. This is a major disadvantage of the vaccine. A Phase I and II clinical trial is investigating the effect of BCG vaccination on infants born to HIV-infected mothers to evaluate whether BCG has any effect on an infant's risk of progression of HIV disease [201].

Trials have reported a diverse range of protective efficacy of BCG between 0 and 90%, with high rates of protection in North America and Northern Europe and no or low protection in tropical areas [11]. In meta-analyses, BCG shows an average of 86% efficacy against miliary and meningeal TB but only heterogenous efficacy against pulmonary TB and improved protection against TB deaths, TB meningitis and disseminated TB compared with total TB cases [12,13]. A quantitative review revealed waning efficacy in seven out of ten trials evaluated and an average of 14% overall efficacy after 10 years [14]. However, long-term studies in American Indians and Alaskan natives demonstrated 82% efficacy after 20 years and 52% efficacy after 50–60 years, showing that the protective efficacy of BCG can be durable in certain populations [15].

Clinical trials evaluating hypotheses for the variable efficacy of BCG

There are a number of hypotheses for the varying efficacy of BCG including genetic variability between different strains, loss of genes essential for protective immunity, a suboptimal $CD8^+$ T-cell response despite good $CD4^+$ T-cell responses, exposure to nontuberculous (environmental) mycobacteria (NTM), chronic helminth infections interfering with the immune response and variations in immunization methods.

BCG strain differences

Although all strains of BCG originate from the originally passaged isolate of *M. bovis*, propagation under different laboratory conditions has led to genetic differences between strains [16]. The WHO has kept lyophilized seed lots of each strain since 1956 in order to prevent further alterations from the original BCG [2]. Approximately 90% of all vaccinations use the French Pasteur 1173 P2 strain, the Danish SSI 1331 strain, the Glaxo 1077 strain and the Japanese Tokyo 172 strain. Different BCG strains induce different immune responses in humans as well as animal models and the same strains perform differently in different locations, but there is no evidence that these phenotypic differences relate to differences in protective immunity between strains [17,18].

Exposure to NTM

One likely explanation for the marked geographical variation in efficacy is in variations in exposure to NTM. In populations where there are high levels of exposure to NTM and poor BCG efficacy, those with lower immune responses to NTM show greater IFN-γ responses to BCG vaccination, suggesting NTM may be inhibiting BCG's effectiveness [19]. There are two hypotheses for the mechanism by which NTM may affect the efficacy of BCG: masking, where BCG cannot further boost the background level of immunity induced by NTMs and blocking, where the ability of BCG to replicate and thereby induce a protective immune response is inhibited by pre-existing immunity. Sensitizing mice with a mixture of NTMs inhibited the replication of BCG and blocked the protective effect of BCG but not of a TB subunit vaccine [20]. Young adults in Malawi with higher levels of prevaccination IFN-γ responses to PPDs from NTM showed lower IFN-γ responses to BCG vaccination than those with lower responses to NTM prevaccination [21]. Subsequent comparisons of IFN-γ responses to NTM and *M. tuberculosis* PPDs before and after BCG vaccination in the UK and Malawi were performed, as BCG has 80% efficacy in the UK but no efficacy in Malawi [22]. Skin test delayed-type hypersensitivity responses to PPD prior to vaccination were higher in adolescents and young adults in Malawi than the UK but increased to a greater extent in the UK following BCG (Glaxo 1077) vaccination, such that postvaccination responses were similar in both populations [22]. Differences in immune responses to BCG vaccination between the UK and Malawi are also seen in infants, 100% of whom respond to PPD from *M. tuberculosis* after BCG vaccination in the UK, but only 53% respond in Malawi [23]. This indicates that the increase rather than magnitude of IFN-γ response correlates with protection, or that the NTM-induced immunity confers some protection against TB and BCG does not improve upon it.

Chronic helminth infection

Chronic helminth infections are common in areas where BCG is less effective and are associated with a shift towards Th2-type immune responses, which are usually associated with impaired antigen-specific and Th1-type responses [24]. Helminth infections also induce regulatory T cells (Tregs), which produce inhibitory cytokines, such as TGF-β, which suppress proinflammatory cytokines. This can impact on an individual's response to vaccinations and ability to withstand infections. Indeed, in Ethiopa, helminth-infected subjects had low TB-antigen-specific immune responses and reduced immune responses to BCG compared with control subjects who had been treated with antihelminthics [24]. The effect of antihelminthic treatment on BCG efficacy requires further evaluation.

