The status of tuberculosis vaccine development

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Tuberculosis represents the leading global cause of death from an infectious agent. Controlling the tuberculosis epidemic thus represents an urgent global public health priority. Epidemiological modelling suggests that, although drug treatments for tuberculosis continue to improve, WHO timelines to control the spread of the disease require a new vaccine capable of preventing tuberculosis, particularly in adolescents and adults. The spread of strains resistant to multiple drugs adds additional urgency to the vaccine development effort yet attempts to develop new vaccines with wider applicability and better, longer-lasting efficacy than BCG—the only tuberculosis vaccine licensed for use globally—have proven challenging. Results from clinical efficacy trials, particularly a completed, phase 2b trial for preventing tuberculosis disease in people infected with *Mycobacterium tuberculosis* using the adjuvanted protein subunit vaccine M72/AS01E give hope. We review the current status of tuberculosis vaccine candidates and outline the diversified vaccine development that are underway.

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Introduction

Developing interventions against tuberculosis represents a crucial global health priority.[1] Tuberculosis is the leading cause of death globally from a single infectious agent, killing approximately 1·45 million people in 2018, 251000 of whom were HIV-infected,[2] illustrating the importance of developing a vaccine capable of preventing tuberculosis in both HIV-uninfected and HIV-infected individuals. Approximately one quarter of people worldwide, an estimated 1·7 billion people, are infected with *Mycobacterium tuberculosis*, the bacterium that causes tuberculosis.[3] Of these infected people, 5–15% will develop tuberculosis during their lifetimes, with much higher risk of disease expected for people with compromised immune systems, such as those with HIV infection, malnutrition or diabetes, people exposed to silica dust or indoor smoke, or people who use alcohol or tobacco.[4]

The spread of tuberculosis caused by *M tuberculosis* strains resistant to multiple drugs represents a growing threat to public health due to poor cure rates,[2] high fatality,[5] and the extraordinary cost of treating patients with multidrug-resistant (MDR) tuberculosis (MDR tuberculosis is defined as *M tuberculosis* isolates resistant to the two first-line treatments for tuberculosis, rifampicin and isoniazid) and extensively drug-resistant (XDR) tuberculosis (XDR tuberculosis is defined as *M tuberculosis* isolates resistant to rifampicin and isoniazid, as well as any fluoroquinolone and at least one of the three injectable second-line drugs [amikacin, kanamycin, or capreomycin]).[6,7] Vaccines capable of preventing tuberculosis due to drug sensitive strains will probably be effective against drug-resistant tuberculosis given that the molecular mechanisms that confer *M tuberculosis* drug resistance should not change the immunological characteristics of the organism against which an effective, vaccine-induced immune response is targeted. Accordingly, tuberculosis vaccines might have important roles in future strategies to control MDR and XDR tuberculosis, particularly as resistance to the new mycobacterial drugs bedaquiline and delamanid has been reported.[8] Accelerating the development of new and effective vaccines represents a crucial component of the global efforts to control the tuberculosis epidemic, including the spread of drug-resistant strains.

Tuberculosis vaccine feasibility

The existence of natural human defences against tuberculosis suggests that it should be possible to discover and develop a preventive vaccine. Approximately 85–95% of people infected with *M tuberculosis* can control the infection, never developing any manifestation of tuberculosis disease.[4] Observational studies have shown that harbouring latent *M tuberculosis* infection provides some protection against tuberculosis disease developing from new exposure to the pathogen.[9,10] Additionally, some individuals never acquire *M tuberculosis* infection despite long-term exposure to people with active pulmonary tuberculosis, such as household contacts and health-care workers, raising the possibility of intrinsic resistance.[11,12] Moreover, the possibility that some become infected with *M tuberculosis* but then naturally clear their infection has been raised by documented accounts of individuals who convert their tuberculin skin tests or interferon-γ release assays (IGRAs), but later revert to negative,[13–15] although these reports should be interpreted with caution.[16] The reality of the ongoing tuberculosis epidemic, however, illustrates that many people are incapable of independently generating sufficient immune control of *M tuberculosis* infection, underlining the importance of developing a vaccine.

