The Immunological Basis for Immunization Series

Module 5: Tuberculosis
Update 2021

World Health Organization

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Contents

Abbreviations and acronyms

ADCC	Antibody-dependent cytotoxic
BCG	Bacille Calmette Guerin
COR	Correlate of risk
DR	Drug-resistant
DS	Drug-sensitive
DURT	Donor unrestricted T (cells)
GTBVP	Global TB Vaccine Partnership
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigen
IAVI	International AIDS Vaccine Initiative
IGRA	Interferon-gamma release assay
ILC	Innate lymphoid cells
ISG	Interferon-stimulated gene
ISG	Interferon-stimulated genes
MAIT	Mucosal-associated invariant T (cells)
mRNA	Messenger ribonucleic acid
NK	Natural killer (cells)
NKT	Natural killer T (cells)
PET CT	Positron emission tomography – computed tomography
QFT	QuantiFERON
RNA	Ribonucleic acid
SAGE	Strategic Advisory Group of Experts on Immunization
TB	Tuberculosis
TBVI	Tuberculosis Vaccine Initiative
TNF	Tumor necrosis factor
TST	Tuberculin skin test

Preface

This module is part of the World Health Organization (WHO) series The immunological basis for immunization, which was initially developed in 1993 as a set of eight modules comprising one module on general immunology and seven modules each devoted to one of the vaccines recommended for the Expanded Programme on Immunization – i.e. vaccines against diphtheria, measles, pertussis, polio, tetanus, tuberculosis and yellow fever. Since then, this series has been updated and extended to include other vaccines of international importance. The main purpose of the modules is to provide national immunization managers and vaccination professionals with an overview of the scientific basis for vaccination against a range of important infectious diseases. The modules developed since 1993 continue to be vaccine-specific, reflecting the biological differences in immune responses to the individual pathogens and the differing strategies employed to create the best possible level of protection that can be provided by vaccination. The modules also serve as a record of the immunological basis for the WHO recommendations on vaccine use, published in the WHO vaccine position papers[1].

[1] See: *http://www.who.int/immunization/documents/positionpapers_intro/en/index.html*, accessed 31 July 2018.

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Conflict of interest

None reported.

The need for immunization against tuberculosis

It is estimated that about one quarter of the global population, approximately 1.7 billion people (2014 estimates), are infected with *Mycobacterium tuberculosis* and between 5–15% of infected people are expected to progress to tuberculosis (TB) disease during their lifetimes.[1] The risk of progressing from infection to TB disease is highest in the very young, HIV- infected people, smokers, people taking immunosuppressive medication and those with diabetes mellitus. The risk of progression from primary infection to TB disease in the absence of preventive therapy is approximately 30–50% in infancy and 10% in the second year of life, but declines to 2–5% between 5 and 10 years of age, increasing thereafter to 10–20% in adolescents and adults.[2] More than half of the lifetime TB risk is thought to occur in the first two years after infection.[3,4] While young children bear the highest risk of progression to disease and the highest risk of severe morbidity in the form of miliary and meningitic disease, the ongoing global TB epidemic is driven by transmission via droplet spread from infectious adolescents and adults with pulmonary TB disease. Therefore, although children bear a disproportionate burden of disease, systematic TB control efforts are directed at halting adolescent and adult disease.

In 2019, an estimated 10 million people worldwide developed TB disease (an incidence rate of 130 per 100 000 of the global population), approximately 8.2% of whom had HIV-associated TB; 12% were children and over 177 000 were provided with treatment for multidrug- or rifampicin resistant TB. TB is the most common identifiable cause of infectious disease mortality. An estimated 1.4 million people died from TB worldwide in 2019, including some 208 000 whose deaths were from HIV-associated TB.[5] Globally, TB incidence has begun to decline at a rate of approximately 2% per year – a modest reduction that must accelerate to 10% per year by 2025 and 17% thereafter to achieve the milestones of the End TB Strategy which aims for a 90% reduction in the TB incidence rate by 2035.[6] This unprecedented fall in incidence is likely to be unachievable without deployment of a new and more effective TB vaccine.

A more effective vaccination strategy against both drug-sensitive (DS-) and drug-resistant (DR-) pulmonary TB among adults has the potential to have a major impact on the global epidemic by interrupting transmission. Immunogenicity and effectiveness of a TB vaccine are unlikely to be affected by the enzymatic mutations underlying resistance to first-line drugs, fluoroquinolones and injectables, which makes TB vaccination a potentially powerful tool against *M. tuberculosis* antimicrobial resistance.[7] WHO has proposed "preferred product characteristics" for new TB vaccines, with the goal of developing both a safe, effective and affordable TB vaccine for adolescents and adults and an affordable TB vaccine for neonates and infants with improved safety and efficacy as compared to Bacille Calmette Guerin (BCG).[8] An ideal TB vaccination strategy might hinge on: 1) a new vaccine or vaccines given in adolescence and/or young

adulthood as a boost for newborn BCG in order to prevent the rapid increase in TB disease notifications occurring in adults; 2) an infant vaccine more effective than BCG with consistent and durable protection lasting into adulthood; or 3) some combination of these pre- and post-exposure approaches. Regional differences in TB burden, mycobacterial exposure and *M. tuberculosis* infection prevalence, comorbidities such as HIV infection, and population-level genetic and sociodemographic differences are all likely to affect the success of these strategies at country level. For instance, in parts of South Africa where up to 50% of adolescents and 80% of adults are *M. tuberculosis*-infected,[9,10] a TB vaccination strategy aimed primarily at the *M. tuberculosis*-uninfected population might target the age group of 9–12 years, whereas a strategy aimed at *M. tuberculosis*-infected persons might target adults aged 18–25 years, to avoid the need for tuberculin skin test (TST) or interferon-gamma release assay (IGRA) screening prior to vaccination.[10]

Bacille Calmette Guerin (BCG) vaccine

BCG is the only vaccine licensed against TB. BCG was first used in 1921 and is given universally at birth in TB-endemic countries.[11] BCG vaccine products in current use appear to have become more attenuated than the original *M. bovis*-derived strain and there are many genetic and proteomic differences among the 14 circulating strains.[12,13,14] However, despite apparent differences in immunogenicity and reactogenicity, there are insufficient efficacy data to recommend one strain over another.[15,16] A global BCG vaccine distribution shortage, magnified by technical manufacturing difficulties at two major producers, began in 2013 and was projected to result in 7433 excess TB deaths in children under 15 years of age.[17] Although the distribution shortages may have been overcome, country-level programmatic reliance on licensed BCG strains often produced by a single external manufacturer remains a concern. Fermentation of BCG has been suggested as one way to combat production shortages.[18] This would require multiple new studies, but it would be an exceptional accomplishment to have one universal strain of BCG for global immunization.

