

Review

**BCG Vaccine and New Tuberculosis Vaccines Against *Mycobacterium tuberculosis*:
A review**

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Abstract: *Mycobacterium tuberculosis* (*M. tuberculosis*) causes tuberculosis (TB) which is a serious infectious disease. Bacteria are spread from person to person through tiny droplets released into the air via sneezing and coughing. Despite global efforts to control TB, the disease is the second most common cause of death after Acquired Immune Deficiency Syndrome (AIDS). Currently, Bacillus Calmette–Guérin (BCG) vaccine is used to prevent tuberculous meningitis and miliary disease, particularly in young children, but its protective efficacy is variable in adults. Therefore, there is an urgent need for the development of alternative TB vaccines. Recently, new TB vaccine development efforts have been advanced in different clinical studies. Most of these vaccines are live-attenuated or recombinant mycobacterium, live viral vector-based, and protein/adjuvant vaccines. This review explains the recapitulation of the current status of new TB vaccines updated with scientific literature references.

Keywords: *Mycobacterium tuberculosis*; new vaccines; clinical studies; BCG; review

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1. Introduction

Tuberculosis is an infectious and disseminated granulomatous disease that is caused by a member of a mycobacteria group called *Mycobacterium tuberculosis* complex (MTBC). When Dr. Robert Koch identified and described the *M. tuberculosis* bacilli, Albert Calmette and Camille Guérin simultaneously discovered Bacillus Calmette–Guérin (BCG) vaccine (Daniel et al., 2006). Although TB is curable with medications and is preventable with the BCG vaccine, it is one of the most dangerous infectious diseases worldwide. Even if the incidence rates of TB considerably dropped recently, TB has been still accepted as a global problem by the World Health Organization (WHO, Global Tuberculosis Report, 2018).

According to WHO 2018 report, it is estimated that TB disease developed in 10 million people (range 9.0–11.1 million) in 2017. Asia region has been most severely affected by TB. Over 95% of TB deaths occur in low or middle-income countries as India, Indonesia, China, the Philippines, and Pakistan. Approximately, 9% of TB cases were among people with Human Immunodeficiency Virus (HIV). In 2017, there are 1.3 million deaths among HIV-negative people, whereas 300,000 deaths were seen among HIV-positive people due to TB disease. On the other hand, there are 1.7 billion people with latent TB infection who have a risk of developing active TB infection along their lifetime (WHO, Global Tuberculosis Report, 2018).

In 2015, WHO declared the End TB Strategy Plan to end TB by 2035. The strategy aimed to reduce TB deaths by 95% and TB incidence by 90% compared with 2015 (WHO, The End TB Strategy 2015). If effective TB vaccines and new medications to decrease TB disease are not developed, it may not be able to achieve this goal (Schrager et al., 2019).

Despite the use of *Mycobacterium bovis* BCG vaccine and global efforts to prevent disease TB continue to remain a serious disease around the world. The more effective new TB vaccines are required to protect people from all kinds of TB forms (Montagnani et al., 2014). Moreover, these persons may have another disease or infection as HIV infection or diabetes mellitus. Research on the novel TB vaccines to prevent TB started over the past decade. Recent research and developments in the new TB vaccine will be summarized in this review.

2. *Bacillus Calmette-Guérin* (BCG) Vaccine

BCG vaccine containing an attenuated strain of *M. bovis* is the only licensed vaccine that has been used to prevent TB for more than 90 years. (WHO Global Tuberculosis Report 2012; Zwerling et al., 2011). At present, WHO recommends a single dose of BCG for neonatal inoculation in the countries with high TB prevalence and incidence (WHO, Biologicals, BCG vaccine). First tested in the year 1921, BCG had highly variable protective efficacy, ranging from 0–80% in the adult population in different settings. BCG vaccination has been indicated to be effective at preventing from disseminated TB disease and meningial TB in children, but it fails to exhibit adequate protection against pulmonary TB, particularly among young adults in high-endemic regions. (Hussey et al., 2007).