Preclinical & immunological evaluation of TB vaccines

Animal models that have been used to evaluate the safety, toxicology and efficacy of TB vaccines include mice, guinea pigs, cows and nonhuman primates (NHPs). Major differences in immunology, TB pathology and BCG vaccine efficacy between humans and small animals limit the application of small animal models for in-depth evaluation of candidate vaccines [25,26]. Cattle are a natural host for TB and are a good model for human TB in many respects, although the infecting organism is *M. bovis* rather than *M. tuberculosis* [27]. NHP are considered the best available animal model but the cost, expertise requirements and ethical implications inhibit their widespread use. There is a great need to validate animal models against efficacy trials in humans to fully understand their application. Until the predictive value of these animal models for vaccine efficacy in humans is determined, candidate TB vaccines need to be moved rapidly from preclinical to clinical trials.

In early clinical trials primarily evaluating the safety of new candidate TB vaccines, a detailed characterization of the immune response to vaccination is often a secondary objective. Protective immunity against *M. tuberculosis* is complex and although much is understood about essential components of a protective immune response, immune correlates that can predict protection remain elusive [28,29]. A recent trial in South African infants has shown that levels of multifunctional T cells, 10 weeks post-BCG vaccination, do not correlate with protection, despite the fact that individually many of the components of this response are known to be essential for protection [30]. The susceptibility to TB disease in a number of contexts highlights the importance of Th1 $CD4^+$ T cells in protection against TB [31–36]. Examples include advanced HIV infection, specific IFN-γ immunodeficiencies following treatment with TNF-α-blocking drugs in the context of LTBI, and in murine knockout, cell depletion and adoptive transfer studies. However, $CD4^+$ T cells are not the only cells required. Protective immunity also depends upon MHC class I-restricted T cells, namely $CD8^+$ T cells and γδ T cells, particularly in preventing reactivation of latent infection [37,38]. Macrophages activated by IFN-γ are essential for inhibiting or killing intracellular *M. tuberculosis* bacilli, which can multiply unchecked within resting macrophages and IFN-γ production by antigen-specific T cells is the best available correlate of protection, although is insufficient alone [22,39–41]. There has been a shift in emphasis towards analysis of polyfunctional T cells, producing TNF-α, IL-2 and IFN-γ [39,42]. The roles of central and effector memory cells, Tregs and B cells require further definition [43].

Exploratory immunology assays currently used in clinical trials focus on evaluating the Th1 cytokine and T-cell axis, particularly IFN-γ detection in response to stimulation with vaccine-specific antigens. Three such assays include whole-blood-cultured IFN-γ enzyme-linked immunosorbent assay, *ex vivo* peripheral blood mononuclear cell IFN-γ enzyme-linked immunosorbent spot (ELISpot), and whole-blood *ex vivo* intracellular staining [22,44–46]. These assays can also be used in conjunction with polyfunctional flow cytometry to evaluate IFN-γ, TNF-α and IL-2-producing T cells and their effector functions (such as degranulation) and surface marker expression [42]. Functional assays measuring *in vitro* mycobacterial growth inhibition are another area of interest and a clinical comparison of four such assays with respect to BCG vaccination of healthy adults in the UK is ongoing [47–50]. MHC tetramer assays are also likely to be valuable for both diagnostic and postvaccination analyses [51]. An

expert panel for the WHO recommended that, while individual investigators continue to select specific assays for evaluating clinical trials, certain assays are harmonized and standardized to allow comparison of vaccine immunogenicity and blood products stored for retrospective analysis as new assays and technologies become available or new markers of protection are described [52].

Clinical trials evaluating strategies to improve the efficacy of BCG immunization

The safety profile of BCG and its proven efficacy in certain locations and against disseminated TB and TB meningitis supports its ongoing use and warrants ongoing clinical trials, evaluating alterations to the current regimen. Outcome measures include TST responses, *in vitro* responses to PPD stimulation or efficacy. However, TST and *in vitro* responses to PPD do not correlate well with BCG efficacy and the effectiveness of novel approaches to vaccination cannot be determined without efficacy studies [53].

Boosting BCG with BCG

There is evidence suggesting that BCG revaccination in adolescence confers protection against TB meningitis [54]. Studies of BCG revaccination in children showed no beneficial effect on TB incidence in Brazil or on mortality in Guinea Bissau [12,55]. Boosting healthy BCG-vaccinated adults in the UK was well tolerated and enhanced PPD-specific $CD4^+$ T-cell responses, but did not induce $CD8^+$ T cells [56]. An ongoing Phase I clinical trial in adults with LTBI in South Africa is evaluating the effect of BCG revaccination alone compared with isonazid therapy followed by BCG revaccination on mycobacterial antigen-specific immune responses [202]. The WHO's current position is that there is no proven benefit of BCG revaccination [2].

