Moving tuberculosis vaccines from theory to practice

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Abstract | Tuberculosis (TB) vaccine research has reached a unique point in time. Breakthrough findings in both the basic immunology of *Mycobacterium tuberculosis* infection and the clinical development of TB vaccines suggest, for the first time since the discovery of the *Mycobacterium bovis* bacillus Calmette–Guérin (BCG) vaccine more than a century ago, that a novel, efficacious TB vaccine is imminent. Here, we review recent data in the light of our current understanding of the immunology of TB infection and discuss the identification of biomarkers for vaccine efficacy and the next steps in the quest for an efficacious vaccine that can control the global TB epidemic.

Incipient TB
A state of *Mycobacterium tuberculosis* infection in which the host is likely to progress to active tuberculosis (TB) disease but has not yet manifested clinical symptoms, radiographic abnormalities or microbiological evidence of active disease. Can be detected using transcriptomic or proteomic biomarkers of inflammation.

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Tuberculosis (TB) is among the top ten causes of death overall and is the leading cause of death owing to infection with a single type of pathogen[1]. It is estimated that almost one-quarter of the global population, between 2 billion and 3 billion people, has been infected with *Mycobacterium tuberculosis* (Mtb) and may be at risk of progression to TB[2]. Up to 80% of the lifetime risk of progression to disease is thought to occur in the first 2 or 3 years after infection[3]. In 2017, an estimated 10 million people developed TB and 1.6 million people died from the disease[1].

It is now appreciated that Mtb infection does not represent a single, uniform state and that the historical division of TB into either latent (latent TB infection (LTBI)) or active TB has gravely underappreciated the complex and dynamic nature of the host–pathogen interactions[4–6]. Progression from Mtb infection to clinical disease appears to transition via a number of continuous asymptomatic infection states that have previously been classified as LTBI, which include the asymptomatic states recently termed incipient TB and subclinical TB, before active, clinical TB disease manifests[7,8]. Further, within an individual host, Mtb-infected lesions in the lung or draining lymph nodes do not develop in a uniform synchronized manner but independently and therefore represent a spectrum of pathology that can span all stages from sterile, calcified granulomas through to caseous, necrotic lesions with exceedingly high bacterial burdens[9]. It is important to consider both the complexity of the human–Mtb interactions and the magnitude and diversity of the global epidemic as a backdrop to the challenges faced by vaccine development. Given that in some high incidence areas the majority of the adult population is infected, classic prophylactic vaccination (before any exposure to the pathogen) is applicable for only a proportion of the individuals in need. Therefore, TB vaccine development efforts are currently focused on developing vaccines for the following administration regimens: prophylactic vaccination, which is a vaccine administered to individuals in order to prevent Mtb infection or clinical disease (prophylactic vaccines can be either priming vaccines such as *Mycobacterium bovis* bacillus Calmette–Guérin (BCG) (see BOX 1) used in neonates or booster vaccines for later administration); postexposure vaccination, which is a vaccine administered to Mtb-infected individuals to prevent the development of active disease (many priming and booster vaccines are currently developed for postexposure administration owing to their ability to boost and supplement the naturally occurring infection-promoted responses); and therapeutic vaccination, which is a vaccine administered to individuals with clinical disease in combination with or after antibiotic treatment to prevent recurrence of disease. Development of both prophylactic and postexposure vaccines is actively being pursued with several candidates in clinical trials, but therapeutic vaccines are also receiving increasing attention.

Five years ago, the results of a large phase IIb efficacy trial of the first TB booster vaccine candidate tested in infants were published. Vaccination of 4–6-month-old infants, who had received neonatal BCG vaccination, with MVA85A induced no additional protection against Mtb infection or active TB beyond that observed in the placebo arm of the study[10]. This result was a great disappointment to both the TB vaccine research community and funders and a call to action for researchers in basic and applied vaccine development. Today, we are witnessing immense progress in both preclinical and clinical TB vaccine research, including the first proof-of-concept study showing that revaccination with BCG can protect adolescents from sustained Mtb infection[11] and that the subunit vaccine M72/AS01$_E$ provides protection against the development of TB disease in Mtb-infected adults[12]. Here, we discuss recent breakthroughs

Box 1 | **The BCG vaccine and variation in its efficacy**

Mycobacterium bovis bacillus Calmette–Guérin (BCG) has been in use for more than 80 years and is the world's most widely administered vaccine. BCG is the collective name for multiple daughter strains of an attenuated strain of *Mycobacterium bovis*[88] (the pathogen responsible for tuberculosis (TB) in cattle) that lacks the genetic segments encoding a number of virulence factors and important T cell antigens, such as ESAT6 and CFP10, which are part of the ESX1 secretion system[89]. Vaccination with BCG results in a localized and self-limiting infection that exposes the immune system to a complex antigen repertoire including mycobacterial proteins, lipids and glycolipids and induces both conventional MHC-restricted and unconventional T cell responses as well as antibody responses and trained immunity[90–92]. Vaccination with BCG consistently provides a high degree of protection in infants and toddlers against highly lethal meningeal TB and miliary TB[93] and appears to last 10–15 years[94], although a small number of studies show much longer persistence of protective effects, up to 40 or 50 years after vaccination[95,96]. However, in the adult population, BCG vaccination provides only highly variable protection against pulmonary TB, ranging from 0% to 80%, which appears to correlate with geographical latitude (vaccination efficacy is poorest in the regions closest to the equator) and may be related to immunological sensitization to atypical, non-tuberculous mycobacteria, which are particularly abundant in tropical environments[97]. A recent systematic review of randomized controlled trials of BCG vaccination shows that prior infection with *Mycobacterium tuberculosis* (Mtb) or sensitization with environmental mycobacteria is associated with a reduced efficacy of BCG vaccination against pulmonary TB[98]. Environmental mycobacteria are likely to induce a low level of anti-Mtb immunity owing to homology between species within the genus *Mycobacterium* that can mask or block the replication of BCG necessary for sufficient induction of immunity[76,99] (FIG. 2). There are hundreds of atypical mycobacteria with different levels of cross reactivity with BCG and with different geographical distributions[100,101]. Furthermore, the focus on these environmental mycobacteria as a reason for the sensitization has distracted the focus from Mtb infection as a source of prior immune sensitization with strong effects on BCG efficacy. In geographical regions with a high prevalence of mycobacteria, where the lowest efficacy of BCG has been reported, Mtb infection represents a very significant source of sensitization, with 50–80% of the population showing immunological sensitization suggestive of Mtb infection[98]. Despite decades of interest in the effects of environmental mycobacteria on BCG vaccine efficacy, this issue remains challenging to investigate given the large number of species in different parts of the world. The availability of well-curated and standardized antigen preparations from different atypical mycobacteria should be prioritized to facilitate such research.

Subclinical TB
A state of *Mycobacterium tuberculosis* infection in which the host has radiographic abnormalities or microbiological evidence of active tuberculosis (TB) disease but has not yet manifested clinical symptoms of active disease.

Priming vaccines
Vaccines that mediate sensitization or stimulation of an immune response with antigen for the first time; that is, the vaccines prime the immune response.

