

Tuberculosis 2019 3



Insights and challenges in tuberculosis vaccine development

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Tuberculosis kills more people than any other pathogen and the need for a universally effective vaccine has never been greater. An effective vaccine will be a key tool in achieving the targets set by WHO in the End TB Strategy. Tuberculosis vaccine development is difficult and slow. Substantial progress has been made in research and development of tuberculosis vaccines in the past 20 years, and two clinical trial results from 2018 provide reason for optimism. However, many challenges to the successful licensure and deployment of an effective tuberculosis vaccine remain. The development of new tools for vaccine evaluation might facilitate these processes, and continued collaborative working and sustained funding will be essential.

Introduction

After the empirical attenuation of *Mycobacterium bovis* by Calmette and Guérin in Lille almost 100 years ago, little progress was made in tuberculosis vaccine development until the turn of the 21st century. To date, bacille Calmette-Guérin (BCG) remains the only licensed vaccine against tuberculosis, and the disease is the leading cause of death from a single infectious agent.¹ The scale of the global tuberculosis epidemic, despite high levels of BCG vaccine coverage throughout the world, shows the urgent need for a more universally effective vaccine.^{1,2} Populations and disease manifestations for which BCG is protective do exist,^{3–5} and insights gained from these settings, together with recent clinical and preclinical data on BCG, could inform the development of a more universally effective vaccine.^{6,7}

Many new tools are needed to contain the current tuberculosis epidemic. Improved diagnostic tests and novel antibiotics are required to improve both diagnosis and treatment, particularly for drug-resistant cases. However, effective vaccination remains the most cost-effective, long-term control measure for any infectious disease, and an effective tuberculosis vaccine will be critical if we are to achieve the WHO End TB Strategy elimination targets.⁸ Mathematical modelling has suggested that a 60% effective vaccine that provides 10 years of protection could prevent 17 million cases of tuberculosis between 2024 and 2050, if delivered to adolescents and adults.⁹ A 2016 systematic review¹⁰ of mathematical models exploring the epidemiological impact of future tuberculosis vaccines concluded that a vaccination for all ages, or one targeted at adolescents or adults, would achieve greater and more rapid epidemiological effects than neonatal vaccination. Adolescents and young adults are the most likely to develop sputum smear-positive disease. Therefore, targeting this age group would have an additional indirect effect via reducing transmission.¹¹ Although other target populations are important for a tuberculosis vaccine to be effective, such as infants and people living with HIV, targeting these groups alone would not have such a substantial effect on transmission.

Another approach to defining key target populations for an effective tuberculosis vaccine is to consider previous mycobacterial exposure. For instance, a conventional vaccine is administered prophylactically before exposure. Alternatively, a vaccine could be delivered post exposure; such a vaccine could be given to the quarter of the world's population that are estimated to be latently infected with *Mycobacterium tuberculosis*. A vaccine could also be delivered therapeutically, as an adjunct to chemotherapy, to people with tuberculosis disease, or it could be given to prevent relapse in patients who have completed treatment.^{11,12}

Although an effective vaccine would certainly be a useful tool in the global effort to control the tuberculosis epidemic, tuberculosis vaccine development is difficult. We do not have defined immunological correlates of protection, the predictive value of preclinical animal models is uncertain, and *M. tuberculosis* has effective mechanisms for evading host immunity. However, there is cause for optimism. The substantial increase in research and development activity in tuberculosis vaccine development over the past two decades is now starting to

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This is the third in a **Series** of three papers about tuberculosis

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Key messages

- Despite considerable scientific challenges, substantial progress is being made in the development of an effective vaccine against tuberculosis
- In 2018, a positive signal of efficacy was shown in two human efficacy trials
- The identification and validation of immune correlates of protection, together with validated preclinical animal models, would greatly facilitate tuberculosis vaccine development
- New tools, such as controlled human infection models and in-vitro functional killing assays, might facilitate vaccine selection, because capacity for human efficacy testing remains low
- Sustained investment in research and development, together with global collaborative working, will ensure this progress continues

	Examples in clinical development	Description of approach	Advantages and disadvantages
Live	BCG-ZMP1, MTBVAC, VPM	Whole organism	Includes many antigens, but might also include antigens that are not protective
Vector	Ad5 Ag85A, TB/Flu04L, ChAdOx1 85A, MVA85A, CMV-6Ag, MVA multiphasic vaccine	Selected protective antigens delivered in a recombinant viral vector	Can be a potent antigen delivery system, but can only include a few antigens
Subunit	H56/CAF01, M72 + AS01, GamTBvac, H56 + IC31, ID93 + GLA-SE	Recombinant protein in combination with an adjuvant	Can be a potent antigen delivery system, but can only include a few antigens
Whole cell	DAR-901, <i>Mycobacterium indicus pranii</i> , RUT1, <i>Mycobacterium vaccae</i>	Whole (killed) organism or fragments of whole organism	Includes many antigens, but might also include antigens that are not protective