The BCG vaccine, first used in 1921, is an attenuated form of *Mycobacterium bovis*, the primary cause of bovine tuberculosis, and is currently the only tuberculosis vaccine that is licensed for use globally. BCG is the most widely used vaccine in history, with more than 4 billion doses administered since that first vaccination.[17] BCG is routinely given to infants within days of birth, or when they first interact with health services, in most countries.[18]

Multiple studies have shown that vaccination of infants with BCG is effective at preventing severe forms of tuberculosis in children.[17] BCG has been shown to protect infants and young children, up to approximately 10 years of age, from developing pulmonary and extrapulmonary

tuberculosis, although the degree and duration of protection has been variable, with increased protection in areas farther from the equator.[19] Some observations also indicate that BCG seems to induce better protection in those who are not tuberculosis skin test positive.[20] Some degree of protection against active tuberculosis might last for up to 20 years following school-aged BCG vaccination[21] and as long as 50–60 years following infant vaccination.[22] Studies of BCG revaccination of adolescents have not consistently shown a protective effect against tuberculosis disease.[23,24] Although a 2018, completed, placebo-controlled study of BCG revaccination in South African adolescents showed a significant degree of protection against sustained, de novo *M tuberculosis* infection as defined by an IGRA conversion of 6 or more months duration relative to the placebo arm,[25] the clinical significance of these results will require further study.

The clinical pipeline of tuberculosis vaccine

The tuberculosis vaccine candidates currently in clinical trials (figure) can be grouped into two general categories: mycobacterial whole cell-derived vaccines (table 1) and subunit vaccines, directed against a limited number of selected antigens (table 2).

Mycobacterial whole cell-derived vaccines

Whole cell vaccines are derived from *M tuberculosis*, BCG, or closely related strains of non-tuberculous mycobacteria. This class of vaccines can be further broken down into live vaccines that have been attenuated through genetic modification (VPM 1002,[26] MTBVAC[27]), or vaccines derived from killed or fractionated whole mycobacteria (*Mycobacterium vaccae* [Vaccae],[28] *Mycobacterium indicus pranii* [MIP],[29] DAR-901,[30] RUTI[31]; table 1).

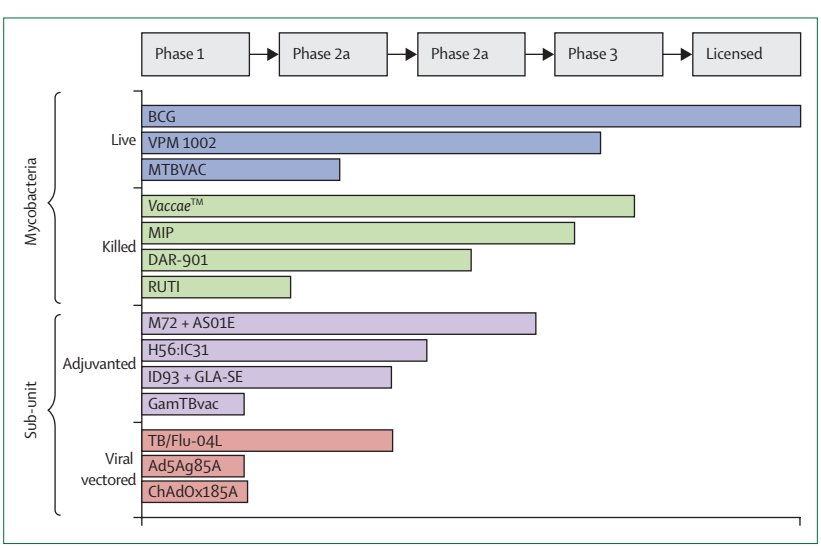

Figure: **Tuberculosis vaccine candidates in clinical development**
The indicated clinical development stages of vaccine candidates are based on an extrapolation from data in ClinicalTrials.gov.

Using a whole cell-derived vaccine represents an attractive vaccine strategy for several reasons. Chief among them is the proven efficacy of BCG, a live, attenuated whole cell vaccine that provides a solid proof-of-concept. Additionally, whole cell-derived vaccines comprise many different antigenic components and offer the possibility of stimulating a more diverse immune response than subunit vaccines due to the presence of non-protein antigens such as lipids[32–34] and glycolipids[35] prevalent in the outer layers of *M tuberculosis*, microbial metabolites, and phosphoantigens.[36]

Subunit vaccines

Subunit vaccines can be further subcategorised into adjuvanted protein subunit vaccines and recombinant viral vectored vaccines. By contrast with whole cell vaccines, subunit vaccines target a small number of selected antigens, usually six or less. Accordingly, a major challenge to developing protein subunit vaccines is the need to identify the optimal antigens to include in such vaccines. Intense efforts have been underway to identify the key antigens, expressed from approximately 4000 genes associated with *M tuberculosis*, that could elicit a protective immune response against the organism.[37,38] Some researchers have specifically selected antigens for inclusion in vaccine candidates according to their expression profile at various stages of mycobacterial infection, on the basis of metabolic activity and protein expression thought to affect vulnerability to the immune system.[39]

