



## Review

## Novel approaches to tuberculosis vaccine development

Stefan H.E. Kaufmann<sup>a,\*</sup>, January Weiner<sup>a</sup>, C. Fordham von Reyn<sup>b</sup><sup>a</sup> Max Planck Institute for Infection Biology, Department of Immunology, Charitéplatz 1, 10117 Berlin, Germany<sup>b</sup> Infectious Disease and International Health, Geisel School of Medicine, Hanover, New Hampshire, USA

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## SUMMARY

Tuberculosis (TB) remains the deadliest infectious disease. The widely used bacille Calmette–Guérin (BCG) vaccine offers only limited protection against TB. New vaccine candidates for TB include subunit vaccines and inactivated whole-cell vaccines, as well as live mycobacterial vaccines. Current developments in TB vaccines are summarized in this review.

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## 1. Introduction

According to a recent analysis, tuberculosis (TB) has killed one billion people over the last 200 years, more victims than from smallpox, malaria, plague, influenza, cholera, and AIDS together.<sup>1</sup> Indeed towards the end of the 19<sup>th</sup> century, one in five of all deaths was caused by TB.<sup>2</sup> Although TB is considered a disease of the past in some circles, it remains the deadliest contagious disease globally. In 2015, 10.4 million new cases of active TB were recorded, resulting in 1.8 million deaths (World Health Organization, WHO).<sup>3</sup>

Approximately two billion people are infected with the causative agent, *Mycobacterium tuberculosis*, but only a small proportion of those individuals living with a latent TB infection (LTBI) are at risk of developing active disease (somewhere in the order of 10% over a lifetime).<sup>4</sup> This is because our immune system is capable of containing the pathogen in a dormant stage.<sup>5</sup> However, since the immune response fails to achieve sterile eradication, individuals with LTBI are at risk of developing TB later in life.

TB reactivation is greatly accelerated by co-infection with HIV.<sup>3</sup> Of the 15 million individuals suffering from co-infection with HIV and *M. tuberculosis*, 1.2 million have developed TB in 2015, rendering HIV co-infection a major driving force in the TB pandemic. An

additional complication is the increasing incidence of multidrug-resistant (MDR)-TB annually; this accounts for half a million new cases with only a 50% chance of cure by drug treatment. Globally some 50 million individuals are already latently infected with MDR *M. tuberculosis*, creating a remarkable resource for future cases of active TB with insufficient treatment options.<sup>3</sup> Nevertheless, the WHO has vowed to reduce TB morbidity by 90% and TB mortality by 95% by 2035.<sup>6</sup> This ambitious goal can only be accomplished successfully if more rapid diagnostics, new drugs for shorter therapy, and new vaccines to prevent pulmonary TB become available.<sup>6</sup> A short up-to-date overview of vaccines is provided here.

## 2. The disease and the pathogen

TB is primarily a disease of the lung, which serves as the port of entry and site of disease manifestation.<sup>7</sup> *M. tuberculosis* is transmitted by aerosol; if these bacteria reach the alveoli in the deeper lung, they are engulfed by alveolar macrophages and interstitial dendritic cells. These antigen-presenting cells transport *M. tuberculosis* to draining lymph nodes, where T lymphocytes are stimulated. Although antibodies are produced abundantly in response to *M. tuberculosis* infection, T-cells are generally considered the main mediators of protection during natural infection.<sup>7</sup> Orchestrated by T-cells, solid granulomas are formed in the lung parenchyma, where *M. tuberculosis* is contained in a persistent stage.<sup>8</sup> Such solid granulomas are present in the two billion individuals with LTBI. Active disease emerges when granulomas lose their sophisticated structure and become necrotic

\* Corresponding author. Tel.: +49 30 28460 502.