Table 1: Approaches used in tuberculosis vaccine development

yield important results (table 1). In 2000, the tuberculosis vaccine pipeline was empty, with almost all tuberculosis research being done in preclinical animal models, which was in stark contrast to the pipelines for HIV and malaria vaccines.^{13,14} By comparison, in 2019, more than a dozen candidate vaccines have been evaluated in clinical trials (figure 1, table 2). The vaccine candidates that have progressed to clinical evaluation include recombinant strains of BCG and attenuated strains of *M tuberculosis*, both designed primarily to replace BCG vaccination in infancy (tables 1, 2).^{15,16} Candidates also include protein and adjuvant combinations and recombinant viral vector subunit vaccines, designed to boost the efficacy of neonatal BCG vaccination (tables 1, 2).^{17–20} Most of the candidates are being developed as prophylactic vaccines to prevent tuberculosis disease. However, some are specifically being developed as therapeutic or postexposure vaccines.^{21,22}

The latest insights in the field of tuberculosis vaccine development, generated from clinical and preclinical studies, are reviewed here. Although considerable progress has been made, many challenges remain to be overcome to achieve the successful development, licensure, and deployment of a universally effective tuberculosis vaccine. Novel tools and models might facilitate this development and improve chances of success.

Insights from clinical trials

In 2018, after a mean follow-up of 2.3 years, Van Der Meeren and colleagues²³ reported the interim results of a phase 2b efficacy trial of a protein and adjuvant candidate tuberculosis vaccine, M72 and AS01e, evaluated in HIV-uninfected adults with *M tuberculosis* latent infection in Africa. M72 is a fusion protein of two mycobacterial antigens, the 32 and 39 kDa antigens from *M tuberculosis*, and is delivered in combination with the adjuvant AS01e.¹⁸ This interim analysis showed a 54.0% (95% CI 2.9–78.2) reduction in bacteriologically confirmed active pulmonary tuberculosis disease in the

M72 plus AS01e group compared with the placebo group ($p=0.04$).²³ This notable result has caused a reawakening of interest in the field of tuberculosis vaccine development because it shows proof of concept and biological feasibility in humans. Of note, an age effect was seen in this trial, whereby the protective efficacy in individuals aged 25 years or younger was 84.4% (95% CI 31.0–96.5). One explanation for this finding is that more recently infected people are easier to protect. However, important questions remain. The durability of this protective effect is unclear, and data from the final analysis will be important in this regard. A key question that will inform the ultimate impact of such a vaccine is whether it is protective in an *M tuberculosis*-uninfected population. Biological samples, taken at enrolment from every participant for immune correlate studies, must now be evaluated to identify immune signatures of protection. Such correlates, once validated in future efficacy trials, would greatly facilitate the development and optimisation of this and other candidate vaccines.

Prevention of tuberculosis disease remains the pivotal efficacy outcome for licensure of a new tuberculosis vaccine. However, prevention-of-disease trials require large numbers of participants, long periods of follow-up, and substantial resources. Efforts to evaluate more exploratory clinical efficacy endpoints, which require smaller clinical trials, are underway. Prevention of *M tuberculosis* infection, as defined by the development of a positive interferon- γ release assay (IGRA) in a subject with a negative IGRA at trial enrolment, has been proposed as a useful clinical endpoint to allow the detection of a biological signal of efficacy in humans.¹¹ The first data from a prevention-of-infection trial was reported in 2018.⁶ In this study in South Africa, 900 participants without *M tuberculosis* infection were randomly allocated to receive BCG revaccination, a boost with a subunit candidate vaccine, H4 and IC31, or placebo. H4 is a fusion protein of the mycobacterial antigens TB10.4 and Ag85B, and is delivered with the adjuvant IC31.²⁴ Neither vaccine group in the study achieved the primary endpoint of a significant reduction in *M tuberculosis* infection, as defined by QuantiFERON-Tuberculosis (QFT; Qiagen, Hilden, Germany) conversion.⁶ However, the BCG revaccination group showed a significant reduction in the exploratory endpoint of sustained QFT conversion. Further trials are needed to confirm this result and to determine the effects of BCG revaccination on tuberculosis disease in this population. An important consideration is whether the underlying immunological mechanisms necessary to prevent infection and disease are the same. Previously, a large BCG revaccination study in Brazil²⁵ found no significant protective effect of BCG revaccination on prevention of tuberculosis disease. A possible explanation for this discrepancy is varying levels of non-tuberculous mycobacteria across the sites where these trials were conducted, which could have interfered with the efficacy