3. Development in TB Vaccines

Currently, there are numerous TB candidate vaccines at different stages in clinical trials. These candidate vaccines may be classified into conventional vaccines, prophylactic vaccines, booster vaccines, therapeutic/post-exposure vaccines and vaccines to prevent reinfection (Table 1) (Gopal et al., 2013; Soundarya et al., 2019; Usman et al., 2017). Some of these candidates are focused to replace BCG or as a boost BCG induced immunity. There are advantages and challenges of each vaccine type (Schito et al., 2015). The conventional TB vaccine BCG which is an attenuated *M. bovis* strain is an only licensed vaccine for TB currently. Also, recombinant BCG (rBCG) developed by using recombinant BCG technology is included in this category. The rBCG has been constructed either by

adding certain genes to BCG or removing specific genes from the natural mycobacterial genome. For that purpose, RDI and RD2 loci that are known as immunodominant *M. tuberculosis*-specific antigens are integrated into BCG. In the rBCG30 is over-expressed the gene Ag85B. Compared to BCG, rBCG30 secretes more over-expressing Ag85B and induces the greater Th1 immune response that inhibits intracellular mycobacteria.

Priming and boosting vaccines should be administered before exposure to TB bacilli. While a priming vaccine is normally applied to newborn infants, a boosting vaccine which is targeted at adolescents and adults are administered to enhance the immune response. In other words, to generate bigger and long-lasting immunity booster vaccines are given. Therapeutic or post-exposure vaccines could be given to already infected individuals or those potentially exposed during TB treatment (Kaufmann et al., 2017; Soundarya et al., 2019). The essential purpose of a therapeutic vaccine is to potentiate chemotherapy and to increase the rate of bacterial clearance (Soundarya et al., 2019). Vaccination for preventing reinfection is recommended during or after the treatment for TB (Orme et al., 2015).

Table 1. Application of different vaccine types against TB

Different Vaccine Types and Application	
Conventional	BCG, rBCG
Prophylactic	VPM 1002, MTBVAC
Booster	Ad5 Ag85A, ChAdOx1 MTB85A
Therapeutic	RUTI, ID93/GLA-SE
Prevent Reinfection	BCG Revaccination, H1/H56:IC31 (in trials)

BCG, Bacillus Calmette-Guérin; rBCG, recombinant BCG.

4. TB Vaccine Candidates in Clinical Phase

At present, many new TB vaccines have been developed in clinical studies. The current state of new TB vaccine candidates is shown in Table 2 and Figure 1.

Figure 1. Clinical phases of current TB vaccine candidates that adapted from AERAS website. Available www.aeras.org/pages/global-portfolio (accessed date: 21 October 2019). BCG, Bacillus Calmette-Guérin; MIP, *Mycobacterium indicus pranii*; TB, tuberculosis.