Booster vaccines
Vaccines that are typically given after an earlier priming vaccine and further stimulate an immune response that already exists to an antigen to increase the response magnitude or modulate the function of the response; that is, the vaccines boost the pre-existing immune response.

in our understanding of the mechanism of protective immune responses, provide an overview of the vaccine candidates in clinical trials and discuss whether it is time to reconsider BCG revaccination as part of a future improved TB vaccine strategy.

Immune responses to Mtb

Mtb can establish infection in susceptible individuals after the inhalation of a single or a few bacteria that are taken up by alveolar macrophages. The pathogen has developed a refined set of evasion mechanisms that delay bacterial transport to the regional lymph node and allow it to evade host cellular immunity, giving it sufficient time to establish a productive infection[13–15]. The result is a delayed onset of the natural adaptive immune response observed both in animal models of TB[16] and in human clinical TB[17,18].

One of the primary roles of vaccination is to establish efficient and long-lived immune memory in order to shorten the interval between infection and the onset of an adaptive immune response at the site of infection, such that the infection can be controlled rapidly and spreading to secondary sites is avoided. In humans, the first exposure to Mtb typically occurs after the immune response has been primed by other mycobacterial encounters, either in the form of BCG vaccination or environmental mycobacteria. As a result, TB vaccine strategies should consider how prior induction of T cells (and other immune responses) by these exposures may influence the function, trafficking and survival of vaccination-induced responses and their effectiveness against Mtb. Furthermore, in settings with very high rates of Mtb infection, such responses must be able to resist the effects of repeated reinfection and long-term continuous antigen exposure from this chronic infection.

The role of CD4⁺ T cells. Recent data in animal models suggest that vaccine-induced CD4⁺ cells of the T helper 17 (T_H17) cell subtype, which naturally traffic to the airways, can accelerate the recruitment of protective T_H1 cells[19–21]. In fact, a recent study of a rhesus macaque model of pulmonary vaccination showed that vaccination with BCG induces pulmonary T_H1 cells and/or T_H17 cells, which co-express IFNγ, TNF, IL-2 and IL-17 and protect against infection upon repeat low-dose challenge with Mtb[22]. When CD4⁺ T cells arrive at the site of infection, they encounter aggregates of Mtb-containing macrophages and other immune cells and together form the tight cellular structure referred to as the granuloma. The CD4⁺ T cells secrete cytokines, which activate infected macrophages to control bacterial growth and attract more immune cells to the granuloma (reviewed elsewhere[23]).

Most vaccine research has focused on T_H1 cells and the effector cytokine IFNγ as a readout for successful vaccination and a potential indicator of vaccine efficacy. However, it is clear from animal studies[22,24,25] that expansion beyond the narrow focus on IFNγ is necessary to identify new biomarkers (also called immune correlates of protection (COP); see BOX 2) to support vaccine evaluation and optimization. This is also supported by conflicting data on the role of IFNγ in human studies. A study of COP in the participants of the MVA85A phase IIb trial suggested that higher frequencies of BCG-reactive IFNγ-secreting cells, as quantified by ELIspot assay, were associated with a reduced risk of developing TB[26]. By contrast, the MVA85A booster vaccine referred to above induced long-lived CD4⁺ T cells that co-expressed IFNγ, TNF and IL-2 (REF.[27]), a functional subset of cells termed polyfunctional by many in the field, but this subset did not afford protection[10]. Similarly, in a study of 10-week-old infants that were vaccinated with BCG at birth, there was no association between the frequencies of BCG-reactive T_H1 cells or the co-expression patterns of IFNγ, TNF and IL-2 of these cells and subsequent risk of developing TB[10,26–28]. Collectively, these studies suggest that T_H1 cell responses are necessary but not sufficient to mediate protection against Mtb and that other functions and characteristics of T cells, and perhaps other arms of immunity, are involved in protection against TB.

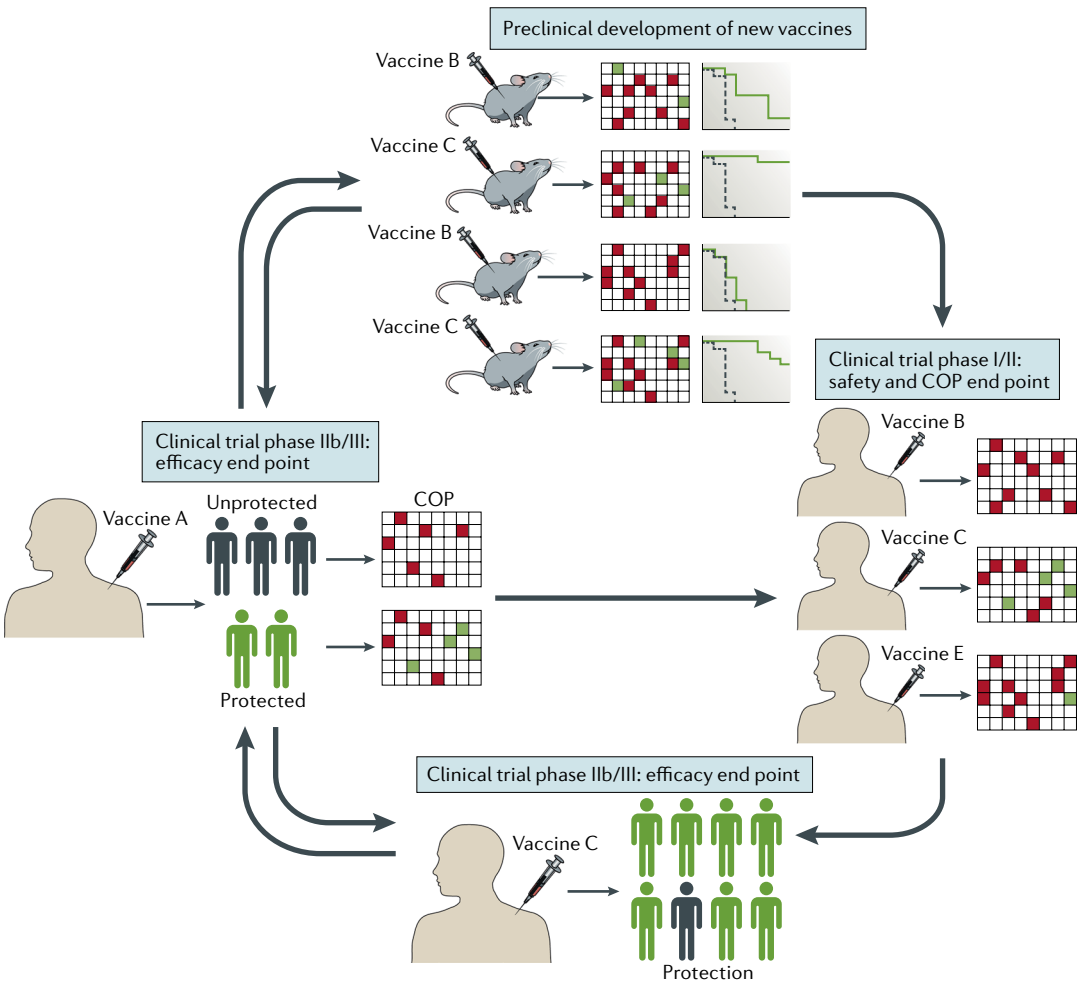
Recently, the expression of CD153, a surface molecule of the TNF superfamily that is expressed by Mtb-specific CD4⁺ T cells during infection, was suggested as a promising marker of protection in animal models. CD153 was also found to be expressed by Mtb-specific

