AMERICAN SOCIETY FOR MICROBIOLOGY | Clinical Microbiology Reviews®

REVIEW

Tuberculosis Vaccine Development: Progress in Clinical Evaluation

Suraj B. Sable,[a] James E. Posey,[a] Thomas J. Scriba[b,c,d]

[a]Division of Tuberculosis Elimination, National Center for HIV/AIDS, Viral Hepatitis, STD, and TB Prevention, Centers for Disease Control and Prevention, Atlanta, Georgia, USA

[b]South African Tuberculosis Vaccine Initiative, Cape Town, South Africa

[c]Institute of Infectious Disease and Molecular Medicine, Cape Town, South Africa

[d]Division of Immunology, Department of Pathology, University of Cape Town, Cape Town, South Africa

SUMMARY Tuberculosis (TB) is the leading killer among all infectious diseases worldwide despite extensive use of the *Mycobacterium bovis* bacille Calmette-Guérin (BCG) vaccine. A safer and more effective vaccine than BCG is urgently required. More than a dozen TB vaccine candidates are under active evaluation in clinical trials aimed to prevent infection, disease, and recurrence. After decades of extensive research, renewed promise of an effective vaccine against this ancient airborne disease has recently emerged. In two innovative phase 2b vaccine clinical trials, one for the prevention of *Mycobacterium tuberculosis* infection in healthy adolescents and another for the prevention of TB disease in *M. tuberculosis*-infected adults, efficacy signals were observed. These breakthroughs, based on the greatly expanded knowledge of the *M. tuberculosis* infection spectrum, immunology of TB, and vaccine platforms, have reinvigorated the TB vaccine field. Here, we review our current understanding of natural immunity to TB, limitations in BCG immunity that are guiding vaccinologists to design novel TB vaccine candidates and concepts, and the desired attributes

Citation Sable SB, Posey JE, Scriba TJ. 2019. Tuberculosis vaccine development: progress in clinical evaluation. Clin Microbiol Rev 33:e00100-19. https://doi.org/10.1128/CMR.00100-19.

Address correspondence to Suraj B. Sable, SSable@cdc.gov.

Published 30 October 2019

of a modern TB vaccine. We provide an overview of the progress of TB vaccine candidates in clinical evaluation, perspectives on the challenges faced by current vaccine concepts, and potential avenues to build on recent successes and accelerate the TB vaccine research-and-development trajectory.

KEYWORDS clinical trials, desired attributes, developmental trajectory, natural immunity, new TB vaccines, tuberculosis

INTRODUCTION

Vaccines are the victories of immunology. Yet the development of effective vaccines that can provide lifelong protection against three of the most deadly infectious diseases, tuberculosis (TB), human immunodeficiency virus (HIV)/AIDS, and malaria, has so far eluded vaccinologists. Pathogen evasion of the host immune response is the shared trait of these diseases. With ~1.6 million deaths annually, TB, an ancient airborne disease caused by *Mycobacterium tuberculosis*, is the top killer among all infectious diseases worldwide (1). Unfortunately, the current strategies are not enough to achieve TB elimination in this century. Increases in the number of drug-resistant TB cases and converging syndemics of TB, HIV, and type 2 diabetes warrant novel prevention and control measures (2). Without effective TB vaccines, shorter treatment regimens, and improved point-of-care diagnostics, we will not be able to end the global TB epidemic.

Historical efforts to develop an effective TB vaccine are long-standing, going back to the 1800s, yet *Mycobacterium bovis* bacille Calmette-Guérin (BCG), a partially effective vaccine developed in 1921, remains the only licensed vaccine against TB (Fig. 1). BCG is a part of the World Health Organization's Expanded Program on Immunization (EPI) and is listed on the WHO's list of essential medicines (233). Even though time-tested and most widely used, BCG has major limitations. Its efficacy against severe and extrapulmonary forms of pediatric TB is well recognized, but highly variable and poor protection at all ages against pulmonary TB remains a major concern (3). Despite the widespread use of BCG, it is estimated that around one-quarter of the world's population currently harbors a latent TB infection (LTBI) (4), and around 3 in every 1,000 people globally carry latent multidrug-resistant *M. tuberculosis* infection (5). Although LTBI is by definition clinically asymptomatic and approximately 90% of individuals with LTBI will not progress to disease, this state in the spectrum of infection is a potential source of disease reactivation and remains a major impediment to TB elimination. A new TB vaccine that has greater protective efficacy than BCG and that can prevent disease in adolescents and adults, thereby interrupting *M. tuberculosis* transmission, is essential for global TB elimination and for achieving the WHO's "End TB" goals of 90 to 95% reductions in TB cases and associated deaths by 2035 (http://www.who.int/tb/strategy/end-tb/en/). The development of a safer and highly efficacious TB vaccine therefore should hold a top-priority position on the global research agenda.

In the last 2 decades, over 20 vaccine candidates have progressed through clinical studies, and 14 are under active evaluation in clinical trials (Fig. 2). Unfortunately, several candidates will not advance through clinical development, as vaccine development has historically been an empirical process. Disappointing results, as exemplified by setbacks in the MVA85A and AERAS-422 trials (6–8), highlight our incomplete understanding of the complexity of the host immune responses to *M. tuberculosis* and challenges associated with developing a vaccine that can elicit lifelong protective immunity. These trials highlight the gap in our knowledge of the correlates of protection or biomarkers that can predict who will control infection and who will develop the disease. However, results from recent path-breaking vaccine trials (9, 10), together with recent advances in the identification of host biomarkers that have improved our understanding of the spectrum of *M. tuberculosis* infection, disease pathogenesis, and disease progression (11–17), promise that effective TB vaccines remain an attainable goal. In this review article, we discuss some of the challenges faced by current TB vaccine concepts, the progress of current vaccine candidates in clinical trials, and

1834	Johann Lukas Schönlein proposes the name "tuberculosis" for the devastating disease.
1865	Jean-Antoine Villemin demonstrates the transmissibility of tuberculosis (TB).
1882	Robert Koch discovers the causative agent of TB and names it *Mycobacterium tuberculosis* in 1883.
1884	Robert Koch expounds his famous "Koch's postulates."
1890	Koch's "therapeutic vaccine," tuberculin derived from killed TB bacilli, is found to be ineffective with extensive immunopathology ("Koch's phenomenon").
1900	Albert Calmette and Camille Guérin begin TB vaccine research at the Pasteur Institute, Lille, France.
1902	Edmond Nocard isolates a virulent *M. bovis* strain from the milk of a cow with a tuberculous udder.
1905	Robert Koch wins the Nobel Prize in medicine for his discoveries related to TB and its etiological agent.
1908	Calmette and Guérin begin subculturing Nocard's *M. bovis* strain on potato-bile-glycerin medium at roughly 3-week intervals.
1912-1920	Calmette and Guérin conduct several animal experiments. After 230 passages, they designate the attenuated bacillus "BCG" in 1920.
1921	The first infant is vaccinated with BCG by an oral route at the Charité hospital Paris by Dr. Weill-Hallé.
1924-1928	More than 114,000 infants are vaccinated with BCG in Europe and North America without serious complications.
1929-1933	Lübeck disaster: 251 neonates are accidently vaccinated with a BCG lot contaminated with *M. tuberculosis*; 173 develop clinical TB and 72 die.
1933-1935	The first organized BCG vaccine trials in Saskatchewan infants and Native American Indians, which later demonstrate long-term BCG efficacy.
1935-1940	Distribution of BCG to several countries for worldwide application. Change of route of administration to mainly via skin.
1950	Major BCG vaccine trials are started by the BMRC and USPHS. BCG shows 80% protective efficacy in the BMRC trial, and a *M. microti* vaccine shows a comparable protective efficacy.
1956	Seed lots of BCG are established under the direction of the WHO following identification of several BCG daughter strains.
1968	The Chingleput trial in South India is started; it later reveals no efficacy of BCG against adult pulmonary TB.
1974	BCG is incorporated into the WHO's Expanded Programme on Immunization (EPI).
1993	The WHO declares TB a global public health emergency, which reinvigorates TB vaccine research.
1995	Increased recognition that the efficacy of BCG against adult pulmonary TB is highly variable.
1996	The first molecular analysis of genetic differences between virulent *M. bovis* and BCG.
2000	Four strains (Glaxo, Danish, Pasteur, and Tokyo) account for 90% of the BCG vaccine administered worldwide.
2004	The WHO BCG guidelines: A single dose of BCG should be given to all healthy infants in high-TB-burden countries as soon as possible after birth.
2007	The revised WHO BCG guidelines: Infants at risk for HIV infection should no longer be vaccinated with BCG*.
2011	The BCG World Atlas reveals universal BCG vaccination in 157 countries with a coverage rate exceeding 80% of all infants on the planet.
2013	A new BCG-booster vaccine MVA85A shows no efficacy in a clinical trial in South African infants.
2015	A worldwide shortage of BCG vaccine with a deficit of more than 17 million doses.
2018	Efficacy signals are observed in phase 2b trials of M72:AS01$_E$ and BCG revaccination in Africa.

FIG 1 Timeline of key milestones in the history of tuberculosis vaccine development and human use. BCG, bacille Calmette-Guérin; BMRC, British Medical Research Council; USPHS, U.S. Public Health Service; WHO, World Health Organization; MVA85A, modified vaccinia virus Ankara vector expressing antigen 85A of *M. tuberculosis*; M72:AS01$_E$, a recombinant fusion protein of *M. tuberculosis* 39a (Rv1196) and *M. tuberculosis* 32a (Rv0125) in the AS01$_E$ adjuvant. *The WHO further updated guidelines on BCG vaccination of infants born to HIV-infected mothers in 2018. According to these guidelines, HIV-infected neonates should delay BCG vaccination until antiretroviral therapy (ART) has been started and they are immunologically stable. If HIV-infected individuals, including children, who are receiving antiretroviral therapy (ART) are clinically well and immunologically stable, they should be vaccinated with BCG. Furthermore, neonates with an unknown HIV status born to HIV-infected women should be vaccinated if they have no clinical evidence suggestive of HIV infection, regardless of whether the mother is receiving ART.

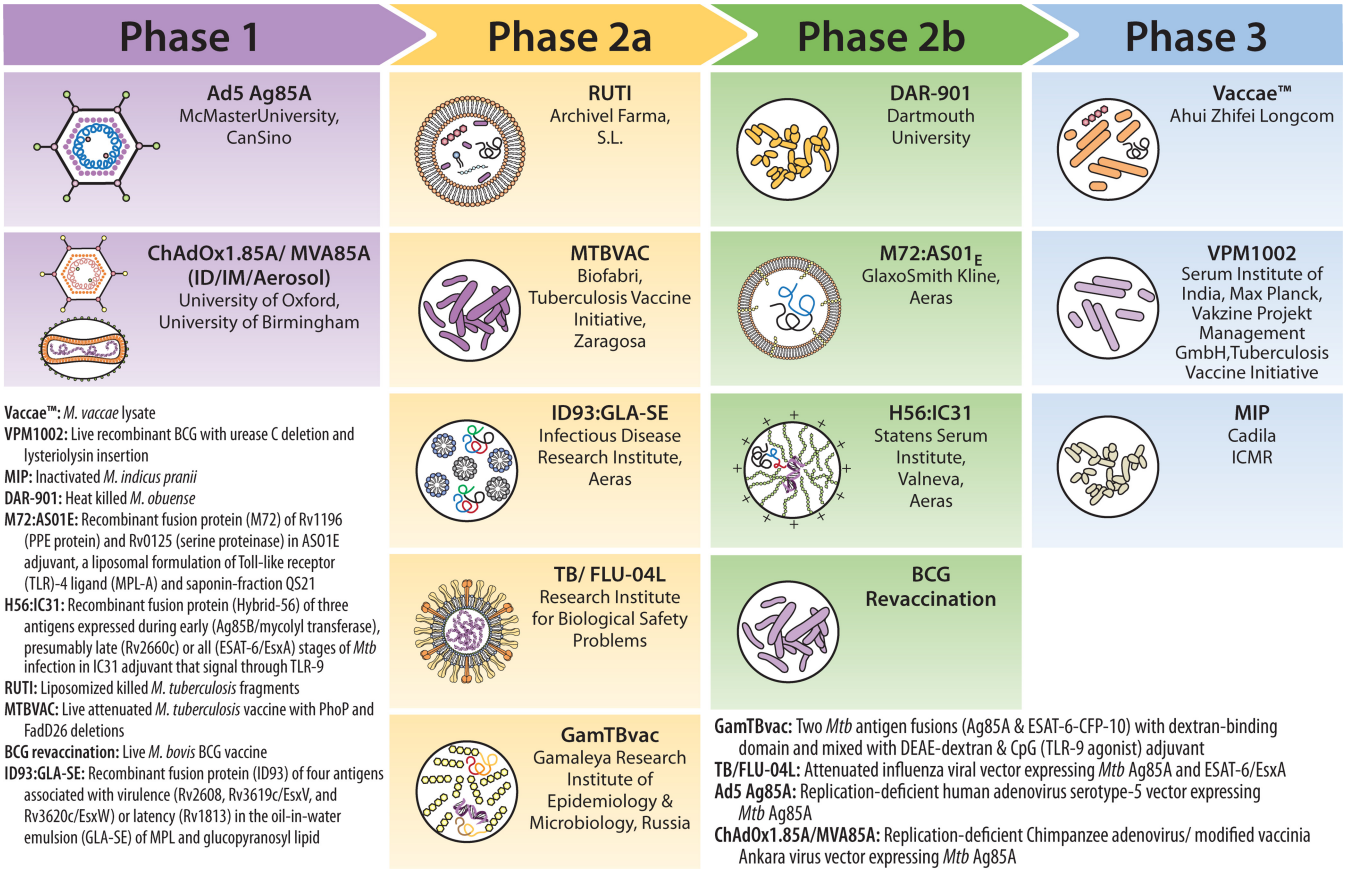

FIG 2 Current global clinical pipeline of TB vaccine candidates. The 2019 global clinical portfolio of TB vaccine candidates includes mycobacterial killed, whole-cell, or extract vaccine candidates (Vaccae, MIP, DAR-901, and RUTI); live-attenuated mycobacterial vaccine candidates (VPM1002, BCG revaccination, and MTBVAC); recombinant live-attenuated or replication-deficient virus-vectored candidates expressing an *M. tuberculosis* protein(s) (TB/FLU-04L, Ad5Ag85A, and ChAdOx1.85A/MVA85A); and a mycobacterial fusion protein(s) in an adjuvant formulation (M72:AS01$_E$, H56:IC31, ID93:GLA-SE, and GamTBvac). See the text for additional information on vaccine candidates. ID, intradermal; IM, intramuscular; *Mtb*, *M. tuberculosis*.

potential avenues to build on recent successes and accelerate the TB vaccine research-and-development (R&D) trajectory.

NATURAL IMMUNITY AND TB VACCINE DEVELOPMENT

The human immune system can contain *M. tuberculosis* infection in most cases, and only 5 to 15% of people with LTBI progress to TB disease during their lifetime (1). These figures, along with the evidence that some people remain negative by *M. tuberculosis* infection tests despite repeated *M. tuberculosis* exposures and that some individuals revert to being test negative after initially testing positive (18–21), suggest that humans can clear *M. tuberculosis* to avoid or abort infection. Furthermore, established LTBI seems to confer protection against subsequent TB disease upon reinfection in a proportion of individuals (22–24), strongly suggesting that infected humans mount protective immunity against *M. tuberculosis*, which can drive "natural-immunity-guided" vaccine development. Likewise, prior infection appears to be protective against reinfection in some animal models (25, 26). This natural immunity to reinfection, termed concomitant immunity, likely requires distinct immune responses other than the long-lived memory immune responses usually targeted by conventional vaccination strategies (27). It may also require innate myeloid cell activation elicited by prior infection or established LTBI (28). Furthermore, in the preantibiotic era, self-healing was reported for some pulmonary TB patients, suggesting that the natural immune response can successfully heal or provide long-term control of clinical disease without antibiotics in some individuals (29). Long-term immunity against *M. tuberculosis* is clearly cell medi-

ated, based on substantial experimental evidence (30). However, there is mounting evidence for a possible role of antibodies in protection (31, 32). Antibodies that may contribute to protection have recently been identified in some individuals who remain healthy despite long-term, heavy *M. tuberculosis* exposures, with or without exhibiting signs of LTBI (33, 34). These findings collectively offer potential evidence for the existence of natural immunity in those immunologically sensitized by *M. tuberculosis* infection and those not sensitized by *M. tuberculosis* infection. Although natural immunity might not be widely generalizable, it clearly can occur in some people (termed "resisters") (35) and may inform rational TB vaccine design. These resisters, who persistently test negative by *M. tuberculosis* infection tests such as the tuberculin skin test (TST) and interferon gamma (IFN-γ) release assays (IGRAs) despite heavy *M. tuberculosis* exposure, harbor antibody responses and non-IFN-γ T-cell responses to *M. tuberculosis*-specific proteins, suggesting that they may have in fact once been infected (or may still be infected) with *M. tuberculosis* (21). Therefore, understanding the immunological footprint and specificity of antigen recognition in these populations, including various high-exposure cohorts of resisters, and improved insights into *M. tuberculosis*-host biology are vital for developing an effective vaccine.

Natural Immunity against TB

M. tuberculosis is a well-adapted intracellular pathogen of human phagocytes, with lung-resident macrophages being the primary conduit of infection and immunity. Macrophages serve as the major niche for infection and as front-line enforcers of protective immunity, both innate and acquired, to control or eliminate *M. tuberculosis*. The recognition of *M. tuberculosis* by various receptors on the cell surface and within macrophages results in the release of an array of innate immune mediators (36). Mouse and human studies have shown that several innate mediators, such as vitamin D, macrophage migration-inhibitory factor (MIF), tumor necrosis factor (TNF), interleukin-1 (IL-1), IL-12, and IL-18, synergize with IFN-γ produced by innate immune cells, such as natural killer (NK) cells (37–41), to activate early antimicrobial activities in macrophages. While macrophage-derived antimicrobial resistance directly inhibits *M. tuberculosis* growth, tolerance mechanisms by macrophages protect the host from the negative impact of tissue damage. Several of these innate mediators are critical for host resistance against *M. tuberculosis*; however, their response needs to be finely balanced and tightly regulated to prevent immunopathology (42).

Bactericidal neutrophils likely also play an important part in limiting early infection, but their excessive buildup, typically seen in progressive lesions in postprimary TB, can contribute to tissue damage and need restraining (43). Innate lymphoid cells (ILCs) producing macrophage-activating granulocyte-macrophage colony-stimulating factor (GM-CSF) (44) and "unconventional" or "donor-unrestricted" T lymphocytes, such as mucosa-associated invariant T (MAIT), CD1-restricted, HLA-E-restricted, and $\gamma\delta$ T lymphocytes that reside in the interstitium, also likely play an important part in early defense upon infection (45, 46). Group 3 ILCs (ILC3s) producing IL-17 and IL-22 rapidly accumulate in the *M. tuberculosis*-infected lung; orchestrate chemokine production, which mediates the accumulation of alveolar macrophages; and induce lymphoid follicle formation. These ILC3s and ectopic lymphoid structures thus appear to participate in early protective immunity against TB (47). Airway-residing innate lymphocytes are ideally located to respond to pathogens very early, but it is unknown whether these cells play any significant role in immediate resistance following natural infection. Infection does not occur in all *M. tuberculosis*-exposed people, suggesting that natural innate immune mechanisms may clear bacteria in some individuals. However, in light of the global prevalence of humans with evidence of immune sensitization to *M. tuberculosis* (4), bacterial evasion of innate responses and the establishment of persistent infection without eradication appear to be hallmarks of *M. tuberculosis* infection.

Following the establishment of primary infection, infected dendritic cells (DCs) and recruited monocytes transport *M. tuberculosis* to draining lymph nodes (LNs), which initiate T-cell priming and acquired immunity (48, 49). The homing of activated T

lymphocytes to the site of infection in the lung interstitium contributes to granuloma structure and the control of *M. tuberculosis* infection (50, 51). T lymphocytes, in particular $CD4^+$ T helper 1 (T_H1) lymphocytes, and IFN-γ produced by these cells are necessary to control *M. tuberculosis* (30). Inherited and acquired immunodeficiency in the constituents of the IFN-γ pathway is associated with exquisite susceptibility to mycobacterial disease, validating the central role of the T_H1 axis in resistance (52). IFN-γ stimulates enhanced antimycobacterial activities in macrophages, whereas TNF synergizes with IFN-γ in restraining *M. tuberculosis* growth and maintaining granuloma architecture (30). Other $CD4^+$ T-lymphocyte subsets, notably those producing IL-17 (T_H17 cells) and IL-21, also participate in host resistance (53–55). While IL-17 aids in recruiting protective T_H1 cells at the site of infection (56), IL-21 augments the $CD8^+$ T-cell response (55). $CD8^+$ T lymphocytes that lyse infected macrophages and kill *M. tuberculosis* by producing cytotoxic molecules may contribute to protective immunity and possibly enforce long-term control (57).

M. tuberculosis infection elicits both effector and memory T cells. In people with LTBI, antigen-specific memory T cells predominantly exhibit a T_H1 phenotype and express multiple cytokines (58). Similarly, B lymphocytes produce antibodies in response to *M. tuberculosis* infection; however, a notion has long prevailed that antibodies and B cells play less significant roles in protection. This has been challenged recently by a number of studies that suggest that antigen-specific antibodies, and their functions, may contribute to immunity against *M. tuberculosis* (33, 34, 59, 60). Following this doctrine, TB vaccine research has focused largely on identifying strong IFN-γ/T_H1-inducing vaccine candidates for several years. Likewise, polyfunctional T cells that coexpress multiple T_H1 cytokines, such as IFN-γ, TNF, and IL-2, have been considered qualitatively superior to T cells expressing a single cytokine by many in the field and are being investigated with much interest in ongoing vaccine trials. However, definitive evidence that these cells are important components of protective immunity to *M. tuberculosis* is lacking (61).