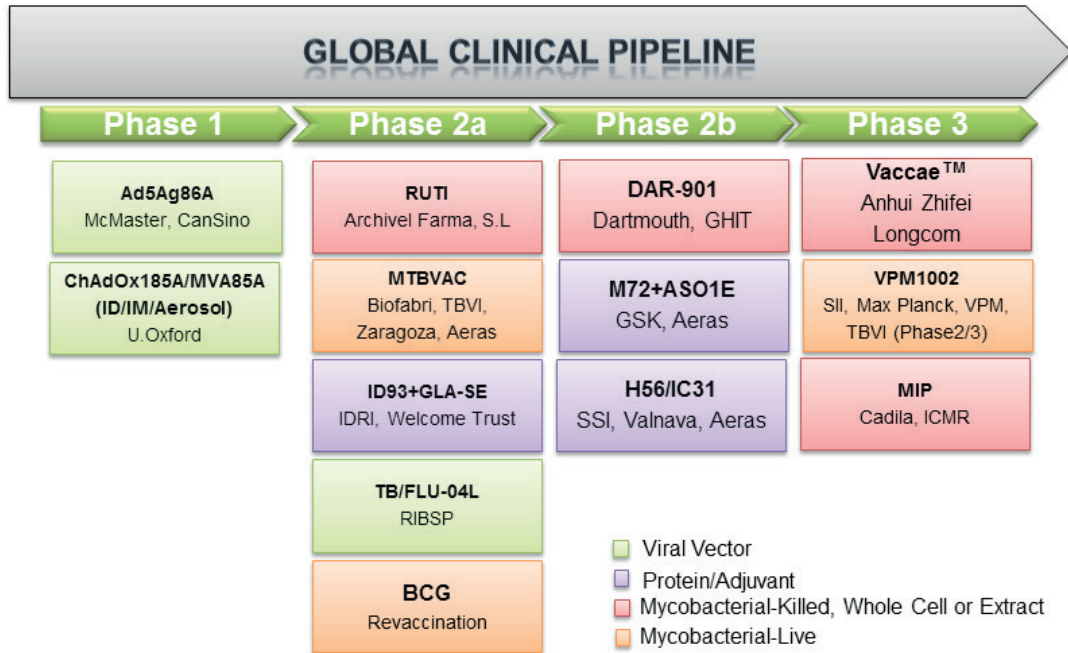


Table 2. Current novel TB vaccines in clinical trials against TB

TB vaccine name	Vaccine Type/Strategy	Clinical trial status	Reference
Ad5 Ag85A	Viral-vectored vaccine/Prime boost	Phase I	Tang et al. 2016
ChAdOx1 85A + MVA85A	Viral vector/Prime boost	Phase I	Tameris et al. 2013
RUTI	Mycobacterial-whole cell or Extract / Fragmented MTB	Phase IIa	Vilaplana et al. 2010
MTBVAC	Live-attenuated vaccine/Priming vaccine	Phase IIa	Clark et al. 2017
ID93 + GLA-SE	Adjuvanted subunit/Prime-Boost	Phase IIa	Penn-Nicholson et al. 2018
TB/FLU-04L	Viral vector/Prime booster	Phase IIa	Safrit et al. 2018
DAR-901	Mycobacterial-whole cell or Extract/ Prime booster	Phase IIb	Panga et al. 2016
M72F:AS01	Protein and adjuvant/prime boost	Phase IIb	Van Der Meeren et al. 2018
Hybrid 56 + IC31	Adjuvanted subunit/Prime-Boost	Phase IIb	Luabeya et al. 2015
<i>Mycobacterium vaccae</i>	Mycobacterial-whole cell or Extract / Boost, Post infection, immunotherapy	Phase III	Hawkridge et al. 2011
VPM1002	Recombinant live/Priming vaccine	Phase III	Nieuwenhuizen et al. 2017
MIP (<i>Mycobacterium indicus pranii</i>)	Heat-killed whole <i>Mycobacterium indicus pranii</i>	Phase III	Kamal et al. 2016

4.1. Phase-I TB Vaccine

Ad5Ag85A

The Ad5Ag85A vaccine is primed with non-replicating viral adenovirus serotype 5 (Ad5) vector expressing *M. tuberculosis* antigen 85A (Usman et al., 2017). Preliminary studies showed that the Ad5Ag85A vaccine candidate protected for TB when it is intranasally administered like BCG booster vaccine in mice. In phase-I trial of this vaccine, safety, immunogenicity and tolerability of vaccine evaluated and found to be safe and well-tolerated. Also this vaccine was capable of inducing polyfunctional CD4⁺ and CD8⁺ T cells immunity in BCG-primed young people (Tang et al, 2016). However, neutralization of the antigen with Ad5 antibodies existed is the major difficulty in performing clinical trials with the Ad5 vector-based vaccine (Orme et al., 2015, Khoshnood et al., 2018). In the circumstances, the antigen is removed from the tissues without providing the necessary immunity and memory cells after vaccination (Soundarya et al., 2019).

