Vaccine research and development: tuberculosis as a global health threat

MOHAMMED MAIKUDI USMAN1,2, SALMAH ISMAIL1, TEOW CHONG TEOH1
1Institute of Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur, Malaysia
2Department of Biotechnology, School of Pure and Applied Sciences, Modibbo Adama University of Technology, Yola, Nigeria

Abstract
One of the aims of the World Health Organisation (WHO) Millennium Development Goals (MDG) is to reduce the number of cases of tuberculosis (TB) infection by the year 2015. However, 9 million new cases were reported in 2013, with an estimated 480,000 new cases of multi-drug resistant tuberculosis (MDR-TB) globally. Bacille Calmette-Guérin (BCG) is the most available and currently used candidate vaccine against tuberculosis; it prevents childhood TB, but its effectiveness against pulmonary TB in adults and adolescents is disputed. To achieve the goal of the WHO MDG, the need for a new improved vaccine is of primary importance. This review highlights several articles that have reported vaccine development. There are about 16 TB vaccines in different phases of clinical trials at the time of writing, which include recombinant peptide/protein, live-attenuated and recombinant live-attenuated, protein/adjuvant, viral-vectorized, and immunotherapeutic vaccine. Further studies in reverse vaccinology and massive campaigns on vaccination are needed in order to achieve the target for TB eradication by 2050.

Key words: tuberculosis, Mycobacterium tuberculosis, vaccine, clinical trials, BCG vaccine.

Introduction
Tuberculosis is a disease caused by an infectious agent called Mycobacterium tuberculosis (Mtb). Despite reports of declining TB cases in recent years, it also remains a leading deadly infectious disease globally, second only to HIV [1]. In 2012, an estimated 8.7 million new infections in the year 2011, out of which 1.4 million people died of tuberculosis, was published by the WHO. The report also showed highest burden in Asia and Africa, while China and India accounted for almost 40% of the total TB cases globally [2]. Although there are great achievements globally regarding the threat of TB, in Sub-Saharan Africa tuberculosis remains a major cause of morbidity and mortality [3]. Some factors are responsible for the growth of TB in Africa, the HIV epidemic being the most important one [4, 5]. An increase in the incidence of MDR-TB is another factor that threatens the efforts towards TB control throughout the world [6]. At latency stage of TB infection the containment of infection is achieved by the host, indicated by survival of mycobacteria in relatively stable numbers [7]. Patients with latent tuberculosis typically do not feel ill and are not infectious [8].

World Health Organisation (WHO) Millennium Development Goals (MDG) are a set of targets aimed at expressing key points of human development [9]. The goals of MDG were generated at the United Nations Millennium Summit in September 2000 [10]. In addition to a target associated with MDG and endorsed by the Stop TB partnership for reducing its prevalence and death as a result by 50%, the target was also aimed at eradication of TB as a worldwide health threat by the year 2050 [11]. Bacille Calmette-Guérin is a vaccine against tuberculosis, developed in 1921 by Albert Calmette and Camille Guérin, caused by attenuation of the Mycobacterium bovis strain [12]. The novel tuberculosis vaccines need to be better in efficacy and safety, or both, than BCG. Therefore, the urgent need for alternative anti-tuberculosis vaccines is of paramount importance. Bacille Calmette-Guérin immunisation is still in used because of the protection it gives against the infant form of tuberculosis [13, 14]. Administration of diverse booster vaccines later to BCG prime strengthens the protection induced by BCG. Also, other vaccines serve as replacements to Bacille Calmette-Guérin for the generation of a superior immune response up-front. In both cases, the aim of vaccination is the generation of long lasting protection against the most prevalent form of pulmonary tuberculosis in all age groups [13, 14].

Complete-genome sequencing, and comparative and system biology lead to new knowledge into the origin and evolution of Mtb and the molecular principle of its pathogenicity. These have vital implications about our perspective of the new vaccine development [15]. Progress in the fields of molecular biology, genomics, proteomics, and
transcriptomics contributed tremendously in the search for a new and enhanced tuberculosis vaccines [16]. Some TB vaccines have entered different stages of clinical trials in recent years. An attempt to review recent research and development of TB vaccines is given below.