E-mail address: [Kaufmann@mpiib-berlin.mpg.de](mailto:Kaufmann@mpiib-berlin.mpg.de) (Stefan H.E. Kaufmann).

or even caseous due to massive cell death. During LTBI, *M. tuberculosis* reduces its metabolic and replicative activity to become dormant.<sup>9</sup> However, in caseous granulomas, *M. tuberculosis* reactivates its metabolism and replicates to reach high numbers. Rupture of a caseous granuloma allows for *M. tuberculosis* dissemination to other tissue sites and to the environment. Expectoration of cellular material containing *M. tuberculosis* serves as the source of disease transmission.<sup>7</sup>

Although TB has long been considered to have two clearly defined states (LTBI and active TB disease), recent evidence suggests the existence of a whole spectrum of disease ranging from LTBI to active TB.<sup>10</sup>

### 3. The current vaccine and future candidates

A vaccine against infant TB was introduced in 1921 by the French scientists Albert Calmette and Camille Guérin, which was accordingly named bacille Calmette–Guérin (BCG). This vaccine is now widely used to prevent severe forms of extrapulmonary TB such as miliary TB in infants.<sup>11,12</sup> However, BCG fails to prevent the most common form of disease – pulmonary TB – at any age.<sup>11,12</sup> BCG is an attenuated strain of *Mycobacterium bovis*, the etiological agent of TB in cattle. Although it is well tolerated, it can disseminate in immunocompromised individuals, notably HIV-infected persons, causing a disease termed BCGosis.<sup>7</sup> Accordingly, BCG is not recommended for HIV-exposed neonates in several countries.

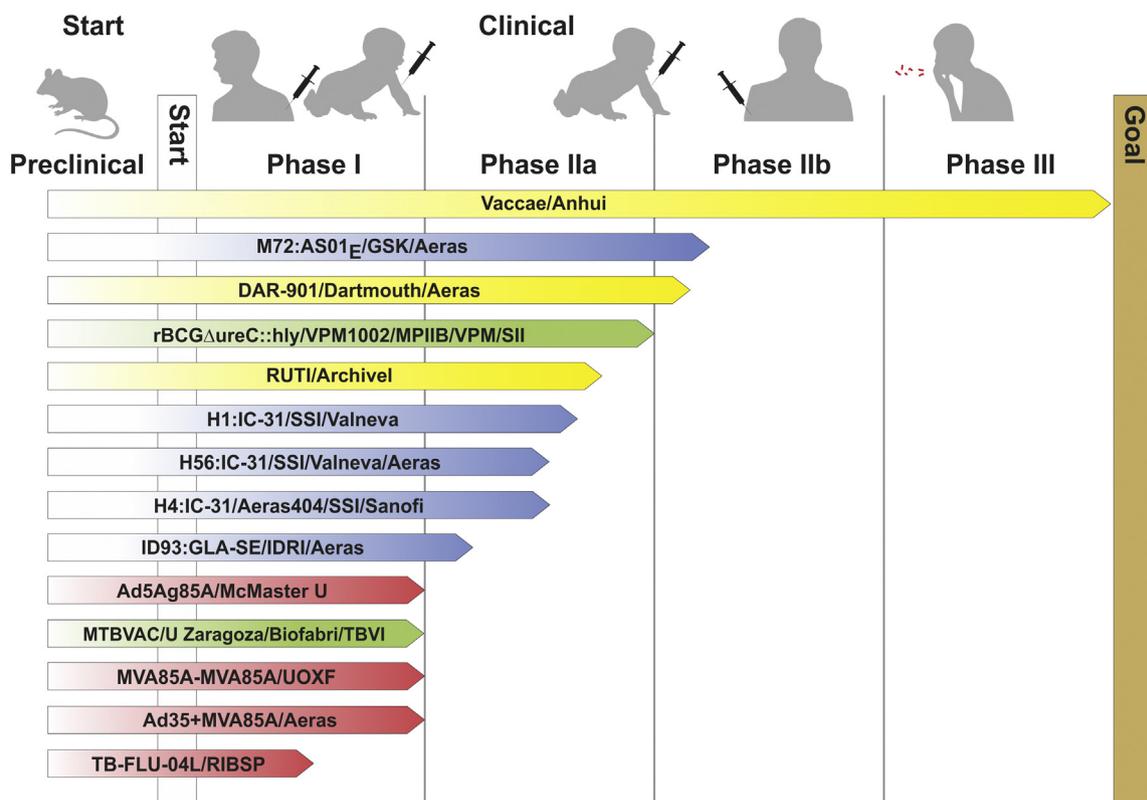
Because of these limitations of BCG, novel TB vaccine candidates have been developed, of which several have reached the clinical trial pipeline. These TB vaccine candidates can be categorized into the following: (1) preventive pre-exposure vaccines, which are administered prior to first exposure to *M. tuberculosis*, typically to