Box 2 | **Immune correlates of vaccine protection**

The discovery of vaccine-associated correlates of protection (COP) is possible only if samples from successful placebo-controlled efficacy trials of a tuberculosis (TB) vaccine are available. This is because COP discovery requires the comparison of immune responses in vaccinated and unvaccinated individuals who are protected against *Mycobacterium tuberculosis* (Mtb) and in those who are not protected against Mtb (for example, individuals with active TB)[102,103]. The lack of such COP has been a major limitation in TB vaccine development. A robust and validated COP could predict vaccine efficacy and allow significantly more efficient testing of vaccine candidates while facilitating the rational design of improved vaccines and strategies[104]. The exciting efficacy signals provided by the phase IIb trial of the subunit vaccine H4:IC31 (REF.[11]) versus *Mycobacterium bovis* bacillus Calmette–Guérin (BCG) revaccination and the phase IIb trial of the subunit vaccine M72/AS01$_E$ (REF.[12]) bring about a new phase in TB vaccinology, as they unlock the possibility of identifying vaccine-associated COP against Mtb infection or TB disease.

In the phase IIb trial of MVA85A in infants, the frequency of HLA-DR$^+$ CD4$^+$ T cells was identified as a correlate of risk of progression to TB, and the level of Ag85A-specific IgG and frequencies of BCG-reactive IFNγ-secreting cells were identified as correlates of non-progression[26], demonstrating that the identification of COP is possible. The figure shows a suggested framework for utilizing newly identified immunological correlates of protection in TB vaccine development. Prospective validation of these COP in future efficacy trials (phase IIb/III) is necessary to confirm their validity as true predictors of vaccine protection (bottom). Translation of the COP to appropriate and highly tractable animal models of TB vaccination (for example, non-human primates and mice), which should be done alongside infectious challenge studies to confirm in vivo protection, would allow the discovery of the mechanistic underpinnings of protective immunity against Mtb (see the figure, top) and faciliate the preclinical selection of vaccine candidates. Measurement of such COP in phase I or II clinical trials of novel vaccine candidates could also be used to accelerate selection of candidates for efficacy trials (see the figure, right). Similarly, the identification of COP will facilitate a more rapid and cheaper readout of efficacy in diverse geographical or epidemiological settings for vaccine candidates for which evidence of efficacy may be limited to one setting.

In the trials of H4:IC31–BCG and M72/AS01$_E$ discussed above[11,12], blood samples for the discovery of COP were collected and stored, and a consortium has been established to develop and execute the analyses. Universal application of newly identified COP may ultimately be more challenging than many expect; it is quite possible that mechanisms of protection induced by distinct vaccines are different or that the translation between species is problematic. Ultimately, newly discovered COP would require prospective validation in future efficacy trials to confirm their validity as predictors of vaccine protection, known as surrogates of protection (reviewed elsewhere[102]).



Correlates of protection
(COP). A measurable feature, often a functional characteristic of an immune response, that associates with protection against becoming infected and/or developing disease.

ELISpot assay
(Enzyme-linked immunosorbent spot assay). A type of immune assay that quantifies the frequency of protein-secreting single cells on the basis of enzyme-linked detection of protein spots on immune-absorbent membranes.

Inducible bronchus-associated lymphoid tissue
A tertiary lymphoid structure that consists of lymphoid follicles in the lungs or bronchus and that is a site for priming immune responses.

$CD4^+$ T cells in humans who successfully control Mtb infection[29]. This molecule is an example of the new candidate biomarkers of protective immunity that should be considered in analyses of immune COP in the context of vaccine trials.

The role of CD8⁺ cells. Mtb-specific $CD8^+$ T cell responses increase during disease progression with kinetics that appear to positively correlate with the bacterial burden[30,31], but their role in protective immunity to Mtb is unclear. Evidence that $CD8^+$ cells may play a protective role comes from a non-human primate (NHP) study in which $CD8^+$ T cells were depleted, which resulted in compromised BCG vaccine-induced immune control of Mtb[32]. However, recent data from murine models suggest that even very high numbers of vaccine-induced $CD8^+$ T cells that are specific for antigens involved in protective immunity fail to recognize Mtb-infected macrophages or affect Mtb proliferation in animal infection studies[33,34]. Similarly, antigen-specific $CD8^+$ T cells induced by an adenovirus-based TB vaccine in humans failed to recognize Mtb-infected dendritic cells in vitro[35]. Although the subject of numerous studies, the role of $CD8^+$ T cell responses in protective immunity against TB therefore still remains unresolved.

The role of B cells. Antigen-specific antibodies, and their functional attributes in immunity against Mtb, have recently received increasing attention. Compelling evidence shows marked differences in the plasma levels of natural mycobacteria-specific IgG or IgA and their glycosylation profiles and Fc functions when comparing individuals with LTBI and patients with active TB[36,37]. Further, a recent study of a rhesus macaque model of pulmonary BCG vaccination showed that high levels of antigen-specific IgA in bronchoalveolar lavage fluid were associated with protection against Mtb infection and disease[22].

Mtb escape mechanisms. In addition to delaying the onset of adaptive immune responses, Mtb has also evolved a set of escape mechanisms aimed at inhibiting $CD4^+$ T cell activation during later stages of the infectious process[38]. Examples include the downregulation of certain target antigens to very low levels[39] and the active transfer of immunodominant antigens to uninfected bystander dendritic cells and macrophages[40]. The outcome of this host–pathogen stand-off is bacterial survival in a state of latent infection. It is notable that established latent infection seems to provide significant protection against reinfection in classical epidemiological studies[41,42] and in NHP models[43], a phenomenon that provides evidence for protective natural immunity against Mtb. We note that it is possible that immune mechanisms necessary for protection against the establishment of Mtb infection may be different from those required for successful long-term containment of an established Mtb infection such that progression to disease is averted. Ongoing studies of vaccine-induced COP, described below, will provide important insights into this.