The antigens included in current vaccines in clinical trials have all been tested for their ability to induce some form of protective immunity against tuberculosis in an animal model of tuberculosis vaccination. However, most of these antigens have been selected for their capacity to induce the release of the Th1 cytokines interferon-γ and tumour necrosis factor. This choice is informed by the clinical observation that individuals with innate or acquired immunodeficiency in these immune pathways are highly susceptible to tuberculosis.[40] Whether these characteristics will result in the generation of protective immunity, however, is uncertain.[41,42] A component of the advancement of these purified protein antigens to clinical trials involves evidence of their ability to elicit classical T-cell responses. Non-classic T cells are intriguing in that they use host molecules that do not vary between individuals, such as CD1, MR1, and HLA-E. The ligands for these molecules are lipid, glycolipid, vitamin metabolite, and peptide and glycopeptide antigens.[43] The T-cell receptors from the non-classic T cells can recognise these ligands presented in the context of the non-classic molecules. How these non-classic T cells might best be used as vaccines remains to be determined. Vaccine strategies targeting the non-classic responses could possibly enhance the development of more traditional T-cell or B-cell responses. Additionally, cells using the γδ T-cell receptor can recognise glycolipid-antigens and phosphoantigens.[43] Tuberculosis vaccination strategies predicated on the generation of antibodies

remain relatively unexplored but represent a valid area for further investigation.[44]

Adjuvanted protein subunit vaccines

Four adjuvanted protein subunit vaccines are in clinical trials: M72/AS01E,[45] ID93+GLA-SE,[46] H56/IC31,[47] and GamTBvac[48] (table 2 and appendix pp 1–4). Adjuvanted protein subunit vaccines consist of an antigenic protein, or a linked series of antigenic proteins, administered along with an adjuvant—an agent that is designed to potentiate immune system stimulation in such a way as to maximise the immunological impact of the antigens incorporated in the vaccine.

A 2018, phase 2 clinical trial compared H4:IC31 vaccine, consisting of a fusion protein of two immunodominant antigens, antigen 85B and TB 10·4, in combination with the IC31 adjuvant, to placebo, and BCG revaccination to placebo, in protecting South African adolescents (uninfected with *M tuberculosis* but at high risk of *M tuberculosis* infection) from developing sustained quantiferon conversion.[25] Results of this trial showed that neither the H4:IC31 vaccine nor revaccination with the BCG vaccine prevented initial quantiferon conversion, the primary outcome (for H4:IC31, efficacy point estimate 9·4%, 95% CI –36·2 to 39·7; p=0·63; for BCG, efficacy point estimate 20·1%, 95% CI –21·0 to 47·2; p=0·29). In light of these results, the clinical development of the H4:IC31 vaccine has been discontinued. BCG re-vaccination, however, reduced the rate of sustained quantiferon conversion, the secondary outcome (efficacy point estimate 45·4%, 95% CI 6·4 to 68·1; p=0·03); the point estimate of efficacy of sustained quantiferon conversion for H4:IC31 was 30·5% (95% CI –15·8 to 58·3; p=0·16).[25] Future studies should be conducted with subunit vaccines comprised of a wider and more diversified array of antigens, and used in combination with more potent adjuvants, in order to assess the full potential of the subunit vaccine strategy for developing a protective tuberculosis vaccine. Additionally, the results of the BCG revaccination arm of this study suggest that further investigation of the potential utility of this strategy is warranted.

Final analysis of a phase 2b clinical trial of M72/AS01E, consisting of a fusion protein of two immunodominant, highly conserved *M tuberculosis* proteins, *M tuberculosis* 32A and adjuvanted with the proprietary adjuvant system AS01E, provided even more robust evidence of protective efficacy of a subunit vaccine.[49] In this randomised, double-blinded, placebo-controlled trial, more than 3500 IGRA-positive, HIV-negative-adults age 18–50 years from South Africa, Zambia, and Kenya, most of whom had received BCG vaccination as infants, were randomly assigned 1:1 to receive two doses of either the vaccine or placebo intramuscularly, 1 month apart. The primary endpoint was progression to bacteriologically confirmed active pulmonary tuberculosis. After 3 years of follow-up, vaccine efficacy was 49·7% (95% CI 2·1–74·2). The results of this proof-of-concept study assessing the potential for a vaccine to prevent the development of tuberculosis disease in *M tuberculosis*-infected adults are unprecedented and increase optimism that developing vaccines capable of preventing pulmonary tuberculosis in adults is possible. Follow-up studies to confirm these results, to obtain a more precise estimate of the extent of protection, and to get a better sense of the duration of the protective effect will be essential. Whether the vaccine can protect individuals who have not been infected with *M tuberculosis* in the past should also be evaluated along with other potential indications for the vaccine, such as prevention of re-current tuberculosis following completion of successful treatment.[50]