neonates; these are also known as priming vaccines; (2) preventive post-exposure vaccines, which are targeted at adolescents and adults with LTBI and prior BCG immunization; these are also known as boosting vaccines; (3) therapeutic vaccines, which are to be administered in adjunct with canonical TB drugs, notably to persons at higher risk of developing recurrent disease.

Figure 1 provides an overview of the major TB vaccine candidates in the clinical pipeline. Preventive vaccines come in three generic types: subunit vaccines, viable whole-cell vaccines, and inactivated whole-cell vaccines.

Subunit vaccines are composed of one or more antigens that are considered protective (Table 1). Often several antigens are combined to improve vaccine efficacy. Yet, protectivity is generally defined loosely and based on protection measured in one or more experimental animal models. To increase protectivity, antigens are either formulated with adjuvant or expressed by a recombinant viral vector (Tables 2 and 3). A number of current vectored vaccine candidates are based on recombinant adenovirus or vaccinia virus, many of which express the antigen 85A (Table 3).<sup>13–22</sup> Another viral vectored vaccine against TB harnesses a replication-deficient influenza virus expressing *M. tuberculosis* antigens. Some vectored vaccines are being developed not only as BCG boosters, but also as prime boost strategies comprising different viral vectors and/or *M. tuberculosis* antigen combinations.<sup>20</sup> The recombinant modified vaccinia Ankara (MVA) vector expressing antigen 85A (MVA85A) was one of the most advanced TB vaccines, but it failed to demonstrate protection in a preventive pre-exposure phase IIb trial.<sup>23</sup> Generally, these subunit vaccines are given as a boost after a BCG prime, with the aim of improving BCG-induced protection, i.e. to increase efficacy and prolong duration.

Since pre-exposure vaccines are mostly confronted with metabolically active *M. tuberculosis*, antigens for this type of



**Figure 1.** Pipeline of major TB vaccines in clinical trials. RUTI and Vaccae are therapeutic vaccines; all other vaccines are preventive. For further explanations of antigens, adjuvants, and genetic modifications of the vaccines, see Tables 1–5. (Abbreviations: GSK, Glaxo Smith Kline; MPIIB, Max Planck Institute for Infection Biology; VPM, Vakzine Projekt Management; SII, Serum Institute India; SSI, Statens Serum Institute; McMaster U, McMaster University; TBVI, Tuberculosis Vaccine Initiative; UOXF, University of Oxford; RIBSP, Research Institute for Biological Safety Problems.)

**Table 1**  
Antigens used in subunit TB vaccines

Vaccine	Antigen	Description
M72	Rv1196 Rv0125	PPE family member Peptidase
H1	ESAT-6	Prominent antigen of Mtb encoded in region of difference 1
H4	Ag85B TB10.4	Mycolyl transferase Prominent TB antigen
H56	H1 + Rv2660c	Dormancy antigen
ID93	Rv2608 Rv3619 Rv3620 Rv1813	PPE family member Virulence factor Virulence factor Dormancy antigen
Ad5Ag85A	Antigen 85A	Mycolyl transferase
MVA85A	Antigen 85A	Mycolyl transferase
Ad35	Antigen 85A TB10.4	Mycolyl transferase Prominent TB antigen
Ag85B	Antigen 85B	Mycolyl transferase
TB-FLU-04L	Antigen 85A	Mycolyl transferase

TB, tuberculosis; PPE, proline, poline, glutamate residues; Mtb, *Mycobacterium tuberculosis*.

**Table 2**  
Adjuvants used for TB vaccines

Vaccine	Name	Composition
H1, H4, H56	IC31	Cationic peptide/TLR9 agonist
H1	CAF01	Cationic liposome/immunomodulatory glycolipid
ID93	GLA-SE	Oil in water emulsion/TLR4 agonist
M72	AS01E	Liposome/TLR4 agonist

TB, tuberculosis; TLR, toll-like receptor.