Natural Immunity and Immunological Balance

Growing evidence suggests that the T_H1 axis, while necessary, is insufficient for protection. Both $CD4^+$ T-cell- and IFN-γ-independent mechanisms of protection against TB exist (62). For example, during the first year after HIV infection, the risk of TB disease increases despite mostly normal $CD4^+$ T-cell counts (63), and some simian immunodeficiency virus (SIV)-TB-coinfected macaques and HIV-TB-coinfected humans maintain LTBI without reactivation despite a severe loss of pulmonary and peripheral $CD4^+$ T cells (64). In mice, $CD4^+$ T cells can provide protection by IFN-γ- and TNF-independent mechanisms after infection or following vaccination (65, 66), and additional $CD4^+$ T-cell effector functions likely account for resistance (67, 68). Resisters, who persistently test negative for *M. tuberculosis* infection and resist the development of classical LTBI and clinical TB disease, harbor non-IFN-γ $CD4^+$ T-cell responses to *M. tuberculosis*-specific proteins, marked by high levels of CD40L/CD154 upregulation (21). These findings suggest that there are unique IFN-γ-independent mechanisms of protection in resisters that are not captured within the current clinical spectrum of disease (21). Although the T_H17 response participates in both primary and recall protection in mice (54, 56), an excess T_H17 response can also promote immunopathology and signal disease severity (69, 70). The same is true for excessive or uncontained IFN-γ expression, which can also promote immunopathology (67, 71). Pathological T_H17 responses may be counterregulated by T_H1 and B cells, whereas T_H2 and T regulatory (T_{Reg}) cells can regulate proinflammatory T_H1 responses (72–74). Thus, while too little inflammation fails to control *M. tuberculosis* growth, too much of a proinflammatory response compromises adaptive immunity and leads to immunopathology. It follows that a fine balance between different immune cell subsets and their pro- and anti-inflammatory mediators is required to control *M. tuberculosis* (42).

Failure of the immune response to eliminate *M. tuberculosis* results in a continuum of infection. Although defined immunosuppressive mechanisms can cause progression

to active TB disease, a large number of apparently immunocompetent individuals also develop TB. In these individuals, sequential immune alterations characterize progression from infection to disease (75). Yet precipitating factors and the causal association of immunological changes that lead to the development of active TB disease are poorly understood. High-resolution lung imaging in people and monkeys has revealed that TB disease is highly heterogeneous, lung granulomas are very dynamic, and *M. tuberculosis* infection results in a broader spectrum of clinical stages than previously appreciated (76–78). However, within-host heterogeneity can be considerable, and the host-pathogen interaction proceeds differently in each individual lesion, exhibiting polymorphic and complex disease presentations. While some lesions become sterilized, others may progress, suggesting that different stage-specific *M. tuberculosis*-expressed antigens are concomitantly present in the lung and that critical immune responses must act at the level of each granuloma, which ultimately determines the clinical outcome of infection (76, 79, 80). A complete understanding of the molecular basis of *M. tuberculosis* clearance, control, or lack thereof within granulomas is thus necessary to inform more-rational development of effective TB vaccines.

Challenges to Natural Immunity

Most cases of TB develop soon, within 1 to 2 years, after an individual becomes infected with *M. tuberculosis* (81, 82), fueling ongoing transmission. However, reactivation many years after asymptomatic infection is an important challenge, and the immune mechanisms that prevent primary TB progression may well be different in nature from those that prevent reactivation TB. It is clearly very important to understand why immunity fails to control initial *M. tuberculosis* infection and why some people fail to maintain long-lasting protective immunity after *M. tuberculosis* infection. *M. tuberculosis* has coevolved with humans and clearly has developed numerous strategies to evade, tolerate, and undermine host immunity to establish persistence for decades so that reactivation at a later time in the form of reactivation pulmonary TB, typified by cavitary disease (83), can ensure transmission. Of course, humans have also adapted to the pathogen, leading to the fine balance between effective immune responses and *M. tuberculosis* survival. However, in some cases, environmental, genetic, and other precipitating factors likely swing the balance in favor of *M. tuberculosis*. How a vaccine can be designed to protect against these factors is difficult to fathom. An alternative (or additional) school of thought is that long-term chronic infection results in aberrant immune activation, dysregulation in immune pathways, and the accumulation of "terminally differentiated" effectors in the vasculature, leading to failed control and disease progression (84–86). Under this model, immunological tolerance likely plays a protective role, but T-cell hyperactivation and hyperproliferation compromise infection tolerance and ultimately precipitate the loss of critical T-cell function (87). Although definitive evidence for the existence of exhausted T cells in people with LTBI is lacking, such immune dysfunction may occur in both the lymphoid and myeloid compartments (84–86, 88). This is supported by mouse studies, which suggest that "less-differentiated" memory $CD4^+$ T cells exert greater control of *M. tuberculosis* than "highly differentiated" effector T cells by their superior ability to proliferate, home to infected tissues, and perform many functions (86, 89, 90). Yet multiple metabolic and anatomical barriers (for example, aberrant angiogenesis and foamy macrophages) can restrict the infiltration of effector T cells into granulomas, and their defective positioning may limit the effectiveness of interactions with infected macrophages (91). However, interpretation of data from these studies and their relevance for human immunopathogenesis should take into account that such animal models typically model primary *M. tuberculosis* infection and do not recapitulate human disease progression with postprimary granulomas and fibrocaseous disease (83, 92).

Furthermore, in *M. tuberculosis*-infected and antibiotic-treated "memory-immune" mice, recall of $CD4^+$ or $CD8^+$ memory T-cell responses following reinfection fails to reduce the bacterial burden long-term or improve survival compared to primary responses (93). In subunit TB10.4 (EsxH)-vaccinated mice, the primary T-cell response

induced by *M. tuberculosis* infection upon challenge outnumbered the secondary memory $CD8^+$ T-cell response, and the memory $CD8^+$ T cells failed to afford protection (94). In humans and macaques, prior infection appears to be protective against reinfection progressing to TB disease (24, 26). However, people with prior TB disease can become *M. tuberculosis* infected again despite successful anti-TB treatment and in fact exhibit a higher risk of developing reinfection disease (95). Whether this failure is due to social, anatomical, or epidemiological factors or inherent or acquired immunological defects is unclear. In light of this, a prevailing notion is that attempts to reproduce responses to natural infection by vaccination will not be successful in these people and that new TB vaccines will likely need to induce "uncommon" or "unnatural" immunity that is superior to natural immunity. An example of such unnatural immunity is that induced following tetanus toxoid vaccination or by successful glycoconjugate vaccines where polysaccharides are covalently linked to proteins (96), since such responses are not observed after natural infection with the offending pathogen. The induction of unconventional HLA-E-restricted and major histocompatibility complex (MHC) class II-restricted $CD8^+$ T-cell responses using genetically programmed cytomegalovirus (CMV)-vectored candidates is an example of uncommon immunity (97). Yet whether vaccine-induced protection over and above that afforded by natural immunity can be achieved by the induction of unnatural immunity remains unclear. It is noteworthy that recent results from the phase 2b trial of M72:$AS01_E$ in *M. tuberculosis*-infected individuals (described below) suggest that vaccine boosting of T-cell and antibody responses primed by infection might protect against TB (10).

BCG, A FRAMEWORK TO UNDERSTAND TB IMMUNITY

The principle behind BCG vaccination entails priming "natural immunity" to mycobacterial antigens, mimicking natural infection of the host, to generate immunological memory that ensures accelerated responses after exposure to *M. tuberculosis*. However, despite inducing a strong T_H1 response, this vaccine has proven insufficient to control global TB epidemics, most likely because BCG does not consistently protect against pulmonary TB. Early studies investigating BCG-elicited protection informed us about T-cell-based mechanisms of immunity. More recently, we have learned about the nonspecific protection that it provides against general morbidity and mortality in infants from resource-limited counties (98). This heterologous beneficial effect against nontargeted diseases has been ascribed to effects on innate immune cell function, termed "trained immunity." It relies, in part, on the epigenetic imprinting of stem cells and innate immune cells, such as monocytes and NK cells, that exhibit memory-like attributes (99–101), providing evidence for innate-immunity-mediated protection.

BCG Immunity and New TB Vaccine Candidates

BCG appears to be more efficacious in low-TB-incidence countries farther from the equator (102). BCG protection typically lasts 10 to 15 years, although two studies in low-incidence settings suggested that protection against pulmonary TB can last for many decades (103, 104). In high-TB-burden countries, the TB incidence peaks dramatically in young adults (105), suggesting an alteration or waning of BCG immunity that is triggered during puberty and persists through late adolescence. One underlying explanation for this effect could be that BCG preferentially induces a T effector memory (T_{EM}) response that is insufficiently long-lived or perhaps prone to attrition following chronic infection or repeated mycobacterial exposures common in these countries (106–108). TB epidemiology and disease diversity vary substantially between different age groups (109). In low-TB-incidence countries such as the United States that do not routinely vaccinate with BCG, the TB incidence also peaks in young adults (110), suggesting alternate, potential physiological or immunological changes or expansion of social networks and potential TB exposures. BCG, being a live vaccine, is generally not considered a good booster immunization, although this has not been systematically investigated in clinical trials. Two large, cluster-randomized clinical trials of BCG revaccination in Brazil and Malawi showed no efficacy against TB disease (111–113).

However, neither trial enrolled participants based on *M. tuberculosis* infection status, and infection status was not accounted for in efficacy analyses. Acquisition of *M. tuberculosis* infection was also not measured as an outcome during the trial periods. Studies of BCG-induced responses have predominantly focused on T cells, yet we do not know which aspects of BCG-elicited immune responses are most important for protection against *M. tuberculosis*. This has thwarted the development of modern TB vaccines.

The growing understanding is that the complex interplay between BCG, the human host, and the environment determines the nature of anti-TB immunity. Host genotypes; nutritional and metabolic status; environmental factors like smoking, microbiota, and coinfections; and the virulence and fitness of prevalent *M. tuberculosis* strains may all impinge on BCG immunity. Widespread environmental exposure to nontuberculous mycobacteria (NTM) appears to explain some of BCG's variable and short-lived protection in certain regions where TB is endemic. It has long been conjectured that prior NTM exposure "blocks" BCG replication and the induction of protective immunity or "masks" any protective effects of BCG, which fails to further boost NTM-induced background immunity (114, 115). A recent meta-analysis suggests that an absence of prior NTM sensitization or *M. tuberculosis* infection is associated with greater BCG efficacy against pulmonary TB (3). The prevailing hypothesis suggests that since NTM share a broad antigen repertoire with BCG and *M. tuberculosis* and elicit phenotypically similar T-cell responses (116), NTM sensitization leads to reduced BCG-induced immunity. In light of the long-standing disputes around this issue (117), defining the exact role of NTM in vaccination against TB is critically important, especially for live mycobacterial whole-cell vaccines.

Another potential limitation of BCG is the heterogeneity of its daughter strains, each of which has evolved in different institutions worldwide due to various mycobacterial culture conditions. One common feature of all BCG strains is the absence of 6-kDa early secretory antigenic target (ESAT-6) secretion system 1 (ESX-1), owing to the deletion of region of difference 1 (RD1) (118). The deletion of the RD1 locus, which encodes key virulence factors such as ESAT-6 and culture filtrate protein 10 (CFP-10), underlies the attenuation of the parental *M. bovis* strain. Five type VII secretion systems like ESX-1 are present in *M. tuberculosis* (118). Considering growing evidence of their role in immune evasion and virulence, these ESX systems are increasingly targeted for the development of live recombinant mycobacterial vaccines (119, 120), and the preferential recognition of ESX antigens by T cells following natural infection has made them attractive candidates for subunit vaccine development (121) (Fig. 2).

Compared with *M. tuberculosis*, ~120 genes are lost in BCG, and about 23% of the known human T-cell epitopes in *M. tuberculosis* are absent in BCG strains (122). Some BCG strains (Japan, Moreau, and Glaxo) also do not produce virulence surface lipids phthiocerol dimycocerosates (PDIMs) or phenolic glycolipids (PGLs). Whether diversity within and between strains contributes to the variable efficacy of BCG in clinical trials is unknown, but these strain differences generate variable immune responses in people and inconsistent protection in animal models (123–125). Therefore, BCG strain heterogeneity may likely have implications for new BCG boosting or supplementation strategies.

The loss of ESX-1 and virulence lipids in BCG has contributed greatly to our understanding of the pathobiology of *M. tuberculosis*, and vaccinologists have used this knowledge to pursue new vaccine leads. ESAT-6, PDIMs, and PGLs execute numerous immune evasion strategies in macrophages, including for phagosome rupture and cytosolic escape of *M. tuberculosis* and upregulation of responses detrimental to the host (for example, aberrant type I IFN levels) (126–128). From the host perspective, however, cytosolic escape of *M. tuberculosis* results in antigen processing through both class I and class II MHC pathways and the induction of $CD8^+$ and $CD4^+$ T-cell responses (129). BCG, which lacks ESX-1, remains restricted to the phagosome and induces a weaker $CD8^+$ T-cell response (129). This knowledge has facilitated the engineering of recombinant BCG (rBCG) candidates with cytosolic-escape capability that appear more immunogenic and safer in immunocompromised hosts (130). The live-attenuated *M.*

tuberculosis vaccine candidate MTBVAC (Fig. 2) instead employs the strategy of attenuation by double deletions of the *phoP* and *fadD26* virulence genes, which leads to the complete abolishment of PDIM biosynthesis and defects in the ESX-1 system. MTBVAC therefore produces ESAT-6 but cannot export it, which results in decreased virulence yet improved protection in animal models (131).

To impede destruction in macrophages, *M. tuberculosis* has also evolved the capacity to inhibit protective mechanisms of innate immunity, such as phagosome acidification, phagosome-lysosome fusion, reactive oxygen and nitrogen species, apoptosis, and autophagy (126, 132). The most advanced rBCG vaccine in clinical trials, VPM1002 (Fig. 2), expresses listeriolysin from *Listeria monocytogenes*, while the urease C gene has been deleted. This promotes phagosome acidification, phagosome membrane perturbation, and the escape of antigens into the cytosol, enhancing immunogenicity and protection compared to parental BCG in mice (133). Deletion of the antiapoptotic gene *nuoG* from VPM1002 further appears to enhance protection against *M. tuberculosis* challenge in mice (134).

In *M. tuberculosis*-exposed people, it takes about 3 to 6 weeks before the tuberculin skin test turns positive (135). After *M. tuberculosis* infection of mice, accumulation of activated $CD4^+$ T cells in the lung is also delayed, occurring between 14 and 21 days postinfection (48, 136–138). This delay is thought to be a part of the immune evasion strategies employed by *M. tuberculosis*, by hampering the migration of antigen-carrying DCs to draining LNs, by inhibiting antigen processing and presentation by antigen-presenting cells, and by inducing the early expansion of ESAT-6-specific T_{Reg} cells that impede naive T-cell priming (48, 136–138). Additional mechanisms are employed by *M. tuberculosis* to limit the efficacy of T cells and the adaptive immune response (139). It was recently suggested that in BCG- or subunit-vaccinated mice, a similar delay occurs in the initiation of recall of existing memory T-cell responses following *M. tuberculosis* challenge (140, 141). Since most humans who are exposed to *M. tuberculosis* already have mycobacterium-specific memory T cells due to neonatal BCG vaccination or NTM exposure, it is not clear to what degree this is an issue in humans.

TB VACCINES AND DESIRED IMMUNE RESPONSES

TB vaccines must induce responses to antigens that are expressed and presented during initial and later stages of *M. tuberculosis* infection. These responses must also reside in the appropriate tissue locations during the relevant infection stages. Unlike HIV and influenza virus, for which antigen variation is a major vaccination challenge, *M. tuberculosis* proteins that comprise the main targets of human T cells do not exhibit significant sequence variation. The implications of the conservation of T-cell epitopes for vaccine development are not fully understood (142, 143). Yet this phenomenon has fueled the theory that these immunodominant antigens deliberately drive persistent, robust T-cell responses which may act as "decoys" and divert the response from targeting the critical antigens (144). Exaggerated T_H1 responses may also benefit *M. tuberculosis*, the theory suggests, by promoting an inflammatory environment that facilitates tissue damage, expectoration, and aerosolization of the pathogen to drive transmission. In HIV-*M. tuberculosis*-coinfected individuals, the occurrence of cavitary disease is associated with larger numbers of circulating $CD4^+$ T cells, supporting this possibility (145). This hypothesis must be considered in light of the knowledge that most *M. tuberculosis*-infected people have strong T_H1-cell responses to the same immunodominant T-cell epitopes, which do not appear to drive pathogenesis.

Taken together, our understanding of the T-cell responses required for effective control of *M. tuberculosis*, while avoiding immunopathology, has advanced considerably in recent years. To achieve control of *M. tuberculosis*, rational vaccine design should aim to elicit a balanced immune response that encompasses multiple components of pulmonary mucosal and systemic responses (Fig. 3). Since a single vaccine may not possess all "desired" attributes, multiple vaccines and combination approaches, such as heterologous boosting, may be required.

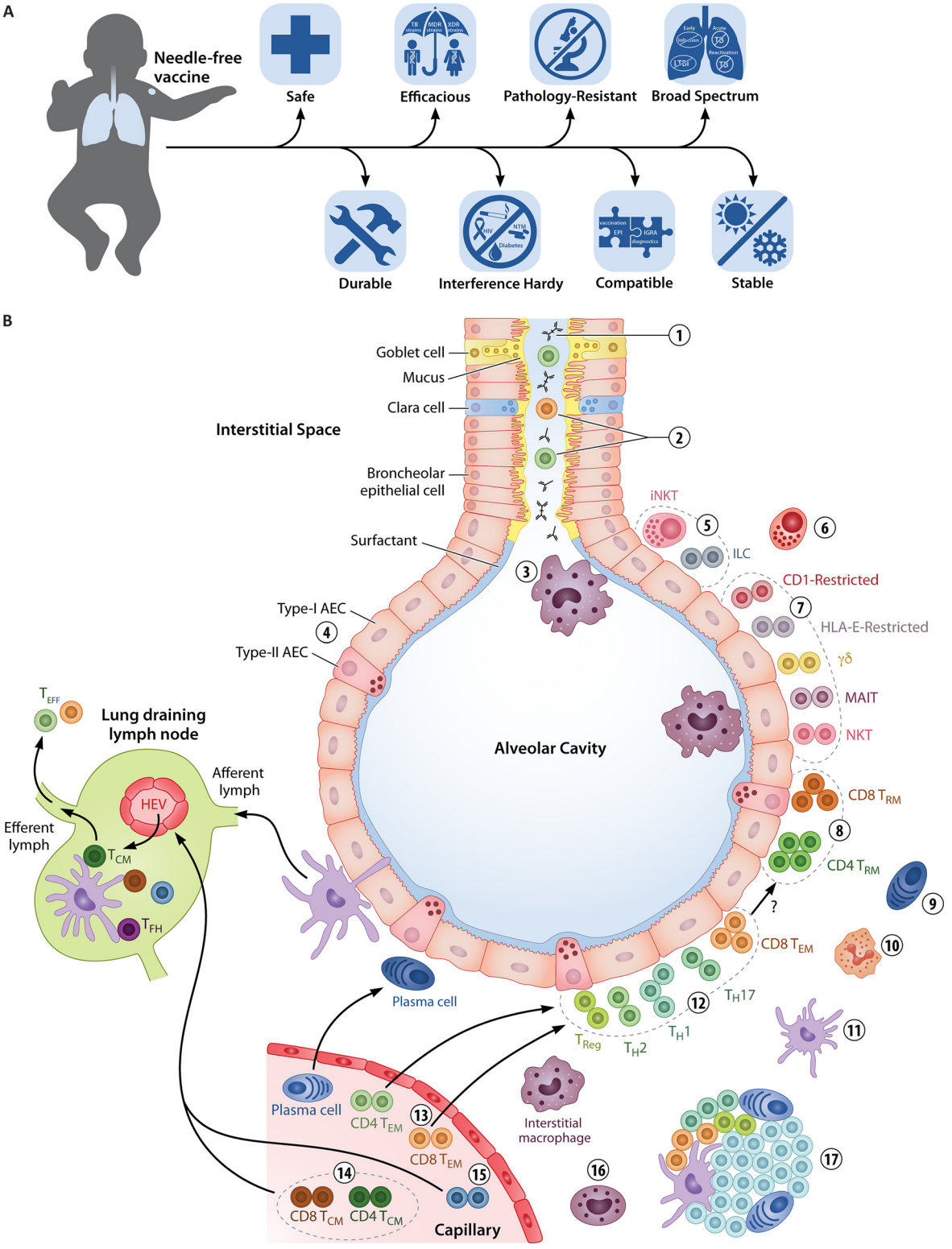

FIG 3 Tuberculosis vaccine and desired attributes. (A) Characteristics of an ideal TB vaccine. (B) An effective TB vaccine will likely need to engage multiple mechanisms and should aim to elicit a comprehensive immune response involving multiple arms of the immune system. Humoral and cell-mediated immunity may act at different points in time, or synergistically, to prevent *M. tuberculosis* infection and TB disease. Vaccine-elicited protective antibodies in the airways can prevent the establishment of infection or limit the acquisition of infection. Cytokines produced by airway-resident innate lymphocytes may equip alveolar macrophages with early bactericidal functions and recruit monocyte-derived macrophages to the site of infection. Yet *M. tuberculosis*, a "robust" intracellular pathogen, frequently succeeds in establishing a long-term infection in macrophages. Vaccine-elicited or trained innate lymphoid cells, NK cells, unconventional T cells, and

(Continued on next page)

C

'Systems Immunology' of 'Desired' TB Vaccine
1. **Antibodies:** Attachment and entry blocking, high-affinity, broadly neutralizing, FC effector function equipped complement-fixing, opsonophagocytic, antibody-dependent cell phagocytosis (ADCP) & antibody-dependent cell cytotoxicity (ADCC)-capable and *Mtb* killing.
2. **Airway T cells:** ↑ 'innate-like' sensing & early effector functions.
3. **Alveolar macrophages:** ↑ microbicidal capacity & ↑ trained-immunity.
4. **Alveolar epithelial cells (AEC):** ↑ surfactant & antimicrobial peptide production.
5. **Innate lymphoid cells (ILC):** ↑ 'innate-like' sensing & early anti-*Mtb* effector functions.
6. **Natural killer (NK) cells:** 'Memory-like' quick recall, ↑ trained-immunity & ↑ killer potential.
7. **Unconventional T cells:** Rapid recall & ↑ early anti-*Mtb* effector functions.
8. **Tissue-resident memory T cells (T_{RM}):** ↑ sensory and early anti-*Mtb* effector functions.
9. **Plasma cells:** Rapid antibody secretion & specificity against critical antigens.
10. **Neutrophils:** ↑↓ frequency and ↑microbicidal capacity.
11. **Dendritic cells (DC):** ↑↓ subset frequency, ↑activation & rapid migratory capacity to draining lymph node (LN). Increased antigen presentation by LN DCs.
12. **Interstitial effector memory T cells (T_{EM}):** Appropriately located, ↑↓ pro & anti-inflammatory subsets, sustained proliferation & ↑↓ multifunctional capacity without exhaustion.
13. **Circulating T_{EM}/T_{EFF}:** Lung and granuloma-homing, ↑↓ pro & anti-inflammatory subsets, sustained proliferation & ↑↓ multifunctional capacity without terminal differentiation.
14. **Circulating central memory T cells (T_{CM}):** Draining LN-homing, long-lived, rapid recall and generation of secondary effectors of crucial specificity that recognize *Mtb*-infected cells with low to intermediate TCR avidity.
15. **Circulating memory B cells:** Draining LN-homing, long-lived, rapid recall and generation of plasma cells of crucial specificity.
16. **Monocytes:** ↑↓ frequency & ↑ 'trained-immunity.'
17. **Lymphoid follicle:** Local antigen-presentation niche?