The diversity of the CD4⁺ T cell response to tuberculosis. For protection against TB, the $CD4^+$ T cell subset is of major interest. $CD4^+$ T cells differentiate into T central memory (T_{CM}) cells that home to secondary lymphoid organs and, on the basis of their expression of adhesion molecules, most likely also to inducible bronchus-associated lymphoid tissue structures in the lung[44]. Upon antigen re-exposure, T_{CM} cells differentiate into T effector memory (T_{EM}) cells and effector cells of either the T_H1 cell or T_H17 cell lineage that migrate to and exert their effector functions in infected tissues (see FIG. 1). A proportion of these T cells subsequently remains in the lung as T tissue-resident memory (T_{RM}) cells. An efficient frontline defence in the lung depends on both T_{RM} cells localized in the lung before infection and newly recruited T effector (T_{EFF}) cells that arrive after infection. However, for a chronic infection such as Mtb, the longevity of the immune response, that is, the ability to withstand the continuous exposure to antigen for very long periods without exhaustion, is likely of equal importance. This is where T stem cell memory (T_{SCM}) cells and T_{CM} cells play a central role because their proliferative potential can maintain the supply of tissue-homing T cells. Designing TB vaccine strategies therefore requires careful consideration of the distribution of different subsets of $CD4^+$ T cells, how they respond to repeated antigen exposure during persistent infection and their ability to traffic to and be retained within the lung, both before and during ongoing Mtb infection.

In humans, Mtb infection promotes the development of $CD4^+$ T cells that span a range of differentiation states, from the most early T_{SCM} $CD4^+$ cells[45] through to fully differentiated T_{EFF} cells that predominantly express IFNγ[46–48]. Many factors are likely to determine where in this range a given specific T cell response will lie, including the expression level of the particular Mtb antigen that is targeted, the stage of disease progression or host–pathogen interaction and the location of the T cell itself. In order to allow a conclusive interpretation of results, it is therefore essential that human studies of T cell function and differentiation clearly and carefully characterize the clinical phenotype of study participants to define infection stage. Overall, an increasing mycobacterial load correlates with progressive differentiation of Mtb-specific $CD4^+$ T cell responses away from T_{CM} cells that secrete IL-2 and towards T_{EFF} cells that secrete predominantly IFNγ[46–49]. In animal models of Mtb infection, ongoing antigen exposure is a significant challenge for the host immune system and results in $CD4^+$ T cell exhaustion through inhibitory receptors such as TIM3 (also known as HAVCR2)[50] and the upregulation of exhaustion markers such as killer-like lectin receptor G1 (KLRG1)[51]. This results in the loss of self-renewing T_{CM} cell subsets and in functional impairment, eventually resulting in uncontrolled growth of Mtb[52]. In TB vaccine studies in the mouse model, it has become clear that T cell responses promoted by an adjuvanted vaccine formulation typically differ from Mtb-induced T cell responses in that they preferentially induce IL-2-producing T_{CM} cells. Compared with T_{EM} cells and T_{EFF} cells induced by continuous exposure to Mtb, these T_{CM} cells are less likely to become terminally

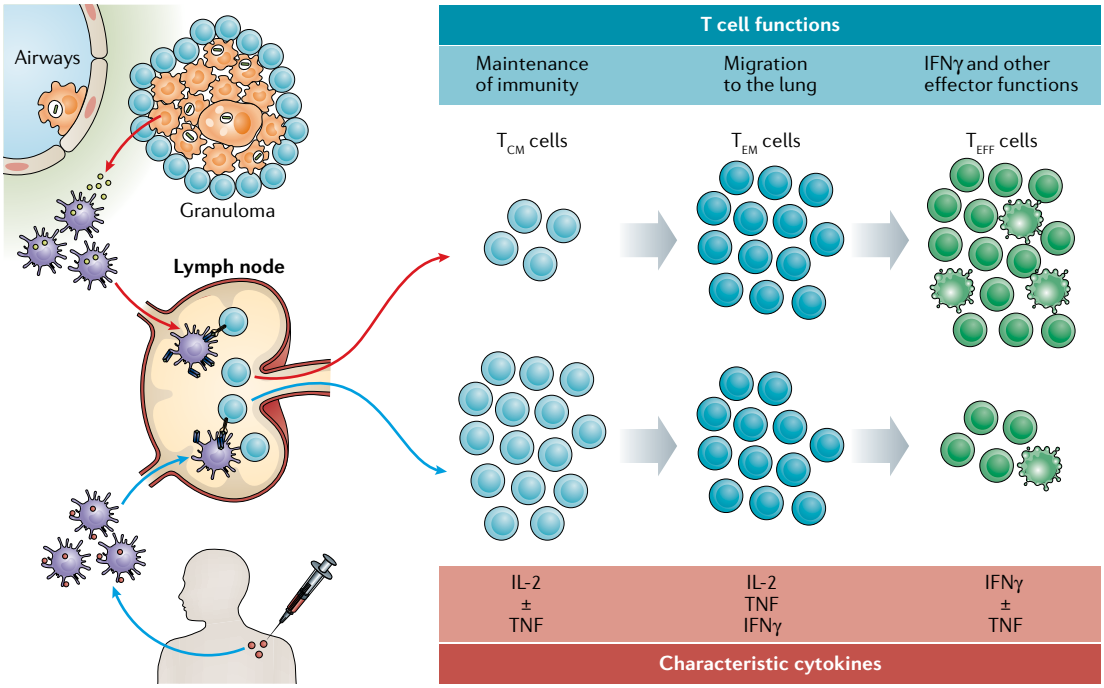


Fig. 1 | **The balance between Mtb-specific T cell differentiation into either T effector memory or T central memory cells.** After *Mycobacterium tuberculosis* (Mtb) infection of alveolar macrophages (red arrows), Mtb is transported to lung-draining lymph nodes by infected dendritic cells, initiating T cell priming or triggering the activation of pre-existing memory T cells, which appears to preferentially drive T cell differentiation towards late-stage T effector memory (T_{EM}) cell and T effector (T_{EFF}) cell responses. Ongoing antigen expression is likely a driver of this T cell differentiation process, which favours primarily IFNγ -expressing and/or TNF-expressing T cells and little IL-2 expression. Vaccine administration in the skin or muscle promotes antigen uptake by dendritic cells, which traffic to draining lymph nodes to prime or activate T cells (blue arrows). In the case of subunit protein-adjuvant vaccines, the resulting T cell responses appear to be dominated by less differentiated T central memory (T_{CM}) cell responses; these cells primarily express IL-2 and/or TNF. Achieving long-lived protective immunity by vaccination may require the establishment of a careful balance between T_{CM} cell and T_{EM} cell responses, such that a durable pool of memory cells resides in secondary lymphoid tissues while an appropriate tissue-resident population with rapid effector function is maintained in peripheral airway tissues.

differentiated, predominantly IFNγ-secreting KLRG1$^+$ T cells (FIG. 1).

Such vaccine-induced T cells therefore have the desirable ability to resist terminal differentiation, which would eventually result in functional impairment and depletion of the Mtb-specific T cell pool[48,49,52]. Animal models of TB that investigated T cell differentiation have demonstrated that BCG vaccination, similarly to Mtb infection, pushes T cell differentiation towards T_{EFF} cells, which results in a failure to efficiently maintain long-term protection against Mtb[53,54].