It is clear that the efficacy of BCG vaccination has varied widely across clinical trials and observational studies,[16] with the greatest efficacy being observed in youngest children without previous immune sensitization to mycobacteria. In a systematic review of 18 clinical trials, protection offered by BCG vaccination was greatest in infants (rate ratio, RR 0.41; 95% confidence interval, CI 0.29–0.58) and in TST-negative children (RR 0.26; 95% CI 0.18–0.37), with little evidence of efficacy observed in older persons (RR 0.81; 95% CI 0.55–1.22). The observation that BCG efficacy was greater in trials conducted further from the equator was attenuated in multivariable analysis that took diagnostic detection bias into account.[16] Similar trends were observed in a systematic review that included observational studies.[19] BCG appears to offer consistent protection against the most severe forms of childhood TB, with efficacy estimated at 73% (95% CI 67–79) against tuberculous meningitis and 77% (58–87) against miliary TB in a meta-analysis of 18 case-control studies. In these studies efficacy also varied geographically, with greater protection observed in studies in Latin America than in Asian studies.[20]

There is some evidence that BCG vaccination may provide a non-specific survival benefit for young children independent of protection against TB,[21,22] although this has been questioned by WHO's Strategic Advisory Group of Experts on Immunization (SAGE).* Non-specific protection against mortality due to other diseases attributed to BCG[23] is thought to be associated with epigenetic imprinting of innate immune cells, known as trained immunity, which enhances their functional capacity to control a variety of infectious organisms.[24] Clinical trials are under way to test the efficacy

* See: https://www.who.int/wer/2014/wer8921.pdf (accessed 26 January 2021).

of BCG vaccination against disease caused by SARS-CoV-2. It may be important to determine whether other live, whole-cell or even certain subunit TB vaccine candidates confer non-specific survival benefits. Other non-specific effects of BCG on diseases such as bladder cancer are well documented,[25] as are the mycobacteria-specific protective benefits conferred by BCG against diseases such as leprosy and Buruli ulcer.[26]

Primary infant BCG vaccination does not seem to offer consistent, durable protection for much longer than 10 years. A review of 10 clinical trials calculated average BCG efficacy beyond 10 years after vaccination at 14% (95% CI -9–32).[19,27] However, in five of these studies there was evidence of measurable protection at least 15 years after BCG vaccination.[19] For example, in the control arm of a BCG revaccination trial, efficacy of BCG was 39% (95% CI 9–58) at age 15–20 years,[28] and in Alaska persistent protection estimated at 40% was observed after 40 years of age.[29] Notwithstanding these isolated reports of more durable protection, the waning of infant BCG-induced immunity suggests there could be a potential benefit from BCG boosting or revaccination approaches before or early in adolescence. However, two randomized controlled trials have shown no overall benefit of BCG revaccination in providing additional protection against TB.[30,31] One very large, open-label, cluster-randomized trial of school-age Brazilian children, in which the disease endpoint was determined by passive follow-up and linkage to health service records, showed 9% efficacy (95% CI -16–29%).[31] Extended trial follow-up over 9 years also showed no benefit, with 12% efficacy overall (95% CI -2–24%). However, 33% efficacy (95% CI 3–54%) was observed in the subgroup of younger children aged 7–11 years of age at one study centre,[32] which some have hypothesized as perhaps due to less prior mycobacterial exposure, including to non-tuberculous mycobacteria. A large, randomized, double-blind, placebo-controlled trial in Malawi also showed no benefit of BCG revaccination against all forms of confirmed TB (RR 1.43; 95% CI 0.88–2.35),[30] although the trial was conducted in a setting where primary BCG vaccination had previously not demonstrated efficacy.[33] Observational studies conducted in Europe and West Africa suggest that primary BCG vaccination of children may offer protection against *M. tuberculosis* infection, defined by IGRA conversion after household or congregate exposure, with overall protective efficacy of 19%.[34] Notably, in six studies in which children were followed for incident disease, overall efficacy was 71%, the combined result of 27% protection against infection and 58% protection against disease.[34] BCG revaccination of *M. tuberculosis*-uninfected adolescents was also recently shown to reduce the rate of sustained IGRA conversion, which may reflect sustained *M. tuberculosis* infection, by 45% in a high-transmission setting.[35] The value of this approach – i.e. BCG revaccination of *M. tuberculosis*-uninfected populations for prevention of subsequent TB disease – remains to be determined in efficacy trials that will be large and costly due to lower disease incidence in this population. However, if effective, BCG revaccination of *M. tuberculosis*-uninfected populations is likely to be cost-effective as a public health intervention.[36]

The host immune response to *M. tuberculosis* infection

It was estimated that 1.7 billion people have been infected with *M. tuberculosis*,[1] and yet most of these individuals successfully control or contain the bacterium and avert progression to TB disease. It follows that, in most infected individuals, the host immune response mounted against *M. tuberculosis* is highly effective and can be characterized to reveal key immunological effector mechanisms that vaccination should aim to invoke. This reasoning is the basis for hundreds of studies into natural immune responses to *M. tuberculosis*. However, such studies cannot reveal which features of the immune response are causally linked to successful immunity against TB, which features have no effect, and which features may actually be drivers of disease pathology. Nevertheless, much can be gleaned from understanding the natural immunity against *M. tuberculosis* in persons with asymptomatic *M. tuberculosis* infection.