BCG vaccination route

BCG can be given by intradermal or subcutaneous injection, percutaneous multipuncture and orally but the WHO currently recommends intradermal delivery into the deltoid region of the arm; the most widely administered route [2]. Japan administers Tokyo 172 BCG by percutaneous multipuncture due to complaints about local reactions and hypertrophic scars following intradermal vaccination [57]. South Africa switched from percutaneous Japanese Tokyo 172 BCG to intradermal Danish SSI 1331 BCG in 2000, but no differences in adverse events were identified [58]. A comparison of TB cases in South African children before and after the change in delivery route revealed no difference in TB cases but a reduced proportion of children with disseminated disease in those given intradermal BCG [59]. Percutaneous Tokyo 172 BCG induced enhanced frequencies of BCG-specific IFN-γ-secreting T cells and Th1 cytokines, compared with intradermal Danish SSI 1331 BCG, but protective efficacy was not assessed [60]. Japanese Tokyo 172 BCG is the only commercially available strain available in both percutaneous and intradermal formulations and was used to compare the efficacy of two routes of delivery in South African neonates [61]. Despite greater antigen-specific T-cell responses after percutaneous vaccination, there were no significant differences in TB incidence, hospital admissions or mortality between the two routes [60,61].

Oral delivery of BCG would potentially be a practical, well-tolerated and inexpensive approach, removing the need for needles and syringes. It is hypothesized that, as *M. tuberculosis* is a mucosal pathogen, TB vaccines may be more effective if delivered mucosally. In healthy adults there were no tolerability differences between oral delivery of Connaught BCG and placebo [62]. Interestingly, oral delivery inhibited TST responses but enhanced *M. tuberculosis*-specific IFN-γ secretion by peripheral blood mononuclear cells. In Brazil, oral BCG was safely given to adults who had received intradermal BCG in childhood or adolescence and boosted IFN-γ ELISpot responses to mycobacterial antigens [63]. A Phase I placebo-controlled study in adults in the USA is currently comparing the safety and immunogenicity of Danish SSI 1331 BCG given orally, intradermally and by both routes combined [203]. Analysis of the protective efficacy of oral BCG is now required to determine if this is a viable vaccination route. One concern about oral delivery of BCG is whether targeting the gut-associated lymphoid tissue induces the required homing molecule expression. Encouragingly, numerous studies in animal models have demonstrated that oral delivery of BCG (usually in a lipid matrix) has induced protection against a respiratory *M. tuberculosis* challenge in mice, guinea pigs, possums, badgers, deer and cattle [64]. In animal models, the route of vaccine delivery determines the location of antigen-specific cells and the location of antigen-specific cells in the airway lumen is important for optimal protection against TB [65–67]. Future clinical studies evaluating airway delivery of a TB vaccine in humans (i.e., targeting bronchoalveolar-associated lymphoid tissue) would be valuable.

BCG vaccination age

The WHO policy recommends that infants should be vaccinated at birth (or at 40 weeks for preterm infants), but the optimal age for vaccination of infants is not known [2]. There are conflicting hypotheses that delaying neonatal BCG in all infants could enhance the vaccine-induced immune response, as the neonatal immune system is immature, or could attenuate the immune response through exposure to NTM. Data supporting both hypotheses has been reported. A randomized controlled trial (RCT) in South Africa showed that delaying vaccination until 10 weeks of age was associated with enhanced polyfunctionality and frequency of BCG-specific IFN-γ, TNF-α and IL-2-producing $CD4^+$ T cells [68]. In The Gambia, delaying vaccination of infants was associated with reduced mycobacterial proinflammatory responses (IFN-γ, IL-6 and IL-17), but significant IL-10 responses were observed compared with vaccination at birth [69]. Mycobacterial T-cell responses were detected in BCG-naive infants at 4 months of age and were hypothesized to skew the immune response to BCG vaccination. However, in this study the Th1 responses were comparable by 9 months of age and the persistent response was a Th2-biased response [69]. Evidence for a nonspecific effect of BCG on reducing all-cause mortality in infants has led to the hypothesis that early vaccination of preterm (usually defined as low birth weight) infants may be associated with

reduced mortality, particularly in females [70]. A Phase IV RCT in Guinea Bissau is evaluating the effect of early vaccination of low-birth-weight infants on adverse events, scar size, PPD reactions, morbidity and mortality [204]. The gender-specific effects of BCG combined with other health interventions in low-birth-weight infants (vitamin A supplementation and oral polio vaccine) are also under evaluation [205].