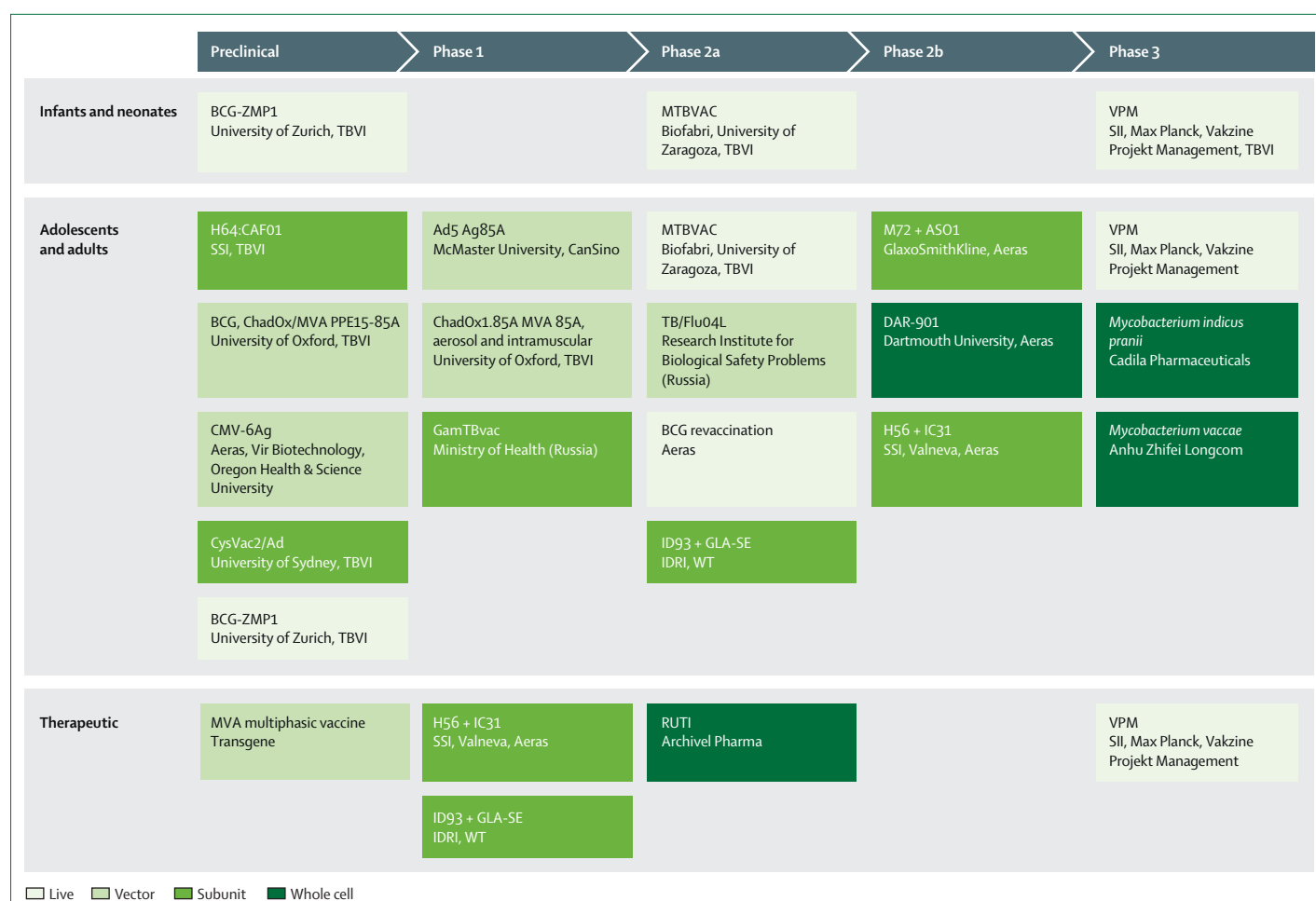


Figure 1: Global tuberculosis vaccine development pipeline

Candidate vaccines in late preclinical and clinical development are included. Figure reproduced with permission of TBVI. TBVI=TuBerculosis Vaccine Initiative. SII=Serum Institute India. SSI=Statum Serum Institute (Denmark). IDRI=Infectious Disease Research Institute (USA). WT=Wellcome Trust.

of BCG vaccination and revaccination.^{26,27} An improved understanding of how exposure to non-tuberculous mycobacteria affects the efficacy of BCG and novel candidate vaccines across different geographical settings is needed. This effort will be facilitated by the development of improved tools to quantify non-tuberculous mycobacteria-specific exposure.²⁸

Other clinically advanced tuberculosis vaccine candidates include a recombinant strain of BCG, which is currently being tested in a phase 2/3 clinical trial to assess efficacy against recurrence of tuberculosis, and a rationally attenuated strain of *M. tuberculosis*, which is currently being evaluated in South African infants.^{29,30} Both of these BCG replacement vaccine candidates have some preclinical evidence of superiority over BCG and have been shown to be safe and at least as immunogenic as BCG in early clinical testing.^{15,16,31,32} Other clinically advanced subunit booster vaccines include protein-adjuvant combinations being evaluated in phase 2 studies.^{33,34} Table 2 summarises ongoing clinical trials.