**Disease burden of tuberculosis**

Tuberculosis is likely to remain in a position of a major public health problem in the coming decades because of its large global load [17]. According to a 2014 (WHO) report on TB, an estimated figure of 1.5 million deaths were recorded in 2013 (0.4 million were HIV-infected people and 1.1 million were HIV-negative) [18]. *Mtb* and HIV infections act in a collaborative manner [19]. The role of CD4 T cells is of paramount importance in *Mtb* infection control; in HIV and TB co-infection there is continuous loss of CD4 T cells, leading to an advancement to active form of TB [20]. Most cases of TB infection and death prevail among men, but the TB burden is also high among women. In 2012, the South-East region and Western Pacific region accounted for about 58% of the world’s TB cases. Also, the African region had nearly one quarter of the world’s cases; India and China recorded the highest number of cases with 29% and 12% of the global total respectively [21]. In Sub-Saharan Africa, TB has increased markedly over the past two decades and there was a reported doubling of annual incidence from 173.6 to 351.7 per 100,000 population between 1990 and 2007 [22]. MDR-TB can be defined as TB found to be resistant to both rifampicin and isoniazid drugs with or without resistance to other anti-TB drugs [23]. MDR-TB is on the increase; extensively drug-resistant tuberculosis (XDR-TB) and totally drug-resistant tuberculosis have already been reported. These obstacles impose rising threats to tuberculosis control [2, 24, 25].

**Bacteriology**

In 1882, Robert Koch discovered *Mtb*, the intracellular pathogen [26]. Mycobacteria are non-motile, aerobic bacteria that have characteristics of acid fastness (Ziehl-Neelsen staining) because of mycolic acid enriched cell wall [27]. *Mtb* are rod-shaped bacilli [28]. The presence of a wide array of complex lipids and lipoglycans on the cell surface of *Mtb* make it unique among the bacterial pathogens [29]. The hydrophobic nature and the complexity of the cell wall, which is composed of the following: arabinogalactan, peptidoglycan, fatty acid (mycolic acids), and glycolipids layered on top of the plasma membrane critically cause *Mtb* to deceive the immune system of the host [30].

**Immune response to tuberculosis infection**

Tuberculosis infection is airborne, its cycle starts when a host inhales infectious airborne particles, usually of less than 5 μm diameter, containing infectious pathogen [31]. Alveolar macrophages engulf mycobacteria when they enter the lung, where *Mtb* reproduces, and inhibit macrophage killing mechanisms. Despite the inhibitory influence of *Mtb*, infected macrophages secrete chemokines and cytokines, leading to the recruitment and activation of many immune cell populations to the lung [32]. The innate immunity activation depends on recognition of *Mtb* components of the cell wall as mycolic acid, mannan, and peptidoglycans through toll-like receptors [33]. Mycobacterial antigen recognition, macrophages, and dendritic cell (DC) activation as well as other cells involved in innate immunity need toll-like receptors [34]. The bacilli is engulfed by macrophage through phagocytic receptors, of which the complement and mannose receptors play an important role [35]. The immune cells enclose the pathogenic bacteria in the first stage of tuberculosis infection, intracellular multiplication occurs, and the bacteria-overloaded cells may traverse the alveolar barrier, affecting other tissues and organs [36]. Survival of *Mtb* in macrophages is achieved by inhibiting acidification of the phagosomal complement and also by inhibition of the fusion of the phagosome with lysosomes [35]. The cell-mediated immunity is effectively involved in regulating *Mtb* limitation in granulomatous lesions of the lungs, usually without eliminating the bacteria that prevail in the latent stage [37]. Besides macrophages, it is understood that DCs also play a role as an important intracellular niche for *Mtb* [38]. Dendritic cells are key regulators of adaptive immunity and are potent antigen presenting cells [39]. Dendritic cells have the unique ability to migrate to draining lymph nodes from the site of infection and afterwards recruit T cells of infection where they effectively activate the acquired immune response [40].