T cells

Antigen-specific T cells are clearly necessary for effective immunity against *M. tuberculosis*, as evidenced from a wealth of human and animal model studies.[37,38] This forms the basis for most current vaccination strategies against TB, which overwhelmingly target antigen-specific T cells and primarily the CD4+ T cell subset that expresses Th1 cytokines. However, beyond the presence of functional *M. tuberculosis*-specific Th1 cells, it is not known which characteristics of such responses are responsible for protective immunity. Much emphasis has been placed on CD4+ T cells that are polyfunctional – i.e. that simultaneously co-express multiple cytokines (e.g. IFN-γ, TNF-α, IL-2) on a single cell level. However, on the basis of current evidence, polyfunctionality of CD4+ T cells is not sufficient to mediate protection against *M. tuberculosis*.[39] Other functional attributes may be important, including effector function, the degree of T cell differentiation, or memory profile, or tissue residence or homing potential. Another important question regarding T cell responses is which *M. tuberculosis* antigens they target. Early work on T cell responses focused primarily on proteins that are actively secreted by *M. tuberculosis*, such as ESAT-6, CFP-10, TB10.4, Ag85A and Ag85B which were found to be relatively immunodominant.[40-44] As a result, such antigens were incorporated into the first new generation TB vaccine candidates.[45,46] However, more recent, genome-wide analyses of CD4+ T cell responses to *M. tuberculosis* antigens in latently infected adults show that a much broader set of proteins is targeted.[47-49] More research is required to determine which of these antigens may be associated with protective immunity and would make the best vaccine antigens.[50]

CD8+ T cells are thought to also contribute to protective immunity against *M. tuberculosis*, although the degree of this contribution is not definitive. Several studies have reported elevated frequencies of antigen-specific CD8+ T cell responses in TB patients compared to healthy *M. tuberculosis*-infected controls.[51-53] The implication of this phenomenon is currently not clear but the finding appears to be reproducible and has been proposed as a diagnostic approach for TB disease.[53] Interestingly, treatment of *M. tuberculosis*-infected persons with the anti-TNF biologic infliximab or biosimilars for autoimmune disease was shown to reduce the numbers of terminally-differentiated, TNF-dependent CD45RA+CD8+ T cells, with a concomitant reduction in antimycobacterial capacity.[54] These data provide evidence that antigen-specific CD8+ T cells are likely to contribute to successful control of *M. tuberculosis* infection. Recently, there has been much interest in so-called donor-unrestricted T (DURT) cells, which include γδ T cells, MR1-restricted mucosal-associated invariant T (MAIT) cells, CD1d-restricted NKT cells and CD1b-restricted lipid-specific T cells.[55] Such cell subsets typically possess high effector function, including cytokine expression and cytotoxicity, and naturally reside at mucosal sites, including the airways.[55] They may, therefore, be ideally located to respond rapidly to *M. tuberculosis* infection. Compared with conventional CD4+ or CD8+ T cells that require priming in lymphoid tissues, must differentiate into effector cells and then traffic to infected tissues, DURT cells may provide early immunity against invading pathogens. They may also contribute to the early inflammatory responses required to recruit memory T cells, thus acting as "immune primers" in a role analogous to that of adjuvants. However, whether such airway-resident DURT populations play a role in resistance to *M. tuberculosis* infection in humans is currently not known and is the focus of ongoing research.

B cells and antibodies

Recent insights about the roles played by B cells and antibodies in immunity against intracellular bacteria have revealed a number of mechanisms that may also mediate control of *M. tuberculosis*. [56-58] Interestingly, antigen-specific antibodies from asymptomatic *M. tuberculosis*-infected individuals had significantly different glycosylation profiles on their constant (Fc) domains compared with those from TB patients, which endowed them with a unique functionality that promoted selective binding to CD16 and allowed intracellular killing of *M. tuberculosis*.[59] Another study showed that, compared with uninfected controls, the function of B cells was impaired in persons with latent *M. tuberculosis* infection and in patients with active TB, suggesting that such B-cell dysfunction compromises cellular host immunity against *M. tuberculosis* infection.[60] Further, higher antibody-dependent cytotoxic (ADCC) responses were observed in *M. tuberculosis*-infected individuals than in uninfected donors, which may contribute to the antigen-specific cytotoxic response and control of *M. tuberculosis* in those with latent infection.[61] Since B cells are found in lung granulomas, it is thought that they may be important antigen-presenting cells to T cells, in addition to secreting antibodies and conferring regulatory functions by modulating inflammation.[58]

Other cell subsets that can be induced or manipulated by vaccination may also contribute to the successful anti- mycobacterial immune response. Although NK cells are not thought to be traditional targets of vaccination owing to their mostly innate behaviour, it has recently become clear in studies of viral infections, such as cytomegalovirus, that NK cells can differentiate to acquire "memory" functions with enhanced antiviral properties.[62,63] Notably, a recent study showed that NK cells and their cytotoxic potential was higher in *M. tuberculosis*-infected persons than in uninfected individuals and that this was associated with elevated ADCC responses.[61] Modulation of these different immune cells by vaccination against TB is discussed in further detail below.

Immune response to BCG

BCG vaccination in infants induces a robust T cell response which comprises predominantly Th1-cytokine- expressing CD4+ T cells.[64] These BCG-specific CD4+ T cells typically express IFN-γ in combination with TNF and IL-2,[64-66] although IL-17 expression by CD4+ T cells has also been reported.[65,57] Antigen-specific IFNγ-expressing CD8+ T cells are also induced by BCG vaccination, but at frequencies much lower than CD4+ T cells.[64-66] BCG-induced CD4+ and CD8+ T cells also display cytotoxic potential, including expression of perforin, granzyme B and granulysin.[66-68] These responses appear to peak in frequency between 6 and 10 weeks of age, followed by waning of the immune responses, although BCG-specific CD4+ and CD8+ T cells remain detectable up to 12 months after vaccination.[66]

BCG vaccination also induces mycobacteria-specific antibody responses, as reported in several studies.[23,24,69,70] However, BCG-induced antibody responses are not universally observed,[71] probably because of pre-existing, high-titre antibodies induced by prior exposure to environmental mycobacteria.[72,73] It is not clear if any of these responses induced by BCG vaccination confer protective immunity against TB. A study of immune correlates in BCG-vaccinated infants, which measured CD4+ and CD8+ T-cell expression of IFN-γ, TNF-α, IL-2 and IL-17 at 10 weeks of age, reported no association between frequencies or cytokine-expression patterns in BCG-specific CD4+ and CD8+ T cells and subsequent risk of TB disease.[65] In contrast, frequencies of cells expressing IFN-γ, measured by ELISpot assay after BCG stimulation, were lower in infants who ultimately developed TB than in those who remained healthy.[89] As observed in infants, intradermal vaccination of adolescents or adults with BCG also induces T cells that predominantly express IFNγ.[35,75–77] In general, immune responses to BCG vaccination in these populations appear to be similar to those reported after infant vaccination provided that the persons have not been pre-sensitized by prior BCG vaccination or *M. tuberculosis* infection. In individuals with prior immune responses to mycobacteria, BCG revaccination appears to boost CD4+ T cell responses, although to a lesser degree than observed in unsensitized individuals.[76,78] It is notable that, in a trial of BCG revaccination in adults with prior *M. tuberculosis* infection, only a transient increase in Th1-cytokine expressing CD4+, CD8+ and γδ T cells was observed; however, BCG revaccination significantly boosted IFN-γ-expressing $CD56^{dim}$ and $CD56^{hi}$ NK cells which remained elevated up to 1 year after revaccination,[78] suggesting induction of memory features in NK cells.