Vitamin A supplementation & BCG

Vitamin A supplementation has been hypothesized to enhance the immune response and efficacy of BCG. This is based upon some evidence for a reduction in all-cause child mortality and boosting of immune responses to measles, oral polio, tetanus and influenza vaccines [71]. The population of Guinea Bissau has moderate-to-severe vitamin A deficiency and the interactions of vitamin A supplementation and BCG vaccination have been studied there. The first RCT showed no long-term effects on TST responses, BCG scar prevalence, IFN-γ responses to PPD or mortality when high-dose vitamin A was given at birth with BCG vaccination [72,73]. In low-birth-weight infants, there was no interaction between vitamin A supplements and BCG vaccination, whether given at birth or delayed [74].

Clinical trials of candidate TB vaccines

A new TB vaccine may be prophylactic and/or therapeutic. A prophylactic vaccine would ideally be safe pre- or post-exposure to *M. tuberculosis* infection and effective in preventing infection, primary disease, latent infection and reactivation of latent infection. A therapeutic vaccine would be considered efficacious if it enabled shortening the course of, or improving the response to, chemotherapy, but there are inherent safety issues involved in evaluating therapeutic vaccines in clinical trials. Candidate prophylactic TB vaccines in development are designed either to replace BCG with an alternative live mycobacterial vaccine or to enhance BCG by boosting with a subunit vaccine. It may be that a novel TB regimen would combine both approaches by priming with a novel live mycobacterial vaccine and boosting with a subunit vaccine. This article focuses upon those candidate vaccines that have progressed to clinical trials.

Live mycobacterial vaccines designed to replace BCG

The rationale for novel live-attenuated mycobacterial vaccines is to build upon the efficacy conferred by BCG against systemic disease while addressing the reasons for its limited efficacy against pulmonary disease. As BCG has been shown to induce protective and durable protection against TB in some geographical regions, it may be possible to enhance this protection with modifications to the existing vaccine. Recombinant BCG vaccines that have reached early clinical evaluation either overexpress immunodominant antigens, are engineered to improve $CD8^+$ T-cell induction or combine both strategies and are described later. An alternative approach to recombinant BCG is to use live-attenuated *M. tuberculosis*, since the antigenic profiles for BCG are different from *M. tuberculosis*. Three attenuated *M. tuberculosis* candidate vaccines have reached an advanced stage of preclinical development. MTBVAC01 is an attenuated strain of *M. tuberculosis* with an inactivated *phoP* gene, which encodes a virulent transcription factor [75]. It confers protection against TB in mice, guinea pigs and NHP and has undergone extended stability and toxicology studies [76–78]. Two auxotrophic strains of *M. tuberculosis* ($mc^2$2020 and $mc^2$2030) are safe and protect mice and NHP against *M. tuberculosis* aerosol challenge but do not prolong survival in NHP compared with BCG [79–81]. The safety of these candidates in immunocompromised mice and safety and efficacy in NHP provide a platform for Phase I clinical trials in healthy human subjects.

Specific regulatory issues to overcome in the clinical development of live mycobacterial vaccines include: evaluating attenuation and length of persistence in preclinical models; justifying the use of antibiotic resistance markers; means of confirming identity; diagnostic tools that can differentiate between vaccination and infection; and genetically modified organism environmental risk assessment [82,83]. Furthermore, initial clinical trials would need to exclude HIV-infected individuals. Considerations for eventual Phase III studies would include the ethics of giving a vaccine candidate in place of BCG and the feasibility of performing non-inferiority efficacy studies where the novel vaccine is compared with BCG.

VPM1002 rBCGΔureC:Hly

A recombinant BCG (rBCG) strain (ΔureC:Hly + rBCG; VPM1002) has been constructed and expresses listeriolysin (Hly) derived from *Listeria monocytogenes* and enables BCG to escape from the endosome. This strain has been made urease-C-deficient to provide the optimal pH for Hly activity [84]. *M. tuberculosis* and BCG are preferentially located within the phagosomes of professional antigen-presenting cells (APCs), therefore their antigens are present through the MHC class II pathway and principally induce $CD4^+$ T cells. It has been hypothesized, however, that *M. tuberculosis* can cross-prime $CD8^+$ T cells by inducing apoptosis of the infected host cell. The apoptotic vesicles contain mycobacterial antigens that are taken up by the bystander APCs and the antigens. As BCG only induces weak apoptosis, the aim of VPM1002 is to enhance $CD8^+$ T-cell production by improving presentation of BCG antigens via the MHC class I pathway. BALB/c mice were significantly better protected against *M. tuberculosis* aerosol challenge than by parental BCG, likely related to improved cross-priming [84,85]. A Phase I clinical trial evaluating the safety and immunogenicity of this vaccine in healthy male subjects has been completed and a dose-escalation RCT comparing the safety and immunogenicity of VPM1002 and BCG in healthy adults in South Africa is ongoing [206,207].