Initially, the main role of less differentiated CD4$^+$ T cells (such as T_{CM} cells and T_{SCM} cells) was thought to relate exclusively to their ability to resist differentiation, replenish T_{EM} cells and maintain long-lived memory both before and after infection. Recent insights into T cell migration patterns have added important new facets to this interpretation. Using an intravascular staining technique, a less differentiated memory subset of T cells, which expresses the checkpoint molecule PD1 and the chemokine receptor CXCR3, was shown to enter the Mtb-infected lung parenchyma[55]. By contrast, the more differentiated T_{EFF} cell subsets, characterized by the expression of KLRG1 and the fractalkine receptor CX_3CR1, lose their ability to enter the lung parenchyma and to protect against Mtb[55]. The negative influence of a highly differentiated and strongly T_H1 cell-polarized response on protection against Mtb is further supported by recent data from experimental animal models that investigate this question from different angles[56–58].

Choosing the best antigens. Two critical and as yet unresolved questions in TB vaccinology are how to select the best antigens and how many antigens to include in a vaccine. The retrospective analysis of the phase IIb clinical trial of the MVA85A booster vaccine, which contains a single antigen (Ag85A), highlights this question[59,60]. Given that earlier studies had shown that Ag85A is expressed at only low levels during chronic Mtb infection in mouse models[61,62], it is possible that the disappointing results were due to poor antigen choice[61,62]. Some of the most vaccine-relevant T cell antigens are virulence factors such as those associated with the ESX1 protein secretion system, which is instrumental for pathogen survival and is expressed at high levels in vivo during both the acute and chronic phase of infection[61–63]. Although one might assume that this high level of expression might indicate suitability as an immune target, immune responses to highly expressed antigens may eventually become exhausted during

chronic infection. An unanswered question is whether repeated exposure or reinfection in humans who live in high-transmission settings also drives T cell exhaustion. Interestingly, most of the immunodominant antigens are conserved, with minimal sequence variation among different clinical strains of Mtb, indicating that the induction of T cell exhaustion may be an integral part of the Mtb survival strategy[64–66]. In a direct comparison between T cell responses specific for the ESAT6 antigen (which shows high and continuous expression during Mtb infection) and Ag85B (which is primarily expressed early during infection), the different expression profiles were found to have a profound influence on the quality of the immune response. Ag85B-specific T cells developed into classical $CCR7^{+}KLRG1^{-}$ T_{CM} cells after primary Mtb infection in mice, whereas ESAT6-specific T cells were maintained in an effector state and gradually increased their expression of KLRG1 and lost IL-2 expression[54,63]. Similar differences between the differentiation state and functional capacity of human ESAT6-specific and Ag85B-specific $CD4^{+}$ T cells were also observed in healthy trial participants with LTBI who were vaccinated with the H1:IC31 or H56:IC31 vaccines, which contain both antigens[63].

These findings are in agreement with previous reports of the influence of antigen persistence and load on the distribution of different subsets of human memory T cells in clinical studies of other infections or vaccines[67]. A vaccine that provides a single exposure to an antigen followed by antigen clearance, such as the tetanus vaccine, primarily induces IL-2-producing T_{CM} cells, whereas pathogens that cause contained infections that provide low but persistent antigen exposure, such as herpes simplex virus 1, induce T_{EM} cells that express IFNγ, TNF and low levels of IL-2 (REF.[67]). T_{EFF} cells that express only IFNγ on the other hand are induced by infections such as cytomegalovirus that provide high and persistent antigen exposure[67].

Striking the balance to achieve longevity and efficacy. As discussed above, excessive induction of T_{EFF} cells by a vaccine modality or its antigen components may lead to impaired maintenance of memory and functional ability of the immune response, and, in the most extreme case, with very high expression of effector cytokines, such as IFNγ or IL-17, the result can be immunopathology[68–71]. TB vaccine strategies therefore need to strike an optimal balance between the self-renewing T_{SCM} cell and T_{CM} cell subsets and the more differentiated T_{EM} cell and T_{EFF} cell subsets that provides an efficient first-line defence in the lung (FIG. 1). This is complicated by the large proportion of individuals with LTBI (in some high-endemic regions more than 50% of the population)[2] and extensive reinfection in high-transmission settings. Individuals with LTBI have an already established T cell response and represent a challenging population in which vaccine modalities and doses intended for initial priming in naive hosts may be suboptimal[72]. A recent analysis of the literature on the pathogenesis of human TB before antibiotics were introduced furthermore suggests that immune responses required to prevent progression to reactivation TB (that is, progression of established LTBI to active disease) are likely to be different from those required to control or prevent the establishment of the primary infection[9]. This follows from the argument that, in the case of progression to reactivation TB, a successful immune response would need to protect against tissue damage and cavitation. A recent interim analysis of the ongoing phase IIb trial of the subunit vaccine $M72/ASO1_{E}$ as a postexposure vaccine, which showed a vaccine efficacy of 54% against progression to TB relative to placebo[12], clearly shows that efficacious vaccine modalities in pre-sensitized populations are possible. Delineating the functional, phenotypic and differentiation characteristics of T cell responses induced by the two antigens in this vaccine (Mtb32A and Mtb39A) will be critical for our understanding of immune COP against TB.

Tuberculosis vaccines in clinical trials

The development of an efficacious TB vaccine strategy relies on a healthy pipeline of TB vaccine candidates that represent a diverse repertoire of formulations and mycobacterial antigens and that induce a broad range of immune responses with different characteristics. Eleven TB vaccine candidates (TABLE 1) are currently in clinical testing for prophylactic, postexposure or therapeutic indications.

Whole cell vaccines — live. Live, attenuated whole cell vaccines were initially developed as prophylactic, priming vaccines with the aim to replace BCG-prime vaccination in infants, but they are now also being assessed as postexposure vaccines in adolescents and adults. Two of these vaccines, the recombinant BCG vaccine VPM1002 and the live, attenuated Mtb vaccine MTBVAC, are currently in clinical trials. Both induce a complex and diverse immune response to many antigens, which may offer an advantage over subunit vaccines that have a response restricted to a few antigens. However, such live vaccines are likely to be subject to the same interference caused by prior immunological sensitization by non-tuberculosis mycobacteria (NTMs) as reported for BCG. VPM1002 is also being assessed as a postexposure vaccine for the prevention of recurrence of active TB.

Whole cell vaccines — inactivated. On the basis of a classical vaccine development paradigm, these products, which include RUTI, *Mycobacterium vaccae*-based vaccines and the *Mycobacterium obuense*-based DAR-901 vaccine, utilize killed whole mycobacterial cells or mycobacterial cell extracts to safely induce complex immune subsets against multiple Mtb antigens. RUTI and *M. vaccae*-based vaccines are primarily being pursued as therapeutic vaccines, while DAR-901 is being developed as both a prophylactic and a therapeutic vaccine.

Adjuvanted protein subunit vaccine. Subunit vaccines are based on protein antigens administered with adjuvants. These are primarily developed as prophylactic or postexposure vaccines that boost responses that were initially primed by BCG or Mtb infection for preventing the establishment of Mtb infection, active TB or recurrent disease. Subunit vaccines that are currently in

Reactivation TB
Also known as post-primary tuberculosis (TB) or secondary TB; TB that typically occurs months to years after the initial infection and is associated with distinct disease manifestation compared to primary TB. Reactivation frequently occurs in the setting of weakened immunity and usually involves the lung apex.