Protective immunity against *Mtb* depends critically on T lymphocytes, due to its intracellular lifestyle [41]. In tuberculosis, cellular responses are the mediators of both pathogenesis and protection, which involves primarily interactions of phagocytes of macrophage lineage and lymphocytes [42]. Production of cytokines like interferon-γ (INF-γ) and tumour necrosis factor (TNF) establish protective immune responses against *Mycobacterium tuberculosis* infection; both cytokines activate macrophage toward *Mtb* control [43]. The cytokines play an essential role in controlling mycobacterium growth by expression of reactive nitrogen and oxygen [44]. In the presence of oxygen, an enzyme associated with macrophage functions in tuberculosis, called nitric oxide synthase-2, catalyses the metabolism of L-arginine into L-citrulline and nitric oxide, which takes part in killing the intracellular pathogen [45]. The *Mtb* immune response consists of great number of different cell kinds, including T cells, neutrophils, B-cells, and natural killer cells, and the roles played by CD4 T helper type 1 cells are the best understood [46]. Other interleukin (IL) producing T lymphocytes such as CD8 T lymphocyte and CD4 cells are likely take part in protective immunity.
After infection, *Mtb* stimulates both CD4 and CD8 T cells and other immune cells; secretion of INF-γ dominates a strong type 1 immune response [48]. During adaptive immune response to *Mtb* infection, CD4 cells are the primary source of INF-γ, which are required for the survival of the host during both phases of acute and chronic infection [49]. The need for INF-γ in immune protection of tuberculosis is well established both in animal models and in humans [50]. Interferon γ is the key cytokine in humans and also in mice, the role of which is to activate the bactericidal actions in the host cell, macrophage [51]. It has been reported that individuals with genetic deficiency in the INF-γ receptor are more likely to be infected with mycobacterial [52]. Several studies have revealed an increased susceptibility to mycobacterial diseases in INF-γ-deficient mice and also in humans having INF-γ receptor abnormalities [38]. INF-γ expresses protein peptides cathelicidin and defensin-β 2, which are delivered to *Mtb* phagosomes via vitamin D-dependent pathway [53], defensins, and a single cathelicidin, LL-37, which are major groups of host defensive peptides in humans. Susceptibility to infectious diseases including tuberculosis has been reported due to alteration in the synthesis of these molecules [53-55].

**Vaccines**

Currently, most tuberculosis vaccines under different stages of clinical trials are focused on either replacement of BCG or as a booster following vaccination with prime BCG [56]. Subsequently to the failure of MVA85A in the last two years, there has been no new, prominent TB vaccine entering clinical testing. There are 16 TB candidate vaccines in clinical testing, classified into priming vaccines, prime boosters, and immunotherapeutic vaccines [57]. Most of these candidates are subunit vaccines; selected antigens of *Mtb* are expressed using recombinant viral vectors or are administered in combination as protein/adjuvant [58]. The developmental pipeline of new TB vaccines is shown in Table 1.

**Bacillus Calmette-Guérin**

BCG vaccine was first developed from a virulent strain [68]. Attenuation of the original BCG strain of *M. bovis* led to the establishment of a BCG vaccine resulting from subcultures in a media aimed at preserving its immuno-genicity [69]. BCG play a protective role against *Mtb* because it induces CD4 (T helper type 1) and CD8