MVA85A

The viral-vector subunit TB vaccine MVA85A expresses immunodominant mycobacterial antigen 85A (Ag85A) (Dockrell et al., 2016). BCG boosted with the MVA85A vaccine induces cellular immunity. In preclinical studies, the MVA85A vaccine indicated that it can improve protective efficacy against TB in mice. In the phase-I clinical studies, intradermal and aerosol formulations of the vaccine are experimented (Khoshnood et al. 2018). When the vaccine is administered as an aerosol, cellular immunity has been induced better than the intradermal route. The immune responses generated against this vaccine are variable in studies performed in different individuals groups. Furthermore, this vaccine did not demonstrate a protective effect against TB in a phase-IIb clinical trial (Tameris et al., 2013). Currently, phase-I clinical trials are continued with both intradermal and aerosol formulations of the vaccine in the United Kingdom (Frick et al., 2015).

4.2. Phase-IIa TB Vaccine

RUTI

RUTI, is a liposomal vaccine produced with detoxified, fragmented *M. tuberculosis* cells. This vaccine is being developed as a therapeutic vaccine for adults. RUTI is designed to shorten the chemotherapy treatment for both LTBI and TB disease. A strong cellular and humoral immune response are generated against *M. tuberculosis* bacilli after the vaccine administration. A phase-I trial of RUTI study demonstrated specific immune responses against *M. tuberculosis* antigens (Vilaplana et al., 2010). In a phase-II trial RUTI was found safe and was indicated that is triggered polyantigenic responses after a short course of chemotherapy administration (Cardona et al., 2006). In recent a study, efficacy of RUTI was found as similar to the BCG vaccine (Vilaplana et al., 2011).

MTBVAC

MTBVAC is constructed by attenuation of the *M. tuberculosis* clinical isolate Mt103. Both the more reliable and impressive vaccine is generated by the deletion of *phoP* and *fadD26* virulence genes existed on the genome of *M. tuberculosis* clinical isolate Mt103 (Clark et al., 2017; Khoshnood et al., 2018). In extensive preclinical studies, MTBVAC showed safety comparable to BCG, with superior immunogenicity and efficacy against TB bacilli in mouse and guinea pig models. When MTBVAC is applied as a booster vaccine to BCG, it supplied a longer-lasting immunity in a guinea pig model (Arbues et al., 2013; Clark et al., 2017). In a phase-Ia study, MTBVAC demonstrated the immunogenicity in without BCG-vaccine adults living in TB non-endemic regions (Spertini et al., 2015). Then, a dose-escalation study is ongoing in newborns in an endemic TB area of South Africa. Dose defining safety and immunogenicity study of MTBVAC, a phase-IIa is planned in 99 newborns non-exposed to HIV in TB-endemic regions of sub-Saharan Africa. Additionally, to evaluate dose-escalation safety and immunogenicity of MTBVAC vaccine is planned as randomized, double-blind, controlled phase-IIa study in 120 healthy South African adults with and without LTBI (Schragar et al., 2019).

ID93/GLA-SE

ID93 is a fusion protein composed of four immunogenic *M. tuberculosis* proteins. Each protein is separated into different categories: Rv2608 is within the PPE protein family outer membrane-associated (Rv2608), while Rv3619 and Rv3620 are the EsX protein family of secreted virulence factors. The other protein Rv1813 is up-regulated under hypoxic conditions. Rv1813 is associated with the latent growth of *M. tuberculosis* whereas the other proteins express the virulence of TB bacilli. GLA-SE is a synthetic adjuvant formulated in squalene oil. Four *M. tuberculosis* fusion proteins and GLA-SE adjuvant are combined. GLA-SE induced antigen-specific Th1 immune responses in mice (Penn-Nicholson et al., 2018). ID93/GLA-SE increased the production of polyfunctional T cell responses in BCG-vaccinated or non-BCG-vaccinated mice and guinea pigs (Baldwin et al., 2012). To evaluate the safety and immunogenicity of ID93/GLA-SE candidate vaccine, Phase-I and phase-II clinical trials have been completed in healthy adults in both the United States and South Africa. In these studies, ID93/GLA-SE induces a broad polyfunctional T-cell response, and there is an enhancement in multifunction antibodies (Penn-Nicholson et al., 2018). In phase-II trial, ID93/GLA-SE provided long-lived immunity due to an increase of CD4+ T-cell responses in humans (Panga et al., 2016). Several new phase-II studies related to the safety, immunogenicity, and efficacy of ID93/GLA-SE for prevention of TB infection is planned with high-risk health care workers in Korea.