An alternative approach undergoing clinical evaluation is the delivery of candidate tuberculosis vaccines direct to the respiratory mucosa, administered by aerosol (NCT02337270, SNCTP000002920).^{35,36} Evidence from preclinical animal models suggests that delivering a vaccine direct to the respiratory mucosa, which mimics the natural route of infection, might be a more protective route of immunisation than conventional intradermal or intramuscular delivery.^{37–39} The location of antigen-specific T cells within the airway is considered important for protection, and studies in mice suggest that the location of these T cells within the lung parenchyma is critical for protection.^{40,41} Furthermore, a needle-free approach has advantages in terms of safety and logistics.⁴² The clinical trials done to date suggest that this route of vaccine delivery is well tolerated and immunogenic. Further studies are needed to determine if such an approach is superior to parenteral immunisation. Experimental medicine approaches such as this, whereby candidate vaccines

	ClinicalTrials.gov identifier	Phase	Location	Participants	Number of participants	Endpoints
VPM1002	NCT03152903	2/3	India	Adult patients with pulmonary tuberculosis who have successfully completed treatment	2000	Percentage of cases with bacteriologically confirmed tuberculosis recurrence; safety assessed by solicited local and regional reactogenicity and unsolicited adverse events and serious adverse events
MTBVAC	NCT03536117	2	South Africa	Neonates	99	Safety and immunogenicity; QFT conversion and reversion
MTBVAC	NCT02933281	1/2	South Africa	Healthy adults with or without latent <i>Mycobacterium tuberculosis</i> infection	144	Safety and immunogenicity
H56 + IC31	NCT03512249	2	South Africa, Tanzania	Adults who are HIV-negative with a diagnosis of drug-susceptible pulmonary tuberculosis	900	Rate of tuberculosis disease recurrence; safety; immunogenicity
ID93 + GLA-SE	NCT03722472	1	USA	Healthy adults	48	Safety and immunogenicity
ID93 + GLA-SE	NCT03806686	2	Korea	Health-care workers	105	Safety; immunogenicity; prevention of <i>M tuberculosis</i> infection
Aerosol ChAdOx1 85A	NA*	1	Switzerland	Healthy adults	39	Safety and immunogenicity
Aerosol Ad5 Ag85A	NCT02337270	1	Canada	Healthy adults	36	Safety and immunogenicity
AEC/BC02	NCT03026972	1	China	Healthy adults with or without latent <i>M tuberculosis</i> infection	135	Safety and immunogenicity
GamTBvac	NCT03878004	2	Russia	Healthy adults	180	Safety and immunogenicity

Data are sponsor-reported information from ClinicalTrials.gov and the Swiss National Clinical Trials Portal. QFT=QuantIFERON-TB. NA=not applicable. *Swiss National Clinical Trials Portal identifier: SNCTP000002920.

Table 2: Ongoing clinical trials of candidate tuberculosis vaccines

are tested in small clinical trials to establish proof-of-concept data, are increasingly being recognised for providing complementary data to those generated by preclinical animal studies.⁴³ Furthermore, parallel animal and human studies, in which study design is carefully aligned, can help to show how representative various animal models are of human vaccination and infection, and will allow prioritisation of the most representative models.

Biomarkers and mechanisms of protective immunity

Rational vaccine development would be facilitated by an improved understanding of protective immunity in humans. Furthermore, the identification and subsequent validation of an immunological correlate of protection would transform tuberculosis vaccine development. In other fields, vaccines have been licensed on the basis of such correlates, reducing the cost and complexity of clinical development programmes.⁴⁴ For a complex pathogen such as *M tuberculosis*, the identification of a simple immune correlate of protection, and licensure on the basis of such a correlate, is unlikely. However, a defined immune correlate would have clear utility in vaccine design and development, dose and regimen optimisation, and the selection of candidate vaccines to progress to large, expensive, field efficacy trials. The optimal samples from which to identify immune correlates are samples taken prospectively from

field efficacy studies. Therefore, every opportunity to take and store such samples must be taken. Prospective samples taken from efficacy studies, even those studies that do not subsequently show efficacy, still provide an opportunity for the identification of immune correlates of risk of tuberculosis disease, *M tuberculosis* infection, or both, depending on the clinical endpoints. With use of samples taken from an infant efficacy trial in which the vaccine under evaluation, MVA85A, was found to be safe but not more protective than BCG alone, three immunological correlates of risk of tuberculosis disease were identified.⁴⁵ Higher levels of activated CD4 T cells, reduced BCG-specific interferon- γ (IFN- γ) enzyme-linked immunosorbent spot assay responses and, in an exploratory analysis, reduced anti-Ag85A IgG, were all associated with an increased risk of development of tuberculosis disease in BCG-vaccinated South African infants.⁴⁵ Such studies can also provide insight into the immune mechanisms of protection. Further evaluation of the samples identified that infants with tuberculosis disease were more likely to have concurrent infection with cytomegalovirus than the matched control infants who did not develop disease.⁴⁶ Increasingly, evidence indicates that the high prevalence of infectious diseases in low-income and middle-income countries can make infants more susceptible to infectious disease. This effect differs by geographical location, and it needs to be taken into consideration in the development of a universally effective tuberculosis vaccine.