AERAS-422 (rBCG)

A second strategy is to genetically modify BCG such that it overexpresses one or more major secretory proteins, early targets for the host immune response against *M. tuberculosis*. A precursor rBCG (Tice® strain [Organon]) vaccine, rBCG30, overexpressed one of the major secretory proteins

of *M. tuberculosis*, antigen 85B, a 30-kDa mycolyl transferase [86]. rBCG30 provided significantly better protection than nonrecombinant BCG against *M. tuberculosis* aerosol challenge in guinea pigs [87]. In a Phase I RCT, rBCG30 was well tolerated and had a comparable safety profile to nonrecombinant Tice BCG. Antigen 85B-specific T-cell proliferation and IFN-γ ELISpot responses were enhanced and antigen-specific $CD4^+$ and $CD8^+$ effector T-cell expansion was demonstrated. The antigen 85B-specific T cells induced were also capable of inhibiting the growth of intracellular mycobacteria [88]. This trial showed proof-of-principle that recombinant BCGs that overexpress immunodominant *M. tuberculosis* antigens are a promising approach. AERAS-422 (research strain AFRO-1) is a recombinant BCG Danish 1331 strain that combines both strategies by expressing perfringolysin O as an endosome escape mechanism to allow entry into the cytoplasm while overexpressing immunodominant and protective *M. tuberculosis* antigens, 85A, 85B and Rv3407 [89]. This rBCG vaccine was safe in severe combined immunodeficiency mice and vaccination of C57BL/6 mice demonstrated enhanced immune responses and prolonged survival against *M. tuberculosis* challenge compared with mice vaccinated with the parental BCG. A Phase I clinical trial in healthy human subjects will soon be starting in the USA [208].

Subunit vaccines designed to enhance BCG

Subunit vaccines that are in clinical evaluation involve the delivery of immunodominant mycobacterial antigens to the immune system, using viral vectors or protein–adjuvant systems. The application of subunit vaccines would be to enhance the effectiveness of BCG. The advantage of this approach is that the regimens would retain BCG vaccination of neonates, overcoming the ethical issues inherent with a replacement BCG vaccine.

Viral-vectored vaccines

Recombinant viral vectors, such as poxviruses and adenoviruses, are a technology with the capacity for cloning large or multiple immunodominant antigens and can be manufactured to high titers, allowing easy scale-up of vaccine production. The wild-type poxvirus, vaccinia, has been used widely as a smallpox vaccine, but its capacity to replicate limits the safety of its use in HIV-infected subjects. Modified vaccinia virus Ankara (MVA) is a replication-deficient strain of vaccinia virus (which was attenuated through multiple passages but retains the ability to express proteins) that was safely administered to 120,000 people as part of the smallpox eradication program and is safe in HIV infection [90,91]. Adenoviruses have type 1 immune adjuvant properties and, if rendered replication-deficient by the deletion of the gene *E1*, induce prolonged, but self-limited high levels of antigen release. Furthermore, adenoviruses have a natural tropism for the respiratory epithelium and induce high levels of $CD8^+$ T cells. A drawback of human adenoviruses, however, is the prevalence of neutralizing antibodies in the human population, through natural exposure to wild-type adenoviruses in childhood [92].

MVA85A

MVA85A is a subunit viral vectored vaccine that uses MVA as a delivery system for the mycobacterial antigen 85A [93]. Boosting with MVA85A can improve BCG-induced protection in guinea pigs, NHP and cattle [77,94,95]. MVA85A has been evaluated in a series of Phase I clinical trials in healthy adults in the UK since 2002, including BCG-vaccinated subjects and subjects with LTBI [45,96,97]. Encouragingly, tolerability is comparable to BCG [56]. The promising safety and immunogenicity of this candidate vaccine in the UK has led to further Phase I and IIa clinical trials in target populations in South Africa, The Gambia and Senegal [98–101]. Importantly, MVA85A has been safely administered to high-risk target populations, namely HIV-infected adults, subjects coinfected with HIV and *M. tuberculosis* and infants [Minassian *et al*, Manuscript Submitted] [98]. The immunogenicity of MVA85A has been well characterized and is promising. High frequencies of antigen-specific IFN-γ-producing polyfunctional $CD4^+$ T cells are induced, including expansion of a memory population, and the frequency of antigen-specific cells remains significantly higher than baseline for at least 1 year after vaccination [102]. Antigen-specific, IFN-γ-producing $CD8^+$ T cells have also been detected [56]. A Phase IIb efficacy trial in BCG-vaccinated South African infants is now underway, which will provide essential data on immune correlates of protection as well as the first efficacy data for a novel TB vaccine candidate [209].