See Online for appendix

	Parental mycobacterium	Modification or inactivation
Live attenuated		
VPM1002 (BCG Δ*ureC*::*hly*)	*Mycobacterium bovis* BCG-Prague	Integration of listeriolysin encoding gene insert, urease gene deletion
MTBVAC	*Mycobacterium tuberculosis*	Transcription factor *phoP* and *fadD26* (phthiocerol dimycocerosate synthesis) gene deletions
Killed or extract		
Vaccae	*Mycobacterium vaccae*	Heat-killed
MIP	*Mycobacterium indicus pranii* (formerly: *Mycobacterium w*)	Heat-killed
DAR-901	*Mycobacterium obuense*	Heat-killed
RUTI	*M tuberculosis*	Cell wall fragments of *M tuberculosis* grown under stress conditions, in liposome suspension

Table 1: **Composition of whole mycobacterial cell-derived vaccines in clinical trials**

	Antigen (antigen description)	Adjuvant	Adjuvant description
Adjuvanted protein			
M72	Rv1196 (PPE family), Rv0125 (peptidase)	AS01	Liposome, TLR4 agonist
H56	ESAT-6 (*Mycobacterium tuberculosis* antigen), Ag85B (mycolyl transferase), Rv2660c (dormancy antigen)	IC31	Antimicrobial peptide KLK + oligodeoxynucleotide ODN1a, TLR9 agonist
ID93	Rv2608 (PPE family), Rv3619 (virulence factor), Rv3620 (virulence factor), Rv1813 (dormancy antigen)	GLA-SE	Glucopyranosyl lipid adjuvant in emulsion of squalene oil-in-water, TLR4 agonist
GamTBvac	Ag85A (mycolyl transferase), ESAT6-CFP10 (virulence antigens)	CpG ODN	Oligodeoxynucleotides, TLR9 agonists
Viral vector			
Ad5Ag85A	Ag85A (mycolyl transferase)	Ad5	Recombinant replication deficient human adenovirus 5 vector
ChAdOx185A + MVA85A boost	Ag85A (mycolyl transferase)	ChAdOx1, MVA	Chimpanzee adenovirus, modified vaccinia ankara virus
TB/Flu-04L	Ag85A (mycolyl transferase)	Flu-04L	Attenuated replication-deficient influenza virus (H1N1)

PPE=proline-proline-glutamic acid. TLR=toll-like receptor.

Table 2: **Composition of subunit vaccines in clinical trials**

Recombinant viral-vectored vaccines

Three viral-vectored subunit vaccines candidates are in clinical trials: TB/Flu04L,[51] Ad5 Ag85A,[52] and ChAdOx185A/MVA85A (NCT03681860) (table 2 and appendix pp 1–4). Recombinant viral-vectored vaccines are similar to adjuvanted protein subunit vaccines as they serve as delivery systems for selected *M tuberculosis* protein antigens thought to be important in generating a protective immune response against tuberculosis. In this vaccine strategy, however, the viral vector is intended to trigger a robust and long-lasting immune response to the antigens being presented, obviating the need for an exogenous adjuvant.

Many of the same issues inherent in developing protein subunit vaccines, including the challenge in identifying a few, critical antigens, also apply to viral-vectored delivery. Additionally, the vector must be safe enough to prevent the occurrence of vector-based pathogenesis. Ensuing safety is particularly challenging when designing viral vector-based vaccines for possible administration to immune suppressed individuals, including people with HIV, in whom a vector proven as non-pathogenic in immune competent individuals might cause disease. A further challenge to a viral vectored vaccine occurs if more than one vaccination is required to reach the targeted degree of immune response. In this situation, immunity to the viral vector, developed during the initial administration of the vaccine, could potentially result in the premature, immune-based destruction of the vector during subsequent vaccine administrations, preventing the expression of the antigen and thereby rendering the vaccine ineffective.[53] A similar situation could arise if the people being vaccinated were infected or might become exposed to the virus being used as a vector during the course of a vaccine administration regimen. These problems might be circumvented using a heterologous prime-boost vaccination strategy, in which the boosting vaccine contains the same antigens as the priming vaccine but uses a delivery strategy different from the vectored vaccine used to deliver the priming vaccination.