| TB vaccine | Vaccine Type/Strategy | Phase | Sponsors | Reference |
|------------|-----------------------|-------|----------|-----------|
| MTBVAC | Live-attenuated vaccine/priming vaccine | Phase I | University of Zaragoza, Biofabri, The Tuberculosis Vaccine Initiative (TBVI) | [59] |
| VPM1002 | Recombinant live/prime | Phase I | Max Planck, Vakzine Projekt management GmbH, The Tuberculosis Vaccine Initiative (TBVI) | [60] |
| Ad5 Ag85A | Viral-vectored vaccine | Phase I | McMaster University, Supported by Tianjin Cansino Biotech. Inc | [61] |
| M72 + AS0 | Protein and adjuvant/prime booster | Phase IIB | GlaxoSmithKline, Aeras | [62] |
| MVA85A | Attenuated Mycobacterium tuberculosis strain | Phase I | The Tuberculosis Vaccine Initiative (TBVI), Zaragoza, Biofabri | [63] |
| Crucell Ad35+ MVA85A | Viral vector/Prime booster | Phase I | Crucell, Oxford University, Aeras | [62] |
| Hybrid 1 + IC31 | Recombinant protein/Prime-boost | Phase I | Statens Serum Institut/Tuberculosis Vaccine Initiative/Intercell | [64] |
| Hybrid 4 + IC31 | Recombinant and adjuvant | Phase I | Statens Serum Institut (SSI), Tuberculosis Vaccine Initiative (TBVI) | [65] |
| Hybrid 56 + IC31 | Adjuvanted subunit/Prime-Boost | Phase II | Statens Serum Institut | [66] |
| ChAdOx1 85A + MVA85A | Viral vector/Prime-boost | Phase I | Oxford University | [62] |
| ID93 + GLA-SE | Adjuvanted subunit/Prime-Boost | Phase I | Infectious Diseases Research Institute | [66] |
| DAR-901 | Mycobacterium-whole cell or Extract | Phase I | Darmouth, Aeras | [63] |
| TB/FLU-041 | Viral vector/prime booster | Phase I | Research Institute for Biological Safety Problems | [63] |
| Mycobacterium vaccae | Therapeutic/Boost, Post infection | Phase III | NIH, Aeras, Immodulon | [60] |
| RUTI | Immunotherapeutic/Fragmented MTB | Phase II | Archivel Farma | [67] |
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actogenic vaccines, with reactogenicity depending on vari-
vaccine, BCG vaccines are considered among the most re-
development in young children [82]. Despite being a safe
mobilization tuberculosis meningitis with a 50% reduced risk of disease
1974, and it provides protection against tuberculosis and
1962 and 1975. Their results
reviewed that the rates of TB in unvaccinated and vacci-
mansions are 3.3 and 1.3 per 1000 person-years,
over the 10 to 20 years with negative tuberculin skin test and vaccination
Th1 response I sand required for protection. Marchant et al.
demonstrated that Th1 memory response is induced at
BCG happens to induce Th1 response and fails to induce
Th17 response in the lung. The ability of BCG to induce
Th1 but not Th17 mostly leads to inferior efficiency of BCG vaccine [75]. Interferon γ is produced as a result of
Th1 response I sand required for protection. Marchant et al.
demonstrated that Th1 memory response is induced at
BCG immunisation in a similar way when admin-
istered later in life [76]. An improved understanding
of the reasons behind variability of BCG efficacy to such
a great extent is important to assess new vaccines against
tuberculosis, which are undergoing clinical trial [77]. The
different levels of protection could be due to variations in
BCG strain from different locations [77]. Immune response in
individuals might be influenced by exposure to other
environmental mycobacteria (EM), which, as a result, in-
terfere with the effectiveness of BCG. Efficacy of BCG
> 70% was reported in populations from nations situated far
away from the equator having no or less prevalence of
EM [78]. The effectiveness of BCG was found to be sig-
ificantly lower in individuals of countries located near to
the equator [79]. It has been reported that BCG has a pro-
tection time ranging from 10 to 20 years in the majority
of cases [80]. Recently, Nguipdop-Djoma Patrick et al.
demonstrated that BCG vaccination exhibited long-last-
ing protective ability against TB in individuals aged 12-50
years with negative tuberculin skin test and vaccination
carried out between the years 1962 and 1975. Their results
revealed that the rates of TB in unvaccinated and vacci-
nated participants are 3.3 and 1.3 per 1000 person-years,
respectively [81]. BCG has been used in neonates since
1974, and it provides protection against tuberculosis and
tuberculosis meningitis with a 50% reduced risk of disease
development in young children [82]. Despite being a safe
vaccine, BCG vaccines are considered among the most re-
actogenic vaccines, with reactogenicity depending on vari-
ation with different strains and the number of viable bacilli
[83]. Immunocompromised children infected with HIV or
immunosuppressed individuals are especially vulnerable to
complications of BCG vaccine [84]. A study was carried
out on 349 BCG-immunised patients having severe com-
bined immunodeficiency in 17 countries; the results indi-
cated a high rate of complications of BCG vaccine [79].

Live attenuated TB vaccines

Improvement of the vaccine depends on strengthening
the immunogenicity and persistence of a genetically mod-
ified recombinant strain of BCG (rBCG). Hence, a geneti-
cally manipulated rBCG could be more efficient compared to
the parental BCG due to introduction of some parts of
DNA (genes) lost during in vitro attenuation [85]. The loss
of the RD1 region is the genetic principal behind BCG
attenuation – the region encoding the machinery needed to
synthesise and export the major T-cell antigen/virulence
factor ESAT-6/CFP-10 [86]. The first recombinant BCG
was generated by Horwitz et al. [87] and Horwitz and
Harth [88]. rBCG3 overexpressed antigen Ag85b, which
induced protection against TB significantly in animals.
Compared with parental BCG, rBCG30 significantly in-
creased Ag85b-specific T cells that inhibit intracellular
mycobacteria [89].