FIG 3 (Continued)
T_{RM} cells at submucosa may act as sensory cells, recruit memory T cells, and/or act as early effectors to increase the kinetics of killing of *M. tuberculosis*-infected cells, leading to abortion of infection. Nevertheless, immune evasion strategies employed by *M. tuberculosis* likely present challenges for the prevention of infection, resulting in the establishment of infection in the interstitium. Induction of lymphoid follicles, as a local antigen presentation site, may be a desired feature of vaccination to reduce the bottleneck in delayed antigen presentation in draining LNs and impediment in the activation of T_{RM} and T_{EM} cells. Activated dendritic cells and other antigen-presenting cells during recall responses may rapidly initiate the activation of T_{CM} cells and memory B cells. TB vaccines will need to elicit long-lived memory T cells, and these memory T cells will need to rapidly expand and generate secondary effectors with a sustained proliferative and "functional" capacity. Primed effectors will need to be specific to critical antigens in the life cycle of *M. tuberculosis*, possess lung-homing potential, traffic to the infection site, recognize *M. tuberculosis*-infected cells, and resist terminal differentiation or exhaustion. Mucosal antibodies may prevent infection or reduce the severity of infection, host-damaging effects, and systemic dissemination. Effector T-cell responses must be capable of eliminating infection, or at least enforcing lifelong control of infection, while preserving delicate anatomical structures. This necessitates appropriately placed, tightly regulated, and highly balanced pro- and anti-inflammatory responses. Although pulmonary mucosal vaccination appears to be capable of inducing a protective local immune response, it must be safe for administration. (C) Desired attributes of immune responses (portrayed and listed in panel B) to be elicited by a "modern TB vaccine." iNKT, invariant natural killer T cells; MAIT, mucosa-associated invariant T cells; HEV, high endothelial venules; FC, fragment crystallizable region of an antibody.

CURRENT TB VACCINE DEVELOPMENT APPROACHES

TB vaccine approaches can be broadly divided into prevention-of-infection (POI), prevention-of-disease (POD), prevention-of-recurrence (POR), or therapeutic vaccines to treat *M. tuberculosis* infection or TB disease. They are divided into live mycobacterial vaccines, subunit vaccines, and killed mycobacterial vaccines based on the platform used.

POI Vaccines

The POI vaccine, given preexposure, can prevent initial or sustained infection and is therefore thought to also protect against disease. As discussed above, several lines of evidence suggest that some people can resist infection despite repeated intense exposures (35), while BCG may also offer partial protection against infection (146, 147), providing the rationale for the POI approach (148). POI trials are smaller, shorter, and

less costly than POD trials, owing to the 8- to 10-fold-higher annual infection rates than TB disease rates in high-transmission settings (149). Such trials thus provide opportunities to study mechanisms of vaccine efficacy signals directly in humans, providing a platform to select lead candidates for further testing (149). A major challenge is that no tests are available to measure the acquisition, persistence, and clearance of asymptomatic *M. tuberculosis* infection directly. Current methods therefore rely on the detection of T-cell responses induced by infection, a process that takes 4 to 8 weeks. Furthermore, commercial IGRAs suffer from assay variability and uncertainty regarding the most effective assay cutoff (150). In addition, a vaccine candidate that cannot prevent infection might still protect against disease progression by inducing successful control of *M. tuberculosis* replication and would have a major impact on TB prevention. Conversely, a candidate that prevents infection in those individuals who, if infected, would in any case not progress to TB disease would have no or little impact on disease, transmission, and the TB epidemic (148). Despite these challenges, a recent landmark POI trial tested the ability of BCG revaccination or H4:IC31 subunit vaccination to prevent *M. tuberculosis* infection in healthy South African adolescents (9). H4:IC31 consists of a recombinant fusion protein (Hyvac-4) of TB10.4/EsxH and antigen 85B (Ag85B) in the IC31 adjuvant that signals through Toll-like receptor 9 (TLR9). Although neither vaccine showed efficacy based on the primary endpoint, namely, prevention of initial infection (QuantiFERON-TB gold in-tube [QFT] conversion at the manufacturer's cutoff), BCG revaccination significantly reduced the rate of sustained infection (QFT conversion followed by two consecutive QFT-positive results, 3 months apart, as a secondary endpoint), with an efficacy of 45.4% (95% confidence interval [CI], 6.4 to 68.1%), which might indicate the ability to help control or clear infection. BCG revaccination also showed an efficacy of 45.1% in preventing initial infection at a higher QFT cutoff of 4 IU/ml. Since QFT values above 4 IU/ml have been associated with a very high risk of TB disease in infants and adults (19, 151), this result further supports the hypothesis that BCG-induced immune responses might have promoted improved control of *M. tuberculosis* replication or perhaps even sterilization. H4:IC31 vaccination showed an efficacy of 30.5% (95% CI, −15.8 to 58.3%) in preventing sustained infection, which did not differ significantly from that of the placebo group (9). Therefore, H4:IC31 is no longer in further clinical evaluation. These results encourage POI trials of other candidates in the pipeline and warrant further evaluation of BCG revaccination in *M. tuberculosis*-uninfected individuals to determine if prevention of infection and subsequent progression to TB disease can be achieved. Potential barriers to BCG revaccination include existing contraindication in HIV-infected individuals and interference by prior NTM exposures in some settings.

POD Vaccines

A POD vaccine is given pre- and postexposure to protect against progression to TB disease. Epidemiological modeling suggests that an effective POD vaccine given to adolescents or young adults will have the fastest and largest impact on the global TB epidemic by interrupting transmission (152). Although most candidates in ongoing clinical trials (Fig. 2) aim to prevent TB disease, POD trials are larger, longer, and more costly than POI trials, owing to the lower rate of TB disease endpoints (149). To address this, the recent phase 2b POD trial of the candidate M72:AS01$_E$ subunit vaccine in South Africa, Kenya, and Zambia was conducted only with IGRA-positive individuals, a population with enriched TB case accrual. In an endpoint-triggered interim analysis of this trial, comprising 3,283 adults, the incidence of pulmonary TB was significantly lower in the M72:AS01$_E$ group than in the placebo group after a mean follow-up period of 2.3 years. The 54% vaccine efficacy reported in this trial establishes for the first time the proof of principle of vaccine-induced protection against clinical TB disease among persons already infected with *M. tuberculosis*. Although the observed confidence intervals are wide (95% CI, 2.9 to 78.2%), this proof-of-concept study supports further evaluation of M72:AS01$_E$. These results defy widespread skepticism of the feasibility of such a vaccine for the POD indication in IGRA-positive people and represent an

important advance. The final analyses of clinical data from this trial are slated to be released in late 2019. Because this trial included *M. tuberculosis*-infected adults who were predominantly BCG vaccinated, it was not possible to determine the extent to which infection-generated or childhood BCG vaccination-elicited responses influenced vaccine efficacy; additionally, the trial was not designed to determine whether M72:$AS01_E$ can protect against *M. tuberculosis* infection. The global vaccination strategy would ideally target both *M. tuberculosis*-infected and -uninfected individuals, avoiding the need for IGRAs. Because the lack of efficacy among IGRA-positive individuals might halt further clinical development of candidates in the pipeline with potential efficacy against infection, inclusion of both uninfected and infected individuals in future trials would be necessary.

POR Vaccines and Therapeutic Vaccines

Vaccines that aim to prevent recurrent TB (POR vaccines) are administered during or after TB treatment to prevent recurrence after cure. Therapeutic vaccines are administered as an adjunct to drug treatment to increase the effectiveness of treatment and shorten the duration of TB treatment. Since treated TB patients are at a severalfold-higher risk of recurrent TB disease than matching community controls, the POR design achieves endpoint accrual with a much smaller sample size, providing a compelling rationale for POR vaccines (95). TB recurrence occurs in about 2 to 8% of TB patients after completion of treatment by relapse or reinfection, depending on the treatment effectiveness and transmission rates, and ~70 to 90% of this recurrent disease occurs within 1 year of treatment completion (153). As recurrent disease accrual is greater and faster, POR trials are usually smaller and shorter than POD trials (149), but these trials are complex in design. TB treatment is 6 to 24 months long, arduous, and very costly, depending on drug-susceptible or -resistant disease. Furthermore, as an immunotherapeutic adjunct to chemotherapy, POR vaccines may simplify, increase the effectiveness of, or possibly shorten the duration of TB treatment. A therapeutic vaccine may also ameliorate disease severity, reduce treatment failures, and have a major impact on the personal, logistical, and financial burden of TB treatment. POR candidates that prevent relapse and reinfection may also prevent reactivation and could signal an expansion of testing from a POR trial into a larger POD trial. Candidates currently being tested for POR include the H56:IC31 and ID93:GLA-SE subunit vaccines, which were shown to prevent reactivation or limit disease severity in nonhuman primates (NHPs) (154, 155), as well as the rBCG vaccine candidate VPM1002 (see below). They are currently in phase 2 or 3 POR trials in TB patients during or after completion of treatment (Fig. 2).

Live Mycobacterial Vaccines

The BCG vaccine is given to healthy neonates at birth in many countries but is contraindicated in HIV-infected infants. New viable mycobacterial vaccines aiming to replace BCG include rBCG strains and recombinant *M. tuberculosis* deletion mutants such as VPM1002 and MTBVAC, respectively. An evaluation of VPM1002 in a phase 2a trial in HIV-exposed infants in South Africa was recently completed, without any safety concerns. This trial was initiated following extensive preclinical evaluations and demonstration of safety and immunogenicity in healthy infants and adults (Fig. 4) (156–158). If proven safer than BCG in HIV-exposed and -unexposed infants in a planned phase 3 trial, VPM1002 may be the first new vaccine to enter the market since BCG, even if it is not found to be more efficacious than BCG. VPM1002 is also being evaluated in cured TB patients in India, to prevent recurrence (ClinicalTrials.gov identifier NCT03152903), a phase 2/3 trial that is slated to conclude in 2020. VPM1002 is also being investigated for immunotherapy in bladder cancer patients in Switzerland (ClinicalTrials.gov identifier NCT02371447).

MTBVAC was found to be safe in a "first-in-human" trial in healthy Swiss adults (159) and in a recently completed phase 2 trial in South African adults and newborns (ClinicalTrials.gov identifier NCT02729571) (160). In the latter trial, MTBVAC immunogenicity and its effects on IGRA conversion and reversion were also investigated in

FIG 4 Distinct features of T-cell responses to TB vaccine candidates and disparities between responses in animal models and humans. TB vaccine candidates elicit CD4+ and CD8+ T-cell responses with distinct functional profiles, although these responses differ between animal studies and clinical

(Continued on next page)

infants, demonstrating a promising induction of mycobacterium-specific $CD4^+$ T cells that expressed IFN-γ, TNF, and IL-2. Two additional phase 2 trials of MTBVAC in South African newborns and adults are under way (ClinicalTrials.gov identifiers NCT03536117 and NCT02933281). Safety is a critically important consideration for such live mycobacterial candidates, since less-attenuated strains may persist longer *in vivo* and exhibit unacceptable adverse events despite being highly immunogenic. For example, the rBCG vaccine AERAS-422, which expresses Ag85A, Ag85B, and Rv3407 together with perfringolysin, faced termination because two vaccine recipients in a phase 1 clinical trial presented with shingles (8) (Fig. 4). Further clinical evaluation of another candidate, rBCG30, which overexpresses Ag85B, was halted despite increased antigen-specific T-cell responses in healthy adults compared to BCG (161), following disappointing results in the MVA85A and AERAS-422 trials. Although live vaccines may induce a broad and relatively enduring immune response, the concern also exists that repeated environmental mycobacterial exposures may block, mask, or alter their effect, as has been reported for BCG (162). Since it would be unethical to withhold BCG, which saves thousands of lives annually by preventing disseminated and severe forms of TB, efficacy trials to evaluate such candidates in infants also pose regulatory and ethical challenges. Yet BCG is the comparator in a planned efficacy trial of VPM1002 in infants, and some of these candidates are being considered for "simultaneous vaccination" with BCG or as a BCG booster vaccine (163).

Subunit Vaccines

Subunit vaccine candidates aim to boost BCG-primed responses and include virus-vectored or adjuvanted recombinant proteins. The first subunit candidate to enter clinical trials was MVA85A, which delivered immunodominant *M. tuberculosis* Ag85A via a modified vaccinia virus Ankara (MVA) vector. Despite being safe and immunogenic in different populations and age groups in early trials (164–167), phase 2b efficacy trials of MVA85A did not demonstrate vaccine efficacy (6, 7). In the first efficacy trial carried out in BCG-vaccinated South African infants, boosting with MVA85A did not show significant improvement over BCG in preventing *M. tuberculosis* infection or TB disease (6) (Fig. 4) despite inducing Ag85A-specific T_H1 and T_H17 responses. MVA85A-induced T_H1 responses were later found to persist for over 6 years, indicating a highly enduring response (168). In the second efficacy trial carried out with HIV-infected adults, MVA85A also enhanced Ag85A-specific T_H1 responses (7) but yet again showed no efficacy against *M. tuberculosis* infection or disease compared to placebo. However, the latter trial was stopped early in light of the infant trial results and thus did not accrue the endpoints required for necessary statistical power. Several factors are speculated to have contributed to the failure of MVA85A to provide protection, including the use of

FIG 4 Legend (Continued)
trials. The sizes of the colored circles indicate relative magnitudes of specific T_H1, T_H17, and $CD8^+$ T-cell responses induced. The quality of the response with the dominant cytokine-producing subset(s) is described. NI, not investigated; PNI, polyfunctionality not investigated; BDL, below the detection limit. In mice and NHPs, parental BCG induces polyfunctional T_H1 and T_H17 responses and affords partial protection against *M. tuberculosis* challenge (108, 199, 224). No correlation was found between the magnitudes or polyfunctional profiles of BCG-specific T cells and protection against pulmonary TB in South African infants (225). Interestingly, a BCG-specific IFN-γ ELISPOT response was found to be associated with a reduced risk of TB in the same settings (171). BCG-elicited immune responses and protection wane over time in settings where TB is endemic (3, 105, 107), but BCG revaccination improves protection against sustained *M. tuberculosis* infection in adolescents (9). The live BCG replacement vaccine candidates VPM1002, AERAS-422, and MTBVAC induce broad immune responses, including increased polyfunctional $CD4^+$ and $CD8^+$ T-cell responses and improved protection in mice relative to BCG (131, 133, 156, 226), but in clinical trials, polyfunctional T-cell responses did not differ significantly from those induced by BCG (8, 157–159). MTBVAC induces antigen-specific polyfunctional $CD4^+$ T-cell responses at magnitudes that exceed those induced by BCG in infants, but no significant $CD8^+$ T-cell responses are induced, and MTBVAC also results in IGRA conversion. BCG booster subunit candidates increase immunogen-specific $CD4^+$ and/or $CD8^+$ T-cell responses in mice and improve protection. However, inconsistent protection in NHPs (MVA85A, Ad35Ag85A/AERAS-402, Ad5Ag85A, and H1/H4/H56) (26, 155, 181, 182, 227, 228) and relatively low-level immune responses in BCG-vaccinated infants (MVA85A, AERAS-402, and M72) (165–167, 177, 229, 230) compared to those in adults were observed for some candidates. In clinical trials, MVA85A failed to improve protection against *M. tuberculosis* infection or disease despite increased polyfunctional T_H1 and T_H17 responses (6, 168), and AERAS-402-induced polyfunctional $CD8^+$ and $CD4^+$ T cells failed to recognize *M. tuberculosis*-infected target cells (180). Unlike BCG-elicited responses, adjuvanted subunit candidates predominantly induce IL-2-coexpressing polyfunctional $CD4^+$ T-cell subsets that correlate with enhanced protection in mice (183, 188, 191), but the role of these subsets in human protection is unclear, and the relevance of varying immunogenicity of antigenic components in subunit vaccine candidates for protection is unknown (231, 232). MIP elicits beneficial effects in *M. tuberculosis*-infected animal models as a therapeutic vaccine, but in clinical trials, it provided ambiguous benefits in TB patients (196–198).

a single antigen, hypoimmune responsiveness in infants, immunological interference by EPI vaccines, boosting at the peak of the BCG response, immunosuppression in HIV-infected adults, decreased Ag85A expression after *M. tuberculosis* infection, and reduced Ag85A availability in the lungs during chronic infection (89, 169–174). Nonetheless, these results ignited intense and valuable debate in the TB field surrounding prevalent paradigms of protective immunity and vaccination strategies for TB. This led to the reconsideration of boosting prior IFN-γ/T_H1 responses with newer TB vaccines (62, 175) and emphasized the need for more-stringent preclinical efficacy data for advancing only "best-in-class" candidates to late-stage clinical trials. Of significance is that it shifted the focus within the vaccine development community to preventing reactivation TB in adolescents/adults, to interrupt *M. tuberculosis* transmission. Additional clinical trials that will address some of these questions are under way, including combination boosting using simian adenovirus (Ad)- and MVA-vectored Ag85A vaccines by the aerosol route (Fig. 2). Combination boosting by the systemic route using simian adenovirus- and MVA-vectored Ag85A vaccines (ClinicalTrials.gov identifier NCT01829490) and alternate aerosol and systemic immunizations using an MVA-vectored Ag85A vaccine (ClinicalTrials.gov identifier NCT01954563) were found to be safe and immunogenic in healthy BCG-vaccinated adults (176). Since BCG elicits a weak $CD8^+$ T-cell response, dominant $CD8^+$ T-cell-response-inducing replication-deficient adenovirus vector platforms also underwent clinical evaluations as novel BCG booster vaccines. These candidates include adenovirus serotype 35 (Ad35) expressing *M. tuberculosis* antigens Ag85A, Ag85B, and TB10.4 (AERAS-402) and human adenovirus serotype 5 expressing Ag85A (Ad5Ag85A) (177–179). Even though preexisting antivector immunity in the trial populations did not dampen the strength of the booster response, Ad-platform-induced $CD8^+$ T cells either failed to recognize *M. tuberculosis*-infected human targets or failed to provide significant protection over and above BCG in NHPs (180–182) (Fig. 4). Consequently, the Ad35 candidate is no longer being perused, but the Ad5 candidate is still in clinical development (179). Another recombinant virus-vectored candidate in clinical development, TB/FLU-04L, employs a live-attenuated influenza A virus vector to express *M. tuberculosis* antigens Ag85A and ESAT-6 (Fig. 2). Similar to the FluMist vaccine, TB/FLU-04L is delivered by the intranasal route. This delivery platform was found to be safe and immunogenic in healthy BCG-vaccinated, QFT-negative adults in a phase 1 trial in Kazakhstan (ClinicalTrials.gov identifier NCT02501421). An additional phase 2a POD trial of TB/FLU-04L is currently planned for QFT-positive adults.

Fusion protein-adjuvant formulations currently in clinical evaluation include M72:$AS01_E$, H56:IC31, ID93:GLA-SE, and GamTBvac (Fig. 2). H56 and an earlier version, comprising Ag85B and ESAT-6 (H1), were safe and immunogenic when combined with the IC31 or CAF01 adjuvant, as pre- or postexposure vaccines (183–186). H1 is no longer in the clinical pipeline. M72:$AS01_E$, H56:IC31, and ID93:GLA-SE are being investigated for multiple indications, including therapeutic/POR, POD, and/or POI. GamTBvac is a new vaccine formulation consisting of two *M. tuberculosis* antigen fusions of Ag85A and ESAT6–CFP-10 with the dextran-binding domain immobilized on dextran and mixed with an adjuvant consisting of a DEAE-dextran core and CpG oligodeoxynucleotides (TLR9 agonist) (187). The safety and immunogenicity of GamTBvac are currently being evaluated in a phase 2a trial in healthy BCG-vaccinated adults (ClinicalTrials.gov identifier NCT03878004), following successful clinical evaluation in a phase 1 trial in Russia (ClinicalTrials.gov identifier NCT03255278). The excellent safety profiles, well-defined molecular compositions, absence of vector-directed immunity facilitating prime-boosting, and depot-forming slow-antigen-release effects that induce durable responses make such subunit formulations attractive. They are amenable to adjustments to influence the type of immune response induced or to modulate preexisting immunity. However, the use of multiple antigens and complex formulations can make them challenging for good manufacturing practice (GMP) and evaluations. Although prior mycobacterial exposure might not block or mask the subunit vaccines, they will need to improve upon the protection afforded by BCG and NTM exposures. These subunit vaccines induce

memory T-cell subsets distinct from those induced by BCG, specifically by inducing IL-2- and TNF-coexpressing T cells that are less differentiated than BCG-induced effector $CD4^+$ T cells, which express mostly IFN-γ and little TNF and IL-2 (183–186, 188–190) (Fig. 4). Murine experiments suggest that these less-differentiated T-cell responses may provide greater efficacy than BCG (188, 191), but the role of these T-cell subsets in human protection remains unclear. In *M. tuberculosis*-infected individuals, however, the frequencies of these IL-2- and TNF-coexpressing $CD4^+$ T-cell subsets decrease, and those of the IFN-γ-expressing subsets increase, suggesting that underlying *M. tuberculosis* infection can impact the attributes of the subunit vaccine-elicited memory T-cell response (192).