Assessing clinical efficacy of tuberculosis vaccines

When assessing the efficacy of tuberculosis vaccine candidates, investigators generally choose one of three possible clinical endpoints: prevention of tuberculosis disease (PoD), prevention of recurrent tuberculosis disease in people completing a course of anti-tuberculous drug therapy (PoR), or prevention of established *M tuberculosis* infection in individuals entering the trial without evidence of pre-existing *M tuberculosis* infection (PoI). A fourth potential use of tuberculosis vaccines, as immunotherapeutic agents to increase the effectiveness and reduce the duration of drug therapy for active tuberculosis disease, is another endpoint of increasing interest to the field but is beyond the scope of this review (the RUTI vaccine is being developed specifically for this purpose).

Preventing tuberculosis disease

Due to the small percentage of people infected with *M tuberculosis* expected to progress to active tuberculosis during their lifetimes, studies to assess vaccine efficacy for a PoD indication face distinct challenges, including the necessity for a large sample size, a trial duration of 3 years or more, and the high costs associated with meeting these conditions. Additionally, an important uncertainty inherent in developing a vaccine for a PoD indication is whether a single vaccine could prevent tuberculosis disease both in people who receive the vaccine before becoming infected with *M tuberculosis* (ie, people who are IGRA-negative), and in people immunised subsequent to becoming latently infected with *M tuberculosis* (people who are IGRA-positive).

Given these challenges, vaccine developers have used other clinical development strategies before embarking on resource-intensive PoD trials. These strategies might be used to bolster confidence in the clinical potential of a vaccine candidate and to explore the possibility of seeking other indications for tuberculosis vaccines.[54]

Preventing recurrent tuberculosis disease

Individuals completing treatment for drug-sensitive tuberculosis are at elevated risk of experiencing recurrent tuberculosis disease, either due to relapse of incompletely treated disease or recurrent *M tuberculosis* infection.[55] Risks of recurrent tuberculosis disease increase with shortened treatment, poor compliance, and in HIV-infected individuals.

A PoR trial is less onerous to conduct than a PoD trial given that the specific population at risk for recurrent disease—those completing drug treatment for tuberculosis—can be identified, and considering the high rate of recurrent disease occurring in a relatively short time (1 or 2 years) after cessation of drug treatment. These factors make it feasible to use smaller sample sizes, and shorter trial durations, than would be required to assess a PoD outcome. PoR trials can thus provide a general indication of clinical protective activity, while also supporting a potential licensable indication. However, some individuals with relapsed or recurrent infection might have immunological abnormalities that make their protection via vaccination a greater challenge. Individuals who have had tuberculosis can possibly also not be protected with an immune-modulating vaccine, given that tuberculosis disease is associated with a reduction in lung function, damage of the lung matrix, and creation of immune-privileged sites. Additionally, uncertainties exist over the optimal timing of PoR vaccine administration, whether the vaccine should be given before or after completion of drug treatment, adding degrees of risk to this strategy.

Preventing established *M tuberculosis* infection

Preventing established *M tuberculosis* infection with a vaccine (indicated by persistent conversion of tuberculin

skin tests or IGRA from negative to positive) would intuitively appear to be an effective way to prevent tuberculosis disease. It remains unclear, however, whether vaccine success or failure against a PoI endpoint would reflect success or failure against a PoD endpoint. It is possible that a vaccine might only prevent infection in people who otherwise would never develop disease.[25] PoI is therefore not universally accepted as an indication for licensure of a tuberculosis vaccine. This endpoint is nevertheless being used by developers in phase 2 trials to gain greater confidence in the likelihood of the candidate's success, before proceeding to expensive and resource intensive, later-stage PoD trials.[25]

Status of the preclinical pipeline

Several promising tuberculosis vaccine candidates are in advanced pre-clinical development. These include vectored vaccines using chimpanzee adenoviruses,[56] a human parainfluenza virus vectored vaccine,[57] the replication-deficient, modified vaccinia Ankara antigen delivery platform,[58,59] a new adjuvanted subunit vaccine (H64) containing six highly expressed *M tuberculosis* proteins,[60] and a recombinant, attenuated BCG.[61] Additionally, early work is proceeding with several live, attenuated *M tuberculosis* vaccines, including concepts with deletions of Δ*sigH*,[62] Δ*mosR*,[63] or Δ*echA7*[63] genes, and with a variety of next-generation vaccines based on BCG.[64] One of the most promising pre-clinical vaccine candidates uses an attenuated cytomegalovirus vector that has been modified with inserted genes for selected *M tuberculosis* antigens thought to be important for stimulating protective immunity.[65] This new cytomegalovirus-vectored tuberculosis vaccine has demonstrated an overall vaccine effect of approximately 70% in rhesus macaques, with complete prevention of tuberculosis disease in more than 40% of the vaccinated animals.[65] A crucial feature of the cytomegalovirus vector is that it persists, perhaps indefinitely, whereas other viral vectors are eliminated within a few replication cycles. The high degree of protection by this vaccine construct might therefore result from its ability to elicit and maintain lung-based immune responses capable of intercepting *M tuberculosis* at the onset of infection rather than at later stages when the infecting *M tuberculosis* organisms are better capable of circumventing host immune responses.[65]

Diversification of the tuberculosis vaccine portfolio

An examination of the current tuberculosis vaccine candidates reveals the need for establishing a broader and more robust portfolio through diversification of antigens, vaccine platforms, administration routes, and types of immune responses induced.

