



Tuberculosis vaccine development: from classic to clinical candidates

Junli Li^{1,2,3,4} · Aihua Zhao⁵ · Jun Tang^{1,2,3,4} · Guozhi Wang⁵ · Yanan Shi^{1,2,3,4} · Lingjun Zhan^{1,2,3,4} · Chuan Qin^{1,2,3,4}

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Abstract

Bacillus Calmette-Guérin (BCG) has been in use for nearly 100 years and is the only licensed TB vaccine. While BCG provides protection against disseminated TB in infants, its protection against adult pulmonary tuberculosis (PTB) is variable. To achieve the ambitious goal of eradicating TB worldwide by 2050, there is an urgent need to develop novel TB vaccines. Currently, there are more than a dozen novel TB vaccines including prophylactic and therapeutic at different stages of clinical research. This literature review provides an overview of the clinical status of candidate TB vaccines and discusses the challenges and future development trends of novel TB vaccine research in combination with the efficacy of evaluation of TB vaccines, provides insight for the development of safer and more efficient vaccines, and may inspire new ideas for the prevention of TB.

Keywords MTB · TB · BCG · Novel TB vaccine · Clinical trial

Introduction

Tuberculosis (TB), caused by the human pathogens *Mycobacterium tuberculosis* (MTB), has plagued humanity for millennia and remains the deadliest infectious disease in the modern world [1]. Causes of TB resurgence are complex and include the emergence and prevalence of drug-resistant strains, low early detection rates, latent infection, poor public health care conditions, and population movements [2, 3]. In 2018, there were more than 1.2 million TB deaths worldwide

and an additional 251,000 deaths of TB-HIV co-infection. New TB cases reached 10 million (equivalent to 133 new cases per 100,000), and about 3.4% of new TB cases and 18% of previously treated cases had multi-drug resistant TB (MDR-TB) or rifampicin-resistant TB (RR-TB). Adult male patients accounted for 57% of all new-onset patients, and children younger than 15 years old and those with HIV co-infection accounted for 11% and 8.6% of new-onset patients, respectively [4].

In 1993, the World Health Organization (WHO) announced that TB is a global health emergency [5]. With social and economic development, TB morbidity and mortality in Western Europe, North America, and other developed regions began to decline in the early twentieth century. Currently, the number of new cases and deaths per 100,000 population are less than ten cases and one case, respectively [6, 7]. However, for many countries, the major public health issue of ending TB remains a distant reality. At present, there are an estimated 1.7 billion latent tuberculosis infections (LTBI) worldwide, accounting for about a quarter of the population [8, 9]. Given the increase of population movements and immigration, the need for prevention and control of TB worldwide is becoming more serious. Therefore, the 2017 G20 Leaders' Declaration listed the "End TB Strategy" as a key priority for global political attention.

Crucial strategies for controlling and eradicating TB include diagnosis, prevention, and treatment. Timely diagnosis and effective treatment play important roles for controlling the epidemic. In order to reduce incidences of TB in 2030 and 2035

✉ Lingjun Zhan
zhanlingjun2008@163.com

✉ Chuan Qin
qinchuan@pumc.edu.cn

¹ NHC Key Laboratory of Human Disease Comparative Medicine, Institute of Laboratory Animal Sciences, Chinese Academy of Medical Sciences (CAMS) and Peking Union Medical College (PUMC), Beijing 100021, People's Republic of China
² Beijing Key Laboratory for Animal Models of Emerging and Reemerging Infectious, Beijing 100021, People's Republic of China
³ Key Laboratory of Human Diseases Animal Model, State Administration of Traditional Chinese Medicine, Beijing 100021, People's Republic of China
⁴ Tuberculosis Center, Chinese Academy of Medical Sciences (CAMS), Beijing 100021, People's Republic of China
⁵ Division of Tuberculosis Vaccines, National Institutes for Food and Drug Control (NIFDC), Beijing 102629, People's Republic of China

by 80 and 90% compared with levels in 2015, its global incidence will need to decline at an unprecedented rate after 2025. We therefore need new interventions, including shorter, less toxic treatments, improved diagnostics, and more effective vaccines. The TB vaccination is not only the most cost-effective method of controlling the disease but also allows TB outbreaks to be controlled at the source and must be a key component of any strategy to eliminate TB's global burden [10].

Classic TB vaccine-BCG

BCG is a live, attenuated vaccine that is obtained by continuous subculture of *Mycobacterium bovis* and is currently the only licensed vaccine for human TB prevention [11]. The BCG vaccine strain was originally distributed by the Institut Pasteur in France. Due to different passages and culture methods over the years, more than 14 BCG strains using different phenotypes and genotypes have been developed around the world [12–14] (Fig. 1). Currently, more than 90% of the BCG used worldwide has been prepared from four major strains, which are BCG Pasteur 1173P2, BCG Danish 1331, BCG Glaxo 1077, and BCG Japan 172 strain. The BCG strain used for production in China is a progeny strain obtained by subculture of the BCG Danish 823 strain, introduced from the Statens Serum Institut in 1948. In 1992, the National Health

and Commission of the People's Republic of China announced that the Bacillus D₂PB302 strain was the only BCG strain being produced in China (Fig. 1).

Since the early 1970s, BCG has been used as part of WHO's Expanded Programme on Immunization (EPI), which has used more than four billion doses to date and is the most widely used vaccine in history. In 1978, China began to carry out child immunization work nationwide, using a timely and effective BCG vaccination for newborn babies in accordance with prescribed immunization procedures. WHO recommends that newborns receive a single-dose BCG vaccination in high-burden countries, while low-burden countries can selectively vaccinate neonates. It is also recommended that BCG should be given to people who live in high-endemic areas or exposed to MDR-TB but are tuberculin-negative and BCG naïve.

BCG has been shown to be effective for preventing disseminated TB such as miliary TB and tuberculous meningitis in children [15]. In addition, BCG is considered to have a non-specific effect, and it is associated with a decrease in infant mortality [16, 17]. However, others have found that BCG is not significant for reducing TB overall incidence worldwide [18]. The effects of BCG vary widely, with 0–80% effectiveness in different settings, and vaccine-induced immunity diminishes over time. BCG provides 10–20 years of protection [19, 20], but does not provide adequate prevention of PTB for adolescents and adults [21–25].