The recently published prevention of infection trial of BCG revaccination in QFT-negative adolescents also reported that BCG can boost CD4+ T cells that express Th1 cytokines IFNγ and IL-2.[35] A detailed analysis of the immune responses induced in this latter trial also revealed that, in addition to CD4+ T cells expressing IFNγ, IL-2 and TNFα, a substantial IL-22- producing CD4 T cell subset was boosted.† It is clear that the broad repertoire of antigens contained in BCG, which includes a multitude of proteins, lipids, carbohydrates and other moieties that may be antigenic, can induce or boost a very broad spectrum of responses which may be important in its protective efficacy.

† Rozot V, Nemes E. Communications Biology (in press).

Immunological correlates of risk for progression to TB disease

Rational vaccine development is hampered by the lack of immunological correlates of protection against TB. However, the first opportunities to identify such immunological correlates of protection now exist. Efficacy was observed in the first prevention of infection efficacy trial, which demonstrated that BCG revaccination of *M. tuberculosis*-uninfected adolescents provided 45% protective efficacy against sustained *M. tuberculosis* infection; and the first prevention of TB disease efficacy trial of an adjuvanted protein subunit vaccine, M72/AS01$_E$, demonstrated 54% protection against progression to confirmed pulmonary TB disease in *M. tuberculosis*-infected adults through two years of follow-up[79] and 50% protection after three years.[80] Until correlates of protection studies are completed using banked blood specimens collected in these trials, we can rely only on clues from studies of biomarkers of risk for TB which have reported a number of such candidate correlates of risk (COR). Whole-blood transcriptomic RNA signatures, discovered by RNA-sequencing of TB progressors and healthy controls, showed that mRNA transcripts – which largely represent interferon-stimulated genes (ISG) – could predict progression from infection to TB disease with promising sensitivity and specificity.[81,82] These transcriptomic COR signatures, which were validated by blind prediction in independent cohorts of progressors, suggest that disease progression is accompanied by elevated systemic inflammation and type I/II interferon signalling[83] – biological pathways that are also markedly elevated in patients with active TB disease.[84–87]

T cell activation has also been shown to correlate with the risk of TB. In BCG-vaccinated infant participants of a phase 2b efficacy trial of the novel TB vaccine MVA85A,[88] those who developed TB disease during follow-up had significantly higher pre-vaccination levels of HLA-DR-positive CD4+ T cells, a biomarker of T cell activation, than those who remained healthy.[89] Elevated CD4+ T cell expression of HLA-DR was also found to correlate with risk of TB in an independent cohort of *M. tuberculosis*-infected adolescent TB progressors and healthy controls.[83,89] Finally, it was shown in the same infant cohort that very high *M. tuberculosis*-specific IFNγ responses, detected by IGRA as plasma IFNγ values >4IU/mL, were associated with tremendously high risk of TB disease in the following months[90] – a finding that was also observed in adults from Norway.[91] It is very likely that elevated T-cell activation, very high IGRA IFNγ values and upregulated blood ISG expression reflect the same axis of immunopathogenesis during TB progression and are indicators of incipient or subclinical disease.[83,92] Although such COR may not directly inform immunological responses that should be targeted by TB vaccination, there is evidence that vaccination of persons with underlying incipient or subclinical disease can result in a different vaccine-induced response. In *M. tuberculosis*-infected adults who received BCG-revaccination, the IL-17 function of BCG-specific T cell responses was reduced in individuals with high ISG expression.[83] The implications of this observation remain unknown but it demonstrates that post- exposure approaches to vaccination should take the newly acknowledged spectrum of *M. tuberculosis* infection into account.[92]

Two other immunological COR that are of more direct relevance to vaccination were also reported in infant participants in the efficacy trial of MVA85A.[88] Fletcher and colleagues identified that infants who remained healthy (controls) during trial follow-up had higher levels of Ag85A-specific IgG antibodies than infants who developed TB (progressors).[89] Pre-vaccination frequencies of BCG-reactive T cells that secreted IFNγ, detected by ELISpot assay, were also found to be elevated in controls compared to TB progressor infants, and were thus associated with reduced risk of TB disease.[89] This finding was surprising in light of the previous immune correlates study in BCG- vaccinated infants, discussed above, which found no associations between frequencies or cytokine-expression patterns of BCG-specific CD4 and CD8 T cells and subsequent risk of TB.[65] However, a number of differences in sample collection, timing and assay processing could explain this discrepancy.

New TB vaccines

Preclinical studies

Preclinical studies have made significant contributions to our understanding of the immune responses elicited by BCG vaccination and have provided insights into the nature of what might be a protective immune response induced by a range of candidate vaccines in animal models. The immune responses elicited by vaccination in preclinical studies reflect those found in human studies but with fundamental species-specific differences in the immune network and cell types. Induction of a Th1-like CD4+ activation profile with IFN-gamma production, along with some CD8+ T cell and antibody activity, appears to be associated with a protective immune phenotype in preclinical studies. The vast majority of preclinical vaccine studies have involved the murine model, with extensive dissection of the immune responses and cytokine profiles in both pre- and post-exposure models, as well as intense dissection of immune responses where "protection" has been seen (recently reviewed by Ernst).[93]

The reasons for undertaking preclinical studies are multiple, namely: 1) to provide insight into the effect of BCG and novel vaccines on complex immune systems; 2) to provide a framework for triaging vaccine strategies; and 3) to provide supporting data for new products for regulatory purposes. Increasing the probability of success through targeted and predictive preclinical immunology and disease studies is critical to the successful development of a candidate vaccine. However, understanding the relevance of preclinical studies to clinical TB vaccine outcomes has been confounded by several fundamental problems. Preclinical protection outcomes for specific vaccine strategies can vary considerably across different species, and even within the same species, depending on the specific protocol used. The degree to which the underlying immunological profile elicited by vaccine interventions drives the clinical outcome is critical to understanding the relevance of preclinical studies to subsequent human studies. In many cases the ability to undertake targeted studies to better understand the concordance (or not) between preclinical models has been hampered by limitations of the immunological tools available.[94–96] A concerted effort should be undertaken to compare and contrast the linkage between phenotypic outcome (degree of protection, modulation of disease or pathology) from a vaccine intervention and the nature of the immune profile generated between preclinical models (Table 1). This will require efforts to harmonize vaccine protocols, taking into account species differences as well as improving the immune tools available.