Cavitation
The formation of a cavity in the centre of a tuberculosis (TB) nodule or area of consolidation, usually in the upper lung or apex. Cavities may be detected by chest radiography or computed tomography and are a characteristic feature of post-primary or adult type TB.

Table 1 | **Tuberculosis vaccine candidates that are currently in clinical trials or have recently completed clinical trials**

Candidate and developers	Antigens (genes and function), vector or formulation	Mode of immunization	Vaccine-induced T cell response	Vaccine-induced antibody response	Efficacy	Development status	Refs
Whole cell vaccines — live							
VPM1002; Max Planck Institute, Vakzine Projekt Management, TBVI, Serum Institute of India	Recombinant BCG (BCG ΔureC::hly: expresses the listeriolysin gene to promote lysosome escape, while the urease C-encoding gene *ureC*, which reduces acidification of the phagosomal compartment, has been deleted)	Prophylactic, postexposure and therapeutic	$CD4^+$ and $CD8^+$ T cells expressing different combinations of IFNγ, TNF or IL-2; unusual subset of IL-17-expressing $CD8^+$ T cells	Not reported	NA	Phase II completed; phase III trial in newborn babies to commence soon	105,106
MTBVAC; Universidad de Zaragoza, BIOFABRI, TBVI	Live, attenuated Mtb vaccine with two independent and stable deletions in genes encoding the virulence factors *phoP* and *fadD26*	Prophylactic and postexposure	$CD4^+$ and $CD8^+$ T cells that express IFNγ, TNF and IL-2	Not tested	NA	Phase II trials in adults and newborn babies ongoing (ClinicalTrials.gov NCT02933281 and NCT03536117)	(Tameris et al., manuscript in preparation) 107–109
Whole cell vaccines — inactivated							
RUTI; Archivel Farma	Detoxified, fragmented Mtb cells delivered in liposomes	Therapeutic	IFNγ-expressing $CD4^+$ T cells directed to different purified mycobacterial antigens	No changes observed in IgG responses to 16 kDa or 38 kDa antigens	NA	Phase II completed	110,111
M. vaccae-based vaccines; Anhui Zhifei Longcom	Whole cell, heat-killed *M. vaccae*	Therapeutic	Not reported	Not reported	Not known	Phase III results expected in 2019 (ClinicalTrials.gov NCT01979900)	
DAR-901; Dartmouth, Geisel School of Medicine, Global Health Innovative Technology Fund	Whole cell, heat-inactivated *Mycobacterium obuense*, a non-tuberculous mycobacterium closely related to *M. vaccae*	Prophylactic, postexposure and therapeutic	Elevated IFNγ levels and lymphoproliferative responses to stimulation with sonicated *M. vaccae*	IgG responses to lipoarabinomannan	39% (95% CI 4–61%) against TB in HIV-positive patients with $CD4^+$ count >200 cells per μl and BCG scar	Phase II; phase IIb trial of DAR-901 for prevention of infection ongoing (ClinicalTrials.gov NCT02712424)	112,113
MIP; Cadila, Indian Council of Medical Research	Whole cell, heat-inactivated *Mycobacterium indicus pranii*	Therapeutic	Not known	Not known	Not known; no efficacy against pericardial TB	Phase III	114,115
Adjuvanted protein subunit vaccine							
M72/AS01$_E$; GlaxoSmithKline, Aeras	Mtb39A (Rv0125; serine protease), Mtb32A (Rv1196; belongs to the PPE family of proteins), adjuvant consisting of liposomes, monophosphoryl lipid A and *Quillaja saponaria* fraction (QS-21)	Booster, prophylactic and postexposure	Efficient induction of $CD4^+$ T cells co-expressing IFNγ, TNF and IL-2; detectable $CD8^+$ T cell responses	High-level antigen-specific IgG	54.0% (95% CI 13.9–75.4%) against pulmonary TB in IGRA-positive adults	Phase IIb ongoing (ClinicalTrials.gov NCT01755598)	12,81,116

Table 1 (cont.) | **Tuberculosis vaccine candidates that are currently in clinical trials or have recently completed clinical trials**

Candidate and developers	Antigens (genes and function), vector or formulation	Mode of immunization	Vaccine-induced T cell response	Vaccine-induced antibody response	Efficacy	Development status	Refs
Adjuvanted protein subunit vaccine (cont.)							
H4:IC31; Sanofi, Statens Serum Institut, Valneva, Aeras	Ag85B (Rv1886c; mycolyl transferase) and TB10.4 (Rv0288; ESAT family protein) and IC31 adjuvant, consisting of positively charged peptide-based particles and the non-CpG immunostimulatory oligonucleotide ODN1a	Prophylactic and postexposure	$CD4^+$ T cells co-expressing TNF and IL-2 or IFNγ, TNF and IL-2; absent or very low $CD8^+$ T cell responses	Not reported	30.5% (95% CI −15.8% to 58.3%) against sustained IGRA conversion	Phase IIb trials completed	11,117,118
H56:IC31; Statens Serum Institut, Valneva, Aeras	Ag85B (Rv1886c; mycolyl transferase), ESAT6 (Rv3875; ESAT family protein) and Rv2660c (hypothesized to be a stress-related protein and IC31 adjuvant)	Prophylactic, postexposure and therapeutic	$CD4^+$ T cells co-expressing TNF and IL-2 or IFNγ, TNF and IL-2. In Mtb-infected individuals, IFNγ, TNF and IL-2 co-expressing $CD4^+$ T cells; absent or very low $CD8^+$ T cell responses	Not reported	NA	Phase II prevention of TB recurrence trial ongoing (ClinicalTrials.gov NCT03512249)	79,119–122
ID93 + GLA-SE; Infectious Disease Research Institute, Quratis, Wellcome Trust	Rv1813 (hypothesized to be a secreted protein), Rv2608 (belongs to the PE and/or PPE family of proteins), Rv3619 and Rv3620 (ESAT6 family members) and GLA-SE (TLR4 agonist) in a squalene-in-water emulsion	Prophylactic, postexposure and therapeutic	$CD4^+$ T cells expressing IFNγ, TNF and IL-2; absent or very low $CD8^+$ T cell responses	High levels of IgG1 and IgG3 responses to Rv1813 (most immunogenic) as well as the other three antigens	NA	Phase II trial in adults with cured TB disease completed (ClinicalTrials.gov NCT02465216)	123,124
Viral vectored vaccines							
MVA85A; Oxford University, Aeras	Ag85A (Rv3804c; mycolyl transferase) and recombinant vaccinia virus	Prophylactic, prophylactic and postexposure	$CD4^+$ T cells co-expressing IFNγ, TNF and IL-2; absent or very low $CD8^+$ T cell responses	Not reported	17.3% (95% CI −31.9% to 48.2%) against TB disease; −3.8% (95% CI −28.1% to 15.9%) against IGRA conversion	Phase II aerosol administration trials ongoing (ClinicalTrials.gov NCT02532036)	10,27,125,126
Ad5Ag85A; McMaster University, CanSino	Ag85A (Rv3804c; mycolyl transferase) and recombinant adenovirus serotype 5	Prophylactic and postexposure	$CD4^+$ and $CD8^+$ T cells expressing T_H1-type cytokines	Not reported	NA	Phase I/II trials ongoing (ClinicalTrials.gov NCT02337270)	127

BCG, *Mycobacterium bovis* bacillus Calmette–Guérin; IGRA, IFNγ release assay; *M. vaccae*, *Mycobacterium vaccae*; Mtb, *Mycobacterium tuberculosis*; NA, not available; TB, tuberculosis; TBVI, Tuberculosis Vaccine Initiative; T_H1, T helper 1.

clinical testing include H4:IC31, H56:IC31, ID93 + GLA-SE and M72/AS01$_E$. Some of these vaccines are also tested as therapeutic vaccines (for example, H56:IC31 and ID93 + GLA-SE) to prevent recurrence in patients who have completed chemotherapy for active TB.