Killed Mycobacterial Vaccines

Killed mycobacterial vaccine preparations in clinical trials include RUTI, DAR-901, *Mycobacterium vaccae*, and *Mycobacterium indicus pranii* (MIP) (Fig. 2). RUTI is a liposomal formulation containing fragmented, detoxified *M. tuberculosis* grown under stress. As a potential therapeutic vaccine, it was found to be safe and immunogenic in persons with LTBI when administered 1 month after isoniazid treatment (ClinicalTrials.gov identifier NCT01136161). It is under evaluation in HIV-infected and non-HIV-infected persons with LTBI for POD (193), and an additional trial in persons with multidrug-resistant TB is planned (ClinicalTrials.gov identifier NCT02711735). DAR-901, a broth-grown preparation of *Mycobacterium obuense*, is currently in a phase 2b POI trial in BCG-vaccinated adolescents in Tanzania. The efficacy data from this trial are expected in 2020. It is also under evaluation in HIV-infected TB patients as a therapeutic vaccine. SRL172, an earlier agar-grown *M. obuense* preparation, was evaluated in the first phase 3 efficacy trial conducted since BCG (194). Results of this trial suggested that multidose SRL172 vaccination provides some protection against HIV-associated TB (39% reduction in culture-confirmed cases; hazard ratio, 0.61 [95% CI, 0.39 to 0.96]) in BCG-vaccinated adults (194). Yet the development of SRL172 faced challenges due to nonscalability. Several studies have also investigated killed *M. vaccae* and lysates thereof as an adjunct to antibiotic treatment, including in HIV-coinfected persons (195), and *M. vaccae* and MIP preparations are currently in phase 3 POD trials in China and India, respectively. Although efficacy data from the multidose *M. vaccae* (Vaccae) vaccine trial in TST-positive adults were expected in 2016, the status of this trial has not been verified in 2 years (ClinicalTrials.gov identifier NCT01979900). While *M. vaccae* is already licensed as an adjunctive therapeutic vaccine in TB patients in China, MIP is licensed as a leprosy vaccine in India. A clinical trial of MIP in household contacts of TB patients for POD indication is under way in India. However, efficacy signals provided by *M. vaccae* and MIP preparations as therapeutic vaccines are not definitive (Fig. 4) (196–198), and results from ongoing trials are eagerly anticipated.

Limitations of Current Vaccine Approaches

A limitation of current vaccine approaches is that they utilize a limited number of concepts and vaccine classes. Most focus on inducing "conventional" T_H1 immunity and include a limited repertoire of immunodominant target antigens, mainly belonging to the Ag85 and ESAT-6 family of secreted proteins (Fig. 2). The observation in mice that antigen availability limits the protective immunity conferred by Ag85B-specific T cells during chronic infection, whereas functional exhaustion limits immunity by ESAT-6-specific T cells (89), highlights challenges in the development of vaccines using these proteins. The functional attributes of memory T-cell responses induced by six-subunit vaccine candidates in clinical trials were highly similar, which suggests a lack of diversity (Fig. 4) (192). Since *M. tuberculosis* epitope-specific natural T-cell responses are highly heterogeneous and more than several dozen antigens are required to cover 80% of the total $CD4^+$ T-cell response, it is possible that current vaccine approaches using few antigens may not induce natural immunity of sufficient strength and breadth (58, 174). On the contrary, poorly recognized antigens during natural infection, termed unnatural antigens by some, may simply not be recognized by the immune system during

infection, and their role in protection is unclear (174). Results from the M72:AS01$_E$ trial in humans and a recent study of pulmonary BCG vaccination in NHPs suggest that meaningful protection can be achieved (10, 199). The NHP study suggests that mycobacterium-specific T_H1 cells in the lung that coexpress IL-17 (termed T_H1/T_H17 cells), along with the expression of IL-10 by lung cells and mycobacterium-specific IgA antibodies, can protect against infection and disease (199). These findings warrant further investigation of the role of mucosal vaccination and provide novel putative correlates of protection that can be tested as hypotheses in human studies. In addition, new vaccine concepts that exploit immunological and antigenic diversity need exploration. Such approaches may include the induction of uncommon immunity mediated by antibodies and/or donor-unrestricted T cells that recognize antigens other than classical peptides (31, 32, 45). The role of uncommon immune responses induced by novel posttranslationally modified antigens in protection versus pathology also needs investigation (200–203). New vaccine concepts that elicit memory T cells capable of sustainable expansion upon encountering *M. tuberculosis*-infected cells in the lung should be explored (140). Since long-lived memory T cells may arise from a subset of effector cells, characterization of such memory precursor effectors is essential (204). A head-to-head comparison of clinical-grade adjuvants and their abilities to induce protective pulmonary responses is also needed. The overall efficacy of M72:AS01$_E$ suggests that the adjuvant may be critical, while the importance of the immunogens is not clear from the M72:AS01$_E$ results. Whether the efficacy of M72 can be increased by the incorporation of additional protective antigens will need further investigation in different populations. In addition to the induction of host resistance, vaccines that induce infection tolerance and preserve lung function also need evaluation. A combination of approaches could also be explored, for example, by combining BCG revaccination in uninfected adolescents with M72:AS01$_E$ boosting to protect against infection and to prevent disease progression. In the absence of a clear target, adaptive trial designs will likely be the most efficient strategy.

KEY UNANSWERED QUESTIONS

A significant obstacle in TB vaccine development is a lack of correlates of protection or host biomarkers that reliably predict the level of protection induced. In the absence of a clear understanding of which immune responses new vaccines should induce for improved protection, assessments of immunogenicity of new vaccines in ongoing trials remain a measure of "vaccine take" rather than protective immunity. Past vaccine development efforts therefore relied mainly on the assessment of parameters that are presumed important for protection (for example, IFN-γ) (62, 175, 205). Previously completed efficacy trials that did not observe vaccine efficacy have led to a better understanding of correlates of risk. For example, a *post hoc* analysis of the MVA85A trial revealed that T-cell activation and differentiation and an elevated blood monocyte/lymphocyte ratio are associated with an increased risk of developing TB disease, whereas elevated Ag85A-specific IgG titers and frequencies of BCG-specific total IFN-γ-expressing cells measured by an enzyme-linked immunosorbent spot (ELISPOT) assay correlated with a reduced risk (171). Recently, a number of correlates of risk have been identified, although none are specific for TB, and many indicate that innate inflammation or immune activation is elevated during disease progression, likely by detecting incipient or subclinical disease (11, 13, 75). This suggests that these correlates of risk are likely different than the antigen-specific correlates of protection (206). Of course, it is expected that such correlates of protection will interact with correlates of risk, especially in the light of the latter being inflammatory signals that could interfere with vaccine take/efficacy or indicators that disease progression is already at an advanced stage. Stored samples from participants who were protected and those who were not in recent vaccine trials with significant efficacy (9, 10) have the potential to reveal correlates of protection in humans.

It is possible, even likely, that correlates of protection for a neonatal vaccine and those for an adolescent/adult vaccine will be different, and two sets of preferred

product characteristics for TB vaccines were recently proposed (207). Also, rather than a single biomarker, a cluster of biomarkers (biosignature) may be better at predicting protective capacity. Putative correlates could also be validated in resisters, who experience repeated, intense *M. tuberculosis* exposures (for example, in health care workers in high-incidence settings and in household contacts) but never convert their skin test or become IGRA positive (35). However, it is important to address whether resisters, who remain TST and IGRA negative and possess non-IFN-γ T-cell responses to *M. tuberculosis*-specific proteins (21), truly resist *M. tuberculosis* infection or whether they still harbor *M. tuberculosis* infection. As discussed above, there is no microbiological standard to measure the acquisition, persistence, and clearance of asymptomatic *M. tuberculosis* infection, and this has implications for the validation of correlates of protection or the design of vaccine clinical trials and other control interventions. The risk of progression to TB in these IGRA-negative resisters with unique adaptive immune responses is likely low, considering the high negative predictive value of the IGRA. Further comprehensive immunological studies in resisters from high-exposure cohorts may likely provide insights into immune correlates of exposure and early clearance (21). Even though such responses can be mimicked by vaccination, mechanisms of protection induced by vaccination may differ from those induced after natural infection.

Other obstacles that thwart TB vaccine progress include the lack of reliable functional assays or surrogates of immunological control, interpretability of animal models, long and expensive licensure trials, and the lack of market incentives to invest in an ailment that primarily burdens low- and middle-income countries. Additional understanding of the impacts of age, sex, geography, coinfections, and comorbidities on vaccine efficacy is required.

ACCELERATING THE TB VACCINE R&D TRAJECTORY: A PERSPECTIVE

Encouraging results in recent TB vaccine clinical trials (9, 10) represent important progress and provide unprecedented opportunities and new hope for eliminating TB. In the next 1 to 5 years, efficacy data are expected from at least six different trials, including the *M. vaccae* (POD), DAR-901 (POI), VPM1002 (POR and POD), ID93:GLA-SE (POR), and H56:IC31 (POR) trials. Insights gained from these trials and future *post hoc* studies using biobanked samples are expected to further advance our understanding of the prognostic ability of current preclinical models, correlates of risk of TB, and correlates of vaccine-induced protection, in addition to an increased understanding of clinical trial design and conduct for different indications. Translation of correlates of protection identified from clinical trials to preclinical animal studies of vaccination (for example, in NHPs) followed by *M. tuberculosis* challenge would likely provide insights into the mechanisms of vaccine-induced protection (208). Despite these breakthroughs, gaining a deeper understanding of human immunity using systems immunology and counting on the experience of initiatives such as the Human Vaccines Project (http://www.humanvaccinesproject.org/) will add value to the TB vaccine program. Diversification of the global clinical pipeline of TB vaccine candidates by advancing distinct and novel preclinical vaccine platforms and concepts in human clinical trials, simultaneously focusing on basic research and preclinical discovery programs, and developing better tools, including improved animal models and human "challenge" models that mimic natural infection (209, 210), represent valuable objectives. Furthermore, it is important that TB vaccine research incorporates novel ideas from other vaccine programs and maximizes scientific and clinical capabilities to accelerate the TB vaccine R&D trajectory.

Ideas from Other Vaccine Development Programs

HIV. Unprecedented scientific efforts that followed the partial efficacy reported in the Rv144 HIV vaccine trial provided a wealth of new information on the potential correlates of protection against HIV-1 acquisition, which include fragment crystallizable (Fc)-mediated antibody effector functions and $CD4^+$ T-cell responses to HIV envelope (211). Since a minority of HIV-infected people produce antibodies that neutralize a broad variety of HIV strains, concerted efforts are also ongoing to develop immunogens

that will elicit broadly neutralizing antibodies that prevent HIV infection following exposure (212, 213). Efforts are also directed at engaging cell-mediated immunity to induce persistent mucosal memory T cells (212, 214). Likewise, in people with LTBI, a protective role for Fc-mediated *M. tuberculosis*-specific antibody effector functions, tuned via differential glycosylation, was recently described (34). *M. tuberculosis* surface-directed potentially neutralizing antibodies, which act in concert with $CD4^+$ T cells and afford partial protection in mouse and *in vitro* models, have been found in some health care workers who are exposed to high doses of *M. tuberculosis* (33). The resisters identified among household contacts of TB patients have been found to possess noncanonical $CD4^+$ T-cell responses, IgM and class-switched IgG antibody responses with enhanced avidity, distinct Fc profiles, and higher-level FcγR3a binding (21), which may enhance *M. tuberculosis* control (34, 215). Identifying targets of such protective antibodies during natural infection, studying the structural mode of target recognition and $CD4^+$ T-cell–B-cell/antibody interactions, and characterizing protective mucosal B- and T-cell responses may provide a blueprint for the rational development of preventive and therapeutic TB vaccines.

Malaria. RTS,S, the first vaccine to demonstrate partial efficacy against malaria, also induces protective antibodies against the protein made by the infectious stage of the malarial parasite, single-celled sporozoites (216). A chemoattenuated sporozoite vaccine administered by the intravenous route was recently reported to afford sterile protection against malaria, and the frequency of specific polyfunctional $CD4^+$ memory T cells rather than antibodies was associated with protection (217). Intravenous or pulmonary administration of improved live-attenuated mycobacterial vaccines, recently shown to afford high-level protection for BCG (199; data from the laboratory of JoAnne Flynn, Robert Seder, and colleagues [234]), may provide information about the route of vaccination in improved protection against TB, potentially by inducing trained immunity, mucosal antibodies, and tissue-resident memory (T_{RM}) cells and/or by eliminating reservoirs and hiding niches.

Cancer. Interventions that restore appropriate immune checkpoint signaling, reprogram host immunometabolic circuits, and act on epigenetic alterations have often been used as an adjunct to cancer vaccines and chemotherapy to induce efficient antitumor responses (218). In *M. tuberculosis*-infected or diseased individuals, traditional vaccination approaches may fail to correct immune imbalance. Because immune responses to malignancies and TB share many similar mechanisms, host-directed interventions along with therapeutic vaccination should be investigated. Examples of these interventions may include modulation of metabolism pathways to generate long-lived T central memory (T_{CM}) cells responses, reduction of T-cell receptor (TCR) avidity to lessen T-cell exhaustion, the use of chimeric TCR or humanized-antibody therapies, and generation of granuloma-infiltrating T cells (219–223). Distinct host-directed therapies during LTBI and TB disease to potentiate vaccine/chemotherapy responses and determining the effective timing of these interventions in the *M. tuberculosis* infection spectrum will be required.

OUTLOOK AND CONCLUSIONS

After decades of research, the clinical development of a TB vaccine is now at a pivotal juncture, with exciting efficacy signals to improve on. Observations from recent TB vaccine clinical trials with efficacy (9, 10) have raised expectations for identifying correlates of protection against TB. The use of animal and human challenge models, harmonization of preclinical and clinical vaccine studies, and evaluation of vaccine candidates in innovative experimental-medicine trials will advance TB vaccine development. This will allow iterative improvements in partially effective candidates until meaningful protection is achieved in diverse populations. Vaccine candidates that demonstrate such efficacy signals should also be investigated for other indications, such as host-directed therapy–chemotherapy–vaccine integrated approaches to cure infection or disease. While advancing TB vaccine candidates, it will be important to manage expectations and maintain the momentum to yield licensed products. By

complementing ongoing global R&D efforts with increased investments, collaboration between all stakeholders and partners, and bringing political leadership under one umbrella, as was achieved to some degree at the recently concluded United Nations General Assembly High-Level Meeting, more breakthroughs in the development of clinically effective vaccines to conquer TB globally can be achieved.

ACKNOWLEDGMENTS

We thank Jennifer Oosthuizen, Creative Services of the CDC, for creating Web-ready figures.

The perspectives and conclusions in this publication are those of the authors and do not necessarily represent the official position of CDC, nor does mention of vaccine candidates or organizations imply endorsement by the U.S. Government.

S.B.S., J.E.P., and T.J.S. have no conflicts of interest to declare. T.J.S. is an investigator at the South African Tuberculosis Vaccine Initiative (SATVI) and the University of Cape Town, South Africa, and is involved with the clinical evaluation of many TB vaccine candidates described in this review that were developed by other investigators and developers/manufacturers. These individuals and organizations have no role in the overview and perspectives presented in this article.

REFERENCES

1. World Health Organization. 2018. Global tuberculosis report 2018. World Health Organization, Geneva, Switzerland. https://www.who.int/tb/publications/global_report/en/.
2. Pai M, Behr MA, Dowdy D, Dheda K, Divangahi M, Boehme CC, Ginsberg A, Swaminathan S, Spigelman M, Getahun H, Menzies D, Raviglione M. 2016. Tuberculosis. Nat Rev Dis Primers 2:16076. https://doi.org/10.1038/nrdp.2016.76.
3. Mangtani P, Abubakar I, Ariti C, Beynon R, Pimpin L, Fine PE, Rodrigues LC, Smith PG, Lipman M, Whiting PF, Sterne JA. 2014. Protection by BCG vaccine against tuberculosis: a systematic review of randomized controlled trials. Clin Infect Dis 58:470–480. https://doi.org/10.1093/cid/cit790.
4. Houben RM, Dodd PJ. 2016. The global burden of latent tuberculosis infection: a re-estimation using mathematical modelling. PLoS Med 13:e1002152. https://doi.org/10.1371/journal.pmed.1002152.
5. Knight GM, McQuaid CF, Dodd PJ, Houben RMGJ. 2019. Global burden of latent multidrug-resistant tuberculosis: trends and estimates based on mathematical modelling. Lancet Infect Dis 19:903–912. https://doi.org/10.1016/S1473-3099(19)30307-X.
6. Tameris MD, Hatherill M, Landry BS, Scriba TJ, Snowden MA, Lockhart S, Shea JE, McClain JB, Hussey GD, Hanekom WA, Mahomed H, McShane H, MVA85A 020 Trial Study Team. 2013. Safety and efficacy of MVA85A, a new tuberculosis vaccine, in infants previously vaccinated with BCG: a randomised, placebo-controlled phase 2b trial. Lancet 381:1021–1028. https://doi.org/10.1016/S0140-6736(13)60177-4.
7. Ndiaye BP, Thienemann F, Ota M, Landry BS, Camara M, Dieye S, Dieye TN, Esmail H, Goliath R, Huygen K, January V, Ndiaye I, Oni T, Raine M, Romano M, Satti I, Sutton S, Thiam A, Wilkinson KA, Mboup S, Wilkinson RJ, McShane H, MVA85A 030 Trial Investigators. 2015. Safety, immunogenicity, and efficacy of the candidate tuberculosis vaccine MVA85A in healthy adults infected with HIV-1: a randomised, placebo-controlled, phase 2 trial. Lancet Respir Med 3:190–200. https://doi.org/10.1016/S2213-2600(15)00037-5.
8. Hoft DF, Blazevic A, Selimovic A, Turan A, Tennant J, Abate G, Fulkerson J, Zak DE, Walker R, McClain B, Sadoff J, Scott J, Shepherd B, Ishmukhamedov J, Hokey DA, Dheenadhayalan V, Shankar S, Amon L, Navarro G, Podyminogin R, Aderem A, Barker L, Brennan M, Wallis RS, Gershon AA, Gershon MD, Steinberg S. 2016. Safety and immunogenicity of the recombinant BCG vaccine AERAS-422 in healthy BCG-naive adults: a randomized, active-controlled, first-in-human phase 1 trial. EBioMedicine 7:278–286. https://doi.org/10.1016/j.ebiom.2016.04.010.
9. Nemes E, Geldenhuys H, Rozot V, Rutkowski KT, Ratangee F, Bilek N, Mabwe S, Makhethe L, Erasmus M, Toefy A, Mulenga H, Hanekom WA, Self SG, Bekker LG, Ryall R, Gurunathan S, DiazGranados CA, Andersen P, Kromann I, Evans T, Ellis RD, Landry B, Hokey DA, Hopkins R, Ginsberg AM, Scriba TJ, Hatherill M, C-040-404 Study Team. 2018. Prevention of *M. tuberculosis* infection with H4:IC31 vaccine or BCG revaccination. N Engl J Med 379:138–149. https://doi.org/10.1056/NEJMoa1714021.
10. Van Der Meeren O, Hatherill M, Nduba V, Wilkinson RJ, Muyoyeta M, Van Brakel E, Ayles HM, Henostroza G, Thienemann F, Scriba TJ, Diacon A, Blatner GL, Demoitie MA, Tameris M, Malahleha M, Innes JC, Hellstrom E, Martinson N, Singh T, Akite EJ, Khatoon Azam A, Bollaerts A, Ginsberg AM, Evans TG, Gillard P, Tait DR. 2018. Phase 2b controlled trial of M72/AS01E vaccine to prevent tuberculosis. N Engl J Med 379:1621–1634. https://doi.org/10.1056/NEJMoa1803484.
11. Zak DE, Penn-Nicholson A, Scriba TJ, Thompson E, Suliman S, Amon LM, Mahomed H, Erasmus M, Whatney W, Hussey GD, Abrahams D, Kafaar F, Hawkridge T, Verver S, Hughes EJ, Ota M, Sutherland J, Howe R, Dockrell HM, Boom WH, Thiel B, Ottenhoff THM, Mayanja-Kizza H, Crampin AC, Downing K, Hatherill M, Valvo J, Shankar S, Parida SK, Kaufmann SHE, Walzl G, Aderem A, Hanekom WA, ACS and GC6-74 Cohort Study Groups. 2016. A blood RNA signature for tuberculosis disease risk: a prospective cohort study. Lancet 387:2312–2322. https://doi.org/10.1016/S0140-6736(15)01316-1.
12. Suliman S, Thompson EG, Sutherland J, Weiner RDJ, Ota MOC, Shankar S, Penn-Nicholson A, Thiel B, Erasmus M, Maertzdorf J, Duffy FJ, Hill PC, Hughes EJ, Stanley K, Downing K, Fisher ML, Valvo J, Parida SK, van der Spuy G, Tromp G, Adetifa IMO, Donkor S, Howe R, Mayanja-Kizza H, Boom WH, Dockrell HM, Ottenhoff THM, Hatherill M, Aderem A, Hanekom WA, Scriba TJ, Kaufmann SHE, Zak DE, Walzl G, GC6-74 and ACS Cohort Study Groups. 2018. Four-gene pan-African blood signature predicts progression to tuberculosis. Am J Respir Crit Care Med 197:1198–1208. https://doi.org/10.1164/rccm.201711-2340OC.
13. Singhania A, Verma R, Graham CM, Lee J, Tran T, Richardson M, Lecine P, Leissner P, Berry MPR, Wilkinson RJ, Kaiser K, Rodrigue M, Woltmann G, Haldar P, O'Garra A. 2018. A modular transcriptional signature identifies phenotypic heterogeneity of human tuberculosis infection. Nat Commun 9:2308. https://doi.org/10.1038/s41467-018-04579-w.
14. Warsinske HC, Rao AM, Moreira FMF, Santos PCP, Liu AB, Scott M, Malherbe ST, Ronacher K, Walzl G, Winter J, Sweeney TE, Croda J, Andrews JR, Khatri P. 2018. Assessment of validity of a blood-based 3-gene signature score for progression and diagnosis of tuberculosis, disease severity, and treatment response. JAMA Netw Open 1:e183779. https://doi.org/10.1001/jamanetworkopen.2018.3779.
15. Weiner J, III, Maertzdorf J, Sutherland JS, Duffy FJ, Thompson E, Suliman S, McEwen G, Thiel B, Parida SK, Zyla J, Hanekom WA, Mohney RP, Boom WH, Mayanja-Kizza H, Howe R, Dockrell HM, Ottenhoff THM, Scriba TJ, Zak DE, Walzl G, Kaufmann SHE, GC6-74 Consortium. 2018. Metabolite changes in blood predict the onset of tuberculosis. Nat Commun 9:5208. https://doi.org/10.1038/s41467-018-07635-7.
16. Warsinske H, Vashisht R, Khatri P. 2019. Host-response-based gene signa-