The previously discussed analysis of infant correlates of risk⁴⁵ identified lower levels of BCG-induced, mycobacteria-specific antibodies as being associated with risk of development of tuberculosis disease. An effective T-helper 1-like cell-mediated adaptive immune response, characterised by the secretion of IFN- γ and tumour necrosis factor (TNF) from antigen-specific CD4 T cells, is known to be necessary but not sufficient for protective immunity.^{47–52} The idea that antibodies and the humoral immune response might also contribute to protection has become popular in the past few years. Latent tuberculosis infection is often considered to be a manifestation of immunological protection, at least against tuberculosis disease, and a 2016 paper highlighted significant differences in antibody function between patients with latent tuberculosis infection and active tuberculosis disease.⁵³ Patients with latent tuberculosis infection were found to have *M tuberculosis*-specific antibodies, which were more effective at phagolysosomal maturation, inflammasome activation, and macrophage killing of intracellular *M tuberculosis* than antibodies from patients with tuberculosis disease. These data suggest a role for Fc-mediated antibody effector functions in anti-*M tuberculosis* protective immunity, which is perhaps mediated via differential antibody glycosylation.⁵³ Although the precise contribution and mechanism of antibodies in different settings has yet to be defined, these and other data suggest that the development of a vaccine that stimulates humoral as well as cell-mediated immunity is more likely to be protective than one that stimulates cell-mediated immunity alone.

A potent innate immune response facilitates early clearance of mycobacteria. Unconventionally restricted T cells, such as MR1-restricted, CD1-restricted and HLA-restricted T cells might play a role during this early stage.⁵⁴ Data from 2012, which showed that BCG can induce some degree of innate immunological memory, also highlighted the importance of this early innate immune response.⁵⁵ The role of the early innate immune response in BCG-induced protection against tuberculosis is unclear but it might explain the so-called non-specific effects of BCG vaccination, such as in the treatment of bladder cancer.⁵⁶ Some evidence also suggests that BCG vaccination confers non-specific protective effects on infant mortality, particularly among infants with low birthweight.^{57,58} These potential non-specific effects should be considered in the development of replacement BCG vaccines, such as novel recombinant strains of BCG or attenuated strains of *M tuberculosis*, and in ensuring that any replacement vaccine is non-inferior in this regard. For subunit vaccines, the adjuvant or viral delivery system might be critical in determining whether a candidate vaccine is efficacious or not, together with the *M tuberculosis*-specific antigens. Several adjuvants and viral vectors are being evaluated as components of tuberculosis vaccine candidates including GLA-SE, AS01e, and vectors based on adenoviruses, poxviruses and cytomegalovirus. Many of these adjuvants

and vectors are also being developed for other pathogens, including malaria (AS01e, adenoviral vectors) and HIV (cytomegalovirus). Studies of vaccines for one pathogen should be examined to inform vaccine development for other pathogens.

Insights from preclinical animal models

In parallel with insights gained from clinical trials and clinical studies, several important advances have been made in preclinical animal studies that have yet to translate into clinical evaluation. For instance, a recombinant cytomegalovirus-vectored tuberculosis vaccine has been developed, which follows on from the use of this vector as a candidate HIV vaccine.⁵⁹ A cytomegalovirus-based HIV vaccine, expressing the simian immunodeficiency virus antigens Gag, Rev-Tat-Nef, and Env, has shown unprecedented levels of protection against simian immunodeficiency virus challenge in non-human primates.⁵⁹ Of note, this protection appears to be associated with the induction of HLA-E-restricted CD8 T cells.⁶⁰ In these studies, not all the animals are protected, and pre-existing humoral immunity to cytomegalovirus does not appear to prevent this protective effect. In tuberculosis, the same cytomegalovirus vectors, expressing six to nine antigens from *M tuberculosis*, confer a similar level of protective efficacy to that seen in the simian immunodeficiency virus study, with the induction of sterilising immunity in some, but not all, animals.⁶¹ In tuberculosis, this protective effect was not associated with the induction of HLA-E-restricted CD8 T cells. Any candidate tuberculosis vaccine that achieves sterilising immunity, particularly in the stringent non-human primate model, requires further evaluation. However, regulatory and manufacturing issues need to be resolved before these persistent cytomegalovirus vectors can be translated into human clinical evaluation. The absence of sterilising immunity being induced by most tuberculosis candidate vaccines to date could be because of delayed activation or recruitment of mucosal dendritic cells, and a subsequent delay in antigen presentation and activation of vaccine-induced CD4 T-cell responses.⁶² An increased understanding of these mechanisms and how to circumvent them could facilitate the development of improved vaccines.