Viral vectored vaccines. Live, attenuated, non-replicating viruses can be engineered to deliver genes encoding the antigens of interest into host cells. Such vaccines allow for the intracellular production of the antigen in vivo and activate cells of the innate immune

Chemotherapy for active TB
Drug-sensitive tuberculosis (TB) disease is typically treated with a 4-drug regimen of rifampicin, isoniazid, pyrazinamide and ethambutol for 2 months (the intensive phase of treatment), followed by isoniazid and rifampicin for 4 months (the continuation phase).

IFNγ release assay
(IGRA). A test for infection with *Mycobacterium tuberculosis* (Mtb) that measures IFNγ release by T cells after stimulation of blood or peripheral blood mononuclear cells with Mtb-specific peptides. IGRA conversion is an efficacy outcome in clinical trials that test prevention of Mtb infection, defined as conversion to a positive test without reversion to negative status in the next 2 consecutive IGRA tests, 3 months apart (that is, 3 consecutive positive IGRA results).

system and therefore do not need to be adjuvanted. Viral vectored vaccines are being developed as both prophylactic vaccines and postexposure vaccines that boost responses primed by BCG or Mtb infection. A potential problem with viral delivery is the induction of vector-specific immunity that can interfere with subsequent booster vaccinations. Two viral vectored TB vaccine candidates, MVA85A and Ad5Ag85A, are currently in clinical testing in prime–boost combinations, including trials of the MVA85A candidate administered by aerosol to the airways.

BCG revaccination — time to reconsider?

Revaccination with BCG at different ages, but primarily in children, was practised for decades in several countries with limited evidence for its clinical value or cost-effectiveness[73]. Two large cluster-randomized controlled trials conducted in Brazil and Malawi evaluated BCG revaccination for the prevention of TB disease. Neither demonstrated efficacy[74,75], resulting in World Health Organization (WHO) recommendations against this policy. Subsequent follow-up studies conducted as part of the REVAC study in Brazil have added a new layer of understanding to this disappointing result and suggest that prior mycobacterial sensitization is a major factor in preventing BCG revaccination efficacy in regions with a high prevalence of environmental mycobacteria exposure[76] (FIG. 2). In the recent trial with the H4:IC31 subunit vaccine or BCG revaccination in Cape Town, South Africa, BCG revaccination provided significant protection (45% efficacy) against sustained Mtb infection (measured as prevention of sustained IFNγ release assay (IGRA) conversion for a follow-up period of 24 months in healthy adolescents who received a BCG prime in infancy)[11]. The readout of prevention of sustained IGRA conversion may be interpreted in different ways, which are all indicative of protective immunity: the prevention of primary infection, the accelerated clearance of the bacilli after infection, the long-term containment of the primary infection below the IGRA cut-off level or even the prevention of reinfection during the observation period. Regardless of the mechanistic interpretation of this readout, BCG revaccination resulted in a surprisingly high efficacy signal in this study. A plausible explanation for this observation most likely relates to the fact that individuals with Mtb-specific responses at enrolment, as measured by IGRA, were rigorously excluded[11,77] and that Cape Town is thought to be an area with low to modest NTM exposure levels[78]. Compared with the REVAC study in Brazil, the main source of potential sensitization in this trial population therefore most likely came from remaining immune responses to neonatal BCG vaccination 12–16 years previously. So, even though pre-existing mycobacteria-specific T cell responses were found to be common in trial participants in the H4:IC31–BCG revaccination study, the results suggest that, without continuous high-level exposure from environmental mycobacteria or LTBI, the levels of immunity that persist from neonatal BCG vaccination are modest and do not block the efficacy of BCG revaccination significantly in adolescents (FIG. 2).

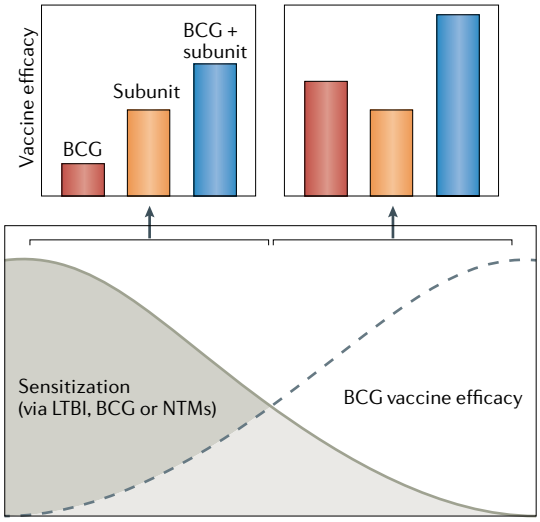


Fig. 2 | **The effects of immunological sensitization to mycobacteria on vaccine efficacy.** The hypothesized interaction between the magnitude of immune sensitization and vaccine efficacy by *Mycobacterium bovis* bacillus Calmette–Guérin (BCG) vaccination (red), adjuvanted protein subunit vaccines (orange) or a BCG-prime, subunit-boost strategy (blue) is shown. According to this model, BCG, and other live whole mycobacterial vaccines, are not efficacious in individuals with substantial prior immunological sensitization owing to latent tuberculosis infection (LTBI), recent BCG vaccination or exposure to atypical non-tuberculous mycobacteria (NTMs) from the environment. Subunit vaccination, by comparison, would be efficacious in such a pre-sensitized population, as would a BCG-revaccination, subunit-boost strategy. In persons with low or no mycobacterial sensitization, the efficacy of BCG vaccination is significantly increased, and the efficacy of subunit vaccination will be largely independent of the levels of sensitization, but the BCG-revaccination, subunit-boost strategy may provide synergistic effects that result in enhanced vaccine efficacy.

This efficacy signal therefore opens up consideration of BCG revaccination in certain settings as part of an overall improved TB vaccination strategy as previously suggested[78]. Samples collected in the H4:IC31–BCG revaccination trial provide a unique opportunity to investigate whether the level of immune sensitization to NTMs at enrolment is associated with differences in the quality of the immune responses to the vaccine and/or protection against Mtb infection.