**Table 3**  
Viral vectors used for TB vaccines

Name	Vector
MVA	Modified vaccinia Ankara virus
Ad5	Adenovirus 5
Ad35	Adenovirus 35
ChAd	Chimpanzee adenovirus
FLU	Replication-deficient influenza virus (H1N1)

TB, tuberculosis.

vaccine are chosen from those expressed during the stages of active replication and metabolism. These include the hybrid H1 and H4 vaccines.<sup>21,22</sup> In contrast, post-exposure vaccines are administered to persons with *M. tuberculosis* in a dormant stage and need to include antigens expressed during latent infection. Ideally, a combination of antigens in the form of fusion proteins is administered to cover both active and latent stages. These so-called multistage vaccines include H56, M72, and ID93 (Table 1).<sup>15,17,24</sup>

Viable vaccines were originally considered as replacement vaccines only and given instead of BCG. Hence, their first target population would be neonates (Table 4). The most advanced vaccine candidate is the recombinant BCG vaccine, VPM1002, which has shown a better safety and efficacy profile than standard BCG in preclinical models.<sup>25</sup> It has completed clinical phase I and phase II trials in adults and neonates and is currently being assessed in HIV-exposed neonates. It was shown that VPM1002

**Table 4**  
Viable TB vaccines

Name	Vaccine	Modification
VPM1002	BCG	Chromosomal integration of listeriolysin encoding gene (perforation of phagosomal membrane); deletion of urease gene (acidification of phagosome)
MTBVAC	<i>Mycobacterium tuberculosis</i>	Deletion of <i>PhoP</i> (transcription factor) and of <i>fadD26</i> (phthiocerol dimycocerosate synthesis)

TB, tuberculosis; BCG, bacille Calmette–Guérin.

**Table 5**  
Inactivated whole-cell mycobacterial vaccines

Name	Inactivated organism	Goal
DAR-901	Non-tuberculous Mycobacterium	Prevention
Mw	<i>Mycobacterium indicus pranii</i>	Therapy
Vaccae	<i>Mycobacterium vaccae</i>	Therapy
RUTI	<i>Mycobacterium tuberculosis</i>	Therapy

was well tolerated in adults with childhood BCG immunization, and protected against TB in an experimental post-exposure mouse model.<sup>26</sup> Hence, this vaccine is currently being developed also as a preventive post-exposure vaccine for adolescents and adults. Accordingly, a vaccination protocol has been submitted to prevent recurrence of TB in previously cured TB patients. Even after successful completion of drug treatment for active TB, over 10% of patients experience recurrence and develop TB for a second time, thus presenting a high risk group for vaccine trials. The second viable vaccine candidate that has successfully completed a phase I clinical trial is a double deletion mutant of *M. tuberculosis* termed MTBVAC.<sup>27</sup>

### 3.1. Inactivated whole-cell mycobacterial vaccines for the prevention of TB

Inactivated whole-cell mycobacterial vaccines used in a multiple-dose schedule were shown to be effective preventive vaccines in experimental models and in humans over 70 years ago, but were not developed further after single-dose BCG became the de facto preventive vaccine of choice.<sup>28,29</sup> More recently SRL172, an inactivated whole-cell vaccine derived from a non-tuberculous Mycobacterium, was shown to be safe, well-tolerated, and immunogenic in phase I and II trials. Efficacy was subsequently documented in a phase III randomized, controlled, booster trial among HIV-infected adults in Tanzania,<sup>30</sup> making it the only new TB vaccine in development for which efficacy in humans has been demonstrated. The SRL172 master cell bank was used to develop scalable manufacturing for the booster vaccine now known as DAR-901. Safety and tolerability were demonstrated in a phase I trial of the DAR-901 booster in adults primed with BCG in childhood. DAR-901 induced both cellular and humoral responses to mycobacterial antigens comparable to those observed with SRL172, but did not result in conversion of the interferon gamma release assay (IGRA). A randomized, controlled phase IIb trial is now underway for the prevention of infection with *M. tuberculosis* among adolescents in Tanzania.