Rather than considering new vaccine candidates, some researchers are investigating the use of BCG delivered by novel routes. In non-human primates, intravenous BCG conferred significantly greater protection against aerosol *M tuberculosis* infection than intradermal BCG.⁷ An intravenous route of immunisation is unlikely to be a deployable strategy for neonates in low-income and middle-income settings. Nevertheless, this preclinical result allows us to identify immune mechanisms of protection; subsequently, safer and more deployable vaccines could be developed to induce those responses. A key question is whether intravenous immunisation induces qualitatively or

quantitatively different immune responses to intradermal immunisation. Carefully controlled human experimental medicine studies, conducted in parallel with non-human primate experiments, will improve our understanding of this question.

Another alternative route of delivery of BCG is by endobronchial administration. In a repeated low-dose aerosol infection model in non-human primates, endobronchial administration of BCG conferred significant protection against *M tuberculosis* infection, as

Panel: Unanswered questions in tuberculosis vaccine development

General questions

- What is the nature and magnitude of the protective immune response needed for a protective vaccine in humans?
- How does the immune response required for protection of disease differ from the immune response required to protect against infection?
- How do co-infections and comorbidities affect vaccine efficacy?

Vaccine-specific questions

- Is M72 plus AS01e protective in individuals without *Mycobacterium tuberculosis* infection?
- Does BCG revaccination protect against tuberculosis disease in the Western Cape?

measured by *M tuberculosis*-specific ESAT6–CFP-10 protein stimulation and an enzyme-linked immunospot-based IGRA.³⁹ Of note, the percentage of purified protein derivative (PPD)-specific CD4 T cells expressing IFN- γ , IL-2, TNF, and IL-17A in the bronchoalveolar lavage fluid, and the percentage of PPD-specific CD8 T cells expressing IFN- γ , were significantly higher in the mucosally vaccinated animals than in the intradermally vaccinated animals.³⁹ This finding illustrates the value of preclinical animal studies for the identification of potential immune correlates of protection that can subsequently be evaluated in human studies.

Challenges

Over the past two decades, substantial progress has been made in the field of tuberculosis vaccine development. More than a dozen candidate vaccines have now been evaluated in clinical trials. However, several important challenges to the successful development, licensure, and deployment of a universally effective tuberculosis vaccine remain; some of the key unanswered questions are presented in the panel. One of the greatest challenges relates to the demonstration of efficacy in human clinical trials. Although showing that a candidate tuberculosis vaccine is well tolerated and immunogenic in early stage clinical trials is relatively straightforward, human efficacy testing requires clinical trials with large sample sizes and long periods of follow-up. Global resources to do field efficacy studies are limited, and the ways in which candidates are selected for progression to efficacy trials needs to be more systematic. A series of stage gates, which define criteria for progression at each stage of tuberculosis vaccine development, from discovery through to licensure, have been agreed by a consortium of funders (figure 2).⁶³ Innovation in clinical trial design has led to the development of prevention-of-infection protocols that might allow demonstration of a biological signal of efficacy in the target species with a relatively small sample size, to generate support for further investment.^{6,11} Efforts to identify early biological signals of efficacy, such as prevention-of-infection trials, can help to bridge the gap between small-scale safety and immunogenicity testing and large-scale efficacy trials, but the predictive value of these surrogate clinical endpoints will only be determined by subsequent efficacy data from prevention-of-disease trials. One of the limitations of prevention-of-infection studies is the absence of precise tools with which to define infection with *M tuberculosis*. IGRAs provide an indirect marker of *M tuberculosis* infection and measure an antigen-specific T cell response to *M tuberculosis* complex organisms.⁶⁴ IGRAs provide more specificity than the tuberculin skin test, and they are not positive after BCG vaccination or exposure to most non-tuberculous mycobacteria. However, IGRAs measure an effector T cell response and might be falsely negative in those with a low-