Table 1: Advantages and disadvantages of preclinical models

Species	Advantages	Disadvantages	Primary use
Mouse	Inexpensive, demonstrates vaccine effect with BCG, significant immunology/gene expression tools available. New cross-breeding strategies have provided additional tools, such as collaborative cross mice, diversity outbred mice as well as ultra-low-dose infection).	Relevance to humans unclear, specific differences in pathology, immunology, receptors and pathways.	Immunogenicity, primary functional screen for a vaccine effect against *M. tuberculosis* infection. These studies can provide supportive data for regulatory purposes.
Guinea pig	Pathology similar to human disease, very susceptible to *M. tuberculosis*, demonstrates robust vaccine effect with BCG. Used in regulatory safety-testing of BCG vaccines.	Relevance to humans unclear, specific differences in pathology, immunology, receptors and pathways; limited immunological tools.	Functional screen for vaccine effect, often used as confirmation of murine observations in vaccine screening.
Goats	Pathology and route of infection similar to humans, animal-to-animal transmission seen; vaccine effect seen with BCG. Potential for field trials with natural transmission.	Limited immunological tools, and relevance to humans unclear. Not inbred so diversity in outcomes is more notable.	Limited use to date. Some field trials of vaccine candidates undertaken; more are planned.
Cattle	Pathology and route of infection similar to humans, animal-to-animal transmission seen, vaccine immunogenicity and efficacy shown with BCG. Potential for field trials with natural transmission.	Constraints on use and vaccination in the European area due to regulations concerning cattle TB and skin test conversion. Expensive, P3 containment need in the EC area. Limited immunological tools, relevance to humans unclear. Not inbred so diversity in outcomes more notable.	Increasing use to date. Experimental infection and some field trials of vaccine candidates ongoing. Limited number of sites with capability to study cattle tuberculosis and vaccine-testing.
Non-human primates	Rhesus and cynomolgus species used. Considered closest to humans, with similar immunology and pathology to humans. However, disease pathogenesis and phenotype differences between rhesus and cynomolgus macaques exist. Ability to use sensitive imaging technologies for screening of disease progression.	Expensive; ethical constraints. BCG effect variable and species dependent (rhesus and cynomolgus lead species used). Not inbred so diversity in outcomes more notable, making statistical powering of studies difficult. Geographical source also may affect vaccine outcome. Limited number of sites with capability to study tuberculosis and vaccine testing. Appropriate imaging technologies and interpretation algorithms not yet harmonized or validated.	Study of tuberculosis infection, vaccine-screening and disease progression.
Others	Rabbits, marmosets, zebra fish, in vitro granuloma, controlled human infection. There are others, often with specific and specialized application (primarily research tools or in development as such).		

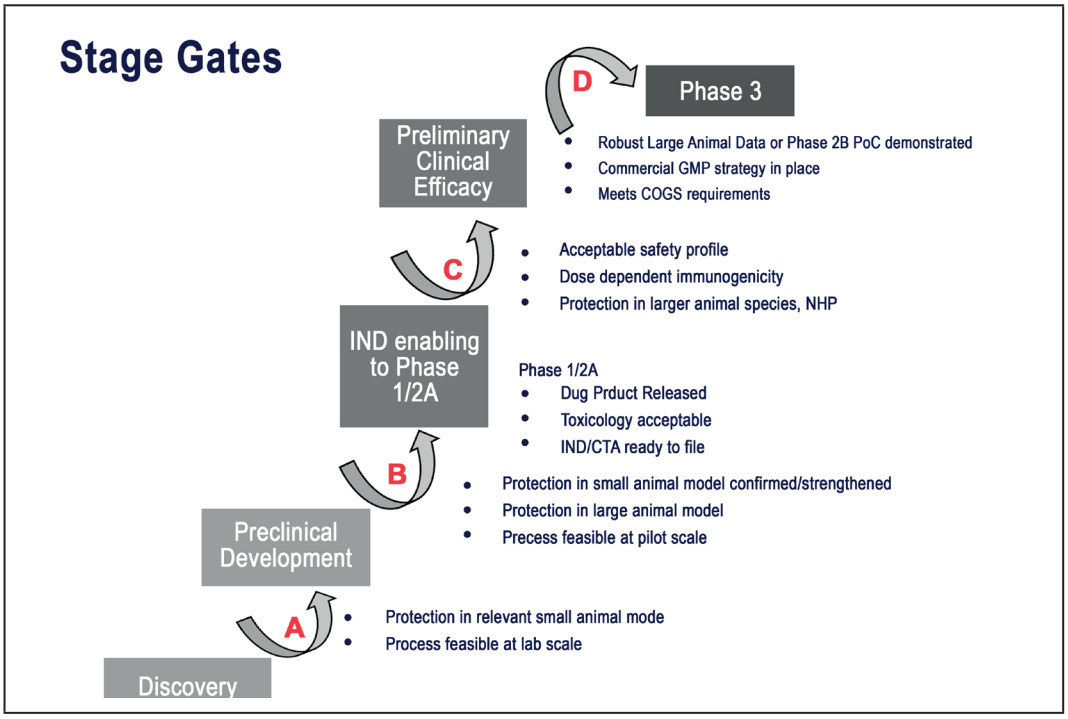
Significant advances in preclinical models have been achieved in monitoring disease progression in non-human primates using PET CT, which have provided new insights into granuloma development, disease progression and vaccine effects in vivo.[97,98] In conjunction with these advances, better understanding of exposure and transmission has enabled the development of natural transmission models in primates, cattle and goats, allowing for field trials of vaccines in real-world, though non-human, cohorts. Low-dose and ultra-low-dose repeated exposure studies have been undertaken to better reflect human disease and thereby to provide tools to better understand vaccine-induced immunity. These models remain under development but are progressing rapidly.[99,100] Murine and small animal models have undergone refinement in technology and immune/gene expression tools and analysis. In addition, improved understanding of the contribution of genetic background can potentially yield significant advances in our understanding of the complex interactions between vaccine interventions, genetics and the subsequent immunological and phenotypic outcomes in terms of protection. The development of genetically characterized, yet genetically diverse, mice (collaborative cross mice) has the potential to reveal new approaches and key points of intervention for vaccine strategies.[101]