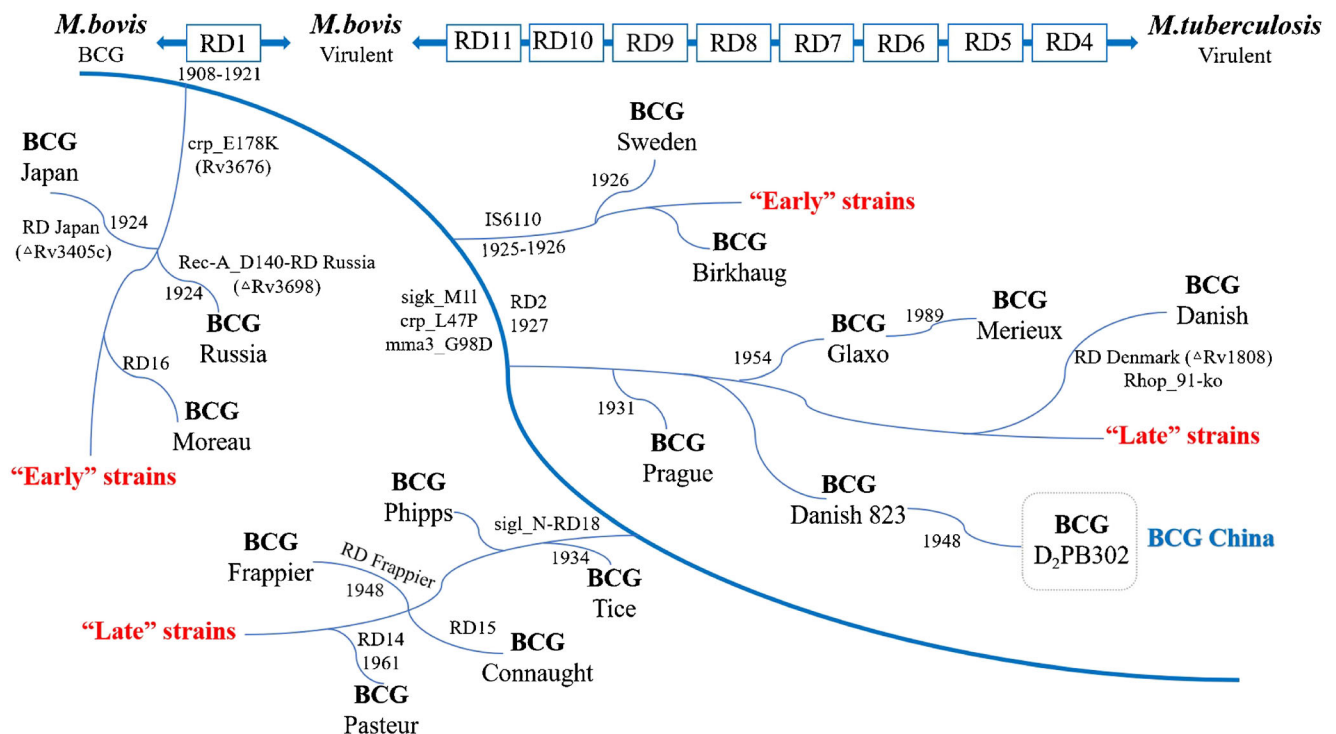


Fig. 1 History and genealogy of BCG substrains. RD1 was missing from all BCG substrains and coincides with the attenuated virulence of *M. bovis*. From the top to the bottom of the timeline, BCG substrains are divided into “Early” strains and “Late” strains. In “Early” strains, the

original characteristics of “authentic Pasteur” were conserved with fewer deletions, insertions, and mutations in the genome of the bacilli than the “Late” strains

In recent years, it has been found that effectiveness evaluation of BCG is influenced by the screening of the tuberculin skin test (TST) prior to immunization. Higher vaccine efficacy was obtained for neonates and adult subjects who were screened by rigorous TST trials. BCG has a 59% protective effect against PTB in newborns (RR 0.41, 95% CI: 0.29–0.58) and 74% in adult subjects who have undergone strict TST screening (RR 0.26, 95% CI: 0.18–0.37). However, the average protection of PTB was significantly reduced in those who did not undergo strict TST screening [21].

BCG is suitable for people who are not sensitized by mycobacteria, although the incidence of LTBI is 60–70% in adults older than 25 in high-endemic areas [26], explaining why the BCG protection effect is low in areas with a high incidence of TB. The early South Indian Chingleput BCG clinical trial failed as most subjects were sensitized by MTB and *non-tuberculous mycobacteria* (NTM) [27, 28]. In short, BCG is suitable for uninfected people, cannot provide lifelong immunity, and is not suitable for HIV-infected patients and its preventive effect on TB needs to be correctly evaluated. However, so far, no new TB vaccine more protective than BCG has passed clinical assessment [29, 30]. Therefore, BCG remains one of the most cost-effective means of preventing TB in countries with high burden of TB for a long time.

Candidate TB vaccines

Despite high rates of BCG vaccination as part of the EPI, the slow decline in global TB highlights the urgency of novel TB vaccines. Under the “End TB Strategy,” developing new vaccines that reduce the prevalence of infection as well as the risk of TB will be a key element of achieving the goal of eradicating TB by 2050. By August 2019, more than 14 TB vaccines had been used in clinical trials, representing a diverse repertoire of formulations and mycobacterial antigens and that induce a wide range of immune responses with different characteristics (Table 1).

Viral vector vaccines

Ad5 Ag85A

This is a type 5 serotype replication-deficient adenoviral vector vaccine, which expresses the MTB Ag85A antigen and is designed to be a heterologous booster vaccine following BCG priming [31–34]. The target population is healthy adults following BCG vaccination. In a mouse model, a single intranasal immunization using Ad5 Ag85A induced an effective protection against the challenge of MTB, while, compared with an intramuscular injection, Ad5 Ag85A intranasal immunization can significantly enhance BCG’s protective effect [35].

Phase I clinical trial ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT00800670) Identifier: NCT00800670) has evaluated the safety and immunogenicity of Ad5 Ag85A in healthy volunteers who had a history of BCG vaccination and those BCG naïve. Overall, intramuscular vaccination was well tolerated and had good safety and immunogenicity for both groups, while a stronger multifunctional T cell response was observed in those with a history of BCG vaccination [36–38]. In September 2017, another phase I clinical trial ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT02337270) Identifier: NCT02337270) is underway to evaluate the safety and immune responses of peripheral blood and lungs after one or two doses of Ad5Ag85A administered by aerosol in healthy volunteers previously immunized with BCG, and the study is expected to be completed on April 2021.

ChAdOx1 85A-MVA85A

ChAdOx1 85A is a *chimpanzee adenovirus* [39], while MVA85A is a modified *vaccinia Ankara virus*, both of which express the MTB antigen Ag85A [40]. In preclinical study, ChAdOx1 85A immunogenicity and protective efficacy against MTB challenge were assessed in a mouse model. Researcher found that intranasally administered ChAdOx1 85A induced stronger CD8⁺ than CD4⁺ T cell immune responses in both lungs and spleens, although it failed to protect mice against aerosol MTB infection. However, further boosting with MVA85A improves immunogenicity and protective efficacy in BCG vaccination [39]. Therefore, novel viral vector vaccines ChAdOx1 85A and MVA85A were used together to form a combined heterologous primary booster regimen and were administered via the mucosal route.

Currently, phase I trial ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT01829490) Identifier: NCT01829490 and NCT03681860) of intramuscular injection of ChAdOx1 85A either alone or in combination with MVA85A for prime-boost regimen have been completed in the UK. Another phase I clinical trial ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT04121494) Identifier: NCT04121494) designed to assess the safety and immunogenicity profile of ChAdOx1 85A given by aerosol inhaled versus intramuscular in adult healthy volunteers was also launched in January 2019 in Switzerland.

TB/FLU-04L

TB/FLU-04L is a replication-deficient attenuated influenza virus mucosal vector vaccine, which expresses the MTB antigens Ag85A and ESAT-6 and was developed by the Research Institute for Biological Safety Problems, Kazakhstan, in collaboration with the Research Institute on Influenza, Russia. It was designed as a preventive booster vaccine for infants, adolescents, and adults. In a preclinical mouse model, an intranasal boost of TB/FLU-04L can significantly improve the protective efficacy of BCG [41]. In phase I clinical trial ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT02501421) Identifier: NCT02501421), 36 healthy

Table 1 Summary of TB vaccine candidates currently under clinical assessment

Strategy	Vaccine candidate	Vaccine type	Phase	Sponsor
Prime	MTBVAC	Live genetically attenuated <i>Mycobacterium tuberculosis</i>	IIa	University of Zaragoza; Biofabri; Tuberculosis Vaccine Initiative
	VPM1002	Live recombinant <i>Mycobacterium bovis</i>	III	Serum Institute of India; Vakzine Projekt Management; Tuberculosis Vaccine Initiative; Max Planck Institute for Infection Biology
Prime-boost	Ad5 Ag85A	Viral vector	I	McMaster University; Canadian Institutes of Health Research; CanSino
	ChAdOx1 85A-MVA85A	Viral vector	I	Oxford University
	ID93 + GLA-SE	Protein/adjuvant	IIa	Infectious Disease Research Institute; Aeras; Wellcome Trust; International AIDS Vaccine Initiative
	TB/FLU-04L	Viral vector	IIa	Research Institute for Biological Safety Problems; Ministry of Health, Kazakhstan; Research Institute of Influenza, Russia
	BCG revaccination (Gates MRI-TBV01-201)	Live attenuated <i>Mycobacterium bovis</i>	IIb	Bill & Melinda Gates Medical Research Institute
	DAR-901 booster	<i>Mycobacterium obuense</i> —whole cell or extract	IIb	Dartmouth University; Global Health Innovative Technology Fund; Aeras
	H56:IC31	Protein/adjuvant	IIb	Statens Serum Institut; Valneva; Aeras
Immunotherapeutic	M72/AS01 _E (GSK 692342)	Protein/adjuvant	IIb	Glaxo-SmithKline; Aeras
	AEC/BC02	Protein/adjuvant	I	AnHui Zhifei Longcom
	RUTI [®]	<i>Mycobacterium tuberculosis</i> —whole cell or extract	III	Archivel Farma S.L.
	MIP/Immuvac	<i>Mycobacterium indicus pranii</i> —whole cell or extract	III	Indian Council of Medical Research; Cadila Pharmaceuticals
	Vaccae [™]	<i>Mycobacterium vaccae</i> —whole cell or extract	III	AnHui Zhifei Longcom

Viral vectors: *Ad5* adenovirus 5, *ChAd* chimpanzee adenovirus, *MVA* modified vaccinia Ankara virus, *FLU* replication-deficient influenza virus (H1N1) Adjuvants: *GLA-SE* oil in water emulsion/TLR4 agonist, *IC31* cationic peptide/TLR9 agonist, *AS01_E* liposome/TLR4 agonist, *BC02* BCG-CpG-DNA and Al (OH)₃ compound adjuvant/TLR9 agonist

adults aged between 18 and 50 were vaccinated with TB/FLU-04L on days 1 and 21, respectively, and results showed good safety and immunogenicity. Currently, the vaccine is undergoing phase IIa clinical trial for LTBI.