tures for tuberculosis diagnosis: a systematic comparison of 16 signatures. PLoS Med 16:e1002786. https://doi.org/10.1371/journal.pmed.1002786.
17. Penn-Nicholson A, Hraha T, Thompson EG, Sterling D, Mbandi SK, Wall KM, Fisher M, Suliman S, Shankar S, Hanekom WA, Janjic N, Hatherill M, Kaufmann SHE, Sutherland J, Walzl G, De Groote MA, Ochsner U, Zak DE, Scriba TJ, ACS and GC6-74 Cohort Study Groups. 2019. Discovery and validation of a prognostic proteomic signature for tuberculosis progression: a prospective cohort study. PLoS Med 16:e1002781. https://doi.org/10.1371/journal.pmed.1002781.
18. Andrews JR, Hatherill M, Mahomed H, Hanekom WA, Campo M, Hawn TR, Wood R, Scriba TJ. 2015. The dynamics of QuantiFERON-TB gold in-tube conversion and reversion in a cohort of South African adolescents. Am J Respir Crit Care Med 191:584–591. https://doi.org/10.1164/rccm.201409-1704OC.
19. Andrews JR, Nemes E, Tameris M, Landry BS, Mahomed H, McClain JB, Fletcher HA, Hanekom WA, Wood R, McShane H, Scriba TJ, Hatherill M. 2017. Serial QuantiFERON testing and tuberculosis disease risk among young children: an observational cohort study. Lancet Respir Med 5:282–290. https://doi.org/10.1016/S2213-2600(17)30060-7.
20. Stein CM, Nsereko M, Malone LL, Okware B, Kisingo H, Nalukwago S, Chervenak K, Mayanja-Kizza H, Hawn TR, Boom WH. 2019. Long-term stability of resistance to latent *M. tuberculosis* infection in highly exposed TB household contacts in Kampala, Uganda. Clin Infect Dis 68:1705–1712. https://doi.org/10.1093/cid/ciy751.
21. Lu LL, Smith MT, Yu KKQ, Luedemann C, Suscovich TJ, Grace PS, Cain A, Yu WH, McKitrick TR, Lauffenburger D, Cummings RD, Mayanja-Kizza H, Hawn TR, Boom WH, Stein CM, Fortune SM, Seshadri C, Alter G. 2019. IFN-gamma-independent immune markers of *Mycobacterium tuberculosis* exposure. Nat Med 25:977–987. https://doi.org/10.1038/s41591-019-0441-3.
22. Vynnycky E, Fine PE. 1997. The natural history of tuberculosis: the implications of age-dependent risks of disease and the role of reinfection. Epidemiol Infect 119:183–201. https://doi.org/10.1017/s0950268897007917.
23. Brooks-Pollock E, Becerra MC, Goldstein E, Cohen T, Murray MB. 2011. Epidemiologic inference from the distribution of tuberculosis cases in households in Lima, Peru. J Infect Dis 203:1582–1589. https://doi.org/10.1093/infdis/jir162.
24. Andrews JR, Noubary F, Walensky RP, Cerda R, Losina E, Horsburgh CR. 2012. Risk of progression to active tuberculosis following reinfection with *Mycobacterium tuberculosis*. Clin Infect Dis 54:784–791. https://doi.org/10.1093/cid/cir951.
25. Lurie MB. 1929. The fate of tubercle bacilli in the organs of reinfected rabbits. J Exp Med 50:747–765. https://doi.org/10.1084/jem.50.6.747.
26. Cadena AM, Hopkins FF, Maiello P, Carey AF, Wong EA, Martin CJ, Gideon HP, DiFazio RM, Andersen P, Lin PL, Fortune SM, Flynn JL. 2018. Concurrent infection with *Mycobacterium tuberculosis* confers robust protection against secondary infection in macaques. PLoS Pathog 14:e1007305. https://doi.org/10.1371/journal.ppat.1007305.
27. Peters NC, Pagan AJ, Lawyer PG, Hand TW, Henrique Roma E, Stamper LW, Romano A, Sacks DL. 2014. Chronic parasitic infection maintains high frequencies of short-lived Ly6C+CD4+ effector T cells that are required for protection against re-infection. PLoS Pathog 10:e1004538. https://doi.org/10.1371/journal.ppat.1004538.
28. Nemeth J, Olson GS, Rothchild A, Jahn A, Mai D, Duffy F, Delahaye J, Srivatsan S, Plumlee C, Urdahl K, Gold E, Diercks A, Aderem A. 2019. Latent *Mycobacterium tuberculosis* infection provides protection for the host by changing the activation state of the innate immune system. bioRxiv. https://doi.org/10.1101/561126.
29. Millington KA, Gooding S, Hinks TS, Reynolds DJ, Lalvani A. 2010. *Mycobacterium tuberculosis*-specific cellular immune profiles suggest bacillary persistence decades after spontaneous cure in untreated tuberculosis. J Infect Dis 202:1685–1689. https://doi.org/10.1086/656772.
30. O'Garra A, Redford PS, McNab FW, Bloom CI, Wilkinson RJ, Berry MPR. 2013. The immune response in tuberculosis. Annu Rev Immunol 31:475–527. https://doi.org/10.1146/annurev-immunol-032712-095939.
31. Li H, Javid B. 2018. Antibodies and tuberculosis: finally coming of age? Nat Rev Immunol 18:591–596. https://doi.org/10.1038/s41577-018-0028-0.
32. Achkar JM, Prados-Rosales R. 2018. Updates on antibody functions in *Mycobacterium tuberculosis* infection and their relevance for developing a vaccine against tuberculosis. Curr Opin Immunol 53:30–37. https://doi.org/10.1016/j.coi.2018.04.004.
33. Li H, Wang XX, Wang B, Fu L, Liu G, Lu Y, Cao M, Huang H, Javid B. 2017. Latently and uninfected healthcare workers exposed to TB make protective antibodies against *Mycobacterium tuberculosis*. Proc Natl Acad Sci U S A 114:5023–5028. https://doi.org/10.1073/pnas.1611776114.
34. Lu LL, Chung AW, Rosebrock TR, Ghebremichael M, Yu WH, Grace PS, Schoen MK, Tafesse F, Martin C, Leung V, Mahan AE, Sips M, Kumar MP, Tedesco J, Robinson H, Tkachenko E, Draghi M, Freedberg KJ, Streeck H, Suscovich TJ, Lauffenburger DA, Restrepo BI, Day C, Fortune SM, Alter G. 2016. A functional role for antibodies in tuberculosis. Cell 167:433–443.e1–e6. https://doi.org/10.1016/j.cell.2016.08.072.
35. Simmons JD, Stein CM, Seshadri C, Campo M, Alter G, Fortune S, Schurr E, Wallis RS, Churchyard G, Mayanja-Kizza H, Boom WH, Hawn TR. 2018. Immunological mechanisms of human resistance to persistent *Mycobacterium tuberculosis* infection. Nat Rev Immunol 18:575–589. https://doi.org/10.1038/s41577-018-0025-3.
36. Ernst JD. 2012. The immunological life cycle of tuberculosis. Nat Rev Immunol 12:581–591. https://doi.org/10.1038/nri3259.
37. Fabri M, Stenger S, Shin DM, Yuk JM, Liu PT, Realegeno S, Lee HM, Krutzik SR, Schenk M, Sieling PA, Teles R, Montoya D, Iyer SS, Bruns H, Lewinsohn DM, Hollis BW, Hewison M, Adams JS, Steinmeyer A, Zugel U, Cheng G, Jo EK, Bloom BR, Modlin RL. 2011. Vitamin D is required for IFN-gamma-mediated antimicrobial activity of human macrophages. Sci Transl Med 3:104ra102. https://doi.org/10.1126/scitranslmed.3003045.
38. Das R, Koo MS, Kim BH, Jacob ST, Subbian S, Yao J, Leng L, Levy R, Murchison C, Burman WJ, Moore CC, Scheld WM, David JR, Kaplan G, MacMicking JD, Bucala R. 2013. Macrophage migration inhibitory factor (MIF) is a critical mediator of the innate immune response to *Mycobacterium tuberculosis*. Proc Natl Acad Sci U S A 110:E2997–E3006. https://doi.org/10.1073/pnas.1301128110.
39. Mayer-Barber KD, Andrade BB, Oland SD, Amaral EP, Barber DL, Gonzales J, Derrick SC, Shi R, Kumar NP, Wei W, Yuan X, Zhang G, Cai Y, Babu S, Catalfamo M, Salazar AM, Via LE, Barry CE, III, Sher A. 2014. Host-directed therapy of tuberculosis based on interleukin-1 and type I interferon crosstalk. Nature 511:99–103. https://doi.org/10.1038/nature13489.
40. Di Paolo NC, Shafiani S, Day T, Papayannopoulou T, Russell DW, Iwakura Y, Sherman D, Urdahl K, Shayakhmetov DM. 2015. Interdependence between interleukin-1 and tumor necrosis factor regulates TNF-dependent control of *Mycobacterium tuberculosis* infection. Immunity 43:1125–1136. https://doi.org/10.1016/j.immuni.2015.11.016.
41. Kupz A, Zedler U, Staber M, Perdomo C, Dorhoi A, Brosch R, Kaufmann SH. 2016. ESAT-6-dependent cytosolic pattern recognition drives noncognate tuberculosis control in vivo. J Clin Invest 126:2109–2122. https://doi.org/10.1172/JCI84978.
42. Orme IM, Robinson RT, Cooper AM. 2015. The balance between protective and pathogenic immune responses in the TB-infected lung. Nat Immunol 16:57–63. https://doi.org/10.1038/ni.3048.
43. Mishra BB, Lovewell RR, Olive AJ, Zhang G, Wang W, Eugenin E, Smith CM, Phuah JY, Long JE, Dubuke ML, Palace SG, Goguen JD, Baker RE, Nambi S, Mishra R, Booty MG, Baer CE, Shaffer SA, Dartois V, McCormick BA, Chen X, Sassetti CM. 2017. Nitric oxide prevents a pathogen-permissive granulocytic inflammation during tuberculosis. Nat Microbiol 2:17072. https://doi.org/10.1038/nmicrobiol.2017.72.
44. Rothchild AC, Jayaraman P, Nunes-Alves C, Behar SM. 2014. iNKT cell production of GM-CSF controls *Mycobacterium tuberculosis*. PLoS Pathog 10:e1003805. https://doi.org/10.1371/journal.ppat.1003805.
45. Godfrey DI, Uldrich AP, McCluskey J, Rossjohn J, Moody DB. 2015. The burgeoning family of unconventional T cells. Nat Immunol 16:1114–1123. https://doi.org/10.1038/ni.3298.
46. Joosten SA, Ottenhoff THM, Lewinsohn DM, Hoft DF, Moody DB, Seshadri C, Collaboration for Tuberculosis Vaccine Discovery-Donor-Unrestricted T-Cells Working Group, Bill and Melinda Gates Foundation. 2019. Harnessing donor unrestricted T-cells for new vaccines against tuberculosis. Vaccine 37:3022–3030. https://doi.org/10.1016/j.vaccine.2019.04.050.
47. Ardain A, Domingo-Gonzalez R, Das S, Kazer SW, Howard NC, Singh A, Ahmed M, Nhamoyebonde S, Rangel-Moreno J, Ogongo P, Lu L, Ramsuran D, de la Luz Garcia-Hernandez M, Ulland TK, Darby M, Park E, Karim F, Melocchi L, Madansein R, Dullabh KJ, Dunlap M, Marin-Agudelo N, Ebihara T, Ndung'u T, Kaushal D, Pym AS, Kolls JK, Steyn A, Zúñiga J, Horsnell W, Yokoyama WM, Shalek AK, Kløverpris HN, Colonna M, Leslie A, Khader SA. 2019. Group 3 innate lymphoid cells mediate early protective immunity against tuberculosis. Nature 570:528–532. https://doi.org/10.1038/s41586-019-1276-2.
48. Wolf AJ, Desvignes L, Linas B, Banaiee N, Tamura T, Takatsu K, Ernst JD. 2008. Initiation of the adaptive immune response to *Mycobacterium*

tuberculosis depends on antigen production in the local lymph node, not the lungs. J Exp Med 205:105–115. https://doi.org/10.1084/jem.20071367.
49. Samstein M, Schreiber HA, Leiner IM, Susac B, Glickman MS, Pamer EG. 2013. Essential yet limited role for CCR2(+) inflammatory monocytes during *Mycobacterium tuberculosis*-specific T cell priming. Elife 2:e01086. https://doi.org/10.7554/eLife.01086.
50. Sakai S, Kauffman KD, Schenkel JM, McBerry CC, Mayer-Barber KD, Masopust D, Barber DL. 2014. Cutting edge: control of *Mycobacterium tuberculosis* infection by a subset of lung parenchyma-homing CD4 T cells. J Immunol 192:2965–2969. https://doi.org/10.4049/jimmunol.1400019.
51. Slight SR, Rangel-Moreno J, Gopal R, Lin Y, Fallert Junecko BA, Mehra S, Selman M, Becerril-Villanueva E, Baquera-Heredia J, Pavon L, Kaushal D, Reinhart TA, Randall TD, Khader SA. 2013. CXCR5(+) T helper cells mediate protective immunity against tuberculosis. J Clin Invest 123:712–726. https://doi.org/10.1172/JCI65728.
52. Boisson-Dupuis S, Bustamante J, El-Baghdadi J, Camcioglu Y, Parvaneh N, El Azbaoui S, Agader A, Hassani A, El Hafidi N, Mrani NA, Jouhadi Z, Ailal F, Najib J, Reisli I, Zamani A, Yosunkaya S, Gulle-Girit S, Yildiran A, Cipe FE, Torun SH, Metin A, Atikan BY, Hatipoglu N, Aydogmus C, Kilic SS, Dogu F, Karaca N, Aksu G, Kutukculer N, Keser-Emiroglu M, Somer A, Tanir G, Aytekin C, Adimi P, Mahdaviani SA, Mamishi S, Bousfiha A, Sanal O, Mansouri D, Casanova JL, Abel L. 2015. Inherited and acquired immunodeficiencies underlying tuberculosis in childhood. Immunol Rev 264:103–120. https://doi.org/10.1111/imr.12272.
53. Scriba TJ, Kalsdorf B, Abrahams DA, Isaacs F, Hofmeister J, Black G, Hassan HY, Wilkinson RJ, Walzl G, Gelderbloem SJ, Mahomed H, Hussey GD, Hanekom WA. 2008. Distinct, specific IL-17- and IL-22-producing CD4+ T cell subsets contribute to the human anti-mycobacterial immune response. J Immunol 180:1962–1970. https://doi.org/10.4049/jimmunol.180.3.1962.
54. Gopal R, Monin L, Slight S, Uche U, Blanchard E, Fallert Junecko BA, Ramos-Payan R, Stallings CL, Reinhart TA, Kolls JK, Kaushal D, Nagarajan U, Rangel-Moreno J, Khader SA. 2014. Unexpected role for IL-17 in protective immunity against hypervirulent *Mycobacterium tuberculosis* HN878 infection. PLoS Pathog 10:e1004099. https://doi.org/10.1371/journal.ppat.1004099.
55. Booty MG, Barreira-Silva P, Carpenter SM, Nunes-Alves C, Jacques MK, Stowell BL, Jayaraman P, Beamer G, Behar SM. 2016. IL-21 signaling is essential for optimal host resistance against *Mycobacterium tuberculosis* infection. Sci Rep 6:36720. https://doi.org/10.1038/srep36720.
56. Khader SA, Bell GK, Pearl JE, Fountain JJ, Rangel-Moreno J, Cilley GE, Shen F, Eaton SM, Gaffen SL, Swain SL, Locksley RM, Haynes L, Randall TD, Cooper AM. 2007. IL-23 and IL-17 in the establishment of protective pulmonary CD4+ T cell responses after vaccination and during *Mycobacterium tuberculosis* challenge. Nat Immunol 8:369–377. https://doi.org/10.1038/ni1449.
57. Chen CY, Huang D, Wang RC, Shen L, Zeng G, Yao S, Shen Y, Halliday L, Fortman J, McAllister M, Estep J, Hunt R, Vasconcelos D, Du G, Porcelli SA, Larsen MH, Jacobs WR, Jr, Haynes BF, Letvin NL, Chen ZW. 2009. A critical role for CD8 T cells in a nonhuman primate model of tuberculosis. PLoS Pathog 5:e1000392. https://doi.org/10.1371/journal.ppat.1000392.
58. Lindestam Arlehamn CS, Gerasimova A, Mele F, Henderson R, Swann J, Greenbaum JA, Kim Y, Sidney J, James EA, Taplitz R, McKinney DM, Kwok WW, Grey H, Sallusto F, Peters B, Sette A. 2013. Memory T cells in latent *Mycobacterium tuberculosis* infection are directed against three antigenic islands and largely contained in a CXCR3+CCR6+ Th1 subset. PLoS Pathog 9:e1003130. https://doi.org/10.1371/journal.ppat.1003130.
59. Chen T, Blanc C, Eder AZ, Prados-Rosales R, Souza AC, Kim RS, Glatman-Freedman A, Joe M, Bai Y, Lowary TL, Tanner R, Brennan MJ, Fletcher HA, McShane H, Casadevall A, Achkar JM. 2016. Association of human antibodies to arabinomannan with enhanced mycobacterial opsonophagocytosis and intracellular growth reduction. J Infect Dis 214:300–310. https://doi.org/10.1093/infdis/jiw141.
60. Abebe F, Belay M, Legesse M, KLMC F, Ottenhoff THM. 2018. IgA and IgG against *Mycobacterium tuberculosis* Rv2031 discriminate between pulmonary tuberculosis patients, *Mycobacterium tuberculosis*-infected and non-infected individuals. PLoS One 13:e0190989. https://doi.org/10.1371/journal.pone.0190989.
61. Lewinsohn DA, Lewinsohn DM, Scriba TJ. 2017. Polyfunctional CD4(+) T cells as targets for tuberculosis vaccination. Front Immunol 8:1262. https://doi.org/10.3389/fimmu.2017.01262.
62. Nunes-Alves C, Booty MG, Carpenter SM, Jayaraman P, Rothchild AC, Behar SM. 2014. In search of a new paradigm for protective immunity to TB. Nat Rev Microbiol 12:289–299. https://doi.org/10.1038/nrmicro3230.
63. Sonnenberg P, Glynn JR, Fielding K, Murray J, Godfrey-Faussett P, Shearer S. 2005. How soon after infection with HIV does the risk of tuberculosis start to increase? A retrospective cohort study in South African gold miners. J Infect Dis 191:150–158. https://doi.org/10.1086/426827.
64. Foreman TW, Mehra S, LoBato DN, Malek A, Alvarez X, Golden NA, Bucşan AN, Didier PJ, Doyle-Meyers LA, Russell-Lodrigue KE, Roy CJ, Blanchard J, Kuroda MJ, Lackner AA, Chan J, Khader SA, Jacobs WR, Jr, Kaushal D. 2016. CD4+ T-cell-independent mechanisms suppress reactivation of latent tuberculosis in a macaque model of HIV coinfection. Proc Natl Acad Sci U S A 113:E5636–E5644. https://doi.org/10.1073/pnas.1611987113.
65. Gallegos AM, van Heijst JW, Samstein M, Su X, Pamer EG, Glickman MS. 2011. A gamma interferon independent mechanism of CD4 T cell mediated control of *M. tuberculosis* infection in vivo. PLoS Pathog 7:e1002052. https://doi.org/10.1371/journal.ppat.1002052.
66. Orr MT, Windish HP, Beebe EA, Argilla D, Huang PW, Reese VA, Reed SG, Coler RN. 2015. Interferon gamma and tumor necrosis factor are not essential parameters of CD4+ T-cell responses for vaccine control of tuberculosis. J Infect Dis 212:495–504. https://doi.org/10.1093/infdis/jiv055.
67. Sakai S, Kauffman KD, Sallin MA, Sharpe AH, Young HA, Ganusov VV, Barber DL. 2016. CD4 T cell-derived IFN-gamma plays a minimal role in control of pulmonary *Mycobacterium tuberculosis* infection and must be actively repressed by PD-1 to prevent lethal disease. PLoS Pathog 12:e1005667. https://doi.org/10.1371/journal.ppat.1005667.
68. Sallin MA, Kauffman KD, Riou C, Du Bruyn E, Foreman TW, Sakai S, Hoft SG, Myers TG, Gardina PJ, Sher A, Moore R, Wilder-Kofie T, Moore IN, Sette A, Lindestam Arlehamn CS, Wilkinson RJ, Barber DL. 2018. Host resistance to pulmonary *Mycobacterium tuberculosis* infection requires CD153 expression. Nat Microbiol 3:1198–1205. https://doi.org/10.1038/s41564-018-0231-6.
69. Cruz A, Fraga AG, Fountain JJ, Rangel-Moreno J, Torrado E, Saraiva M, Pereira DR, Randall TD, Pedrosa J, Cooper AM, Castro AG. 2010. Pathological role of interleukin 17 in mice subjected to repeated BCG vaccination after infection with *Mycobacterium tuberculosis*. J Exp Med 207:1609–1616. https://doi.org/10.1084/jem.20100265.
70. Jurado JO, Pasquinelli V, Alvarez IB, Pena D, Rovetta AI, Tateosian NL, Romeo HE, Musella RM, Palmero D, Chuluyan HE, Garcia VE. 2012. IL-17 and IFN-gamma expression in lymphocytes from patients with active tuberculosis correlates with the severity of the disease. J Leukoc Biol 91:991–1002. https://doi.org/10.1189/jlb.1211619.
71. Barber DL, Sakai S, Kudchadkar RR, Fling SP, Day TA, Vergara JA, Ashkin D, Cheng JH, Lundgren LM, Raabe VN, Kraft CS, Nieva JJ, Cheever MA, Nghiem PT, Sharon E. 2019. Tuberculosis following PD-1 blockade for cancer immunotherapy. Sci Transl Med 11:eaat2702.
72. Cruz A, Khader SA, Torrado E, Fraga A, Pearl JE, Pedrosa J, Cooper AM, Castro AG. 2006. Cutting edge: IFN-gamma regulates the induction and expansion of IL-17-producing CD4 T cells during mycobacterial infection. J Immunol 177:1416–1420. https://doi.org/10.4049/jimmunol.177.3.1416.
73. Kozakiewicz L, Chen Y, Xu J, Wang Y, Dunussi-Joannopoulos K, Ou Q, Flynn JL, Porcelli SA, Jacobs WR, Jr, Chan J. 2013. B cells regulate neutrophilia during *Mycobacterium tuberculosis* infection and BCG vaccination by modulating the interleukin-17 response. PLoS Pathog 9:e1003472. https://doi.org/10.1371/journal.ppat.1003472.
74. Green AM, Mattila JT, Bigbee CL, Bongers KS, Lin PL, Flynn JL. 2010. CD4(+) regulatory T cells in a cynomolgus macaque model of *Mycobacterium tuberculosis* infection. J Infect Dis 202:533–541. https://doi.org/10.1086/654896.
75. Scriba TJ, Penn-Nicholson A, Shankar S, Hraha T, Thompson EG, Sterling D, Nemes E, Darboe F, Suliman S, Amon LM, Mahomed H, Erasmus M, Whatney W, Johnson JL, Boom WH, Hatherill M, Valvo J, De Groote MA, Ochsner UA, Aderem A, Hanekom WA, Zak DE, Other Members of the ACS Cohort Study Team. 2017. Sequential inflammatory processes define human progression from M. tuberculosis infection to tuberculosis disease. PLoS Pathog 13:e1006687. https://doi.org/10.1371/journal.ppat.1006687.
76. Lin PL, Ford CB, Coleman MT, Myers AJ, Gawande R, Ioerger T, Sacchettini J, Fortune SM, Flynn JL. 2014. Sterilization of granulomas is com-