Stage gates for TB vaccine development

The TB Vaccine Development Pathway (stage gates for TB vaccine development) was established by a team of scientific and technical experts from the Tuberculosis Vaccine Initiative (TBVI) and Aeras (now the International AIDS Vaccine Initiative, or IAVI) with the input of the TB vaccine community. The tool has been developed on behalf of the Global TB Vaccine Partnership (GTBVP), an alliance of organizations working to make novel TB vaccines a reality, and is funded by the Bill & Melinda Gates Foundation.‡ The stage-gate process is based on an established principle in the pharmaceutical industry, by which the best current science and understanding feed into a series of gating criteria that are expected to be met for further product development at critical stage-gate decision points from laboratory to phase 3 trial and beyond (Figure 1). Agreement and input from across the TB vaccine development community has provided a robust methodology and framework to facilitate acceleration of TB vaccine development from discovery to clinic. A well understood difficulty in using this approach to TB vaccine development has been navigating the gap between our understanding of immunology, preclinical outcomes, and application to clinical outcomes in humans. The primary application of the stage-gate process has been in translational and preclinical processes to make key Stop/Go decisions in mid- to late-stage preclinical development. However, the processes and decisions made once a vaccine achieves clinical development remain an important aspect of this strategy, but are more clearly defined by established clinical practice and the requirements of the regulatory pathway for acceptance. The stage-gate strategy allows transparent decision-making on whether the available immunological or preclinical outcome data distinguish a candidate from others that are further ahead in development, or whether factors such as evidence of inconsistency in manufacture or yield on purification would make further development more difficult,[102,103] as well as harmonizing clinical development decisions across different vaccine candidates.

‡ See: *https://www.tbvacpathway.org/* (accessed 16 November 2020).

Figure 1: Stage gates



Clinical development of new TB vaccines

In the past decade, more than 15 candidates containing a variety of *M. tuberculosis* antigens have moved into human clinical studies. Candidates in the TB vaccine development pipeline[§] include inactivated whole cell or whole cell extracts (*M. vaccae*, RUTI and DAR-901); viral-vectored vaccines (ChAdOx1.85A, Ad5Ag85A, TB/Flu-04L); fusion protein subunits with adjuvants (M72/AS01E, H4:IC31, H56:IC31 and ID93 + GLA-SE); and live recombinant *M. tuberculosis* or BCG vaccines (MTBVAC and VPM-1002).

Results of proof-of-concept efficacy trials against both *M. tuberculosis* infection and TB disease have been reported for H4:IC31, BCG revaccination, and M72/AS01E.[35,79,80] Live vaccines such as the listeriolysin-altered BCG vaccine VPM-1002 are in phase 2b trials in South Africa and India,[104,105] although an earlier recombinant BCG expressing three *M. tuberculosis* antigens did not progress from phase 1 due to a safety signal.[106] MTBVAC, the first live *M. tuberculosis* vaccine to reach clinical testing, is currently in phase 2 trials.[107,108]

Several novel approaches that seek to accelerate innovation in TB vaccine development are also underway, most of them in preclinical stages and proof-of-concept studies in animal models. For instance, a number of recent non-human primate studies have tested novel routes of BCG delivery, such as aerosol and intravenous administration.[109] Intravenous BCG administration, which induced very high levels of antigen-specific CD4+ and CD8+ T cell responses in the lungs, demonstrated particularly impressive protection against *M. tuberculosis* challenge.[110] Other approaches seek to expand the antigen repertoire included in vaccines, often in novel vaccine designs or constructs. A live, recombinant cytomegalovirus vaccine that expresses up to 9 *M. tuberculosis* antigens, induced and maintained *M. tuberculosis*-specific CD4+ and CD8+ T cell responses that were highly differentiated and tissue-resident in rhesus macaques.[111] This vaccine also achieved very impressive protection against TB disease upon *M. tuberculosis* challenge. Refinement of antigen combinations and formulation with novel adjuvant systems is also underway, as is development of RNA vaccines, yet another innovative approach that allows accelerated testing of novel antigens. As illustrated through project "Warp-speed" for SARS-CoV-2 vaccine development, RNA vaccine technology allows rapid vaccine design and manufacture and RNA-based TB vaccines are in preclinical testing.

§ See: *https://www.tbvi.eu/what-we-do/pipeline-of-vaccines/* (accessed 16 November 2020).

Immune responses to novel TB vaccine candidates in humans

A robust Th1 cellular immune response, possibly accompanied by antibodies, is likely to be required for protection against TB disease. Currently, most candidate TB vaccines contain various combinations of vectors, adjuvants and antigens that induce classical Th1 CD4+ and CD8+ T cell responses that produce cytokines such as IFNγ and TNF. Methods, assays and protocols employed in different trials to measure immune responses induced by different vaccine candidates vary widely[112] but most clinical trials have reported antigen-specific CD4+ and/or CD8+ cells expressing Th1 cytokines IFNγ, TNF or IL-2 as the immunogenicity outcome.

Viral vectored vaccine candidates

The viral vectored vaccine candidates MVA85A and Aeras-402 were the first to reach phase 1-2 clinical testing. Safety and immunogenicity of MVA85A were tested in adults, adolescents, children and infants in multiple countries and in *M. tuberculosis*-infected and -uninfected individuals.[45,113–117] MVA85A strongly boosted antigen- specific CD4+ T cells that predominantly expressed IFNγ, TNF and IL-2 in these studies, while some expression of IL- 17 or GM-CSF was also reported. [45,113–117] Antigen-specific CD8+ T cell responses were very infrequent. A long-term follow-up of MVA85A recipients demonstrated that Ag85A-specific CD4+ T cell responses were sustained 3–5 years after vaccination, suggesting that MVA85A establishes a long-lived memory response.[118] Despite these promising results, MVA85A administration at 4–6 months of age in BCG-vaccinated, South African children did not result in significant protection against TB disease or *M. tuberculosis* infection.[88] MVA85A was also found to be well-tolerated and immunogenic in HIV-infected persons[117,119] but did not demonstrate protective efficacy against TB disease in a small trial of HIV-infected adults.[120] Administration of MVA85A by aerosol, which aims to establish a tissue-resident T cell response in the lung that may afford better protective immunity than the systemic response induced by intradermal MVA85A, is currently under investigation.[121]