Building on recent subunit vaccine success

For the first time since the introduction of universal BCG vaccination into the WHO expanded programme for immunization in 1974, we have encouraging efficacy signals in trials of a TB vaccine[11,12]. In a phase IIb trial of healthy adolescents who received BCG in infancy but tested negative in the IGRA, vaccination with H4:IC31 was associated with 30.5% efficacy against sustained Mtb infection. This result was of statistical significance at the pre-defined statistical threshold of an 80% CI (3.0–52.0%), but not at a more rigorous 95% CI (–15.8%

to 58.3%)[11]. Moreover, interim results of the ongoing phase IIb trial of M72/AS01$_E$, conducted in 3,575 IGRA-positive adults, clearly illustrate that booster vaccination with a subunit vaccine can protect against TB disease in highly sensitized individuals with Mtb infection. This trial, which was conducted in HIV-negative adults from Kenya, Zambia and South Africa, most of whom received neonatal BCG, accrued 10 patients with microbiologically confirmed pulmonary TB into the M72/AS01$_E$ group and 22 patients into the placebo group, translating to an overall vaccine efficacy of 54.0% (95% CI 13.9–75.4%)[12]. An important consideration when interpreting the trial results of M72/AS01$_E$ is how the level of Mtb exposure and reinfection of trial participants may have influenced the efficacy of the subunit vaccine. A recent analysis of the incubation period of TB suggested that the vast majority of clinical TB cases occur early, within 1–2 years following Mtb infection or reinfection. This is different from reactivation of Mtb, which typically happens much later after a long period of latent infection[3]. The recent efficacy trial was conducted in settings with high Mtb infection rates where reinfection with Mtb is likely to be a frequent occurrence. Understanding whether the vaccine protected against reactivation disease or progression to disease following reinfection is likely to be important and will no doubt be the subject of investigation in coming years.

Whether the levels of efficacy observed in the H4:IC31–BCG and M72/AS01$_E$ trials are sufficient and persist long enough for programmatic implementation of the vaccines in their present form is an important question, especially because the reports from each trial followed trial participants for only 2 years. However, combined into a prime–boost strategy with BCG vaccination, novel subunit vaccines may promote a robust response that could significantly add to the efficacy of the BCG vaccine and compensate for its failures in sensitized populations. In accordance with the model in FIG. 2, induction of immunity by BCG is either blocked or masked by high levels of pre-existing T cell-mediated immune responses. By contrast, comparison of H56:IC31 or M72/ASO1$_E$ vaccination in naive versus LTBI individuals indicated that these subunit vaccines can markedly boost BCG-induced and Mtb-primed immune responses[79–81]. Therefore, a combined BCG revaccination and/or subunit vaccine strategy may have great potential in adult and/or adolescent populations.

Conclusion and future perspectives

The large number of different vaccine candidates and their advanced stages in clinical development denote a unique and exciting phase in TB vaccine research. There are also a large number of novel vaccine candidates in preclinical development, including more recently developed vaccine formats such as DNA vaccines, new adjuvants and delivery systems and combination vaccines. It is important that the most promising of these new candidates are advanced to efficacy studies in animals and clinical testing to augment the pipeline of TB vaccine candidates and concepts.

However, a notable limitation of the current clinical development landscape is a lack of inter-trial harmonization or standardization, which precludes a direct comparison of the immunological outcomes of different TB vaccine candidates. A recent analysis attempted to tackle this problem by comparing antigen-specific CD4$^+$ and CD8$^+$ T cell responses induced by BCG and six of the novel TB vaccine candidates, including MVA85A, AERAS-402, H1:IC31, H56:IC31, M72/AS01$_E$ and ID93 + GLA-SE. The investigators retrieved published data on antigen-specific T cell responses from clinical trials completed in adolescents or adults at a single trial site in South Africa[82]. The results show that the magnitude of vaccine-induced T$_H$1 cell-polarized CD4$^+$ T cell responses measured several months after vaccination was the T cell response feature that diverged the most between the different candidates. Unlike the response magnitude, co-expression profiles of IFNγ, TNF and IL-2 by CD4$^+$ T cells suggested a relative lack of functional diversity in responses induced by the different vaccine candidates (see TABLE 1). Interestingly, the analyses suggested that M72/AS01$_E$ induced the highest antigen-specific memory CD4$^+$ cell response among the candidates. Unfortunately, the study did not include results from whole cell or live vaccine candidates, which are known to induce a more diverse and broader repertoire of immune responses.

Overall, the recent positive clinical trial data referred to above represent a very important milestone in international efforts to develop a novel efficacious TB vaccine. These successes illustrate that TB vaccine research is on the right track and will be able to deliver a much-needed improved vaccine strategy that is so critical for controlling the global TB epidemic. It is critical that the field moves forward with urgency towards phase III licensure trials so that an impact on the epidemic can be achieved swiftly. These findings therefore signal the end of the 'post-MVA85A period' of fundamental doubts about both the usefulness of the TB vaccine research strategy and the TB animal models in vaccine discovery[83]. The M72/ASO1$_E$ vaccine contains only two antigens, and the next generation of vaccines may be improved by adding more antigens to increase immune coverage and avoid the risk of escape. The efficacy signal observed with M72/ASO1$_E$ will likely also establish the M72/ASO1$_E$-induced protection as a minimum benchmark in preclinical animal models. As discussed above, many subunit vaccine candidates appear to induce a response that is typically characterized by early differentiated CD4$^+$ T$_{CM}$ and T$_{EM}$ cells, whereas it seems that both viral and live mycobacterial vectors promote a more differentiated CD4$^+$ T$_{EFF}$ cell response[27,52,54,82,84,85]. It will therefore be important to agree on a standard set of parameters that would allow an accurate comparison between studies and vaccines to determine whether this is a reproducible pattern in clinical trials. Recent results of preclinical studies using a recombinant human cytomegalovirus encoding several Mtb T cell antigens have shown impressive protection in an NHP model, where prophylactic vaccination prevented infection in one-third of experimentally infected rhesus macaques[86]. Because cytomegalovirus vectored vaccines establish a persistent lifelong infection and induce a high level of antigen-specific CD4$^+$ and CD8$^+$ T cell responses, these

findings suggest that, in addition to T cell differentiation as discussed above, the sheer size of the pool of Mtb-specific CD4$^+$ T cells has an impact on protection. This is also supported by the M72/ASO1$_E$ trial results, as this vaccine promotes a high frequency of antigen-specific T cells. However, more is not always better, and there is a risk of less protection and insufficient immune memory or even of inflicting immunopathology with vaccines that induce very strong T cell responses, particularly when used in the postexposure setting in individuals with LTBI. With an efficacy signal in young adolescents and/or adults from both BCG revaccination and subunit vaccine studies, it is intriguing to speculate whether the combination of both, administered sequentially or simultaneously[87], may pave the way for a vaccination strategy that protects both uninfected and infected people while providing the possibility of a synergistic effect for inducing more diverse and broader-ranging immune responses. If an additive effect can be demonstrated, the combination of BCG and subunit vaccines may represent a new strategy that elevates the efficacy signal into a range that triggers clinical implementation.

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