Protein/adjuvant subunit vaccines

AEC/BC02

AEC/BC02 is a freeze-dried recombinant TB vaccine developed by the National Institutes for Food and Drug Control, China, and manufactured by Anhui Zhifei Longcom Biologic Pharmacy Co., Ltd. It is composed of MTB subunit protein, Ag85B; fusion protein, ESAT6-CFP10; and a compound adjuvant, BC02, based on BCG-derived unmethylated cytosine-phosphate-guanine (CpG) DNA fragment and aluminum salt. It is mainly used for preventive treatment of LTBI. Preclinical studies have found that AEC/BC02 can induce long-term antigen-specific cellular immune responses in mice, produce a therapeutic effect, and reduce the risk of Koch phenomenon in guinea pig LTBI model [42]. In April 2018, AEC/BC02 began

a phase I clinical trial ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT03026972) Identifier: NCT03026972) of human tolerance in four different populations. (1). Population I has 25 subjects who received TB-PPD skin test and IFN- γ detection whose results are both negative. (2). Population II has 30 subjects who received TB-PPD and ESAT6-CFP10 skin test in different arms and IFN- γ detection whose results are all negative. (3). Population III as uninfected TB-PPD positive population, this group screened 30 subjects whose ESAT6-CFP10 skin test and IFN- γ detection results are both negative, but TB-PPD skin test positive. (4). Population IV has 50 subjects whose three kinds of detection results are all positive (TB-PPD, ESAT6-CFP10, and IFN- γ). Currently, clinical volunteers are being recruited.

H56: IC31

H56: IC31 (AERAS-456) is a protein adjuvant vaccine consisting of MTB early secretory proteins Ag85B and ESAT-6 and latent infection-associated protein Rv2660c in combination with IC31 adjuvant. It is designed to induce immunity against new infections and reactivation of LTBI [43].

When compared with BCG, H56/IC31 vaccine can significantly reduce lung pathological damage and MTB extrapulmonary transmission while inducing a strong memory immune response. More importantly, H56: IC31-vaccinated non-human primates did not reactivate host latent-infected MTB following anti-TNF antibody treatment [44]. The vaccine has been used in three phase I clinical trials. Two of these were carried out with adults who were HIV-negative, with or without latent TB infection ([ClinicalTrials.gov Identifier: NCT01967134](https://clinicaltrials.gov/ct2/show/study/NCT01967134) and [NCT02378207](https://clinicaltrials.gov/ct2/show/study/NCT02378207)) [43, 45, 46]. Another was conducted with HIV-negative, post-treatment patients with drug-susceptible PTB ([ClinicalTrials.gov Identifier: NCT02375698](https://clinicaltrials.gov/ct2/show/study/NCT02375698)), and it was found that the vaccine is safe and has good immunogenicity at all study doses. In addition, phase II trial ([ClinicalTrials.gov Identifier: NCT03512249](https://clinicaltrials.gov/ct2/show/study/NCT03512249)) assessing H56:IC31 for reducing the rate of TB disease recurrence in HIV-negative adults successfully treated for drug-susceptible PTB is ongoing in South Africa and the United Republic of Tanzania.

ID93 + GLA-SE

This is a protein adjuvant vaccine developed by the American Infectious Disease Research Institute in collaboration with Aeras. It consists of four MTB antigens that are associated with virulence (Rv2608, Rv3619, and Rv3620) or latent (Rv1813) as well as TLR-4 ligand GLA-SE adjuvant [47, 48]. In preclinical studies, the ID93 + GLA-SE vaccine has been found to be effective for preventing infection of sensitive and resistant MTB in mice and guinea pigs [49–52]. As a therapeutic vaccine, it can significantly improve the effect of anti-TB antibiotics in mice and non-human primates [53]. Additionally, the study also found that guinea pigs immunized with ID93 + GLA-SE showed a negative DTH response to intradermal PPD, which, in turn, did not interfere with the auxiliary diagnosis of a PPD skin test [54].

The vaccine has been used in two phase I clinical trials in the USA and South Africa. A phase I clinical trial ([ClinicalTrials.gov Identifier: NCT01599897](https://clinicaltrials.gov/ct2/show/study/NCT01599897)) in the USA included 60 healthy adults with no history of BCG vaccination who were negative for QuantiFERON-TB Gold and were intramuscularly injected with 3-needle ID93 and ID93 + GLA-SE vaccine. Researcher found that all dose levels of ID93 and ID93 + GLA-SE were acceptably safe. Compared with ID93 alone, this vaccine elicited higher titers of ID93-specific antibodies, preferentially increasing IgG1 and IgG3 antibody subclass and significantly inducing a multifunctional cytokine profile of CD4⁺ T cells [55]. Another phase Ib trial ([ClinicalTrials.gov Identifier: NCT01927159](https://clinicaltrials.gov/ct2/show/study/NCT01927159)) from South Africa also found that ID93 + GLA-SE was well tolerated, with no serious vaccine-related adverse events and vaccination-induced long-lasting antigen-specific IgG and Th1 cell responses, and that effects peaked after a second vaccination. At the same time, vaccine doses did not

affect the frequency or severity of adverse events, while mild injection-site adverse reactions and flu-like symptoms were common in MTB-infected subjects compared with those not infected with MTB [56]. In addition, the USA ([ClinicalTrials.gov identifier: NCT03722472](https://clinicaltrials.gov/ct2/show/study/NCT03722472)) and South Korea ([ClinicalTrials.gov identifier: NCT03806699](https://clinicaltrials.gov/ct2/show/study/NCT03806699)) are also undergoing two phase I clinical trials in healthy adults and BCG-vaccinated adolescent, both of which are expected to be completed in 2020. In January 2017, a phase IIa clinical trial ([ClinicalTrials.gov Identifier: NCT02465216](https://clinicaltrials.gov/ct2/show/study/NCT02465216)) of the ID93 + GLA-SE vaccine was carried out in South Africa to assess the safety and immunogenicity of TB-HIV co-infected adults following treatment completion. Phase IIb clinical trial in the same population intended to prevent TB recurrence are being prepared.