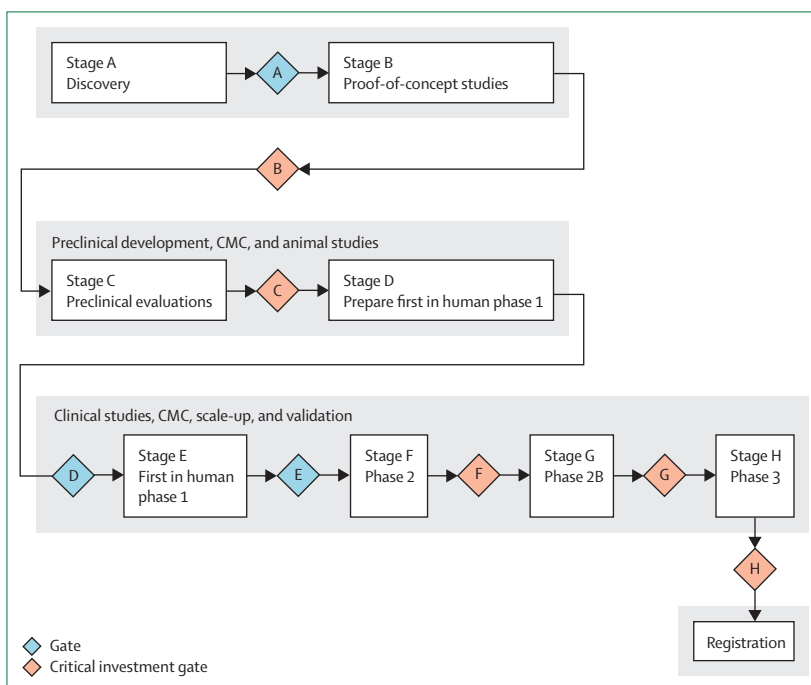


Figure 2: Tuberculosis vaccine development pathway

At each gate, specific criteria are used to decide whether the research should proceed to the next stage. At a critical investment gate, a substantial increase in funding is required. Figure reproduced with permission of the TuBerculosis Vaccine Initiative. POC=proof-of-concept. CMC=chemistry, manufacturing, and control.

frequency effector response or a memory response. The clinical significance of IGRA reversion is unclear.^{65,66} As a clinical endpoint, *M tuberculosis* infection is unlikely to be robust enough for vaccine registration and licensure. Furthermore, a vaccine licensed for this indication would take many decades to have an impact on the global tuberculosis epidemic. However, as a surrogate clinical endpoint, *M tuberculosis* infection is an important biological signal of efficacy in the target species.

Demonstration of efficacy in at least one preclinical animal model is considered an essential prerequisite for progression to clinical evaluation (figure 2).⁶³ Although progressing a candidate vaccine to clinical testing without some evidence of biological effect in an animal model would be considered unethical, the design of animal studies often does not mimic the clinical scenario. For instance, evidence of efficacy in animal models is usually defined as a 0·5–1·0 log reduction in mycobacterial load compared with the relevant control group, which is considerably smaller than the magnitude of effect required in clinical efficacy testing, which is typically powered to detect at least a 60% improvement in protection compared with BCG alone or placebo.⁶⁷ Highly passaged laboratory strains of mycobacteria are routinely used in preclinical animal models, rather than more representative clinical isolates. In general, animals are infected using a large single bolus of mycobacteria, delivered to the lungs by aerosol or direct intratracheal installation, whereas in a clinical setting, the infection is often caused by repeated low-dose exposure.⁶⁷ Efforts to make the preclinical animal models more representative by repeated low-dose exposure, natural transmission models, use of clinical isolates, and a requirement for larger effect sizes, could make animal models more representative, and perhaps more predictive, of the effects of a vaccine candidate in humans.⁶⁸ However, we are unlikely to be able to model the real complexity of human disease, with co-infections, geographical variability, nutritional aspects, and many other factors, in animals. As human efficacy data become available for candidate vaccines, reviewing the preclinical data for those vaccines will be important to establish the predictive ability of animal models.

A further challenge in tuberculosis vaccine development is the diversity of the populations in need of an effective vaccine. One of the most important insights from early BCG studies is the variability in the efficacy of BCG across different populations, an effect that is probably mediated in part by exposure to non-tuberculous mycobacteria.^{69,70} In addition, exposure to helminths, cytomegalovirus, and other co-infections, and factors such as diabetes and monocyte–lymphocyte ratio, might contribute to tuberculosis susceptibility and vaccine efficacy within a population.^{46,70–73} Identifying a single vaccine that protects in all populations, in both a pre-exposure and postexposure setting, and potentially also in a therapeutic setting, might not be feasible. A globally effective tuberculosis vaccine should remain as the ultimate goal but we need to be

realistic. Iterative improvements and increases in understanding based on careful preclinical and clinical studies, open data sharing of positive and negative results, and sustained funding for the long term, are all necessary if we are to achieve this goal.