VPM1002 is the second recombinant BCG vaccine
candidate [79], formed because of two variations of live
Mtb. The gene encoding for Listeriolysin (Hly) from Listeria monocytogenes incorporated into the genome of BCG
[59], rBCGUre:Chly*, conferred high protection against
Mycobacterium tuberculosis challenge through aerosol.
This improved protection was because of efficient perfor-
 ration of the phagocyte phagosomal membrane by Listeri-
olysin (Hly) [90]. rBCGUre:Chly* is now in the phase
of clinical trial due to its enhanced protection against tu-
berculosis [91]. Recombinant rBCGUre:Chly* constructs
movement from endosomes to cytosol due to the activity of
Listeriolysin with concomitant deletion of the urease
gene. Loss of the urease gene leads to improved mycobac-
terial antigen processing via MHC I pathway as well as
improved CD8 cytotoxic T cell activity [92].

BCG::ESAT-28A/L29S improved BCG strain with
modifications at amino acid residues. Leu28-Leu29 of the
ESAT molecule showed strong attenuation in mice and
high protective efficiency both in mouse and guinea-pig
vaccination-infection models [93]. Chun Wang et al.,
2012 [94] constructed three recombinant BCG strains that
overexpressed immunodominant antigens of Mycobacte-
rium tuberculosis, Ag85B (rBCG::85B) and Ag85A (rBC-
G::85A). Both recombinants (rBCG::AB) provided stronger
and longer-lasting protection compared to the BCG
containing vector without insert pMV261(rBCG::261)
using mice.

In January 2013 MTBVAC entered a phase I clinical
trial, and it is the live-attenuated Mycobacterium tubercu-
losis vaccine that entered the phase I trial. It is a derivative
of attenuated strain SO2 obtained by insertion of a kanamycin-resistance cassette in the phoP (phoP is a transcription regulator) gene of \textit{Mtb} transcription. Mutation of \textit{phoP} causes a lack of expression of several genes, including ESAT6, a virulence factor [59]. In preclinical studies it was found that MTBVAC showed the same safety and biodistribution profiles as BCG and indicated superior protection [86]. The satisfactory safety of MTBVAC could be explained based on the following factors: lack of front-line lipids, loss of ESAT-6 expression, and down-expression of the PhoP regulon, essentially for pathogenicity and virulence of \textit{Mycobacterium tuberculosis} [86]. Highly attenuated MTBVAC could be a potential vaccine for populations with high-risk immunosuppression, due to inactivation of an additional gene-generated repeated protein (Erp) [63].

**Subunit and viral vector-based vaccines**

Live-based vaccines are not products chosen by most manufacturers because of safety considerations, especially in immunosuppressed individuals, and technical challenges regarding reproducibility [16]. The main reasons for developing a recombinant protein-based vaccine are as follows: they develop less reactogenicity and are considered more potent, safer, and better characterised vaccines [95]. \textit{Mtb} secretes proteins during \textit{in vitro} growth. One of the possible ways of improvement towards a tuberculosis vaccine would involve use of such secreted proteins. Some of these proteins are immunogenic; these proteins or their agreeing genes could serve as a major part of either a DNA-based vaccine or a subunit vaccine. Identification of antigens secreted in the culture fluid is important for establishing protective immune response against \textit{TB} [85]. Several studies carried out have shown promising results for DNA vaccination against tuberculosis. DNA vaccines express different \textit{Mtb} antigens; these include: Ag85A, Ag85B, ESAT-6, MTP-64, PstS-3, and 65kDa heat-shock protein. These proteins were all found to be effective in inhibiting the growth of \textit{Mtb}-infected mice [96]. We produced a potential peptide/protein vaccine (Myl272-3) from a clone contracted by shotgun cloning in the University of Malaya Molecular Bacteriology and Toxicology laboratory. The protein has an approximate molecular mass of 10.58kDa, which conformed to the computed MW by EXPASY MW bioinformatics tool. Both protein blast and MALTI-TOF analysis indicated homology with phenolpthiocerol synthase I PpSa of \textit{Mtb}. \textit{In silico} analysis of the protein also indicated non-allergenicity and antigenicity of the query protein sequence, which serves as a good guide for the design of a vaccine against \textit{TB}.