A heat-inactivated whole-cell vaccine derived from *Mycobacterium vaccae* ('Vaccae') is also being studied for the prevention of TB after already being approved in China for the adjunctive treatment of TB.<sup>31</sup> The prevention trial was initiated in 2013 and was designed to enroll 10 000 subjects with a positive tuberculin skin test, but additional public information is not presently available (Table 5).

Viable live-attenuated vaccines and inactivated vaccines are termed whole-cell vaccines.<sup>32</sup> Since it is not known what antigens will induce protective immunity in humans, these polyantigenic

vaccines have a greater likelihood than subunit vaccines of including the critical epitopes required for protective efficacy.

### 3.2. Vaccines for the treatment of TB

Several vaccines are being developed to improve treatment outcomes in active TB (reduce mortality or relapse rates). This is a particular challenge in MDR-TB and extensively drug-resistant (XDR)-TB with extremely low cure rates of less than 50%, providing a greater opportunity for identifying the therapeutic efficacy of an investigational vaccine. The biological hypothesis is that additional stimulation with mycobacterial antigens may further enhance the immune response and improve bacterial killing. However, there are experimental data suggesting that certain types of excessive immune response might be detrimental in the immune control of TB in humans.<sup>33,34</sup>

*Mycobacterium indicus pranii* (Mw) is an inactivated non-tuberculous mycobacterial vaccine that has been studied as an adjunct to therapy for leprosy<sup>35</sup> (Table 5). A phase II study of Mw as an adjunct to therapy for TB has been completed and is being analyzed. A preclinical study of Mw administration by the aerosol route will examine immune responses in guinea pig and mouse models.

RUTI is a vaccine being developed to improve the outcomes in the treatment of both LTBI and TB disease and to reduce exposure to antibiotics (Table 5). Its mechanism of action is based on the induction of a polyantigenic cellular response to non-replicating bacilli contained in detoxified cell wall nano-fragments of *M. tuberculosis*. A phase I trial demonstrated safety and immunogenicity and a phase II trial showed safety and immunogenicity in both HIV-negative and HIV-positive volunteers with LTBI.<sup>36</sup> A phase IIa trial is planned to investigate the safety and immunogenicity of RUTI therapeutic immunization in patients with MDR-TB.

### 4. Concluding remarks

Until recently the development of new vaccines against TB was directed towards containing *M. tuberculosis* by prolonging LTBI and blocking active TB disease.<sup>4,5</sup> Although an effective vaccine to prevent TB disease would be an applaudable achievement, the sterile elimination or prevention of *M. tuberculosis* infection would ultimately be preferred. Although the biological mechanisms that might lead to sterile *M. tuberculosis* elimination or the prevention of *M. tuberculosis* infection are not known, recent evidence suggests that BCG immunization is capable of preventing infection at least in part.<sup>5,37</sup> As a result, some new vaccine candidates are now being tested to determine whether they have efficacy in preventing *M. tuberculosis* infection.<sup>38–40</sup> These prevention of infection trials employ IGRAs, which are based on a simple blood test to detect infection.

Because TB is a poverty-related disease, cost matters. Hence, it is critical to accelerate clinical trials and at the same time reduce their cost. One option towards this goal is stratification based on high-risk groups.<sup>41</sup> These include miners, who are at a markedly elevated risk of developing TB, and patients with successfully treated TB who have a high rate of recurrent TB, as described above. Alternatively, biomarkers that predict progression towards active TB would allow the stratification of study participants at greatest risk of developing active TB disease within the duration of a standard clinical trial.<sup>41,42</sup> Indeed, biosignatures that can predict progression to active TB are currently being developed. These signatures comprise changes in the gene expression of defined markers at high sensitivity so that they most likely diagnose subclinical incipient TB.<sup>43</sup>

Even though the development of an improved vaccine against TB presents major challenges on several fronts, it is a goal worth

pursuing. After all, an effective vaccine that prevents pulmonary TB could make a major contribution to the goal of reducing TB morbidity and mortality by 90% and 95%, respectively, by the year 2035.

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**Conflict of interest:** SHEK is co-inventor of the TB vaccine VPM1002. CFvR is the sponsor for DAR-901.

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