Diversification of antigens and vaccine platforms

Most tuberculosis vaccine candidates are variations of vaccine subunit technology or whole cell vaccines based on non-tuberculous mycobacteria. Only one vaccine in clinical testing, MTBVAC, is derived directly from *M tuberculosis*.[27] The vectored and adjuvanted protein vaccines in clinical trials express, at most, four antigens, whereas the pre-clinical cytomegalovirus-vectored tuberculosis vaccines expresses six.[65] The need to generate a interferon-γ producing Th1 type response has typically been a primary consideration for selection of antigens, but antigen identification has recently sought to select *M tuberculosis* antigens that would result in a diversification of immune responses beyond CD4 Th1. Particular efforts have focused upon identifying antigens capable of stimulating non-CD4 T-cell responses, mostly MHC-I-restricted, CD8 T-cell mediated responses.[66–68] Identification of antigens capable of engendering B-cell responses and subsequent antibody expression, are also drawing attention.[44] Ongoing research into the functions of various *M tuberculosis* genes, and the effect of their deletion to allow safe administration to humans while preserving critical components of the vaccine candidate's immunogenicity, has the potential to identify promising new antigens. Nucleic acid-based vaccines, including DNA and RNA vaccines, represent another route to vaccine diversification.[69]

Diversification of immune response types

M tuberculosis is an intracellular pathogen, infecting pulmonary-based antigen presenting cells such as macrophages and dendritic cells before progressing to a stage of established infection within granulomas. Tuberculosis vaccine development has therefore focused on stimulation of cell mediated immune responses. Accordingly, immunogenicity assessments essential to the advancement of tuberculosis vaccine candidates—whole cell and subunit—currently in clinical trials focused on their ability to stimulate interferon-γ, interleukin 2, and tumour necrosis factor responses in CD4 T cells obtained from vaccinated animals and humans, following ex-vivo exposure of these T cells to tuberculin protein, tuberculosis peptides, or other tuberculosis vaccine antigens. Although the T-cell interferon-γ response is believed to be essential in protecting against *M tuberculosis* disease, it has not been confirmed to be either a vaccine-induced correlate or surrogate of protection against tuberculosis.[70]

Attention has focused on the potential benefits of assessing immune responses generated via non-classic presentation of non-protein antigens with limited polymorphism and molecules that are unique to *M tuberculosis*.[71] Examples include HLA-E (which can present peptide and glycopeptide antigens),[67,72] group 1 (CD1a-c) restricted T cells (which recognise CD1 glycolipid and lipid antigens found within the outer mycobacterial membrane),[34,73] mucosal associated invariant T cells (which are enriched in the lungs),[74,75] natural killer T cells (which recognise glycolipids in the context of CD1d),[34] and γδ T cells.[76] As non-classic presentation molecules are highly conserved, they offer the advantage that a vaccine targeting these responses would work across all

populations. Developing vaccines capable of stimulating these and other so-called unconventional T-cell responses, along with conventional CD4 and CD8 T-cell responses, represents a new frontier in tuberculosis vaccine research.

The role of antibody-mediated protection against tuberculosis has gained renewed interest.[77] Indirect effects of humoral immunity can have a key role in increasing the efficiency of cell mediated immune control of pathogens hiding within cells, as *M tuberculosis* does within macrophages and dendritic cells.