AERAS-402/Crucell Ad35

AERAS-402 is a nonreplicating adenovirus (Ad) type 35 expressing a fusion protein of mycobacterial antigens 85A, 85B and TB10.4 [103]. Ad35 was selected owing to low level of pre-existing immunity and low frequency of neutralizing antibodies [104–106]. Intramuscular and intranasal delivery of the vaccine protected two strains of mice (BALB/c and C57BL/6) against intranasal *M. tuberculosis* challenge, mediated by IFN-γ-producing $CD4^+$ and $CD8^+$ T cells [103]. In BCG-vaccinated, *M. tuberculosis*-uninfected adults in South Africa, intramuscular administration of AERAS-402 was well tolerated and induced polyfunctional $CD4^+$ T cells and IFN-γ-producing $CD8^+$ T cells in response to antigen stimulation with antigens 85A, 85B and TB10.4 [107]. AERAS-402 is now being evaluated in target populations. A Phase II trial in South Africa is recruiting HIV-infected, BCG-vaccinated adults and assessing the safety (including effect on CD4 count) and immunogenicity [210]. In Kenya, a Phase I and II safety, immunogenicity and efficacy trial in BCG-vaccinated, HIV-uninfected infants is ongoing [211].

Ad5Ag85A

Ad5Ag85A consists of a nonreplicating Ad5-expressing mycobacterial antigen 85A. Ad5Ag85A, when administered intranasally but not intramuscularly, afforded better protection against *M. tuberculosis* aerosol challenge than cutaneous BCG and enhanced protection when given as a boost to BCG in both BALB/c mice and guinea pigs [65,66,108]. A Phase I clinical

trial in healthy BCG-vaccinated and unvaccinated subjects in Canada is currently evaluating the safety and immunogenicity of Ad5Ag85A in humans for the first time [212]. However, there are some concerns relating to the use of Ad5 as a vaccine vector. Neutralizing Ad5 antibodies are frequent, especially in developing countries where BCG is less effective, with significant levels detected in up to 90% of the sub-Saharan African and 45% of the US population [109]. Furthermore, the STEP trial, using an Ad5-vectored prophylactic HIV vaccine, unexpectedly revealed a nonsignificant trend towards higher acquisition rate of HIV in Ad5-positive men, raising concerns that Ad5 vaccination in previously Ad5-seropositive individuals may enhance the risk of acquiring HIV infection [110].

Protein–adjuvant vaccines

Protein-based subunit vaccines can be produced to high levels of purity in bulk, but require adjuvants in order to induce a potent and durable immune response. Adjuvants may be antigen-delivery systems, such as aluminum-based adjuvants or emulsions, or have immunopotentiating properties, such as Toll-like receptor ligands, saponins, cytokines and bacterial toxins [111]. Only aluminum-based adjuvants are licensed for widespread human use and these induce humoral immunity [112]. The recent development of a number of candidate immunostimulant adjuvants that induce cell-mediated immunity has made a protein–adjuvant subunit TB vaccine a feasible approach.

M72

M72 consists of a 72-kDa polyprotein formulated in the adjuvant AS02A. The polyprotein, Mtb72F, was produced by fusion of two proteins, Mtb32 and Mtb39, which were selected based on their ability to induce IFN-γ production by both $CD4^+$ and $CD8^+$ T cells in healthy, PPD-positive people [113]. Mtb72F was initially evaluated as a recombinant protein formulated in two different adjuvants, AS02A and AS01B, and as a naked DNA vaccine. These formulations, when given intramuscularly to C57BL/6 mice and guinea pigs, afforded protection against aerosol challenge with *M. tuberculosis* [113]. The recombinant polyprotein has subsequently been formulated with AS02A and designated M72. AS02A is an immune-potentiator and contains an oil-in-water emulsion and the immunostimulants 3-deacylated monophosphoryl lipid A (MPL) and QS-21, a detergent purified from the bark of *Quillaja saponaria* [114]. The leading candidate malaria vaccine RTS,S/AS02A, a recombinant malaria antigen formulated in AS02A, is safe, well tolerated and protective against Falciparum malaria in children in Mozambique and The Gambia [115]. M72, given as a boost to BCG, demonstrated improved survival in guinea pigs and NHP [116,117]. The vaccine was well tolerated in a Phase I trial of PPD^- healthy adult subjects in the USA and induced antigen-specific IFN-γ and IL-2 production and $CD4^+$ T cells [118]. Phase IIa trials in TST-positive healthy adults in a TB-endemic area (South Africa) and of different formulations in the Philippines have also been completed [213,214].