mon in active and latent tuberculosis despite within-host variability in bacterial killing. Nat Med 20:75–79. https://doi.org/10.1038/nm.3412.
77. Esmail H, Lai RP, Lesosky M, Wilkinson KA, Graham CM, Coussens AK, Oni T, Warwick JM, Said-Hartley Q, Koegelenberg CF, Walzl G, Flynn JL, Young DB, Barry CE, III, O'Garra A, Wilkinson RJ. 2016. Characterization of progressive HIV-associated tuberculosis using 2-deoxy-2-[18F]fluoro-D-glucose positron emission and computed tomography. Nat Med 22:1090–1093. https://doi.org/10.1038/nm.4161.
78. Drain PK, Bajema KL, Dowdy D, Dheda K, Naidoo K, Schumacher SG, Ma S, Meermeier E, Lewinsohn DM, Sherman DR. 2018. Incipient and subclinical tuberculosis: a clinical review of early stages and progression of infection. Clin Microbiol Rev 31:e00021-18. https://doi.org/10.1128/CMR.00021-18.
79. Gideon HP, Phuah J, Myers AJ, Bryson BD, Rodgers MA, Coleman MT, Maiello P, Rutledge T, Marino S, Fortune SM, Kirschner DE, Lin PL, Flynn JL. 2015. Variability in tuberculosis granuloma T cell responses exists, but a balance of pro- and anti-inflammatory cytokines is associated with sterilization. PLoS Pathog 11:e1004603. https://doi.org/10.1371/journal.ppat.1004603.
80. Marakalala MJ, Raju RM, Sharma K, Zhang YJ, Eugenin EA, Prideaux B, Daudelin IB, Chen PY, Booty MG, Kim JH, Eum SY, Via LE, Behar SM, Barry CE, III, Mann M, Dartois V, Rubin EJ. 2016. Inflammatory signaling in human tuberculosis granulomas is spatially organized. Nat Med 22:531–538. https://doi.org/10.1038/nm.4073.
81. Esmail H, Barry CE, III, Young DB, Wilkinson RJ. 2014. The ongoing challenge of latent tuberculosis. Philos Trans R Soc Lond B Biol Sci 369:20130437. https://doi.org/10.1098/rstb.2013.0437.
82. Behr MA, Edelstein PH, Ramakrishnan L. 2018. Revisiting the timetable of tuberculosis. BMJ 362:k2738. https://doi.org/10.1136/bmj.k2738.
83. Hunter RL. 2018. The pathogenesis of tuberculosis: the early infiltrate of post-primary (adult pulmonary) tuberculosis: a distinct disease entity. Front Immunol 9:2108. https://doi.org/10.3389/fimmu.2018.02108.
84. Jayaraman P, Jacques MK, Zhu C, Steblenko KM, Stowell BL, Madi A, Anderson AC, Kuchroo VK, Behar SM. 2016. TIM3 mediates T cell exhaustion during *Mycobacterium tuberculosis* infection. PLoS Pathog 12:e1005490. https://doi.org/10.1371/journal.ppat.1005490.
85. Shen L, Gao Y, Liu Y, Zhang B, Liu Q, Wu J, Fan L, Ou Q, Zhang W, Shao L. 2016. PD-1/PD-L pathway inhibits M.tb-specific CD4+ T-cell functions and phagocytosis of macrophages in active tuberculosis. Sci Rep 6:38362. https://doi.org/10.1038/srep38362.
86. Sallin MA, Sakai S, Kauffman KD, Young HA, Zhu J, Barber DL. 2017. Th1 differentiation drives the accumulation of intravascular, non-protective CD4 T cells during tuberculosis. Cell Rep 18:3091–3104. https://doi.org/10.1016/j.celrep.2017.03.007.
87. Tzelepis F, Blagih J, Khan N, Gillard J, Mendonca L, Roy DG, Ma EH, Joubert P, Jones RG, Divangahi M. 2018. Mitochondrial cyclophilin D regulates T cell metabolic responses and disease tolerance to tuberculosis. Sci Immunol 3:eaar4135. https://doi.org/10.1126/sciimmunol.aar4135.
88. Joosten SA, van Meijgaarden KE, Del Nonno F, Baiocchini A, Petrone L, Vanini V, Smits HH, Palmieri F, Goletti D, Ottenhoff TH. 2016. Patients with tuberculosis have a dysfunctional circulating B-cell compartment, which normalizes following successful treatment. PLoS Pathog 12:e1005687. https://doi.org/10.1371/journal.ppat.1005687.
89. Moguche AO, Musvosvi M, Penn-Nicholson A, Plumlee CR, Mearns H, Geldenhuys H, Smit E, Abrahams D, Rozot V, Dintwe O, Hoff ST, Kromann I, Ruhwald M, Bang P, Larson RP, Shafiani S, Ma S, Sherman DR, Sette A, Lindestam Arlehamn CS, McKinney DM, Maecker H, Hanekom WA, Hatherill M, Andersen P, Scriba TJ, Urdahl KB. 2017. Antigen availability shapes T cell differentiation and function during tuberculosis. Cell Host Microbe 21:695–706.e1–e5. https://doi.org/10.1016/j.chom.2017.05.012.
90. Woodworth JS, Cohen SB, Moguche AO, Plumlee CR, Agger EM, Urdahl KB, Andersen P. 2017. Subunit vaccine H56/CAF01 induces a population of circulating CD4 T cells that traffic into the *Mycobacterium tuberculosis*-infected lung. Mucosal Immunol 10:555–564. https://doi.org/10.1038/mi.2016.70.
91. Kauffman KD, Sallin MA, Sakai S, Kamenyeva O, Kabat J, Weiner D, Sutphin M, Schimel D, Via L, Barry CE, III, Wilder-Kofie T, Moore I, Moore R, Barber DL. 2018. Defective positioning in granulomas but not lung-homing limits CD4 T-cell interactions with *Mycobacterium tuberculosis*-infected macrophages in rhesus macaques. Mucosal Immunol 11:462–473. https://doi.org/10.1038/mi.2017.60.
92. Hunter R, Actor J. 2019. The pathogenesis of post-primary tuberculosis. A game changer for vaccine development. Tuberculosis (Edinb) 116S:S114–S117. https://doi.org/10.1016/j.tube.2019.04.018.
93. Jung YJ, Ryan L, LaCourse R, North RJ. 2005. Properties and protective value of the secondary versus primary T helper type 1 response to airborne *Mycobacterium tuberculosis* infection in mice. J Exp Med 201:1915–1924. https://doi.org/10.1084/jem.20050265.
94. Carpenter SM, Nunes-Alves C, Booty MG, Way SS, Behar SM. 2016. A higher activation threshold of memory CD8+ T cells has a fitness cost that is modified by TCR affinity during tuberculosis. PLoS Pathog 12:e1005380. https://doi.org/10.1371/journal.ppat.1005380.
95. Verver S, Warren RM, Beyers N, Richardson M, van der Spuy GD, Borgdorff MW, Enarson DA, Behr MA, van Helden PD. 2005. Rate of reinfection tuberculosis after successful treatment is higher than rate of new tuberculosis. Am J Respir Crit Care Med 171:1430–1435. https://doi.org/10.1164/rccm.200409-1200OC.
96. De Gregorio E, Rappuoli R. 2014. From empiricism to rational design: a personal perspective of the evolution of vaccine development. Nat Rev Immunol 14:505–514. https://doi.org/10.1038/nri3694.
97. Hansen SG, Zak DE, Xu G, Ford JC, Marshall EE, Malouli D, Gilbride RM, Hughes CM, Ventura AB, Ainslie E, Randall KT, Selseth AN, Rundstrom P, Herlache L, Lewis MS, Park H, Planer SL, Turner JM, Fischer M, Armstrong C, Zweig RC, Valvo J, Braun JM, Shankar S, Lu L, Sylwester AW, Legasse AW, Messerle M, Jarvis MA, Amon LM, Aderem A, Alter G, Laddy DJ, Stone M, Bonavia A, Evans TG, Axthelm MK, Fruh K, Edlefsen PT, Picker LJ. 2018. Prevention of tuberculosis in rhesus macaques by a cytomegalovirus-based vaccine. Nat Med 24:130–143. https://doi.org/10.1038/nm.4473.
98. Goodridge HS, Ahmed SS, Curtis N, Kollmann TR, Levy O, Netea MG, Pollard AJ, van Crevel R, Wilson CB. 2016. Harnessing the beneficial heterologous effects of vaccination. Nat Rev Immunol 16:392–400. https://doi.org/10.1038/nri.2016.43.
99. Kleinnijenhuis J, Quintin J, Preijers F, Joosten LA, Ifrim DC, Saeed S, Jacobs C, van Loenhout J, de Jong D, Stunnenberg HG, Xavier RJ, van der Meer JW, van Crevel R, Netea MG. 2012. Bacille Calmette-Guerin induces NOD2-dependent nonspecific protection from reinfection via epigenetic reprogramming of monocytes. Proc Natl Acad Sci U S A 109:17537–17542. https://doi.org/10.1073/pnas.1202870109.
100. Suliman S, Geldenhuys H, Johnson JL, Hughes JE, Smit E, Murphy M, Toefy A, Lerumo L, Hopley C, Pienaar B, Chheng P, Nemes E, Hoft DF, Hanekom WA, Boom WH, Hatherill M, Scriba TJ. 2016. Bacillus Calmette-Guerin (BCG) revaccination of adults with latent *Mycobacterium tuberculosis* infection induces long-lived BCG-reactive NK cell responses. J Immunol 197:1100–1110. https://doi.org/10.4049/jimmunol.1501996.
101. Kaufmann E, Sanz J, Dunn JL, Khan N, Mendonca LE, Pacis A, Tzelepis F, Pernet E, Dumaine A, Grenier JC, Mailhot-Leonard F, Ahmed E, Belle J, Besla R, Mazer B, King IL, Nijnik A, Robbins CS, Barreiro LB, Divangahi M. 2018. BCG educates hematopoietic stem cells to generate protective innate immunity against tuberculosis. Cell 172:176–190.e1–e11. https://doi.org/10.1016/j.cell.2017.12.031.
102. Fine PE. 1995. Variation in protection by BCG: implications of and for heterologous immunity. Lancet 346:1339–1345. https://doi.org/10.1016/s0140-6736(95)92348-9.
103. Aronson NE, Santosham M, Comstock GW, Howard RS, Moulton LH, Rhoades ER, Harrison LH. 2004. Long-term efficacy of BCG vaccine in American Indians and Alaska Natives: a 60-year follow-up study. JAMA 291:2086–2091. https://doi.org/10.1001/jama.291.17.2086.
104. Nguipdop-Djomo P, Heldal E, Rodrigues LC, Abubakar I, Mangtani P. 2016. Duration of BCG protection against tuberculosis and change in effectiveness with time since vaccination in Norway: a retrospective population-based cohort study. Lancet Infect Dis 16:219–226. https://doi.org/10.1016/S1473-3099(15)00400-4.
105. Abubakar I, Pimpin L, Ariti C, Beynon R, Mangtani P, Sterne JA, Fine PE, Smith PG, Lipman M, Elliman D, Watson JM, Drumright LN, Whiting PF, Vynnycky E, Rodrigues LC. 2013. Systematic review and meta-analysis of the current evidence on the duration of protection by bacillus Calmette-Guerin vaccination against tuberculosis. Health Technol Assess 17:1–372. https://doi.org/10.3310/hta17370.
106. Orme IM. 2010. The Achilles heel of BCG. Tuberculosis (Edinb) 90:329–332. https://doi.org/10.1016/j.tube.2010.06.002.
107. Soares AP, Kwong Chung CK, Choice T, Hughes EJ, Jacobs G, van Rensburg EJ, Khomba G, de Kock M, Lerumo L, Makhethe L, Maneli MH, Pienaar B, Smit E, Tena-Coki NG, van Wyk L, Boom WH, Kaplan G, Scriba TJ, Hanekom WA. 2013. Longitudinal changes in CD4(+) T-cell memory

responses induced by BCG vaccination of newborns. J Infect Dis 207:1084–1094. https://doi.org/10.1093/infdis/jis941.
108. Nandakumar S, Kannanganat S, Posey JE, Amara RR, Sable SB. 2014. Attrition of T-cell functions and simultaneous upregulation of inhibitory markers correspond with the waning of BCG-induced protection against tuberculosis in mice. PLoS One 9:e113951. https://doi.org/10.1371/journal.pone.0113951.
109. Donald PR, Marais BJ, Barry CE, III. 2010. Age and the epidemiology and pathogenesis of tuberculosis. Lancet 375:1852–1854. https://doi.org/10.1016/S0140-6736(10)60580-6.
110. Iqbal SA, Winston CA, Bardenheier BH, Armstrong LR, Navin TR. 2018. Age-period-cohort analyses of tuberculosis incidence rates by nativity, United States, 1996-2016. Am J Public Health 108:S315–S320. https://doi.org/10.2105/AJPH.2018.304687.
111. Anonymous. 1996. Randomised controlled trial of single BCG, repeated BCG, or combined BCG and killed *Mycobacterium leprae* vaccine for prevention of leprosy and tuberculosis in Malawi. Karonga Prevention Trial Group. Lancet 348:17–24. https://doi.org/10.1016/S0140-6736(96)02166-6.
112. Rodrigues LC, Pereira SM, Cunha SS, Genser B, Ichihara MY, de Brito SC, Hijjar MA, Dourado I, Cruz AA, Sant'Anna C, Bierrenbach AL, Barreto ML. 2005. Effect of BCG revaccination on incidence of tuberculosis in school-aged children in Brazil: the BCG-REVAC cluster-randomised trial. Lancet 366:1290–1295. https://doi.org/10.1016/S0140-6736(05)67145-0.
113. Barreto ML, Pereira SM, Pilger D, Cruz AA, Cunha SS, Sant'Anna C, Ichihara MY, Genser B, Rodrigues LC. 2011. Evidence of an effect of BCG revaccination on incidence of tuberculosis in school-aged children in Brazil: second report of the BCG-REVAC cluster-randomised trial. Vaccine 29:4875–4877. https://doi.org/10.1016/j.vaccine.2011.05.023.
114. Andersen P, Doherty TM. 2005. The success and failure of BCG—implications for a novel tuberculosis vaccine. Nat Rev Microbiol 3:656–662. https://doi.org/10.1038/nrmicro1211.
115. Barreto ML, Pilger D, Pereira SM, Genser B, Cruz AA, Cunha SS, Sant'Anna C, Hijjar MA, Ichihara MY, Rodrigues LC. 2014. Causes of variation in BCG vaccine efficacy: examining evidence from the BCG REVAC cluster randomized trial to explore the masking and the blocking hypotheses. Vaccine 32:3759–3764. https://doi.org/10.1016/j.vaccine.2014.05.042.
116. Lindestam Arlehamn CS, Paul S, Mele F, Huang C, Greenbaum JA, Vita R, Sidney J, Peters B, Sallusto F, Sette A. 2015. Immunological consequences of intragenus conservation of Mycobacterium tuberculosis T-cell epitopes. Proc Natl Acad Sci U S A 112:E147–E155. https://doi.org/10.1073/pnas.1416537112.
117. von Reyn CF. 2014. BCG, latitude, and environmental mycobacteria. Clin Infect Dis 59:607–608. https://doi.org/10.1093/cid/ciu331.
118. Groschel MI, Sayes F, Simeone R, Majlessi L, Brosch R. 2016. ESX secretion systems: mycobacterial evolution to counter host immunity. Nat Rev Microbiol 14:677–691. https://doi.org/10.1038/nrmicro.2016.131.
119. Sayes F, Pawlik A, Frigui W, Groschel MI, Crommelynck S, Fayolle C, Cia F, Bancroft GJ, Bottai D, Leclerc C, Brosch R, Majlessi L. 2016. CD4+ T cells recognizing PE/PPE antigens directly or via cross reactivity are protective against pulmonary *Mycobacterium tuberculosis* infection. PLoS Pathog 12:e1005770. https://doi.org/10.1371/journal.ppat.1005770.
120. Sweeney KA, Dao DN, Goldberg MF, Hsu T, Venkataswamy MM, Henao-Tamayo M, Ordway D, Sellers RS, Jain P, Chen B, Chen M, Kim J, Lukose R, Chan J, Orme IM, Porcelli SA, Jacobs WR, Jr. 2011. A recombinant *Mycobacterium smegmatis* induces potent bactericidal immunity against *Mycobacterium tuberculosis*. Nat Med 17:1261–1268. https://doi.org/10.1038/nm.2420.
121. Knudsen NP, Norskov-Lauritsen S, Dolganov GM, Schoolnik GK, Lindenstrom T, Andersen P, Agger EM, Aagaard C. 2014. Tuberculosis vaccine with high predicted population coverage and compatibility with modern diagnostics. Proc Natl Acad Sci U S A 111:1096–1101. https://doi.org/10.1073/pnas.1314973111.
122. Copin R, Coscolla M, Efstathiadis E, Gagneux S, Ernst JD. 2014. Impact of in vitro evolution on antigenic diversity of *Mycobacterium bovis* bacillus Calmette-Guerin (BCG). Vaccine 32:5998–6004. https://doi.org/10.1016/j.vaccine.2014.07.113.
123. Ritz N, Dutta B, Donath S, Casalaz D, Connell TG, Tebruegge M, Robins-Browne R, Hanekom WA, Britton WJ, Curtis N. 2012. The influence of bacille Calmette-Guerin vaccine strain on the immune response against tuberculosis: a randomized trial. Am J Respir Crit Care Med 185:213–222. https://doi.org/10.1164/rccm.201104-0714OC.
124. Zhang L, Ru HW, Chen FZ, Jin CY, Sun RF, Fan XY, Guo M, Mai JT, Xu WX, Lin QX, Liu J. 2016. Variable virulence and efficacy of BCG vaccine strains in mice and correlation with genome polymorphisms. Mol Ther 24:398–405. https://doi.org/10.1038/mt.2015.216.
125. Tran V, Ahn SK, Ng M, Li M, Liu J. 2016. Loss of lipid virulence factors reduces the efficacy of the BCG vaccine. Sci Rep 6:29076. https://doi.org/10.1038/srep29076.
126. Cambier CJ, Falkow S, Ramakrishnan L. 2014. Host evasion and exploitation schemes of *Mycobacterium tuberculosis*. Cell 159:1497–1509. https://doi.org/10.1016/j.cell.2014.11.024.
127. Augenstreich J, Arbues A, Simeone R, Haanappel E, Wegener A, Sayes F, Le Chevalier F, Chalut C, Malaga W, Guilhot C, Brosch R, Astarie-Dequeker C. 2017. ESX-1 and phthiocerol dimycocerosates of *Mycobacterium tuberculosis* act in concert to cause phagosomal rupture and host cell apoptosis. Cell Microbiol 19:e12726. https://doi.org/10.1111/cmi.12726.
128. Cambier CJ, O'Leary SM, O'Sullivan MP, Keane J, Ramakrishnan L. 2017. Phenolic glycolipid facilitates mycobacterial escape from microbicidal tissue-resident macrophages. Immunity 47:552–565.e1–e4. https://doi.org/10.1016/j.immuni.2017.08.003.
129. Simeone R, Majlessi L, Enninga J, Brosch R. 2016. Perspectives on mycobacterial vacuole-to-cytosol translocation: the importance of cytosolic access. Cell Microbiol 18:1070–1077. https://doi.org/10.1111/cmi.12622.
130. Groschel MI, Sayes F, Shin SJ, Frigui W, Pawlik A, Orgeur M, Canetti R, Honore N, Simeone R, van der Werf TS, Bitter W, Cho SN, Majlessi L, Brosch R. 2017. Recombinant BCG expressing ESX-1 of *Mycobacterium marinum* combines low virulence with cytosolic immune signaling and improved TB protection. Cell Rep 18:2752–2765. https://doi.org/10.1016/j.celrep.2017.02.057.
131. Aguilo N, Gonzalo-Asensio J, Alvarez-Arguedas S, Marinova D, Gomez AB, Uranga S, Spallek R, Singh M, Audran R, Spertini F, Martin C. 2017. Reactogenicity to major tuberculosis antigens absent in BCG is linked to improved protection against *Mycobacterium tuberculosis*. Nat Commun 8:16085. https://doi.org/10.1038/ncomms16085.
132. Saini NK, Baena A, Ng TW, Venkataswamy MM, Kennedy SC, Kunnath-Velayudhan S, Carreno LJ, Xu J, Chan J, Larsen MH, Jacobs WR, Jr, Porcelli SA. 2016. Suppression of autophagy and antigen presentation by *Mycobacterium tuberculosis* PE_PGRS47. Nat Microbiol 1:16133. https://doi.org/10.1038/nmicrobiol.2016.133.
133. Nieuwenhuizen NE, Kulkarni PS, Shaligram U, Cotton MF, Rentsch CA, Eisele B, Grode L, Kaufmann SHE. 2017. The recombinant bacille Calmette-Guerin vaccine VPM1002: ready for clinical efficacy testing. Front Immunol 8:1147. https://doi.org/10.3389/fimmu.2017.01147.
134. Gengenbacher M, Nieuwenhuizen N, Vogelzang A, Liu H, Kaiser P, Schuerer S, Lazar D, Wagner I, Mollenkopf HJ, Kaufmann SH. 2016. Deletion of *nuoG* from the vaccine candidate *Mycobacterium bovis* BCG Δ*ureC*::*hly* improves protection against tuberculosis. mBio 7:e00679-16. https://doi.org/10.1128/mBio.00679-16.
135. Wallgren A. 1948. The time-table of tuberculosis. Tubercle 29:245–251. https://doi.org/10.1016/s0041-3879(48)80033-4.
136. Wolf AJ, Linas B, Trevejo-Nunez GJ, Kincaid E, Tamura T, Takatsu K, Ernst JD. 2007. *Mycobacterium tuberculosis* infects dendritic cells with high frequency and impairs their function in vivo. J Immunol 179:2509–2519. https://doi.org/10.4049/jimmunol.179.4.2509.
137. Blomgran R, Desvignes L, Briken V, Ernst JD. 2012. *Mycobacterium tuberculosis* inhibits neutrophil apoptosis, leading to delayed activation of naive CD4 T cells. Cell Host Microbe 11:81–90. https://doi.org/10.1016/j.chom.2011.11.012.
138. Shafiani S, Dinh C, Ertelt JM, Moguche AO, Siddiqui I, Smigiel KS, Sharma P, Campbell DJ, Way SS, Urdahl KB. 2013. Pathogen-specific Treg cells expand early during *Mycobacterium tuberculosis* infection but are later eliminated in response to interleukin-12. Immunity 38:1261–1270. https://doi.org/10.1016/j.immuni.2013.06.003.
139. Ernst JD. 2018. Mechanisms of *M. tuberculosis* immune evasion as challenges to TB vaccine design. Cell Host Microbe 24:34–42. https://doi.org/10.1016/j.chom.2018.06.004.
140. Carpenter SM, Yang JD, Lee J, Barreira-Silva P, Behar SM. 2017. Vaccine-elicited memory CD4+ T cell expansion is impaired in the lungs during tuberculosis. PLoS Pathog 13:e1006704. https://doi.org/10.1371/journal.ppat.1006704.
141. Griffiths KL, Ahmed M, Das S, Gopal R, Horne W, Connell TD, Moynihan KD, Kolls JK, Irvine DJ, Artyomov MN, Rangel-Moreno J, Khader SA. 2016. Targeting dendritic cells to accelerate T-cell activation overcomes a bottleneck in tuberculosis vaccine efficacy. Nat Commun 7:13894. https://doi.org/10.1038/ncomms13894.