Diversification of administration routes

All tuberculosis vaccines currently beyond phase 1 in the clinical pipeline are administered via injection, either intramuscularly or intradermally. The initial site of *M tuberculosis* infection, however, occurs in the lung, and delivering a tuberculosis vaccine via aerosol, potentially stimulating both mucosal and peribronchial immune responses against *M tuberculosis*, is being investigated. Several phase 1 studies are exploring the safety and immunogenicity of administering various tuberculosis vaccines, including BCG and MVA85A, via aerosol,[78,79] while preclinical studies explore the efficacy of aerosol application, either administered alone or in combination with an injected tuberculosis vaccine.[80]

Older and more recent experiments in rhesus macaques that used intravenous route of BCG administration showed superior protection than BCG administered intradermally or through the aerosol route.[81,82] The heightened protective effect of intravenous administration might therefore be due to the generation of immunological control through increased levels of CD4 effector T-cell activity within the lung or increased activation of innate components of the cellular immune response.[82]

Diversification of administration strategies

In addition to diversifying routes of vaccine administration, the potential utility of homologous and heterologous prime-boost approaches is also being explored. In a homologous prime-boost strategy, the same vaccine platforms, delivering the same tuberculosis antigens, are used in the priming and boosting phases of vaccine administration; in a heterologous prime-boost strategy, different vaccine platforms are used to deliver the same antigens.[83] The heterologous prime-boost strategy has become increasingly attractive given the potential for stimulating a broader immune response when delivering antigens in different ways, and as means to overcome the problem of anti-vector immunity, a known challenge to vectored vaccine platforms requiring a number of administrations over time.[83]

Critical tools are missing

Developing better tools for testing and selecting vaccine candidates represent, together with diversification of the portfolio, another crucial goal towards developing a tuberculosis vaccine.

Biomarkers and biosignatures

Although certain elements of effective immunity to tuberculosis are well understood, the full picture of an immune response capable of protecting against *M tuberculosis* infection or tuberculosis disease remains obscure.[70] A better understanding of correlates of protection—a measurable immune response, or group of responses, demonstrated to protect against *M tuberculosis* infection or tuberculosis disease—would be a major step forward.[84] This knowledge could then guide antigen selection and accelerate vaccine development by early selection of promising candidates in smaller clinical trials, including bridging studies across geographical areas, age groups, and risk groups. Many of the most advanced biometric techniques, including transcriptomics, proteomics, and metabolomics, are being used, individually and in combination, to identify potential biosignatures, combinations of a variety of biomarkers, of immune protection.[84,85] However, applying these techniques requires that samples from clinical trials are collected, bio-banked, and available and this aspect is not always adequately addressed or budgeted for in clinical trials.

Animal models

The tuberculosis vaccines in clinical development have all undergone prior testing in animal models, mostly mice, guinea pigs, and non-human primates. The testing has typically involved an aerosol exposure to *M tuberculosis* from hundreds to more than 1000 colony-forming units, but such supraphysiological conditions have created an unnaturally difficult challenge for vaccine candidates to overcome. Recent efforts to improve animal challenge models for tuberculosis vaccine testing have attempted to better approximate the low level of exposure occurring in natural human infection. Most animal challenge models are now using low-dose, aerosolised *M tuberculosis* inocula, ranging from 25 to ten colony-forming units, or so-called ultra-low-dose approaches, where fewer than ten colony-forming units of *M tuberculosis* are administered.[86,87]

Mice have typically been used as a first screening model in vivo due to the relatively inexpensive and rapid results, but the extent to which the results of challenge experiments in mice predict human outcomes of vaccination remains uncertain.[88] Guinea pigs have not been used as extensively as mice for *M tuberculosis* challenge experiments, but they are noted for their exquisite sensitivity to low-dose *M tuberculosis* infection.[89] These experiments often represent the final step in preclinical assessment, given the similarities in genetics, tuberculosis pathophysiology and overall immunology, between non-human primates and humans.[90] A substantial variability of the outcome of *M tuberculosis* infection among non-human primate species has been noted, emphasising the importance of selecting the most appropriate species for challenge experiments based on

the ultimate study goals.[91] Developments in scanning technologies, particularly a combination of PET and CT scanning, offer the potential of making quantifiable comparisons between vaccinated and unvaccinated non-human primates of the development of pulmonary tuberculosis infection and disease within days to weeks after challenge.[92] This comparison might provide an opportunity to obtain earlier assessments of vaccines at far lower cost than were possible before.[93]

Controlled human infection model

The development of a controlled human infection model of tuberculosis would represent a breakthrough for tuberculosis vaccine research. These models have played important roles in advancing vaccine development efforts, particularly for malaria and influenza.[94] Controlled human infection models permit a carefully controlled administration of pathogens to humans in a safe manner to assess the clinical effect of vaccines or drugs at an early stage of vaccine development and at relatively low cost. Optimally, a controlled human infection model for *M tuberculosis* would permit an assessment of vaccine protection against a low dose *M tuberculosis* exposure via the aerosol route. Developing a challenge strain of live *M tuberculosis* sufficiently safe for experimental aerosol administration to humans, and detectable by sensitive assays in serum, urine, or in the breath, remains a primary goal for the controlled human infection model of tuberculosis. Additionally, a model using intradermal BCG assessed via punch biopsy and PCR and colony-forming units quantification has been used in humans and is undergoing further evaluation.[95]