Novel in-vivo and in-vitro tools in vaccine development

Vaccine development for other complex pathogens for which immune correlates are not easy to define has been facilitated by the development of controlled human infection models (CHIMs). In such studies, healthy vaccinated volunteers are deliberately infected with the pathogen in question, and vaccine efficacy, as determined by either presence or absence of established infection or degree of infection, is assessed. Well established CHIMs exist for many pathogens, including liver-stage and blood-stage malaria, influenza, and typhoid.^{74–77} Deliberately infecting healthy volunteers with virulent *M tuberculosis* would not be ethical, because of the duration and potential toxicity of treatment, and because there is currently no method of proving that treatment has eradicated infection. However, work is underway to develop safe and ethical alternative mycobacterial strains for a mycobacterial CHIM. BCG is a replicating strain of mycobacteria that is licensed for human use, and it might be a suitable strain. The demonstration of biological validity is key to the use of a CHIM as a tool to aid vaccine development and selection. Ultimately, this validity can only be shown by a correlation in vaccine-induced protection between a CHIM and a field efficacy study. In the absence of data from a field efficacy study, another way to demonstrate biological validity is to show that a CHIM can detect a known vaccine effect. An intradermal BCG challenge model has been shown to detect a significant protective effect of BCG vaccination in UK adults, a population for which BCG is known to be protective.^{78,79} Although the ultimate validation against virulent aerosol *M tuberculosis* challenge cannot be done in humans, it can be done in preclinical animal models. In mice, non-human primates, and cattle, a BCG vaccine effect can be detected using an intradermal BCG challenge model, and the magnitude of this protective effect is similar to the known protective effect of BCG vaccination against virulent aerosol *M tuberculosis* or *M bovis* challenge in these animal models.^{80–82} Efforts are now underway to develop an aerosol BCG challenge model, to better mimic the natural route of exposure (NCT02709278, NCT03912207). A key aspect of these studies is the detection and quantification of BCG from the airways. Other efforts in this area include the development of a safe, labelled, mycobacterial strain that would allow non-invasive detection, and a repeated measures model that could potentially enhance sensitivity.⁸³ Once established, a CHIM would also allow the identification of potential immune correlates of protection, which could be subsequently validated in field efficacy studies.

Search strategy and selection criteria

References for this Review were identified through searches of PubMed for articles published in the English language from Jan 1, 1977, to June 30, 2019, by use of the terms "vaccine", "tuberculosis", "clinical trial", "non-human primate", and "mouse". Articles resulting from these searches and relevant references cited in those articles were reviewed. Ongoing and unpublished clinical trials were identified by searching ClinicalTrials.gov and the Swiss National Clinical Trials Portal using the vaccine names as listed in the TuBerculosis Vaccine Initiative pipeline.

In the absence of defined, validated immune correlates of protection, researchers are increasingly interested in the development of functional mycobacterial growth inhibition assays (MGIA), which measure the sum of the parts of the host immune response responsible for protection. In the past, these assays have been limited by poor reproducibility and transferability. Such assays have the potential to be used as in-vitro challenge models to demonstrate a vaccine effect and can also be used to determine immune mechanisms of protection. Similar to the in-vivo human challenge models, MGIA require evidence of biological validity, shown by the detection of a known vaccine effect. A peripheral blood mononuclear cell-based MGIA using the BACTEC MGIT system (Becton Dickinson, Franklin Lakes, NJ, USA) has been extensively optimised for reproducibility and for detection of a BCG vaccine effect. With this assay, a BCG vaccine effect can be detected in UK adults,⁸⁴ and it has now been successfully transferred to two independent laboratories.⁸⁴ The biological validity of the assay has also been shown in mice, non-human primates, and humans; a BCG vaccine effect can be detected after in-vivo and in-vitro infection (Tanner R, University of Oxford, personal communication).⁸⁵ The MGIA has the potential to reduce the number of virulent *M. tuberculosis* in-vivo challenge experiments performed in animal studies, if only those vaccine candidates that show a signal of efficacy in in-vitro MGIA are progressed to virulent *M. tuberculosis* challenge experiments.⁸⁶ Furthermore, such functional assays might facilitate vaccine development, dose and regimen optimisation, and vaccine selection in early human studies.

Conclusions

In the past two decades, considerable progress has been made in tuberculosis vaccine development. A biological signal of efficacy has been shown in two efficacy trials. Promising candidate vaccines at an earlier stage of development are being evaluated in preclinical animal models. Furthermore, progress has been made in the understanding of the protective immune response and in the identification of immune correlates of protection. New tools, such as functional in-vitro assays and CHIMs, could

further facilitate vaccine development and selection. However, many unanswered questions remain. Advancing promising candidate vaccines into well designed human efficacy trials is crucial, in parallel with preclinical animal studies and more basic science to define protective immune mechanisms. The effects of co-infections and comorbidities on disease susceptibility and vaccine efficacy require further evaluation. After decades in which little funding was provided for tuberculosis vaccine research and development, and most activity was focused on murine models, we are now in a period of momentum in tuberculosis vaccine development. This momentum must be continued to encourage the next generation of clinicians and scientists to enter this field. Sustained, collaborative models of funding and working, together with open data sharing, are needed if we are to build on recent progress and ultimately develop, license, and deploy a universally effective tuberculosis vaccine. Only then will we achieve the long overdue mission of eradicating tuberculosis as a global epidemic.

Declaration of interests

I declare no competing interests.

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