The adenovirus-35 vaccine, Aeras-402, which contains Ag85A, Ag85B and TB10.4, has also been assessed in adults and infants from several settings and was aimed at boosting BCG-primed or *M. tuberculosis*-induced T cell responses.[46,122-125] Similarly to MVA85A, Aeras-402 vaccination boosted antigen-specific CD4+ T cells that predominantly expressed IFNγ, TNF and IL-2. However, Aeras-402 also induced high-frequency antigen-specific IFN-γ-expressing CD8+ T cell responses. [46,122-125] Antigen-specific antibody responses were not typically assessed in the early trials of viral vectored vaccine candidates, although antibodies to Ag85A, Ag85B and TB10.4 were induced in infants who received either two or three doses of Aeras-402 vaccination at 16–26 weeks of age.[123]

Ad5Ag85A is an adenovirus 5-vectored vaccine that encodes the Ag85A antigen. Ad5Ag85A has completed phase 1 testing in two trials in Canadian BCG-naïve and BCG-vaccinated adults and was shown to have an acceptable safety profile.[126,127] Ad5Ag85A induced Th1 cytokine-expressing CD4+ and CD8+ T cell responses, even in individuals with pre-existing anti-Ad5 antibody responses,[26] while induced CD8+ T cell responses were shown to by cytotoxic.[127] Aerosol administration of Ad5Ag85A is currently being investigated in a phase 1 study in healthy adults in Canada (ClinicalTrials.gov Identifier: NCT02337270).

Protein subunit vaccine candidates

Several protein-subunit vaccine candidates have been developed and are in clinical testing. The M72/AS01$_E$ candidate, which comprises two antigens – Mtb32A (pepA) and Mtb39A (PPE18) – has been assessed in several clinical trials in Europe and Africa in *M. tuberculosis*-infected and -uninfected persons.[128-130] The vaccine induces robust, polyfunctional CD4+ T cells expressing IFNγ, TNF and IL-2, antigen-specific IgG antibody responses as well as detectable CD8+ T cell responses. [128-130] A placebo-controlled phase 2b trial to evaluate the protective efficacy of two doses of M72/AS01E against pulmonary TB in 3573 *M. tuberculosis*-infected adults aged 18–50 years from Kenya, South Africa and Zambia recently reported results of the primary analysis through two years of follow-up *(ClinicalTrials.gov Identifier: NCT01755598)*. M72/AS01E provided 54% protection against progression to confirmed pulmonary TB disease overall; and 84% efficacy in the subgroup of participants younger than 25 years of age, which is possibly a function of more recent *M. tuberculosis* infection.[79] Final results through three years showed 50% protection overall and post hoc analysis did not suggest any age effect on vaccine efficacy; concentrations of M72-specific antibodies and frequencies of M72-specific CD4+ T cells were sustained throughout the follow-up period.[80] Efficacy of M72/AS01E when administered to *M. tuberculosis*-uninfected populations, durability of protection beyond three years, and efficacy in other geographical settings and risk groups, including HIV-infected persons, are important concerns that should be answered in future trials.

ID93+GLA-SE is a fusion protein comprising Rv1813c, Rv2608, Rv3619c and Rv3620c, in the adjuvant formulation GLA-SE, which has been assessed in early-phase trials in both uninfected and *M. tuberculosis*-infected persons in South Africa and the USA.[¶] [131] This vaccine candidate was shown to induce a predominantly polyfunctional, IFNγ, TNF and IL-2-expressing CD4+ T cell response and high-titre antigen-specific IgG1 and IgG3 antibody responses.[131] Antigen-specific CD8+ T cell responses were very low or not detected. A phase 2 trial in which ID93+GLA-SE was administered to TB patients upon completion of successful TB treatment was recently completed. This study assessed safety and immunogenicity in preparation for prevention of recurrent TB disease trials. Results are expected in 2020.[**]

[¶] ClinicalTrials.gov Identifier: NCT02508376 and NCT01599897.
[**] ClinicalTrials.gov Identifier: NCT02465216.

Three related but different fusion protein vaccines (H4, H1 and H56), all adjuvanted in IC31, were developed by Statens Serum Institut in Copenhagen, Denmark, and are in different stages of clinical development. H4:IC31, which contains a fusion protein of Ag85B and TB10.4, was developed as a pre-exposure vaccine candidate (i.e. for individuals not infected with *M. tuberculosis*). H4:IC31 was the first of these products to reach clinical testing in phase 1 trials in *M. tuberculosis*-uninfected adults from Finland and Sweden,[132] after which it was tested in infected and uninfected adults from South Africa.[133] This vaccine candidate induced a T cell response predominated by CD4+ T cells that expressed TNF and IL-2 or these two cytokines plus IFNγ. No notable antigen-specific CD8+ T cell responses have been reported. H4:IC31 was tested for prevention of *M. tuberculosis* infection in 990 QFT-negative adolescents in the recently published phase 2b trial.[35] H4:IC31 demonstrated no efficacy against initial QFT conversion. However, protection against sustained QFT conversion was 30.5% (95% CI -15.8–58.3%), which was significant at a lower, protocol-defined, statistical threshold (80% CI, 3–50.2%).[35] Although these results did not provide definitive evidence for vaccine efficacy of H4:IC31, they suggest that related protein subunit vaccines may have a biological effect against *M. tuberculosis* in humans, a result that provides impetus for the development of H56:IC31.

H1:IC31 and H56:IC31, which both comprise fusion proteins of ESAT-6 and Ag85A and were designed as pre- and post-exposure vaccine candidates, differ in that the H56 polyprotein also includes Rv2660c.[134] Early human trials assessed the safety and immunogenicity of H1:IC31[135-137] but results from animal models suggested that H56:IC31 conferred superior protection to H1:IC31 and consequently H56:IC31 was favoured.[134,138] Safety and immunogenicity of H56:IC31 has been assessed in four clinical trials in South Africa.[††139,140] H1:IC31 and H56:IC31, administered twice or three times, induced predominantly TNF+IL-2+ or IFNγ+TNF+IL-2+ CD4 T cell responses and little-to-no antigen-specific CD8+ T cell responses, as has been reported for H4:IC31. Vaccine-induced CD4+ T cell responses target both the Ag85B and ESAT-6 components, while responses to Rv2660c have been very low and not detected in all vaccinees. [139,140] A recent phase 2 trial assessed the safety and immunogenicity of H56:IC31 in adults who recently completed successful treatment of TB[##] which was conducted in preparation for a phase 2b proof of concept trial that aims to determine the efficacy of H56:IC31 in protecting against recurrent TB.