Tuberculosis vaccine development: a global effort

Ambitious goals for ending the global epidemic of tuberculosis and vastly reducing tuberculosis mortality on or before the year 2035 have been set by the UN within its Sustainable Development Goals, the WHO End TB Strategy, and the End TB Goals of the Stop TB Partnership.[2] The first United Nations High Level Meeting on tuberculosis was held on Sept 26, 2018, and called for accelerated and concerted efforts against the disease in a declaration that was subsequently adopted by the UN General Assembly.[96] None of these ambitious goals, however, can be met without the development of new and better drug treatments for tuberculosis, better point-of-care diagnostics, and the implementation of an effective vaccine.[2]

Economic models have suggested an extraordinary cost-effectiveness from tuberculosis vaccines on both health system and societal perspectives.[97] In one such modelling exercise, a tuberculosis vaccine targeting adolescents and adults in low-income countries, with 60% efficacy over a 10-year duration could prevent 17 million cases of tuberculosis (range 11–24 million) by 2050.[98] Even if the resultant vaccines demonstrated efficacy rates as low as 20%, assuming a duration of effect of 10 years, intermittent mass vaccination campaigns would be logistically and economically feasible.

New information about the molecular structure and mechanisms driving the disease-producing activity of *M tuberculosis*, the immunological responses to *M tuberculosis* infection, the basic pathogenesis of tuberculosis disease in humans and animal models, and the efficacy or lack thereof, shown for a variety of preclinical and clinical vaccine candidates, point the way to several notable, interrelated directions for future tuberculosis vaccine research and development. At the same time, considerable efforts are being made to establish a global framework for optimal development of a new vaccine. Organisations central to global tuberculosis vaccine development such as the TuBerculosis Vaccine Initiative, the International AIDS Vaccine Initiative, the European Commission, the European Investment Bank, the European and Developing Countries Clinical Trials Partnership, and the Bill and Melinda Gates Foundation have formed the Global TB Vaccine Partnership and created a set of mutually agreed stage gate criteria for advancing early-stage tuberculosis vaccine concepts into animal testing and subsequently into progressive clinical development phases.[99] Adherence to these criteria is intended to optimise the use of resources by proposing a predefined prioritisation framework, and thereby decrease the risk of candidate failure in expensive, later-stage clinical efficacy trials. In parallel with this, WHO has developed preferred product characteristics for tuberculosis vaccines that will provide additional guidance regarding characteristics and efficacy targets to tuberculosis vaccine development efforts.[100]

Search strategy and selection criteria

We searched PubMed for articles published in English from Jan 1, 2000, to June 30, 2019 to identify papers on tuberculosis vaccine development, by use of the terms "tuberculosis", "vaccine", "Mycobacterium", "immunization", or "vaccination". We further searched the ClinicalTrials.gov database, provided by the US National Library of Medicine, for relevant entries using the search terms "tuberculosis" and "vaccine". Additionally, we reviewed relevant articles cited in references from the above search extracts and included them as primary sources when appropriate. We reviewed articles resulting from these searches and their references. The number of relevant studies was too large for all to be cited, but we considered all prioritising clinical trials, antigen identification, and pre-clinical validation records.

Conclusion

Developing a tuberculosis vaccine remains a critical global health priority given the deadly nature of the ongoing tuberculosis epidemic and the spread of drug-resistant *M tuberculosis* strains. The significant degree of protection against tuberculosis disease shown

by the M72/AS01E candidate in a phase 2b clinical trial strongly suggests that tuberculosis vaccines are feasible and the encouraging preclinical results from advanced vaccine candidates, such as a cytomegalovirus-vectored tuberculosis vaccine construct, offer the prospect of further progress. Diversifying tuberculosis vaccine-generated immune responses, routes of administration, and vaccine platforms represent novel strategies likely to increase the chance of future vaccine success. Parallel improvements in preclinical and clinical testing strategies will permit greater information about potential vaccine efficacy to be collected in a shorter time and at less cost. Success in this endeavour is not optional given the health burden and cost of the tuberculosis epidemic; a persistent, robust, global effort must be maintained until a safe, efficacious tuberculosis vaccine is achieved.

Contributors

All authors contributed to the conception of the Review. LKS and OO conducted the literature search, data extraction and data synthesis, created the tables and figure, and wrote the manuscript. All authors contributed to the interpretation of the data and revision of the manuscript.

Conflicts of interests

We declare no conflict of interests.

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