Wu Li \textit{et al.} reported a recombinant adenovirus (Ad5-CEAB) expressing \textit{Mtb} antigens Ag85A, Ag85B, CFP10, and ESAT6 proteins combined in a mixture [97]. Ad5-CEAB resulted in a strong antigen-specific immune response as well as heightened humoral responses with a dramatically antigen-specific serum immunoglobulin (IgG).

**Viral vector**

Ad5Ag85A is a viral vectored adenovirus serotype 5 vector vaccine expressing Ag85A developed by McMaster University and supported by Tianjin CanSino Biotechnology Inc. The vaccine went through Phase I trial in 24 Canadian adults: 12 from BCG naïve and 12 from previously BCG-vaccinated, healthy adults. No vaccine-related serious adverse effects were recorded. Ad5Ag85A had immunogenicity in both groups with stimulation of polyfunctional T-cell responses, but found more effectively boosted CD4 and CD8 T-cell immunity in a group of previously-immunised subjects compared to a BCG-naïve group, which is reassuring for its further clinical development serving as a booster vaccine candidate after BCG priming [61].

A phase I trial involving MVA85A combined with Crucell Ad35 (Crucell Ad35 + MVA85A) was carried out among 40 adult participants at Oxford University [62]. Research Institute for Safety Problems and the Research Institute on Influenza, in Russia, developed a recombinant influenza vaccine called TB/FLU-04L, which is composed of influenza virus strain \textit{A/Puerto Rico/8/34 H1N1} and \textit{Mtb} antigens Ag85A and ESA6. A phase IIa trial is being planned for this vaccine candidate, and a phase I trial was completed [63]. ChAdOx1.85A is another adenovirus vaccine that expresses \textit{Mtb} antigen Ag85A; a phase I clinical study is currently testing the safety of ChAdOx1.85A vaccination along with infusion with MVA85A in adults vaccinated with BCG in the United Kingdom [62].

**Subunit adjuvant**

Adjuvants includes compounds and molecules/molecular complexes capable of boosting the potency and effective duration of specific immunological response to antigens [98]. The major hindrance in developing vaccines against bacteria has been attributed to a lack of adjuvant that adequately stimulates cell-mediated immunity [99]. It is therefore essential to administer subunit vaccines with an adjuvant to enhance immune responses to subunit vaccines. The adjuvants approved for human use include Aluminum salts, AS03/04 and MF59. They are primarily promoters of a humoral or Th2 rather than Th1 response [99].

Hybrid 1 + IC31 is a subunit adjuvant vaccine developed by the Statens Serum Institute, TBV1, and Intacell. It is a hybrid of ESAT6 and Ag85B antigen with IC31, the components of the adjuvant system are oligodeoxynucleotide ODN1a and the cationic protein polyamino acid KLK [63]. Reither \textit{et al.} [100] evaluated vaccine candidate H1/IC31in 48 patients infected with HIV, and the results showed durable Th1 immune responses.

Hybrid 4 + IC31 vaccine has a fusion of \textit{Mtb} antigens (Ag85B and TB10.4) with adjuvant IC31, owned by Valneva. In 2014, a three-arm, phase IIa study was announced by Aeras in order to determine the safety and immunogenicity of H4+IC31 and BCG revaccination in approximately 1000 BCG-immunised, non-HIV adolescent
The main targets of therapeutic vaccine design are to prevent latent infection or to reduce the need of chemotherapy [104]. RUTI is one of the therapeutic vaccines made of detoxified, fragmented Mtb cells delivered in liposomes. A previous study revealed that RUTI showed efficacy in controlling latent form of TB infection in mice and guinea pigs, inducing a combined Th1/Th2/Th3 polyantigenic response after a short period of chemotherapy [105]. Preclinical studies with mice revealed that IDR93 vaccine is protective almost at BCG levels, and in guinea pigs a combination of ID93 and BCG reduced the mortality rate [47].

**Immunotherapeutic vaccines**

The Infectious Disease Research Institute came up with the ID93 vaccine, which is the most recent tuberculosis vaccine entering clinical trials, and it was designed to target both forms of active and latent tuberculosis [47]. IDR93 is a protein/adjuvant vaccine that combined four novel sets of antigens including Rv2608, Rv3619, Rv3620, and Rv1813 in addition to the adjuvant (synthetic MPL formulated in a glucopyranosyl lipid stable emulsion). Preclinical studies with mice revealed that IDR93 vaccine is protective almost at BCG levels, and in guinea pigs a combination of ID93 and BCG reduced the mortality rate [47].

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