M72/AS01_E (GSK 692342)

This is a protein adjuvant vaccine developed by Glaxo-SmithKline Biologicals, USA from MTB recombinant fusion protein (Mtb32 and Mtb39) and AS01_B, AS02_A, or AS01_E adjuvant, which is designed to promote BCG-induced immune responses [57–62]. In preclinical studies, GSK 692342 has been shown to induce more efficient immune responses in animal models than either Mtb32 or Mtb39 alone [63] as well as more efficient protection than BCG alone in mice, guinea pigs, and non-human primate [63, 64]. In clinical phase II trials, GSK 692342 has been shown to have clinically acceptable safety and to induce strong humoral and cellular immunity in healthy and HIV-infected populations ([ClinicalTrials.gov Identifier: NCT01262976](https://clinicaltrials.gov/ct2/show/study/NCT01262976)), TB-infected adults ([ClinicalTrials.gov Identifier: NCT01424501](https://clinicaltrials.gov/ct2/show/study/NCT01424501)), and adolescents with PPD negative or positive skin reactivity ([ClinicalTrials.gov Identifier: NCT00397943](https://clinicaltrials.gov/ct2/show/study/NCT00397943), [NCT00621322](https://clinicaltrials.gov/ct2/show/study/NCT00621322) and [NCT00950612](https://clinicaltrials.gov/ct2/show/study/NCT00950612)) [58, 65–72]. At present, M72/AS01_E has been tested in completed phase IIb clinical trial ([ClinicalTrials.gov Identifier: NCT01755598](https://clinicaltrials.gov/ct2/show/study/NCT01755598)) in Kenya, South Africa, and Zambia. HIV-negative LTBI adults were randomized to receive two doses of M72/AS01_E or placebo to study the occurrence of active PTB unrelated to HIV infection and its safety or immunogenicity. Ten patients in the vaccine group were confirmed to have active PTB by bacteriology compared with 22 from the placebo group. The primary analysis of this trial showed a 54% (90% CI: 14–75; $P = 0.04$) point estimate of vaccine efficacy over about 2 years of follow-up [73].

Whole cell/extract vaccines

RUTI[®]

This is an inactive, multi-antigen vaccine based on MTB whole cell extracts [74]. It is hoped that this could be used as a therapeutic vaccine in combination with short-term anti-tuberculosis chemotherapy for prophylactic treatment of

patients with MDR-TB. In preclinical experiments, short-term chemotherapy in mice and guinea pigs using RUTI[®] can effectively control LTBI, significantly induce the Th1/Th2/Th3 complex immune response, and enhance local accumulation of specific CD8 T cells [74]. Phase I studies with healthy volunteers ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT00546273) Identifier: NCT00546273) [75] and phase II studies with LTBI ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT01136161) Identifier: NCT01136161) [76] have found that the vaccine is safe and tolerable and has good immunogenicity at all study doses. Currently, a phase IIa clinical trial ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT02711735) Identifier: NCT02711735) is being conducted by the University Medical Center Groningen to investigate the safety and immunogenicity of a RUTI[®] therapeutic vaccination for patients with MDR-TB following successful intensive-phase treatment.

DAR-901 booster

This is a whole-cell, heat-killed NTM vaccine produced from a large-scale broth growth culture method from the SRL-172 master cell bank [77]. In animal models, using DAR-901 as a heterologous booster significantly induced a strong cellular, humoral immune response in C57BL/6 mice and enhanced aerosol challenge protection against MTB when compared with BCG homologous potentiation [78]. Phase I study ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT02063555) Identifier: NCT02063555) from the Dartmouth-Hitchcock Medical Center in the USA found that subjects (BCG-immunized adults with or without HIV) were safe and able to tolerate three doses of DAR-901 and that this effectively induced the formation of cellular and humoral immune responses [79–81]. A randomized, placebo-controlled, double-blind phase IIb clinical trial ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT02712424) Identifier: NCT02712424) has recently been initiated with adolescents who had previously received BCG in the United Republic of Tanzania.

MIP/Immuvac

MIP, also known as Immuvac, is a heat-killed *Mycobacterium indicus pranii* (*Mycobacterium w*) vaccine. It has been approved by the drug controller general of India and the FDA as an immunotherapeutic and immunoprophylactic agent for treating multibacillary leprosy patients (as an adjunct to standard multidrug therapy), and for preventing the development of leprosy among close contacts of leprosy patients [82–84]. *Mycobacterium w* shares an antigen with both *Mycobacterium leprae* as well as MTB and is also found to be useful in the prevention of TB in experimental animals [85, 86]. Previous studies on the efficacy of *Mycobacterium w* as an immunomodulator in PTB patients have shown higher sputum conversion rates in patients given *Mycobacterium w* as an adjuvant therapy along with standard anti-TB treatment, and it has faster and remarkable sputum converting capacity [87].

Similar studies conducted in PTB category-II patients have shown improved cure rates [88]. As of now, the investigators are investigating MIP for its efficacy in category-II PTB ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT00265226) Identifier: NCT00265226) and category-I PTB ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT00341328) Identifier: NCT00341328) patients in a “double-blind placebo-controlled randomized clinical control trial.”

Vaccae™

This is a non-cell lysate of *Mycobacterium vaccae*, which was jointly developed by National Institutes for Food and Drug Control, China, and the 309th Hospital of the People's Liberation Army (Beijing, China) and is manufactured by Anhui Zhifei Longcom Biologic Pharmacy Co., Ltd. (Anhui, China). In 1999, Vaccae™ got the “New Drug Certificate” and approved by the National Medical Products Administration of China (NMPA) as an immunotherapeutic agent [89], which played an important key role in improving immunity, promoting phagocytosis, regulating bidirectional immunoreaction, and reducing pathological damage. It is therefore used as a combination therapy for the adjuvant treatment of TB chemotherapy and to shorten anti-tuberculosis treatment for patients with drug-sensitive TB. As a new indications for Vaccae™, NMPA approved of the plan “Phase III clinical study of efficacy and safety of Vaccae™ to prevent TB in high risk groups of TB infection” in December 2012. Currently, Vaccae™ was evaluated in phase III clinical trial ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT01979900) Identifier: NCT01979900) as an LTBI immunopreventive vaccine in 2018, and data on its safety and efficacy of TB prevention are being summarized.

Attenuated/recombinant live vaccines

MTBVAC

This is a live attenuated vaccine derived from the clinical isolate of MTB by deletion of *phoP* and *fadD26* genes [90]. It was developed by the University of Zaragoza, Pasteur Institute, and Biofabri with support from TBVI. MTBVAC is the only TB vaccine based on attenuated MTB, which is entering clinical trials [91, 92]. It is intended to serve as a preventive vaccine for newborns and replace BCG as well as to be a booster vaccine for adolescents and adults. However, it is likely to be subject to the same interference caused by prior immunological sensitization by NTMs as reported for BCG. Phase I clinical trial ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT02013245) Identifier: NCT02013245) conducted in Switzerland in 2013 found that, among 18- to 45-year-old healthy adults, the MTBVAC vaccine had similar levels of safety to BCG and that no significant adverse events were caused by different doses. All vaccinated people were negative for IGRA at follow-up after 210 days of inoculation [93]. In May 2018, phase Ib clinical

trials ([ClinicalTrials.gov](https://clinicaltrials.gov) Identifier: NCT02729571) were completed in adults and infants. The first stage includes 18 HIV-uninfected, QFT-negative, BCG-vaccinated adult participants, randomized 1:1 to receive BCG Vaccine SSI or MTBVAC at equivalent dose, and the second stage was commence in 36 HIV-unexposed, BCG-naïve newborn infants, randomized 1:3 to receive BCG Vaccine SSI or MTBVAC at one of three different dose levels. Two phase Ib/IIa clinical trials ([ClinicalTrials.gov](https://clinicaltrials.gov) Identifier: NCT03536117 and NCT02933281) and epidemiological studies ([ClinicalTrials.gov](https://clinicaltrials.gov) Identifier: NCT03536117) have recently been carried out by Aeras and Biofabri with adults and/or infants in South Africa.

BCG revaccination (Gates MRI-TBV01-201)

BCG vaccine is given at or shortly after birth in many developing countries to prevent TB. However, it does not provide protection against PTB. For unknown reasons, a BCG revaccination or “booster,” while not toxic, does not provide much additional protection. In 1995, the WHO issued the “Global Tuberculosis Program and Global Program on Vaccine: Statement on BCG Revaccination for the Prevention of Tuberculosis,” which concluded that the revaccination effect of BCG could not be confirmed. WHO does not advocate to revaccinate, and it is not necessary to vaccinate anyone for multiple times [94]. After the announcement of the statement, the countries that implemented the BCG revaccination policy stopped the strategy, and China also stopped the revaccination policy of BCG in 1997. At present, only a few countries in the world still implement revaccination strategies, and some even inoculate many times. With the emergence of a large number of MDR-TB and TB-HIV co-infection at the end of the twentieth century, WHO issued another position paper on BCG in 2004 [95]. The position paper states that revaccination of BCG in individuals who are positive for TST (whether or not the positive result is caused by NTM, MTB infection, or BCG vaccination) does not improve its immunity against TB. But, if the TST is negative and inevitably close to MDR-TB, BCG may be considered. However, the 2018 [96] position paper replaces the 2004 WHO position paper on BCG vaccine and the 2007 [97] WHO revised BCG vaccination guidelines for infants at risk for HIV infection. It incorporates recent developments in the TB field, provides revised guidance on the immunization of children infected with HIV, and re-emphasizes the importance of the birth dose. The 2018 position paper shows minimal or no evidence of any additional benefit of repeat BCG vaccination against TB or leprosy. Therefore, revaccination is not recommended even if the TST reaction or result of interferon- γ release assays (IGRA) is negative. In addition, the absence of a BCG scar after vaccination is not indicative of a lack of protection and is not an indication for revaccination. But, BCG vaccination of

unvaccinated, TST-negative or IGRA-negative school children is recommended for those coming from or moving to high incidence/burden settings, as well as older groups at risk through occupational exposure.