TB/FLU-04L

Recombinant vaccine TB/FLU-04L, expressing Ag85A/ESAT6 proteins of TB bacilli has developed by using replication-deficient influenza virus strain A/Puerto Rico/8/34 H1N1 (Safrit et al., 2016). In a phase-I trial study performed with TB/FLU-04L vaccine, increasing antigen-specific IFN- γ response has been demonstrated in experimental animals (mice and cynomolgus monkeys). The vaccine was well-tolerated without serious adverse events. When the vaccine tested as a nasal spray, nasal cytokines (IL-1b, TNF α , and IL2) were detected after vaccination (Barry et al., 2016). After phase-I trials with TB/FLU-04L designed as a mucosal boost vaccine completed, a phase-II clinical trial is currently planned for this vaccine candidate (Soundarya et al., 2019).

4.3. Phase-IIb TB Vaccine

DAR-901

DAR-901 is a heat-inactivated, whole-cell preparation derived from *Mycobacterium obuense*. *M. obuense*, non-tuberculous mycobacterium (NTM), has identical multiple antigens with *M. tuberculosis*. Thus, this vaccine candidate may provide cross-protection against TB. DAR-901 is developed to be a booster vaccine for the prevention of TB infection in adolescents and adults. In a phase-I study, the safety and tolerability of the DAR-901 were displayed among adults with BCG (Panga et al., 2016). Also, assessment of tolerability and immunogenicity of DAR 901 at different doses has been accomplished in HIV-infected and HIV-uninfected adults who received the BCG vaccine (Khoshnood et al., 2018). The results indicated that DAR-901 induced a Th1 immune response and boosted protection against TB. Phase-IIb trial to prevent TB among adolescents is underway in Tanzania (Soundarya et al., 2019).

M72/AS01E

M72/AS01E is another subunit vaccine consisting of Mtb39A and Mtb32A antigens of *M. tuberculosis*. Two immunogenic proteins are incorporated with the liposome-based AS01 adjuvant system (Van Der Meeren et al., 2018). Mtb39a and Mtb32a proteins can stimulate peripheral blood mononuclear cells (PBMCs) again in healthy individuals who are positive for Tuberculin Skin Test. A membrane-associated protein, Mtb39a, and a fundamentally expressed protein and a serine protease, Mtb32a were selected by cell antigen screening. Both of them induce Th1 responses.

They are only expressed in *M. tuberculosis* and BCG but not in other mycobacteria (Schrager et al., 2019). Phase-I and phase-II trials have been completed to test the safety and immunogenicity of this vaccine. Overall, M72/AS01E was well-tolerated and induced a cell-mediated and humoral immune response in the recruited populations (Leroux-Roels et al., 2013). A phase-IIb study of the M72/AS01E candidate vaccine has been carried out in HIV uninfected adults in clinics in South Africa, Kenya, and Zambia (Van Der Meeren et al., 2018).

H1/H56:IC31

Subunit adjuvant vaccine, H1/H56:IC31 developed as an adjuvanted fusion protein of three immunogenic *M. tuberculosis* antigens. Ag85B, 6-kDa early secretory antigenic target (ESAT-6) and Rv2660c formulated with the IC31 adjuvant (Luabeya et al., 2015). After TB bacilli are phagocytosed by macrophages at the beginning of infection, both Ag85B, and ESAT-6 antigens of *M. tuberculosis* are thought to be important for the survival of bacteria. When H1/H56:IC31 candidate vaccine administered either priming or booster, it was indicated highly immunogenic in preclinical vaccine studies that were done with mice and guinea pigs (Kamath et al., 2008). Phase-I and phase-II studies were completed for safety and immunogenicity in adults and adolescents that cured the active TB. In these studies, H1/H56:IC31 has been proved that it is a dependable vaccine. Now, a phase-IIb trial of H1/H56:IC31 is planned to test for the prevention of TB infection and prevention of the risk of relapse in previously treated TB patients (Schrager et al., 2019; Soundarya et al., 2019).