142. Coscolla M, Copin R, Sutherland J, Gehre F, de Jong B, Owolabi O, Mbayo G, Giardina F, Ernst JD, Gagneux S. 2015. *M. tuberculosis* T cell epitope analysis reveals paucity of antigenic variation and identifies rare variable TB antigens. Cell Host Microbe 18:538–548. https://doi.org/10.1016/j.chom.2015.10.008.
143. Comas I, Chakravartti J, Small PM, Galagan J, Niemann S, Kremer K, Ernst JD, Gagneux S. 2010. Human T cell epitopes of *Mycobacterium tuberculosis* are evolutionarily hyperconserved. Nat Genet 42:498–503. https://doi.org/10.1038/ng.590.
144. Baena A, Porcelli SA. 2009. Evasion and subversion of antigen presentation by *Mycobacterium tuberculosis*. Tissue Antigens 74:189–204. https://doi.org/10.1111/j.1399-0039.2009.01301.x.
145. Kwan CK, Ernst JD. 2011. HIV and tuberculosis: a deadly human syndemic. Clin Microbiol Rev 24:351–376. https://doi.org/10.1128/CMR.00042-10.
146. Roy A, Eisenhut M, Harris RJ, Rodrigues LC, Sridhar S, Habermann S, Snell L, Mangtani P, Adetifa I, Lalvani A, Abubakar I. 2014. Effect of BCG vaccination against *Mycobacterium tuberculosis* infection in children: systematic review and meta-analysis. BMJ 349:g4643. https://doi.org/10.1136/bmj.g4643.
147. Verrall AJ, Alisjahbana B, Apriani L, Novianty N, Nurani AC, van Laarhoven A, Ussher JE, Indrati A, Ruslami R, Netea MG, Sharples K, van Crevel R, Hill PC. 12 July 2019. Early clearance of *Mycobacterium tuberculosis*: the INFECT case contact cohort study in Indonesia. J Infect Dis. https://doi.org/10.1093/infdis/jiz168.
148. Hawn TR, Day TA, Scriba TJ, Hatherill M, Hanekom WA, Evans TG, Churchyard GJ, Kublin JG, Bekker LG, Self SG. 2014. Tuberculosis vaccines and prevention of infection. Microbiol Mol Biol Rev 78:650–671. https://doi.org/10.1128/MMBR.00021-14.
149. Hatherill M, Tait D, McShane H. 2016. Clinical testing of tuberculosis vaccine candidates. Microbiol Spectr 4:TBTB2-0015-2016. https://doi.org/10.1128/microbiolspec.TBTB2-0015-2016.
150. Nemes E, Rozot V, Geldenhuys H, Bilek N, Mabwe S, Abrahams D, Makhethe L, Erasmus M, Keyser A, Toefy A, Cloete Y, Ratangee F, Blauenfeldt T, Ruhwald M, Walzl G, Smith B, Loxton AG, Hanekom WA, Andrews JR, Lempicki MD, Ellis R, Ginsberg AM, Hatherill M, Scriba TJ, C-040-404 Study Team, Adolescent Cohort Study Team. 2017. Optimization and interpretation of serial QuantiFERON testing to measure acquisition of *Mycobacterium tuberculosis* infection. Am J Respir Crit Care Med 196:638–648. https://doi.org/10.1164/rccm.201704-0817OC.
151. Winje BA, White R, Syre H, Skutlaberg DH, Oftung F, Mengshoel AT, Blix HS, Brantsæter AB, Holter EK, Handal N, Simonsen GS, Afset JE, Bakken Kran AM. 2018. Stratification by interferon-gamma release assay level predicts risk of incident TB. Thorax 73:652–661. https://doi.org/10.1136/thoraxjnl-2017-211147.
152. Knight GM, Griffiths UK, Sumner T, Laurence YV, Gheorghe A, Vassall A, Glaziou P, White RG. 2014. Impact and cost-effectiveness of new tuberculosis vaccines in low- and middle-income countries. Proc Natl Acad Sci U S A 111:15520–15525. https://doi.org/10.1073/pnas.1404386111.
153. Nunn AJ, Phillips PP, Mitchison DA. 2010. Timing of relapse in short-course chemotherapy trials for tuberculosis. Int J Tuberc Lung Dis 14:241–242.
154. Coler RN, Bertholet S, Pine SO, Orr MT, Reese V, Windish HP, Davis C, Kahn M, Baldwin SL, Reed SG. 2013. Therapeutic immunization against *Mycobacterium tuberculosis* is an effective adjunct to antibiotic treatment. J Infect Dis 207:1242–1252. https://doi.org/10.1093/infdis/jis425.
155. Lin PL, Dietrich J, Tan E, Abalos RM, Burgos J, Bigbee C, Bigbee M, Milk L, Gideon HP, Rodgers M, Cochran C, Guinn KM, Sherman DR, Klein E, Janssen C, Flynn JL, Andersen P. 2012. The multistage vaccine H56 boosts the effects of BCG to protect cynomolgus macaques against active tuberculosis and reactivation of latent *Mycobacterium tuberculosis* infection. J Clin Invest 122:303–314. https://doi.org/10.1172/JCI46252.
156. Grode L, Seiler P, Baumann S, Hess J, Brinkmann V, Eddine AN, Mann P, Goosmann C, Bandermann S, Smith D, Bancroft GJ, Reyrat JM, van Soolingen D, Raupach B, Kaufmann SHE. 2005. Increased vaccine efficacy against tuberculosis of recombinant *Mycobacterium bovis* bacille Calmette-Guerin mutants that secrete listeriolysin. J Clin Invest 115:2472–2479. https://doi.org/10.1172/JCI24617.
157. Grode L, Ganoza CA, Brohm C, Weiner J, III, Eisele B, Kaufmann SH. 2013. Safety and immunogenicity of the recombinant BCG vaccine VPM1002 in a phase 1 open-label randomized clinical trial. Vaccine 31:1340–1348. https://doi.org/10.1016/j.vaccine.2012.12.053.
158. Loxton AG, Knaul JK, Grode L, Gutschmidt A, Meller C, Eisele B, Johnstone H, van der Spuy G, Maertzdorf J, Kaufmann SH, Hesseling AC, Walzl G, Cotton MF, VPM Study Group. 2017. Safety and immunogenicity of the recombinant *Mycobacterium bovis* BCG vaccine VPM1002 in HIV-unexposed newborn infants in South Africa. Clin Vaccine Immunol 24:e00439-16. https://doi.org/10.1128/CVI.00439-16.
159. Spertini F, Audran R, Chakour R, Karoui O, Steiner-Monard V, Thierry AC, Mayor CE, Rettby N, Jaton K, Vallotton L, Lazor-Blanchet C, Doce J, Puentes E, Marinova D, Aguilo N, Martin C. 2015. Safety of human immunisation with a live-attenuated *Mycobacterium tuberculosis* vaccine: a randomised, double-blind, controlled phase I trial. Lancet Respir Med 3:953–962. https://doi.org/10.1016/S2213-2600(15)00435-X.
160. Tameris M, Mearns H, Penn-Nicholson A, Gregg Y, Bilek N, Mabwe S, Geldenhuys H, Shenje J, Luabeya AKK, Murillo I, Doce J, Aguilo N, Marinova D, Puentes E, Rodríguez E, Gonzalo-Asensio J, Fritzell B, Thole J, Martin C, Scriba TJ, Hatherill M, MTBVAC Clinical Trial Team. 2019. Live-attenuated Mycobacterium tuberculosis vaccine MTBVAC versus BCG in adults and neonates: a randomised controlled, double-blind dose-escalation trial. Lancet Respir Med 7:757–770. https://doi.org/10.1016/S2213-2600(19)30251-6.
161. Hoft DF, Blazevic A, Abate G, Hanekom WA, Kaplan G, Soler JH, Weichold F, Geiter L, Sadoff JC, Horwitz MA. 2008. A new recombinant bacille Calmette-Guerin vaccine safely induces significantly enhanced tuberculosis-specific immunity in human volunteers. J Infect Dis 198:1491–1501. https://doi.org/10.1086/592450.
162. Lindenstrom T, Moguche A, Damborg M, Agger EM, Urdahl K, Andersen P. 2018. T cells primed by live mycobacteria versus a tuberculosis subunit vaccine exhibit distinct functional properties. EBioMedicine 27:27–39. https://doi.org/10.1016/j.ebiom.2017.12.004.
163. Clark S, Lanni F, Marinova D, Rayner E, Martin C, Williams A. 2017. Revaccination of guinea pigs with the live attenuated *Mycobacterium tuberculosis* vaccine MTBVAC improves BCG's protection against tuberculosis. J Infect Dis 216:525–533. https://doi.org/10.1093/infdis/jix030.
164. McShane H, Pathan AA, Sander CR, Keating SM, Gilbert SC, Huygen K, Fletcher HA, Hill AV. 2004. Recombinant modified vaccinia virus Ankara expressing antigen 85A boosts BCG-primed and naturally acquired antimycobacterial immunity in humans. Nat Med 10:1240–1244. https://doi.org/10.1038/nm1128.
165. Scriba TJ, Tameris M, Mansoor N, Smit E, van der Merwe L, Isaacs F, Keyser A, Moyo S, Brittain N, Lawrie A, Gelderbloem S, Veldsman A, Hatherill M, Hawkridge A, Hill AV, Hussey GD, Mahomed H, McShane H, Hanekom WA. 2010. Modified vaccinia Ankara-expressing Ag85A, a novel tuberculosis vaccine, is safe in adolescents and children, and induces polyfunctional CD4+ T cells. Eur J Immunol 40:279–290. https://doi.org/10.1002/eji.200939754.
166. Scriba TJ, Tameris M, Mansoor N, Smit E, van der Merwe L, Mauff K, Hughes EJ, Moyo S, Brittain N, Lawrie A, Mulenga H, de Kock M, Gelderbloem S, Veldsman A, Hatherill M, Geldenhuys H, Hill AV, Hussey GD, Mahomed H, Hanekom WA, McShane H. 2011. Dose-finding study of the novel tuberculosis vaccine, MVA85A, in healthy BCG-vaccinated infants. J Infect Dis 203:1832–1843. https://doi.org/10.1093/infdis/jir195.
167. Scriba TJ, Tameris M, Smit E, van der Merwe L, Hughes EJ, Kadira B, Mauff K, Moyo S, Brittain N, Lawrie A, Mulenga H, de Kock M, Makhethe L, Janse van Rensburg E, Gelderbloem S, Veldsman A, Hatherill M, Geldenhuys H, Hill AV, Hawkridge A, Hussey GD, Hanekom WA, McShane H, Mahomed H. 2012. A phase IIa trial of the new tuberculosis vaccine, MVA85A, in HIV- and/or *Mycobacterium tuberculosis*-infected adults. Am J Respir Crit Care Med 185:769–778. https://doi.org/10.1164/rccm.201108-1548OC.
168. Tameris M, Geldenhuys H, Luabeya AK, Smit E, Hughes JE, Vermaak S, Hanekom WA, Hatherill M, Mahomed H, McShane H, Scriba TJ. 2014. The candidate TB vaccine, MVA85A, induces highly durable Th1 responses. PLoS One 9:e87340. https://doi.org/10.1371/journal.pone.0087340.
169. Shey MS, Nemes E, Whatney W, de Kock M, Africa H, Barnard C, van Rooyen M, Stone L, Riou C, Kollmann T, Hawn TR, Scriba TJ, Hanekom WA. 2014. Maturation of innate responses to mycobacteria over the first nine months of life. J Immunol 192:4833–4843. https://doi.org/10.4049/jimmunol.1400062.
170. Ota MOC, Odutola AA, Owiafe PK, Donkor S, Owolabi OA, Brittain NJ, Williams N, Rowland-Jones S, Hill AVS, Adegbola RA, McShane H. 2011. Immunogenicity of the tuberculosis vaccine MVA85A is reduced by coadministration with EPI vaccines in a randomized controlled trial in

Gambian infants. Sci Transl Med 3:88ra56. https://doi.org/10.1126/scitranslmed.3002461.
171. Fletcher HA, Snowden MA, Landry B, Rida W, Satti I, Harris SA, Matsumiya M, Tanner R, O'Shea MK, Dheenadhayalan V, Bogardus L, Stockdale L, Marsay L, Chomka A, Harrington-Kandt R, Manjaly-Thomas ZR, Naranbhai V, Stylianou E, Darboe F, Penn-Nicholson A, Nemes E, Hatheril M, Hussey G, Mahomed H, Tameris M, McClain JB, Evans TG, Hanekom WA, Scriba TJ, McShane H. 2016. T-cell activation is an immune correlate of risk in BCG vaccinated infants. Nat Commun 7:11290. https://doi.org/10.1038/ncomms11633.
172. Shi L, North R, Gennaro ML. 2004. Effect of growth state on transcription levels of genes encoding major secreted antigens of *Mycobacterium tuberculosis* in the mouse lung. Infect Immun 72:2420–2424. https://doi.org/10.1128/iai.72.4.2420-2424.2004.
173. Egen JG, Rothfuchs AG, Feng CG, Horwitz MA, Sher A, Germain RN. 2011. Intravital imaging reveals limited antigen presentation and T cell effector function in mycobacterial granulomas. Immunity 34:807–819. https://doi.org/10.1016/j.immuni.2011.03.022.
174. Lindestam Arlehamn CS, McKinney DM, Carpenter C, Paul S, Rozot V, Makgotlho E, Gregg Y, van Rooyen M, Ernst JD, Hatherill M, Hanekom WA, Peters B, Scriba TJ, Sette A. 2016. A quantitative analysis of complexity of human pathogen-specific CD4 T cell responses in healthy *M. tuberculosis* infected South Africans. PLoS Pathog 12:e1005760. https://doi.org/10.1371/journal.ppat.1005760.
175. Andersen P, Woodworth JS. 2014. Tuberculosis vaccines—rethinking the current paradigm. Trends Immunol 35:387–395. https://doi.org/10.1016/j.it.2014.04.006.
176. Manjaly Thomas ZR, Satti I, Marshall JL, Harris SA, Lopez Ramon R, Hamidi A, Minhinnick A, Riste M, Stockdale L, Lawrie AM, Vermaak S, Wilkie M, Bettinson H, McShane H. 2019. Alternate aerosol and systemic immunisation with a recombinant viral vector for tuberculosis, MVA85A: a phase I randomised controlled trial. PLoS Med 16:e1002790. https://doi.org/10.1371/journal.pmed.1002790.
177. Abel B, Tameris M, Mansoor N, Gelderbloem S, Hughes J, Abrahams D, Makhethe L, Erasmus M, de Kock M, van der Merwe L, Hawkridge A, Veldsman A, Hatherill M, Schirru G, Pau MG, Hendriks J, Weverling GJ, Goudsmit J, Sizemore D, McClain JB, Goetz M, Gearhart J, Mahomed H, Hussey GD, Sadoff JC, Hanekom WA. 2010. The novel tuberculosis vaccine, AERAS-402, induces robust and polyfunctional CD4+ and CD8+ T cells in adults. Am J Respir Crit Care Med 181:1407–1417. https://doi.org/10.1164/rccm.200910-1484OC.
178. van Zyl-Smit RN, Esmail A, Bateman ME, Dawson R, Goldin J, van Rikxoort E, Douoguih M, Pau MG, Sadoff JC, McClain JB, Snowden MA, Benko J, Hokey DA, Rutkowski KT, Graves A, Shepherd B, Ishmukhamedov S, Kagina BMN, Abel B, Hanekom WA, Scriba TJ, Bateman ED. 2017. Safety and immunogenicity of adenovirus 35 tuberculosis vaccine candidate in adults with active or previous tuberculosis. A randomized trial. Am J Respir Crit Care Med 195:1171–1180. https://doi.org/10.1164/rccm.201603-0654OC.
179. Smaill F, Jeyanathan M, Smieja M, Medina MF, Thanthrige-Don N, Zganiacz A, Yin C, Heriazon A, Damjanovic D, Puri L, Hamid J, Xie F, Foley R, Bramson J, Gauldie J, Xing Z. 2013. A human type 5 adenovirus-based tuberculosis vaccine induces robust T cell responses in humans despite preexisting anti-adenovirus immunity. Sci Transl Med 5:205ra134. https://doi.org/10.1126/scitranslmed.3006843.
180. Nyendak M, Swarbrick GM, Duncan A, Cansler M, Huff EW, Hokey D, Evans T, Barker L, Blatner G, Sadoff J, Douoguih M, Pau MG, Lewinsohn DA, Lewinsohn DM. 2016. Adenovirally-induced polyfunctional T cells do not necessarily recognize the infected target: lessons from a phase I trial of the AERAS-402 vaccine. Sci Rep 6:36355. https://doi.org/10.1038/srep36355.
181. Darrah PA, Bolton DL, Lackner AA, Kaushal D, Aye PP, Mehra S, Blanchard JL, Didier PJ, Roy CJ, Rao SS, Hokey DA, Scanga CA, Sizemore DR, Sadoff JC, Roederer M, Seder RA. 2014. Aerosol vaccination with AERAS-402 elicits robust cellular immune responses in the lungs of rhesus macaques but fails to protect against high-dose *Mycobacterium tuberculosis* challenge. J Immunol 193:1799–1811. https://doi.org/10.4049/jimmunol.1400676.
182. Jeyanathan M, Shao Z, Yu X, Harkness R, Jiang R, Li J, Xing Z, Zhu T. 2015. AdHu5Ag85A respiratory mucosal boost immunization enhances protection against pulmonary tuberculosis in BCG-primed non-human primates. PLoS One 10:e0135009. https://doi.org/10.1371/journal.pone.0135009.
183. Aagaard C, Hoang T, Dietrich J, Cardona PJ, Izzo A, Dolganov G, Schoolnik GK, Cassidy JP, Billeskov R, Andersen P. 2011. A multistage tuberculosis vaccine that confers efficient protection before and after exposure. Nat Med 17:189–224. https://doi.org/10.1038/nm.2285.
184. van Dissel JT, Joosten SA, Hoff ST, Soonawala D, Prins C, Hokey DA, O'Dee DM, Graves A, Thierry-Carstensen B, Andreasen LV, Ruhwald M, de Visser AW, Agger EM, Ottenhoff THM, Kromann I, Andersen P. 2014. A novel liposomal adjuvant system, CAF01, promotes long-lived *Mycobacterium tuberculosis*-specific T-cell responses in human. Vaccine 32:7098–7107. https://doi.org/10.1016/j.vaccine.2014.10.036.
185. Luabeya AK, Kagina BM, Tameris MD, Geldenhuys H, Hoff ST, Shi Z, Kromann I, Hatherill M, Mahomed H, Hanekom WA, Andersen P, Scriba TJ, H56-032 Trial Study Group, Schoeman E, Krohn C, Day CL, Africa H, Makhethe L, Smit E, Brown Y, Suliman S, Hughes EJ, Bang P, Snowden MA, McClain B, Hussey GD. 2015. First-in-human trial of the post-exposure tuberculosis vaccine H56:IC31 in *Mycobacterium tuberculosis* infected and non-infected healthy adults. Vaccine 33:4130–4140. https://doi.org/10.1016/j.vaccine.2015.06.051.
186. Mearns H, Geldenhuys HD, Kagina BM, Musvosvi M, Little F, Ratangee F, Mahomed H, Hanekom WA, Hoff ST, Ruhwald M, Kromann I, Bang P, Hatherill M, Andersen P, Scriba TJ, THYB04 Study Group. 2017. H1:IC31 vaccination is safe and induces long-lived TNF-alpha(+)IL-2(+)CD4 T cell responses in *M. tuberculosis* infected and uninfected adolescents: a randomized trial. Vaccine 35:132–141. https://doi.org/10.1016/j.vaccine.2016.11.023.
187. Tkachuk AP, Gushchin VA, Potapov VD, Demidenko AV, Lunin VG, Gintsburg AL. 2017. Multi-subunit BCG booster vaccine GamTBvac: assessment of immunogenicity and protective efficacy in murine and guinea pig TB models. PLoS One 12:e0176784. https://doi.org/10.1371/journal.pone.0176784.
188. Lindenstrom T, Knudsen NP, Agger EM, Andersen P. 2013. Control of chronic *Mycobacterium tuberculosis* infection by CD4 KLRG1-IL-2-secreting central memory cells. J Immunol 190:6311–6319. https://doi.org/10.4049/jimmunol.1300248.
189. Day CL, Tameris M, Mansoor N, van Rooyen M, de Kock M, Geldenhuys H, Erasmus M, Makhethe L, Hughes EJ, Gelderbloem S, Bollaerts A, Bourguignon P, Cohen J, Demoitie MA, Mettens P, Moris P, Sadoff JC, Hawkridge A, Hussey GD, Mahomed H, Ofori-Anyinam O, Hanekom WA. 2013. Induction and regulation of T-cell immunity by the novel tuberculosis vaccine M72/AS01 in South African adults. Am J Respir Crit Care Med 188:492–502. https://doi.org/10.1164/rccm.201208-1385OC.
190. Penn-Nicholson A, Geldenhuys H, Burny W, van der Most R, Day CL, Jongert E, Moris P, Hatherill M, Ofori-Anyinam O, Hanekom W, Vaccine Study Team, Bollaerts A, Demoitie MA, Kany Luabeya AK, De Ruymaeker E, Tameris M, Lapierre D, Scriba TJ. 2015. Safety and immunogenicity of candidate vaccine M72/AS01E in adolescents in a TB endemic setting. Vaccine 33:4025–4034. https://doi.org/10.1016/j.vaccine.2015.05.088.
191. Woodworth JS, Aagaard CS, Hansen PR, Cassidy JP, Agger EM, Andersen P. 2014. Protective CD4 T cells targeting cryptic epitopes of *Mycobacterium tuberculosis* resist infection-driven terminal differentiation. J Immunol 192:3247–3258. https://doi.org/10.4049/jimmunol.1300283.
192. Rodo MJ, Rozot V, Nemes E, Dintwe O, Hatherill M, Little F, Scriba TJ. 2019. A comparison of antigen-specific T cell responses induced by six novel tuberculosis vaccine candidates. PLoS Pathog 15:e1007643. https://doi.org/10.1371/journal.ppat.1007643.
193. Nell AS, D'Lom E, Bouic P, Sabate M, Bosser R, Picas J, Amat M, Churchyard G, Cardona PJ. 2014. Safety, tolerability, and immunogenicity of the novel antituberculous vaccine RUTI: randomized, placebo-controlled phase II clinical trial in patients with latent tuberculosis infection. PLoS One 9:e89612. https://doi.org/10.1371/journal.pone.0089612.
194. von Reyn CF, Mtei L, Arbeit RD, Waddell R, Cole B, Mackenzie T, Matee M, Bakari M, Tvaroha S, Adams LV, Horsburgh CR, Pallangyo K, DarDar Study Group. 2010. Prevention of tuberculosis in bacille Calmette-Guerin-primed, HIV-infected adults boosted with an inactivated whole-cell mycobacterial vaccine. AIDS 24:675–685. https://doi.org/10.1097/QAD.0b013e3283350f1b.
195. Groschel MI, Prabowo SA, Cardona PJ, Stanford JL, van der Werf TS. 2014. Therapeutic vaccines for tuberculosis—a systematic review. Vaccine 32:3162–3168. https://doi.org/10.1016/j.vaccine.2014.03.047.
196. Gupta A, Ahmad FJ, Ahmad F, Gupta UD, Natarajan M, Katoch V, Bhaskar S. 2012. Efficacy of *Mycobacterium indicus* pranii immunotherapy as an adjunct to chemotherapy for tuberculosis and underlying