In the mouse model, mice vaccinated with a repeat dose of BCG demonstrated an increased titer of anti-BCG IgG [98, 99], as well as revaccination of cattle with BCG 2 years after first vaccination restored protection from *M. bovis* challenge compared with calves receiving only a single vaccination 2.5 years previously [100]. Interestingly, Use of an in vitro model of human PBMC induced with a repeat dose of BCG at 24 and 72 h of cell culture demonstrated increased anti-BCG IgG levels in supernatants compared with the pre-boost and control groups [101]. Several human clinical studies have performed an analysis of the effects of BCG revaccination in the HIV-1-uninfected ([ClinicalTrials.gov](https://clinicaltrials.gov) Identifier: NCT02378207 and NCT02075203), BCG-naïve healthy adults ([ClinicalTrials.gov](https://clinicaltrials.gov) Identifier: NCT03175380), and LTBI ([ClinicalTrials.gov](https://clinicaltrials.gov) Identifier: NCT01119521). Now, a phase IIb ([ClinicalTrials.gov](https://clinicaltrials.gov) Identifier: NCT04152161) study of the vaccine by Aeras, in collaboration with partners including the South African Tuberculosis Vaccine Initiative (SATVI) and the Emavundleni Research Centre found that, while BCG revaccination did not demonstrate efficacy in preventing initial MTB infection (defined as QFT conversion at an IFN- γ level of ≥ 0.35 IU per ml after day 84), it did result in significantly reduced rates of sustained QFT conversion (defined as three consecutive positive QFT results after day 84) [102]. These findings have fuelled renewed interest in the potential utility of BCG revaccination and prompted consideration of BCG revaccination in certain settings as part of an overall improved TB vaccination strategy, although it is not clear to what extent cellular and/or humoral immunity contribute to this protection.

VPM1002

VPM1002 (rBCG Δ UreC::hly) is a recombinant BCG vaccine developed by the Max Planck Institute for Infectious Biology, which uses the Listeriolysin O (LLO)-encoding gene of *Listeria monocytogenes* (Hly) to replace the urease C encoding gene (UreC) and so improve BCG immunogenicity [93, 103]. LLO is a cholesterol-dependent cytolysin that forms a transmembrane β -barrel-like pore in the phagocytic lysosomal membrane, allowing *Listeria monocytogenes* to escape to the cytosol [104, 105]. Urease inhibits Phagocytic lysosome maturation and improves the survival of MTB in macrophages [106, 107]. Studies have found that the elimination of urease C promotes phagocytic lysosomal fusion while providing optimal pH for LLO stability [104]. Therefore, expression of LLO in VPM1002 leads to the release of bacterial antigens and DNA into the cytosol, ultimately triggering autophagy and apoptosis [103].

In preclinical studies, the VPM1002 vaccine showed superior protection to BCG in MTB aerosol challenge mice and was safe in animal models including immunodeficient mice, guinea pigs, rabbits, and non-human primates [108]. In 2009 and 2011, phase I and phase Ib clinical trials of safety and immunogenicity were carried out with adults in Germany ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT00749034) Identifier: NCT00749034) and South Africa ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT01113281) Identifier: NCT01113281). It was found that VPM1002 is safe and can effectively stimulate the proliferation of IFN- γ -secreting multi-factor T cells [109]. Phase II clinical trials to assess safety and immunogenicity in healthy neonates ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT01479972) Identifier: NCT01479972) as well as HIV exposed and unexposed neonates ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT02391415) Identifier: NCT02391415) have also been carried out in South Africa [110]. Additionally, a phase II/III trial ([ClinicalTrials.gov](https://clinicaltrials.gov/ct2/show/study/NCT03152903) Identifier: NCT03152903) of the vaccine for the prevention of TB recurrence in adults has been initiated in India.

Evaluation of TB vaccines' efficacy

Preclinical evaluation

Preclinical evaluations of the efficacy and safety evaluation of novel TB vaccines are the most direct means of determining whether a vaccine can enter a clinical trial. Different types of novel TB vaccines have different preclinical evaluation methods, meaning there is no unified preclinical efficacy evaluation standard for novel TB vaccines.

Researcher who are preclinically evaluating BCG typically immunize tested animals with BCG, then infect them with MTB, and finally evaluate the number of bacteria and lesion index of liver, spleen, and lung MTB as well as animal survival within a certain period. However, the types of animals used, infected strains, doses of infection, and routes of infection vary. Similar to preclinical efficacy assessment for maternal BCG, the preclinical efficacy of the recombinant BCG vaccine VPM1002 was assessed as an aerosol infection of BALB/c or C57BL6 mice by H37Rv or Beijing/W genotype family clinical isolates. Bacteriological and immunological parameters of infected mice are assessed, such as lung bacterial load, type 1 and 17 cytokine responses, $\gamma\delta$ T cells, central memory cells (TCM), follicular helper T cells (TFH), and antimycobacterial antibodies [111–116]. Safety evaluations were simultaneously performed in mice (including RAG1^{-/-} immunodeficient mice [112], severely combined immunodeficiency mice [112, 116]), guinea pigs, rabbits, and non-human primates [108, 117] to assess rates of VPM1002 dissemination between the lungs, spleen, and other tissues as well as the ability to resist lymph node dissection [114, 116].

M72/AS01_E is one of the novel TB vaccines of subunit protein adjuvant that has been highly expected in clinical

trials. Its preclinical evaluation method differs from recombinant live vaccine VPM1002. Researcher of a mouse model evaluated the number of Mtb72F-specific CD8 T cells, secretion level of γ -interferon and granzyme B, and expression abundance of CD45RB and interleukin-7 receptor α chain on cell surfaces in the lungs of mice after immunization with M72/AS01_E vaccine [118]. In the guinea pig model, effects of the M72/AS01_E vaccine on survival rate, PTB granuloma, and airway remodeling and reconstruction were evaluated [119]. The rabbit model of tuberculous meningitis evaluates the protective effect of the M72/AS01_E vaccine on the central nervous system through clearance rates of TB in cerebrospinal fluid, leukocyte level in cerebrospinal fluid, and pathological damage in brain and lung, as well as changes in body weight [120]. For the cynomolgus monkey, the clinical parameters, pathology, long- and short-term survival rates, Th1 cytokines (IFN- γ , TNF, IL-2, and so on), and innate cytokine IL-6 changes were mainly evaluated [121].

However, there is a consensus that the international evaluation of preclinical efficacy and safety of novel TB vaccines using mouse, guinea pig/rabbit, and non-human primate three-level evaluation models is consistent, although evaluation of different animal types has its advantages and limitations (Table 2).

Clinical evaluation

Clinical trials are the only way to research and develop vaccines. Vaccines that have been preclinically evaluated need to undergo phase I, II, and III clinical trials before obtaining registration approval. It may even be necessary to design phase IV clinical trials to comprehensively evaluate the safety and efficacy of post-marketing vaccines in practical populations. The clinical evaluation of novel TB vaccines is based on TB incidence in phase III clinical results. Vaccine efficacy is evaluated by comparing the difference in TB incidence between the vaccine and placebo groups. The WHO required novel TB vaccines to have a protective power of at least 50%. Phase I and II clinical trials are a relatively simple means of evaluating the safety and immunogenicity of novel TB vaccines. However, due to long-term and delayed recurrence of MTB infection, efficacy evaluation of TB vaccines in phase III clinical trials typically requires a follow-up of at least 2–5 years for large samples.