Comparing immune responses induced by viral vector and protein subunit vaccines

A notable limitation in the clinical vaccine development landscape is that distinct TB vaccine candidates have typically been assessed in unrelated trials with no inter-trial harmonization or standardization. This precludes direct comparison of results obtained for different vaccine candidates, including immunological outcomes. A recent study sought to compare BCG and six novel TB vaccine candidates – MVA85A, AERAS-402, H1:IC31, H56:IC31, M72/AS01E and ID93+GLA-SE – by their induced antigen-specific CD4 and CD8 T cell responses. To address this, Rodo et al. compared published immunogenicity data from clinical trials completed in adolescents or adults at a single site in South Africa.[141] The results suggest that CD4+ T cell response magnitude was

†† ClinicalTrials.gov Identifiers: NCT02378207 and NCT02375698.
ClinicalTrials.gov Identifier: NCT02375698.

the T cell response feature which most differentiated between the different candidates, while analysis of cytokine-co-expression profiles suggested a lack of diversity in responses induced by the different vaccines. M72:AS01E appeared to be the most immunogenic of the candidates since it induced the highest antigen-specific memory CD4+ T cell response. These findings suggest that the likelihood of finding a highly protective vaccine by empirical testing of TB vaccine candidates would be increased by the addition of candidates that induce distinct immune characteristics to the ones already in clinical trials. This comparative analysis did not include data from whole cell or live vaccine candidates, which do appear to induce more diverse, and a broader repertoire of, immune responses.

Whole cell vaccine candidates

Attenuation or inactivation of infectious organisms has been a successful strategy for developing live attenuated vaccines which confer long-lived protective immunity against many different diseases.[142] It is thought that live attenuated or killed whole cell vaccines may thus have potential advantages against *M. tuberculosis* compared with viral vector or protein subunit vaccine candidates on the basis of their broad antigen composition that includes proteins, lipids, carbohydrates and other moieties that may be antigenic and trigger donor unrestricted T cell responses, B cell responses and possibly also NK and ILC responses.

Whole cell vaccines in development include an *M. vaccae* lysate administered to tuberculin-positive adult subjects in a phase 3 study in China to prevent progression from latency to active disease (the published results are not available at present). DAR-901 derived from *M. obuense*, which was shown to provide partial protection against progression to microbiologically-confirmed TB disease in HIV-infected persons in Tanzania using a multi-dose regimen, was recently tested in a proof-of-concept prevention of infection trial.[143,144] A 3-dose DAR-901 regimen did not prevent initial or persistent IGRA conversion, although lower-than-expected event rates limit generalization of the findings.[145] The *M. tuberculosis* lysate RUTI is being developed as a therapeutic vaccine targeted at patients with multidrug-resistant disease.[146]

The live attenuated vaccine candidate at the most advanced stage of clinical development is VPM-1002, a recombinant BCG. The urease C subunit-encoding gene ureC, which reduces acidification of the phagosomal compartment, has been deleted in VPM-1002, while this bacterium expresses the listeriolysin protein from Listeria monocytogenes, which functions to perforate the phagosomal membrane. VPM-1002 is therefore designed to enter the cytosol of host cells, where it releases antigens and increases apoptosis and xenophagy, as shown by in vitro experiments.[147,148] Tolerability and immunogenicity of VPM-1002 have been assessed in two phase 1 trials in adults[104] and two phase 2a trials in healthy infants,[105] one of which was in newborns born to HIV-infected and uninfected mothers (NCT 02391415). VPM-1002 induced a diverse specific T cell response that included CD4+ and CD8+ T cells expressing different combinations of IFNγ, TNF and IL-2. An interesting feature of the VPM-1002-induced response was the emergence of an unusual IL-17-expressing CD8+ T cell subset 16–24 weeks after vaccination.[105] A phase 3 trial of VPM-1002 in patients who have completed TB treatment is under way in India, while a phase 3 trial in infants from South Africa is planned.

MTBVAC is an attenuated *M. tuberculosis* vaccine candidate which contains two independent stable mutations in phoP and fadD26, which are both virulence factors. These deletions result in reduced production of immunomodulatory cell-wall lipids and no secretion of ESAT-6.[149] A phase 1 trial of MTBVAC in healthy adults was completed in Switzerland[150] and a phase 2 study was recently completed in South African adults and infants.[108] MTBVAC induces antigen-specific CD4+ and CD8+ T cells that express IFNγ, TNF and IL-2. Although antigen-specific responses to ESAT- 6 and CFP-10 were not durable to the end of the study in Swiss adults,[150] a dose-dependent IGRA-positive response to MTBVAC that was maintained for up to 12 months was observed in a large proportion of infants who received MTBVAC.[108] The results suggest promising immunogenicity of MTBVAC in infants, but also highlight the importance of developing methods for differentiating between vaccine-induced and *M. tuberculosis* infection-induced IGRA conversion. Two phase 2 dose-finding trials to study safety, tolerability and immunogenicity of MTBVAC are in progress in South Africa, one in infants and another in adults.[§§]

[§§] ClinicalTrials.gov Identifiers: NCT02933281 and NCT03536117.

Conclusion

Recent advances in proof-of-concept efficacy trials against sustained *M. tuberculosis* infection in an *M. tuberculosis*-uninfected population (BCG revaccination)[35] and against progression to TB disease in a *M. tuberculosis*-infected population (M72/AS01E)[79,80] demonstrate that a novel and efficacious vaccine against TB is possible and should serve to provide much-needed impetus to vaccine development efforts. These milestones also offer the opportunity to discover vaccine-mediated correlates of immunity against both sustained infection and progression from infection to TB disease. Discovery and validation of such immune correlates, as applicable across all vaccine products, is critical to accelerating the testing of increased numbers of novel vaccine candidates through phase 1–2 trials. Until such tools become available, critical questions – such as whether BCG revaccination or M72/ASO1E vaccination of *M. tuberculosis*-uninfected populations has meaningful efficacy against subsequent progression to TB disease – can be answered only by large clinical trials in TB-endemic countries. The durability of such protection, and its efficacy in other geographical settings and risk groups, including HIV-infected persons, are other important questions that must be answered in future trials.

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