immune responses in the lung. PLoS One 7:e39215. https://doi.org/10.1371/journal.pone.0039215.
197. Sharma SK, Katoch K, Sarin R, Balambal R, Kumar Jain N, Patel N, Murthy KJR, Singla N, Saha PK, Khanna A, Singh U, Kumar S, Sengupta A, Banavaliker JN, Chauhan DS, Sachan S, Wasim M, Tripathi S, Dutt N, Jain N, Joshi N, Penmesta SRR, Gaddam S, Gupta S, Khamar B, Dey B, Mitra DK, Arora SK, Bhaskar S, Rani R. 2017. Efficacy and safety of *Mycobacterium indicus* pranii as an adjunct therapy in category II pulmonary tuberculosis in a randomized trial. Sci Rep 7:3354. https://doi.org/10.1038/s41598-017-03514-1.
198. Mayosi BM, Ntsekhe M, Bosch J, Pandie S, Jung H, Gumedze F, Pogue J, Thabane L, Smieja M, Francis V, Joldersma L, Thomas KM, Thomas B, Awotedu AA, Magula NP, Naidoo DP, Damasceno A, Chitsa Banda A, Brown B, Manga P, Kirenga B, Mondo C, Mntla P, Tsitsi JM, Peters F, Essop MR, Russell JB, Hakim J, Matenga J, Barasa AF, Sani MU, Olunuga T, Ogah O, Ansa V, Aje A, Danbauchi S, Ojji D, Yusuf S, IMPI Trial Investigators. 2014. Prednisolone and *Mycobacterium indicus* pranii in tuberculous pericarditis. N Engl J Med 371:1121–1130. https://doi.org/10.1056/NEJMoa1407380.
199. Dijkman K, Sombroek CC, Vervenne RAW, Hofman SO, Boot C, Remarque EJ, Kocken CHM, Ottenhoff THM, Kondova I, Khayum MA, Haanstra KG, Vierboom MPM, Verreck FAW. 2019. Prevention of tuberculosis infection and disease by local BCG in repeatedly exposed rhesus macaques. Nat Med 25:255–262. https://doi.org/10.1038/s41591-018-0319-9.
200. Aerts L, Selis E, Corbiere V, Smits K, Van Praet A, Dauby N, Petit E, Singh M, Locht C, Dirix V, Mascart F. 2019. HBHA-induced polycytotoxic CD4(+) T lymphocytes are associated with the control of *Mycobacterium tuberculosis* infection in humans. J Immunol 202:421–427. https://doi.org/10.4049/jimmunol.1800840.
201. Nandakumar S, Kannanganat S, Dobos KM, Lucas M, Spencer JS, Fang S, McDonald MA, Pohl J, Birkness K, Chamcha V, Ramirez MV, Plikaytis BB, Posey JE, Amara RR, Sable SB. 2013. O-mannosylation of the *Mycobacterium tuberculosis* adhesin Apa is crucial for T cell antigenicity during infection but is expendable for protection. PLoS Pathog 9:e1003705. https://doi.org/10.1371/journal.ppat.1003705.
202. Nandakumar S, Kannanganat S, Dobos KM, Lucas M, Spencer JS, Amara RR, Plikaytis BB, Posey JE, Sable SB. 2016. Boosting BCG-primed responses with a subunit Apa vaccine during the waning phase improves immunity and imparts protection against *Mycobacterium tuberculosis*. Sci Rep 6:25837. https://doi.org/10.1038/srep25837.
203. Harriff MJ, Wolfe LM, Swarbrick G, Null M, Cansler ME, Canfield ET, Vogt T, Toren KG, Li W, Jackson M, Lewinsohn DA, Dobos KM, Lewinsohn DM. 2017. HLA-E presents glycopeptides from the *Mycobacterium tuberculosis* protein MPT32 to human CD8(+) T cells. Sci Rep 7:4622. https://doi.org/10.1038/s41598-017-04894-0.
204. Youngblood B, Hale JS, Kissick HT, Ahn E, Xu X, Wieland A, Araki K, West EE, Ghoneim HE, Fan Y, Dogra P, Davis CW, Konieczny BT, Antia R, Cheng X, Ahmed R. 2017. Effector CD8 T cells dedifferentiate into long-lived memory cells. Nature 552:404–409. https://doi.org/10.1038/nature25144.
205. Bhatt K, Verma S, Ellner JJ, Salgame P. 2015. Quest for correlates of protection against tuberculosis. Clin Vaccine Immunol 22:258–266. https://doi.org/10.1128/CVI.00721-14.
206. Walzl G, Ronacher K, Hanekom W, Scriba TJ, Zumla A. 2011. Immunological biomarkers of tuberculosis. Nat Rev Immunol 11:343–354. https://doi.org/10.1038/nri2960.
207. Schrager LK, Chandrasekaran P, Fritzell BH, Hatherill M, Lambert PH, McShane H, Tornieporth N, Vekemans J. 2018. WHO preferred product characteristics for new vaccines against tuberculosis. Lancet Infect Dis 18:828–829. https://doi.org/10.1016/S1473-3099(18)30421-3.
208. Andersen P, Scriba TJ. 2019. Moving tuberculosis vaccines from theory to practice. Nat Rev Immunol 19:550–562. https://doi.org/10.1038/s41577-019-0174-z.
209. Minhinnick A, Harris S, Wilkie M, Peter J, Stockdale L, Manjaly-Thomas ZR, Vermaak S, Satti I, Moss P, McShane H. 2016. Optimization of a human bacille Calmette-Guerin challenge model: a tool to evaluate antimycobacterial immunity. J Infect Dis 213:824–830. https://doi.org/10.1093/infdis/jiv482.
210. Kaufmann SH, Fortune S, Pepponi I, Ruhwald M, Schrager LK, Ottenhoff TH. 2016. TB biomarkers, TB correlates and human challenge models: new tools for improving assessment of new TB vaccines. Tuberculosis (Edinb) 99(Suppl 1):S8–S11. https://doi.org/10.1016/j.tube.2016.05.010.
211. Corey L, Gilbert PB, Tomaras GD, Haynes BF, Pantaleo G, Fauci AS. 2015. Immune correlates of vaccine protection against HIV-1 acquisition. Sci Transl Med 7:310rv7. https://doi.org/10.1126/scitranslmed.aac7732.
212. Stephenson KE, D'Couto HT, Barouch DH. 2016. New concepts in HIV-1 vaccine development. Curr Opin Immunol 41:39–46. https://doi.org/10.1016/j.coi.2016.05.011.
213. Xu L, Pegu A, Rao E, Doria-Rose N, Beninga J, McKee K, Lord DM, Wei RR, Deng G, Louder M, Schmidt SD, Mankoff Z, Wu L, Asokan M, Beil C, Lange C, Leuschner WD, Kruip J, Sendak R, Do Kwon Y, Zhou T, Chen X, Bailer RT, Wang K, Choe M, Tartaglia LJ, Barouch DH, O'Dell S, Todd JP, Burton DR, Roederer M, Connors M, Koup RA, Kwong PD, Yang ZY, Mascola JR, Nabel GJ. 2017. Trispecific broadly neutralizing HIV antibodies mediate potent SHIV protection in macaques. Science 358:85–90. https://doi.org/10.1126/science.aan8630.
214. Hansen SG, Piatak M, Jr, Ventura AB, Hughes CM, Gilbride RM, Ford JC, Oswald K, Shoemaker R, Li Y, Lewis MS, Gilliam AN, Xu G, Whizin N, Burwitz BJ, Planer SL, Turner JM, Legasse AW, Axthelm MK, Nelson JA, Fruh K, Sacha JB, Estes JD, Keele BF, Edlefsen PT, Lifson JD, Picker LJ. 2013. Immune clearance of highly pathogenic SIV infection. Nature 502:100–104. https://doi.org/10.1038/nature12519.
215. Roy Chowdhury R, Vallania F, Yang Q, Lopez Angel CJ, Darboe F, Penn-Nicholson A, Rozot V, Nemes E, Malherbe ST, Ronacher K, Walzl G, Hanekom W, Davis MM, Winter J, Chen X, Scriba TJ, Khatri P, Chien YH. 2018. A multi-cohort study of the immune factors associated with M. tuberculosis infection outcomes. Nature 560:644–648. https://doi.org/10.1038/s41586-018-0439-x.
216. Kazmin D, Nakaya HI, Lee EK, Johnson MJ, van der Most R, van den Berg RA, Ballou WR, Jongert E, Wille-Reece U, Ockenhouse C, Aderem A, Zak DE, Sadoff J, Hendriks J, Wrammert J, Ahmed R, Pulendran B. 2017. Systems analysis of protective immune responses to RTS,S malaria vaccination in humans. Proc Natl Acad Sci U S A 114:2425–2430. https://doi.org/10.1073/pnas.1621489114.
217. Mordmuller B, Surat G, Lagler H, Chakravarty S, Ishizuka AS, Lalremruata A, Gmeiner M, Campo JJ, Esen M, Ruben AJ, Held J, Calle CL, Mengue JB, Gebru T, Ibanez J, Sulyok M, James ER, Billingsley PF, Natasha KC, Manoj A, Murshedkar T, Gunasekera A, Eappen AG, Li T, Stafford RE, Li M, Felgner PL, Seder RA, Richie TL, Sim BK, Hoffman SL, Kremsner PG. 2017. Sterile protection against human malaria by chemoattenuated PfSPZ vaccine. Nature 542:445–449. https://doi.org/10.1038/nature21060.
218. Yarchoan M, Johnson BA, III, Lutz ER, Laheru DA, Jaffee EM. 2017. Targeting neoantigens to augment antitumour immunity. Nat Rev Cancer 17:209–222. https://doi.org/10.1038/nrc.2016.154.
219. Pearce EL, Walsh MC, Cejas PJ, Harms GM, Shen H, Wang LS, Jones RG, Choi Y. 2009. Enhancing CD8 T-cell memory by modulating fatty acid metabolism. Nature 460:103–107. https://doi.org/10.1038/nature08097.
220. Buck MD, O'Sullivan D, Pearce EL. 2015. T cell metabolism drives immunity. J Exp Med 212:1345–1360. https://doi.org/10.1084/jem.20151159.
221. Caserta S, Kleczkowska J, Mondino A, Zamoyska R. 2010. Reduced functional avidity promotes central and effector memory CD4 T cell responses to tumor-associated antigens. J Immunol 185:6545–6554. https://doi.org/10.4049/jimmunol.1001867.
222. Sadelain M, Riviere I, Riddell S. 2017. Therapeutic T cell engineering. Nature 545:423–431. https://doi.org/10.1038/nature22395.
223. Gautam US, Foreman TW, Bucsan AN, Veatch AV, Alvarez X, Adekambi T, Golden NA, Gentry KM, Doyle-Meyers LA, Russell-Lodrigue KE, Didier PJ, Blanchard JL, Kousoulas KG, Lackner AA, Kalman D, Rengarajan J, Khader SA, Kaushal D, Mehra S. 2018. In vivo inhibition of tryptophan catabolism reorganizes the tuberculoma and augments immune-mediated control of *Mycobacterium tuberculosis*. Proc Natl Acad Sci U S A 115:E62–E71. https://doi.org/10.1073/pnas.1711373114.
224. Cruz A, Torrado E, Carmona J, Fraga AG, Costa P, Rodrigues F, Appelberg R, Correia-Neves M, Cooper AM, Saraiva M, Pedrosa J, Castro AG. 2015. BCG vaccination-induced long-lasting control of *Mycobacterium tuberculosis* correlates with the accumulation of a novel population of CD4(+)IL-17(+)TNF(+)IL-2(+) T cells. Vaccine 33:85–91. https://doi.org/10.1016/j.vaccine.2014.11.013.
225. Kagina BM, Abel B, Scriba TJ, Hughes EJ, Keyser A, Soares A, Gamieldien H, Sidibana M, Hatherill M, Gelderbloem S, Mahomed H, Hawkridge A, Hussey G, Kaplan G, Hanekom WA, Other Members of the South African Tuberculosis Vaccine Initiative. 2010. Specific T cell frequency and cytokine expression profile do not correlate with protection against tuberculosis after bacillus Calmette-Guerin vaccination of newborns. Am J Respir Crit Care Med 182:1073–1079. https://doi.org/10.1164/rccm.201003-0334OC.
226. Velmurugan K, Grode L, Chang R, Fitzpatrick M, Laddy D, Hokey D,

Derrick S, Morris S, McCown D, Kidd R, Gengenbacher M, Eisele B, Kaufmann SH, Fulkerson J, Brennan MJ. 2013. Nonclinical development of BCG replacement vaccine candidates. Vaccines (Basel) 1:120–138. https://doi.org/10.3390/vaccines1020120.

227. Sharpe SA, McShane H, Dennis MJ, Basaraba RJ, Gleeson F, Hall G, McIntyre A, Gooch K, Clark S, Beveridge NE, Nuth E, White A, Marriott A, Dowall S, Hill AV, Williams A, Marsh PD. 2010. Establishment of an aerosol challenge model of tuberculosis in rhesus macaques and an evaluation of endpoints for vaccine testing. Clin Vaccine Immunol 17:1170–1182. https://doi.org/10.1128/CVI.00079-10.
228. Billeskov R, Tan EV, Cang M, Abalos RM, Burgos J, Pedersen BV, Christensen D, Agger EM, Andersen P. 2016. Testing the H56 vaccine delivered in 4 different adjuvants as a BCG-booster in a non-human primate model of tuberculosis. PLoS One 11:e0161217. https://doi.org/10.1371/journal.pone.0161217.
229. Kagina BM, Tameris MD, Geldenhuys H, Hatherill M, Abel B, Hussey GD, Scriba TJ, Mahomed H, Sadoff JC, Hanekom WA, 018-402 Clinical Lab Study Team, Mansoor N, Hughes J, de Kock M, Whatney W, Africa H, Krohn C, Veldsman A, Kany AL, Douoguih M, Pau MG, Hendriks J, McClainc B, Benko J, Snowden MA, Hokey DA. 2014. The novel tuberculosis vaccine, AERAS-402, is safe in healthy infants previously vaccinated with BCG, and induces dose-dependent CD4 and CD8T cell responses. Vaccine 32:5908–5917. https://doi.org/10.1016/j.vaccine.2014.09.001.
230. Idoko OT, Owolabi OA, Owiafe PK, Moris P, Odutola A, Bollaerts A, Ogundare E, Jongert E, Demoitie MA, Ofori-Anyinam O, Ota MO. 2014. Safety and immunogenicity of the M72/AS01 candidate tuberculosis vaccine when given as a booster to BCG in Gambian infants: an open-label randomized controlled trial. Tuberculosis (Edinb) 94:564–578. https://doi.org/10.1016/j.tube.2014.07.001.
231. Suliman S, Luabeya AKK, Geldenhuys H, Tameris M, Hoff ST, Shi Z, Tait D, Kromann I, Ruhwald M, Rutkowski KT, Shepherd B, Hokey D, Ginsberg AM, Hanekom WA, Andersen P, Scriba TJ, Hatherill M, H56-035 Trial Group. 2019. Dose optimization of H56:IC31 vaccine for tuberculosis-endemic populations. A double-blind, placebo-controlled, dose-selection trial. Am J Respir Crit Care Med 199:220–231. https://doi.org/10.1164/rccm.201802-0366OC.
232. Penn-Nicholson A, Tameris M, Smit E, Day TA, Musvosvi M, Jayashankar L, Vergara J, Mabwe S, Bilek N, Geldenhuys H, Luabeya AK, Ellis R, Ginsberg AM, Hanekom WA, Reed SG, Coler RN, Scriba TJ, Hatherill M, TBVPX-114 Study Team. 2018. Safety and immunogenicity of the novel tuberculosis vaccine ID93 + GLA-SE in BCG-vaccinated healthy adults in South Africa: a randomised, double-blind, placebo-controlled phase 1 trial. Lancet Respir Med 6:287–298. https://doi.org/10.1016/S2213-2600(18)30077-8.
233. World Health Organization. 2019. World Health Organization model list of essential medicines, 21st list. World Health Organization, Geneva, Switzerland. https://apps.who.int/iris/bitstream/handle/10665/325771/WHO-MVP-EMP-IAU-2019.06-eng.pdf?ua=1.
234. White A. 27 August 2019. Methods of analysis on ^{18}FDG PET/CT imaging to evaluate *M. tuberculosis* infection, prevention, and treatment in non-human primates. Presented at NIAID conference "Visualizing the Dynamics of TB Pathology using Molecular Imaging: Advancing Fundamental Knowledge and Developing Precision Tools for Diagnosis, Monitoring and Drug Development," Rockville, MD.

Suraj B. Sable, D.V.M., Ph.D., received his D.V.M. from Nagpur Veterinary College, M.V.Sc. from the Indian Veterinary Research Institute, and Ph.D. from the Postgraduate Institute of Medical Education and Research, India. He was an American Society for Microbiology (ASM) Postdoctoral Fellow at the Centers for Disease Control and Prevention (CDC), Atlanta, GA. He served as an Immunology Activity Leader and Principal Scientist for ASM and ORISE fellowships at the Division of Tuberculosis Elimination, CDC. He is currently a Senior Scientist, Immunology, in the Laboratory Branch, Division of Tuberculosis Elimination, CDC, Atlanta, GA. Dr. Sable's research interests include immunology and cell biology of *Mycobacterium tuberculosis* infections, animal models of TB, and development of new vaccines and host-directed therapies against TB. He has worked in the field of TB immunology for 20 years and investigated several vaccine candidates in preclinical models.

James E. Posey, Ph.D., received his doctorate at the University of Georgia. He is currently the lead of the Applied Research Team (ART) within the Division of TB Elimination at the Centers for Disease Control and Prevention, Atlanta, GA. He directs the research portfolio of ART that focuses on genotyping of *Mycobacterium tuberculosis*, mechanisms of drug resistance, and immunology and cell biology of tuberculosis. His team is currently applying whole-genome sequencing to understand the transmission dynamics of TB and search for new mechanisms of drug resistance and established the National Tuberculosis Molecular Surveillance Center for performing universal whole-genome sequencing of *Mycobacterium tuberculosis*. The team has discovered several new mechanisms of drug resistance to kanamycin, streptomycin, capreomycin, and fluoroquinolones. His team is also using the latest technologies to investigate latent TB and host-directed therapies for treating TB.

Thomas J. Scriba, Ph.D., is Professor at the University of Cape Town, South Africa, and Deputy Director, Immunology, of the South African Tuberculosis Vaccine Initiative (SATVI), a clinical research organization situated in a setting where TB is endemic. He directs the SATVI Clinical Immunology Laboratory, a team of scientists, postdoctoral fellows, postgraduate students, and technicians. Dr. Scriba's research focuses on TB vaccine development; immunopathogenesis of human TB; immunological biomarkers of *M. tuberculosis* infection, disease progression, and treatment response; as well as identification of correlates of protection against TB. He has been coinvestigator on two dozen phase 1/2/2b clinical trials of novel TB vaccines, led multiple studies that have identified immune correlates of the risk of TB, and developed blood biomarkers with prognostic and diagnostic utility for TB.