The lack of intertrial harmonization or standardization for the clinical evaluation of different novel TB vaccine candidates (Table 3) precludes a direct comparison of those immunological outcomes. Researcher of phase I and phase II clinical studies of vaccines for neonates or uninfected individuals have tended to find that vaccine groups can induce cytokine production as well as enhancing anti-TB immune response. However, when clinically evaluating the LTBI adult prophylactic vaccine, immunized subjects were infected with MTB,

Table 2 Advantages and disadvantages of animal models in preclinical evaluation of TB vaccine

Animal model	Advantages	Disadvantages
Mouse	<ul style="list-style-type: none"> • Small size, easy to operate and low purchase and feeding costs [122]. • Rich in immunoassay reagents and has a clear genetic background and can be used to study the pathogenesis of TB [123, 124]. • Humanized mouse model can be constructed for the study of TB-HIV co-infection [125]. 	<ul style="list-style-type: none"> • Without obvious clinical manifestation of TB infection [126]. • Produces a weak late-type hypersensitivity reaction and induces TB granulomas, but the structure of TB granulomas is different from that of humans [127, 128]. • The sensitivity to MTB is low, and the pathological changes and bacterial load in the organs are uneven [129].
Guinea pig	<ul style="list-style-type: none"> • Susceptibility to MTB and low dose infection can cause significant TB lesions [130]. • Producing a strong delayed type hypersensitivity response and inducing caseous necrosis [130]. • Miliary nodules are observed in the lungs, liver, and spleen, and TB granulomas are very similar to humans [130, 131]. • Anti-TB drugs and vaccines respond well and are commonly used models for TB skin test assays [132]. 	<ul style="list-style-type: none"> • Lack of general clinical manifestations of TB infection [132]. • Limited availability of immune reagents [130, 132]. • Easy to form a wide range of diffuse lesions and cannot spontaneously latent infection [130].
Rabbit	<ul style="list-style-type: none"> • TB granulomas with caseous necrosis and liquefaction, and easy to form cavitation [133]. • Pathological model of bone TB, TB meningitis, and skin TB liquefaction can be constructed [134–139]. 	<ul style="list-style-type: none"> • Higher purchase and feeding costs [133]. • Limited availability of immune reagents [133]. • Easy to form haematogenous spread and death [133].
Non-human primate	<ul style="list-style-type: none"> • General clinical manifestations of TB are consistent with humans (low fever, emaciation, cough, and dyspnea) [140–143]. • Can mimic LTBI and various forms of active TB [144, 145]. • Structure of TB granuloma is similar to human [142]. 	<ul style="list-style-type: none"> • Higher purchase and feeding costs and containment facilities [142]. • Limited availability of immune reagents [142]. • High variation within groups, making it difficult to evaluate the effectiveness of vaccines [142].

and sensitization states and immune markers of the body were found to differ from those who were uninfected. A series of immune indicators commonly used to evaluate vaccines for neonates or uninfected individuals have been positive prior to vaccination. Whether changes in these indicators following vaccination can be applied to the clinical evaluation of LTBI anti-vaccination vaccines remains unknown. Therefore, it is even more rare if a standard set of parameters allows accurate comparisons between studies and vaccines to determine whether this is a reproducible in clinical trials. At present, novel vaccines for TB in phase III clinical trials are MIP/Immuvac, Vaccae™, and VPM1002, and no relevant research data have been published [146, 147].

Challenges of researching TB vaccines

The ideal TB vaccine will have a strong protective efficacy, stable and long-lasting immunogenicity, and no adverse reactions. It would prevent TB by single or several immunizations. However, there is no vaccine that is more protective or sustainable than BCG. While some novel vaccine candidates have achieved certain effects in animal models, how to apply them to humans and whether they can replace BCG still requires a long period of experimentation, follow-up, and demonstration. MVA85A is the first new TB vaccine to receive much attention in the past 90 years. In preclinical animal models, MVA85A was found to significantly enhance BCG

immunogenicity priming in guinea pigs, rhesus monkeys, and cattle [148]. Its safety and tolerability have also been validated in phase I and phase IIa clinical trials in healthy adults and TB- and HIV-infected infants, children, and adolescents [40, 149–156]. However, in a phase IIb clinical trial conducted in South Africa in 2013, it was found that healthy infants who had been vaccinated with BCG and immunized with MVA85A did not have additional protection [157]. This result was a great disappointment to both the TB vaccine research community and funders and a call to action for researchers in basic and applied vaccine development. At the same time, this lesson suggests that some of the results obtained in preclinical animal models do not align with results of clinical trials. Therefore, the development of novel TB vaccines continues to face many challenges.

- (1) Lack of clear protective antigens: MTB has approximately 4000 genes encoding thousands of proteins [158]. The proteins used in TB vaccine design are mainly proliferation-associated antigens (that is, six of the eight subunit vaccines entering clinical studies contain Ag85A or Ag85B proteins). Critical and unresolved questions in TB vaccinology are how to select the best antigens and how many antigen to include in a vaccine. However, limited clinical trial data makes it difficult to support the effectiveness of this antigen or any other single antigen against TB. It is therefore necessary to select more effective antigens to construct a vaccine from the

Table 3 Clinical outcome measures for novel TB vaccine

Vaccine candidate	Phase	Start and completion date	Enrollment	Subjects	Route	Primary outcome measures	Secondary outcome measures
A45 Ag85A	I	Jun. 2009–Jul. 2013	48	Healthy BCG-vaccinated adults	IM	<ul style="list-style-type: none"> Local and systemic symptoms and laboratory toxicity. Number of participants reporting AEs. 	<ul style="list-style-type: none"> The level and quantity of Ag85A-specific T cells be measured by interferon ELISA and ELISPOT assay. Ag85A-specific T cells in BAL and WB be measured by interferon ELISPOT, cytokine production, and ICS assay. Immunogenicity of inhaled administration compared with IM. Immune responses measured from induced sputum compared with BAL. Number of participants developing a positive interferon release assay for TB. Immune response to vaccine correlated with dose and preexisting anti-adenoviral antibodies. Systemic and mucosal cellular immunogenicity by comparing laboratory markers of cell mediated immunity in WB.
ChAdOx1 85A-MVA85A	I	Jul. 2013–Apr. 2016	42	Healthy BCG-vaccinated adults	IM	<ul style="list-style-type: none"> Solicited and unsolicited AEs be recorded for the duration of the study. 	<ul style="list-style-type: none"> Characterization of cell mediated and humoral immune markers. Antibodies to Ag85A in serum. B-cells and neutrophils surface cell markers. Gene expression array and MTB killing assay. None
TB/FLU-04L	I	Oct. 2013–Feb. 2015	36	Healthy BCG-vaccinated adults	Inhal	<ul style="list-style-type: none"> Frequency, incidence, and nature of AEs and SAEs. Immediate reactions occurring within 2 h of administration of any dose. 	<ul style="list-style-type: none"> Antibodies to Ag85A in serum. B-cells and neutrophils surface cell markers. Gene expression array and MTB killing assay. None
AEC/BC02	I	Apr. 2018–	135	Healthy adults	IM	<ul style="list-style-type: none"> Solicited and unsolicited AEs be recorded for the duration of the study. T cell IFN-γ ELISPOT response to antigen Ag85A. The number of participants with skin and local AEs measured by vital signs, routine blood, liver and kidney function, electrocardiography, and chest X-ray detection. Number and percentage of participants with AEs or SAEs. 	<ul style="list-style-type: none"> IFN-γ and antibody level be measured by ICS in WB before and after the immune.
H56:IC31	I	Nov. 2011–Jun. 2013	25	LTBI adults	IM	<ul style="list-style-type: none"> Number and percentage of participants with AEs or SAEs. 	<ul style="list-style-type: none"> Percent Ag85B and ESAT6-specific T cells in WB be measured by ICS. IFN-γ ELISPOT response for Ag85B.