Hybrid 1

Hybrid 1 is based upon a fusion protein of immunodominant antigens 85B and ESAT-6 [119]. In murine models, Hybrid 1 was adjuvanted with dimethyl dioctadecylammonium bromide/MPL (DDA/MPL) and induced comparable protective immunity to BCG [119,120]. In a prime–boost regimen with BCG, Hybrid 1 protected guinea pigs against aerosol *M. tuberculosis* challenge [121]. Antigen 85B formulated with two different adjuvants, AS02A and DDA/MPL, also induced protective immune responses in NHP [122]. IC31® (Intercell)is a synergistic combination of a single-stranded oligodeoxynucelotide and an immunopotentiating peptide ($KLKL_5KLK$) which induces potent antigen-specific cellular immunity via the Toll-like receptor-9/MyD88 signaling pathway [123]. Hybrid 1, adjuvanted with IC31, was protective against *M. tuberculosis* challenge in neonatal and adult mouse models and guinea pigs [124,125]. In the first clinical evaluation of both fusion protein and adjuvant in a Phase I trial in PPD-negative healthy adults, 85B-ESAT-6 adjuvanted with IC31 was associated with transient vaccine-site tenderness and antigen-specific T-cell responses. The T-cell responses were maintained for 2 years [126]. Three of the 15 subjects developed positive ESAT-6/CFP-10 QuantiFERON®-TB Gold (Cellestis) diagnostic test results following vaccination. Although the false-positive results were transient in two of the three, this highlights a concern with incorporating the antigen ESAT-6 into a TB vaccine. Hybrid 1 has also been formulated with a novel liposomal adjuvant, cationic adjuvant formulation (CAF01), giving rise to durable, multifunctional Th1 T-cell responses and protection against *M. tuberculosis* aerosol challenge in mice [127–129]. CAF01 with Hybrid 1 is now in an open-label Phase I clinical trial in The Netherlands [215].

HyVac4

In view of the use of ESAT-6 in diagnostic tests, the immunodominant antigen, TB10.4, was substituted for ESAT-6 in a fusion protein with antigen 85B. This candidate vaccine (HyVac4), adjuvanted with MPL, showed improved protection over 85B-ESAT-6 in mice, associated with $CD4^+$ T-cell production [130,131]. HyVac4 formulated in IC31 and given in a prime–boost regimen with BCG was immunogenic and offered enhanced protection to *M. tuberculosis* aerosol challenge over BCG in guinea pigs [132]. HyVac4 with IC31 (AERAS-404) is currently in ongoing Phase I clinical trials [208].

Therapeutic vaccines in clinical trials

Mycobacterium vaccae

An inactivated whole-cell strain of *Mycobacterium vaccae* was developed initially as a therapeutic TB vaccine candidate [133]. Variable results have been obtained in different geographical locations. There was no difference between treatment and placebo groups in a double-blind RCT in South Africa [134]. A single dose of *M. vaccae* in patients with smear-positive TB in addition to standard chemotherapy in Zambia and Malawi was well tolerated but had no effect on mortality or bacteriological results in the HIV-infected population, and only a trend towards benefit

in the HIV- population [135]. In HIV-negative, sputum-positive TB patients, a single dose in Uganda and a triple-dose regimen in Argentina were both associated with faster bacteriological and radiological improvement compared with placebo [136,137]. *M. vaccae* has since been evaluated as a prophylactic vaccine. One RCT of five doses of *M. vaccae* in BCG-vaccinated, HIV-infected patients in Tanzania demonstrated significant protection against the secondary end point of definite (culture positive) TB, although not against the primary end point of disseminated (bacteremic) disease or against the other secondary end point, probable TB [138].

RUTI®

RUTI® (Archivel Farma) is a candidate therapeutic vaccine based upon detoxified liposomal cellular fragments of *M. tuberculosis* bacilli. In a double-blind Phase I RCT in BCG-naive healthy men in Spain, RUTI was well tolerated and associated with modestly enhanced responses to PPD and mycobacterial antigens, including ESAT-6 and 85B [139]. The next stage in the development of this vaccine would be its evaluation in subjects infected with *M. tuberculosis*.