4.4. Phase III TB Vaccine

Mycobacterium vaccae

Mycobacterium vaccae is an environmental saprophyte mycobacterial species found in the soil. This mycobacterium is a rapidly growing species that is estimated to have immunogenic properties increasing the host's immune response. Initially, *M. vaccae* vaccine is improved as an immunotherapeutic vaccine rather than a preventive vaccine by using heat-killed preparations of *M. vaccae* strain. The safety and immunogenicity of *M. vaccae* vaccine were demonstrated in HIV-infected adults previously vaccinated with BCG in Phase-I and II clinical trials performed in Finland, and Zambia (Vuola et al., 2003). This vaccine was also found as safe and immunogenic and to ensure prominent protection against TB infection in phase-III clinical trial in Tanzania (Von Reyn et al., 2010). While the vaccine protects against the disease in some geographical settings, it may not ensure in the other places. This inconsistency is a major drawback of *M. vaccae* (Usman et al., 2017).

VPM1002

Another recombinant BCG vaccine VPM 1002 is being evaluated either replacement for BCG vaccination for newborn or prevention of TB in adolescents and adults (Nieuwenhuizen et al., 2017). In VPM1002, a listeriolysin (hly) encoding gene from the facultative anaerobic bacterium *Listeria monocytogenes* was added to the BCG genome and the gene encoding urease-C (ureC) was deleted. (Montagnani et al., 2014). Perforation of the phagosomal membrane by listeriolysin allows the release of recombinant antigens into the cytosol of the host. Also, the loss of ureC gene provides an acidic pH of 5.5 inside the phagosome for the optimal listeriolysin function (Orme et al., 2013). By gene modification, microbial antigens are released into cytosol with better CD8+ T-cell stimulation (Hesseling et al., 2007). In early clinical trials, VPM1002 tested in adults with BCG was found as safe and immunogenic in a phase-Ib trial in South Africa. In recent years, a phase-II trial has been concluded for assessment of the safety and tolerability of the vaccine in HIV-exposed and HIV-unexposed newborns in sub-Saharan Africa. To assess of the immunogenicity and safety of VPM1002 in 10,000 South African infants, a phase-III trial is programmed to begin in 2019 and it is planned to complete in 2021 (Schrager et al., 2019).

Mycobacterium indicus pranii

Mycobacterium indicus pranii (MIP) (also known as *Mycobacterium w.*) is a heat-inactivated non-tuberculosis mycobacterial vaccine for patients undergoing chemotherapy (Gupta et al., 2012). MIP contains antigens similar to *Mycobacterium leprae* (Gupta et al., 2012). In phase-II study of MIP has been shown to have immunotherapeutic influence on both leprosy and TB diseases (Kamal et al., 2016). Currently, two phase-III trials were conducted to test the efficiency and safety of MIP in pulmonary TB patients. In these studies, MIP was found that it does not have adverse effects and it plays a significant role in the elimination of the mycobacterium (Sharma et al., 2017).

Conclusion

A safe, efficient, and affordable vaccine is one of the most essential ways of protection against many infectious diseases. The improvement of new and effective TB vaccines is inevitable to control and end to TB. Especially, it is an urgent need to prevent the spread of drug-resistant strains of *M. tuberculosis*. To find a new, more effective vaccine as a booster vaccine for BCG or to replacement of BCG with a better alternative is a priority of the TB vaccine research. Recently, this research has been focused on the recombinant vaccine production using both BCG and *M. tuberculosis* strains. New TB vaccines are developed as preventive, post-exposure, and even therapeutic. In the past several years, tremendous progress has been made in clinical trials with several vaccine candidates. Although TB is considered as a poverty-related disease, it still occurs in developing countries, or among those of high socioeconomic status. Therefore, the expectation is the development of better, more protective TB vaccines in a short span of time. Without new TB vaccines, TB will neither be controlled nor eliminated.

Conflict of Interests

Author declares no conflict of interests.

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