Table 3 (continued)

Vaccine candidate	Phase Start and completion date	Enrollment	Subjects	Route	Primary outcome measures	Secondary outcome measures
I	Nov. 2014–Oct. 2016	22	PTB adults	IM	<ul style="list-style-type: none"> Solicited and unsolicited AEs or SAEs. 	<ul style="list-style-type: none"> Positive for MTB infection on Day 210 be measured by QFT. Changes of CD4 T-cell in PBMC be measured by ICS Assay.
Ib	May. 2015–Dec. 2016	84	Healthy adolescents	IM	<ul style="list-style-type: none"> Number and percentage of participants with AEs or SAEs. Specific CD4+ and CD8+ T-cell responses using the ICS assay. 	<ul style="list-style-type: none"> Humoral response measured by multiplex antibody assay or ELISA. Transcriptional analysis of antigen-stimulated PBMCs. Changes of lymphocyte, dendritic cells, monocytes, and granulocytes. MHC-restricted T-cell responses (CD1-restricted and MR1-restricted). T-cell response be measured by ICS and ELISPOT assay.
I/IIa	Aug. 2013–Nov. 2015	98	LTBI adults	IM	<ul style="list-style-type: none"> Solicited and unsolicited AEs or SAEs. 	<ul style="list-style-type: none"> T-cell response be measured by ICS and ELISPOT assay.
II	Jan. 2019–	900	PTB adults	IM	<ul style="list-style-type: none"> Rate of TB disease relapse or reinfection was confirmed by sputum culture. 	<ul style="list-style-type: none"> Number and percentage of participants with AEs or SAEs. Antigen specific cell mediated immune responses by whole blood ICS assay. Humoral immune responses by IgG ELISA of plasma samples.
ID93 + GLA-SE	I Aug. 2012–May. 2014	60	Healthy adults	IM	<ul style="list-style-type: none"> Safety and tolerability of ID93 alone or in combination with GLA-SE. 	<ul style="list-style-type: none"> ID93 + GLA-SE compared with ID93 alone by evaluating IgG antibody and T-cell responses at specified timepoints.
I	Oct. 2015–Aug. 2017	70	Healthy adults	IM	<ul style="list-style-type: none"> Number of subjects experiencing solicited injection site and systemic AEs. 	<ul style="list-style-type: none"> IgG antibody responses to ID93. Percentage of CD4 and CD8 T cells producing IFN-γ, TNF, and IL-2 measured by ICS. Th1 and Th2 cytokine production in PBMCs.
I	Oct. 2018–	48	Healthy adults	IM	<ul style="list-style-type: none"> Solicited and unsolicited AEs and SAEs be recorded for the duration of the study. 	<ul style="list-style-type: none"> IgG antibody response rate and magnitude. Number of IFN-γ and IL-10 cytokine secreting cells in PBMCs be measured by ELISPOT. Percentage of CD4 and CD8 T cells producing IFN-γ, TNF, and IL-2 in PBMCs measured by ICS.
I	Apr. 2019–	36	Healthy BCG-vaccinated adolescents	IM	<ul style="list-style-type: none"> Solicited and unsolicited AEs and SAEs be recorded for the duration of the study. 	<ul style="list-style-type: none"> Humoral and cellular responses to ID93 + GLA-SE at specified timepoints.
Ib	Sep. 2013–Jul. 2015	66	Healthy BCG-vaccinated adults	IM	<ul style="list-style-type: none"> Solicited and unsolicited AEs and SAEs be recorded for the duration of the study. 	<ul style="list-style-type: none"> Humoral and cellular responses to ID93 + GLA-SE at specified timepoints.

Table 3 (continued)

Vaccine candidate	Phase Start and completion date	Enrollment	Subjects	Route	Primary outcome measures	Secondary outcome measures
Ila	Jun. 2015–Jan. 2017	60	PTB adults	IM	<ul style="list-style-type: none"> Solicited and unsolicited AEs and SAEs be recorded for the duration of the study. Solicited and unsolicited AEs and SAEs be recorded for the duration of the study. 	<ul style="list-style-type: none"> Humoral and cellular responses to ID93 + GLA-SE at specified timepoints. Humoral and cellular responses to ID93 + GLA-SE at specified timepoints. Positive response rate for latent tuberculosis infection from QFT assay. Anti-MTB M72-specific antibody concentrations. Number of seroconverted subjects for M72-specific antibody. Frequency of M72-CD4⁺ and CD8⁺T-cells expressing IL-2, IFN-γ, TNF-α, and CD40-L. Concentrations of IFN-γ produced in serum samples.
M72/AS01 _E (GSK 692342)	Nov. 2006–Dec. 2009	110	Healthy adults	IM	<ul style="list-style-type: none"> Number of subjects with grade 3 solicited local or general symptoms and unsolicited AEs or SAEs. Number of subjects with normal or abnormal hematological and biochemical levels. Levels of C-reactive protein and immunoglobulin E. 	<ul style="list-style-type: none"> Anti-MTB M72-specific antibody concentrations. Number of seroconverted subjects for M72-specific antibody. Frequency of M72-CD4⁺ and CD8⁺T-cells expressing IL-2, IFN-γ, TNF-α, and CD40-L. Concentrations of IFN-γ produced in serum samples.
II	Feb. 2008–Dec. 2008	45	Healthy adults	IM	<ul style="list-style-type: none"> Number of subjects with solicited local or general symptoms and unsolicited AEs or SAEs. Hematological and biochemical levels. 	<ul style="list-style-type: none"> Cytokine expression by M72-specific CD4⁺/CD8⁺ T cells by flow cytometry. Antibody titers to M72 measured by ELISA.
II	Jun. 2008–May. 2009	37	HIV ⁺ adults	IM	<ul style="list-style-type: none"> Number of subjects with solicited local or general symptoms and unsolicited AEs or SAEs. Number of subjects with normal or abnormal hematological and biochemical levels. 	<ul style="list-style-type: none"> Anti-MTB M72-specific antibody concentrations. Frequency of M72-CD4⁺ and CD8⁺T-cells expressing IL-2, IFN-γ, TNF-α, and CD40-L. Number of subjects with significant highly active antiretroviral therapy changes.
II	Apr. 2008–Apr. 2009	180	Healthy adults	IM	<ul style="list-style-type: none"> Number of subjects with grade 3 solicited local or general symptoms and unsolicited AEs or SAEs. Number of subjects with different biochemical and hematological levels. 	<ul style="list-style-type: none"> Anti-MTB M72-specific antibody concentrations. Frequency of M72-CD4⁺ and CD8⁺T-cells expressing IL-2, IFN-γ, TNF-α, and CD40-L.
II	Dec. 2009–Sep. 2010	60	Healthy adolescents	IM	<ul style="list-style-type: none"> Number of subjects with grade 3 solicited local or general symptoms and unsolicited AEs or SAEs. Number of subjects with normal or abnormal hematological and biochemical levels. 	<ul style="list-style-type: none"> Anti-MTB M72-specific antibody concentrations. Frequency of M72-CD4⁺ and CD8⁺T-cells expressing IL-2, IFN-γ, TNF-α, and CD40-L.
II	Nov. 2011–Apr. 2014	142	TB adults	IM	<ul style="list-style-type: none"> Number of subjects with grade 3 solicited local or general symptoms and unsolicited AEs or SAEs. 	<ul style="list-style-type: none"> Anti-MTB M72-specific antibody concentrations.