Expert commentary & five-year view

An effective TB vaccine is an essential component of global TB control strategies. Clinical trials evaluating the efficacy of the existing vaccine, BCG, show that effective and durable vaccination against TB is possible. However, its contraindication in immunocompromised individuals and heterogenous efficacy limit its effectiveness. Improvements in the methodology of BCG administration, modifications to the vaccine itself or enhancement of its effectiveness with a novel TB vaccine are all avenues under current clinical evaluation. To date, no clear conclusions regarding BCG immunization methods, which would warrant changes to the current policies, can be drawn.

A number of TB vaccine candidates are now in clinical trials. In Phase I studies, the primary end points are local and systemic safety. New candidates are first evaluated in mycobacterially naive, then BCG-vaccinated young adults before being tested in adults living in endemic areas. For TB vaccines, there is a particular emphasis on early testing in high-risk groups, such as HIV infection, LTBI and infants as well as dose-escalation studies and studies of different formulations and regimens. Phase I and IIa trials also provide an opportunity to perform detailed immunological assays in small numbers of subjects as a secondary end point. Whilst the development of novel exploratory assays remains important, a single, simple, harmonized assay would allow comparisons between candidates to be made [52]. As it would not be possible or ethical to test every new TB vaccine candidate in large-scale efficacy trials, there is an urgent need for validated preclinical and clinical models to evaluate the vaccine candidates currently in advanced preclinical development and early stage clinical testing. Efficacy trials will, however, improve our understanding of the applicability of animal models and immunological biomarkers [140].

The most advanced candidates are now progressing to efficacy trials in high-burden countries. The implementation of trial sites for efficacy testing poses a major challenge to investigators. Phase IIb and III trials require a robust clinical and laboratory environment with established field resources, where extremely large cohorts may be enrolled and followed up for lengthy time periods [83]. The most advanced clinical trial site with the capacity to perform Phase II and III TB vaccine trials is in Worcester, South Africa and is run by the South African TB Vaccine Initiative of the University of Cape Town. A newer site also being used for efficacy trials is in Kisumu, Kenya at the Kenya Medical Research Institute/CDC field station. There is a major requirement for new TB vaccine trial sites, particularly within the African and Asian continents, to allow multicenter Phase III licensure trials of candidate vaccines. Capacity building of new sites includes documenting the population characteristics, including the incidence of TB cases and other infections and developing clinical, laboratory and field resources [82,141]. Specialist regulatory experience from developed countries will need to be combined with specialist TB knowledge in high-burden countries where studies will be conducted. Local procedures for regulatory and ethical approval and monitoring of clinical trials need to be implemented. Local factors that can impact on the capability to conduct clinical trials include language, cultural perceptions, power and technology and economic instability. General considerations in efficacy study design also involve identifying a viable placebo immunization and the need for validated immunological assays. No assay yet meets the requirements for licensure trials of being discriminatory, sensitive, specific and reproducible [82].

An improved TB vaccine regimen is an essential part of achieving effective global TB control and realizing the Stop TB targets. While it may be possible to enhance the effectiveness of BCG by modifications to the current regimen, it is likely that alterations to the BCG vaccine itself or a novel vaccine that can be given as a boost to BCG will be required. Preclinical TB vaccine development is focusing upon identifying additional vaccine targets and adjuvants and validating animal models of TB. Novel candidates, adjuvants and mucosal vaccination routes are likely to be evaluated in forthcoming Phase I clinical trials. Taking TB vaccine candidates to advanced stages of clinical evaluation will rely upon the identification of immunological correlates of protection and the further development of field sites.

Financial & competing interests disclosure

Helen McShane is a named inventor in a patent filing related to MVA85A and is a shareholder in a joint venture, Oxford Emergent Tuberculosis Consortium, formed for the future development of this vaccine. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

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Key issues

- The existing TB vaccine, bacille Calmette–Guérin (BCG), is only partially effective and is contraindicated in HIV infection.
- A novel TB vaccine or regimen is a key component of the Stop TB Strategy.
- The target population for a TB vaccine are adults, adolescents, children and infants in high-burden countries including those with HIV and latent TB infection.
- Ongoing clinical trials of BCG are evaluating modifications to the current vaccination methods.
- Prophylactic TB vaccines in clinical trials are live mycobacterial vaccines designed to replace BCG or subunit vaccines designed to boost BCG.
- The most advanced candidate vaccines are currently in Phase IIb trials.
- Clinical development of a new TB vaccine is dependent upon validation of animal models for safety, toxicology and efficacy testing and the identification of biomarkers of protection.
- Field sites in high-burden countries are required for large-scale efficacy and licensure trials.

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