Table 3 (continued)

Vaccine candidate	Phase	Start and completion date	Enrollment	Subjects	Route	Primary outcome measures	Secondary outcome measures
II		Jan. 2011–Jun. 2015	240	HIV ⁺ adults	IM	<ul style="list-style-type: none"> Number of subjects with grade 3 solicited local or general symptoms and unsolicited AEs or SAEs. Number of subjects with grade 3 and 4 hematological and biochemical levels. 	<ul style="list-style-type: none"> Number of seroconverted subjects for M72-specific antibody. Frequency of M72-CD4⁺ and CD8⁺T-cells expressing IL-2, IFN-γ, TNF-α, and CD40-L. Anti-MTB M72-specific antibody concentrations. Number of seroconverted subjects for M72-specific antibody. Frequency of M72-CD4⁺ and CD8⁺T-cells expressing IL-2, IFN-γ, TNF-α, and CD40-L.
IIb		Aug. 2014–Nov. 2018	3 595	LTBI adults	IM	<ul style="list-style-type: none"> Incident cases of definite pulmonary TB disease not associated with HIV-infection. 	<ul style="list-style-type: none"> Incident cases of definite Xpert MTB/RIF pulmonary TB disease not associated with HIV-infection. Occurrence of solicited local and general AEs. Frequency of M72-specific CD4⁺/CD8⁺ T cells expressing TNF-α, IFN-γ, IL-2, and CD40L. M72-specific antibody titers and seropositivity rates. Occurrence of grade ≥ 2 hematological and biochemical levels in the safety and immune sub-cohort.
RUTI®	I	Apr. 2007–Jun. 2008	24	Healthy adults	IM	<ul style="list-style-type: none"> Visual analogic scale pain score. Occurrence, intensity, and relationship to vaccination of local and systemic AEs. 	<ul style="list-style-type: none"> Evaluation of the immunogenicity of the different doses of the vaccine tested.
	II	Jun. 2010–May. 2011	95	HIV ⁺ and LTBI adults	IM	<ul style="list-style-type: none"> Focal and systemic tolerability: site of injection for redness, pain, swelling, and induration, hilar lymph nodes for inflammation, ECG parameters, and other vital Signs and physical examination. 	<ul style="list-style-type: none"> Cellular mediated immunity be measured by ELISPOT assay. Antibody-mediated immunity against MTB antigens.
	II	Jan. 2019–	27	MDR-TB adults	IM	<ul style="list-style-type: none"> Safety evaluation be measured by physical examination, SAEs, routine laboratory, and chest radiography. 	<ul style="list-style-type: none"> IFN-γ release and MTB growth inhibition assay of PBMCs in an ex vivo system.
DAR-901 booster	I	Feb. 2014–Jun. 2016	59	BCG vaccinated-HIV ⁺ adults	IM	<ul style="list-style-type: none"> Solicited and unsolicited injection site and systemic AEs. 	<ul style="list-style-type: none"> Cellular and humoral responses to the vaccine antigen.

Table 3 (continued)

Vaccine candidate	Phase	Start and completion date	Enrollment	Subjects	Route	Primary outcome measures	Secondary outcome measures
MIP/Immuvac	IIb	Mar. 2016–	650	Healthy BCG-vaccinated adolescents	IM	<ul style="list-style-type: none"> New infection with MTB based on conversion of IGRA. 	<ul style="list-style-type: none"> New positive IGRA that is also positive on repeat ≥ 3 months later.
	III	Mar. 2005–Mar. 2011	1020	Category-II PTB adults	IM	<ul style="list-style-type: none"> Clinical AEs. The time of sputum conversion, cure rate and relapse in patients of category-II PTB. 	<ul style="list-style-type: none"> The patient's and physician's global assessment of the clinical cure.
	III	Mar. 2007–Mar. 2012	300	Category-I PTB adults	IM	<ul style="list-style-type: none"> Clinical AEs. The time of sputum conversion, cure rate, and relapse in patients of category-I PTB. 	<ul style="list-style-type: none"> The patient's and physician's global assessment of the clinical cure.
Vaccae™	III	Oct. 2013–Nov. 2017	10,000	LTBI adults	IM	<ul style="list-style-type: none"> TB incidence after injection of Vaccae™. 	<ul style="list-style-type: none"> Lesion degree (bacteriology indicators, cavity) of patients. Systemic and local reactions and AEs. The relation between skin test results and paroxysm of TB-PPD.
MTBVAC	I	Jan. 2013–Nov. 2014	36	Healthy adults	ID	<ul style="list-style-type: none"> Safety and reactogenicity be measured by hematological and biochemical. 	<ul style="list-style-type: none"> Kinetics of CD4 T-cell responses by tracking the expression of IFNγ, TNFα, and IL-2.
	I	Sep. 2015–Mar. 2018	54	Healthy infants and adults	ID	<ul style="list-style-type: none"> Injection site, systemic, and regional AEs. 	<ul style="list-style-type: none"> Frequencies and co-expression patterns of CD4 and CD8 T cells expressing specific cytokines in infants WB.
BCG revaccination	Ib/Ila	Jan. 2019–	120	LTBI adults	ID	<ul style="list-style-type: none"> Solicited and unsolicited AEs and SAEs in injection site, systemic, and regional. 	<ul style="list-style-type: none"> CD4 and CD8 T cells were measured by ICS assay. Percentage conversion and reversion rates from QFT assay.
	Ila	Feb. 2019–	99	Newborn infants	ID	<ul style="list-style-type: none"> Solicited and unsolicited AEs and SAEs in injection site, systemic, and regional. 	<ul style="list-style-type: none"> CD4 and CD8 T cells expressing specific cytokines in WB.
BCG revaccination	I	Oct. 2010–Jul. 2013	82	LTBI adults	ID	<ul style="list-style-type: none"> Adverse reactions/events. Direct Ex-vivo gene expression. Effector and memory T cell function be measured by ICS or ELISA. Precursor frequencies of MTB antigen-specific T cells be measured by ELISPOT. 	<ul style="list-style-type: none"> None
	IIb	Oct. 2019–	1800	Healthy BCG-vaccinated adolescents	ID	<ul style="list-style-type: none"> Number of participants with sustained QFT conversion from a negative to positive test (cut-off value of 0.35 IU/mL). 	<ul style="list-style-type: none"> Number of participants with solicited and unsolicited AEs, SAEs, and serious adverse drug reactions.

Table 3 (continued)

Vaccine candidate	Phase Start and completion date	Enrollment	Subjects	Route	Primary outcome measures	Secondary outcome measures
VPM1002	I Sep. 2008–Dec. 2009	80	Healthy BCG-vaccinated male	ID	<ul style="list-style-type: none"> Safety and tolerability be measured by physical examination, vital signs, ECG, liver sonography, chest X-ray, hematology, coagulation, clinical chemistry, and urinalysis. 	<ul style="list-style-type: none"> Primary QFT conversion from a negative to positive test (cut-off value of 0.35 IU/mL and 4 IU/mL). ELISA and ELISPOT for the number of IFN-γ secreting PBMCs after stimulation with PPD. IFN-γ, TNF-α, and IL-2 in CD4 and CD8 T cells be measured by ICS. Serum antibodies against PPD or Ag85B. ELISA and ELISPOT for the number of IFN-γ secreting PBMCs after stimulation with PPD. IFN-γ, TNF-α and IL-2 in CD4 and CD8 T cells be measured by ICS. Serum antibodies against PPD or Ag85B.
Ib	Apr. 2010–Mar. 2011	24	Healthy adults	ID	<ul style="list-style-type: none"> Safety and tolerability be measured by physical examination, vital signs, electrocardiogram, liver sonography, and laboratory safety parameters. 	<ul style="list-style-type: none"> ELISA and ELISPOT for the number of IFN-γ secreting PBMCs after stimulation with PPD. IFN-γ, TNF-α and IL-2 in CD4 and CD8 T cells be measured by ICS. Serum antibodies against PPD or Ag85B.
II	Nov. 2011–Nov. 2012	48	Newborn infants	ID	<ul style="list-style-type: none"> Safety and tolerability be measured by physical examination, vital signs, and standard laboratory safety parameters, including hematology, clinical chemistry, and urinalysis. 	<ul style="list-style-type: none"> Concentration of IFN-γ after WB stimulation was measured by ELISA. Number of CD4 and CD8 T cells and was measured by ICS assay. None
II	Jun. 2015–Nov. 2017	416	HIV ⁺ newborn infants	ID	<ul style="list-style-type: none"> The incidence of grade 3 and 4 adverse drug reactions and IMP-related ipsilateral or generalized lymphadenopathy of 10 mm or greater. 	<ul style="list-style-type: none"> Percentage of overall TB recurrence either bacteriologically confirmed or clinically diagnosed. Safety assessed by solicited local and regional reactogenicity.
II/III	Dec. 2017–	2000	PTB adults	ID	<ul style="list-style-type: none"> Percentage of bacteriologically confirmed TB recurrence cases. 	<ul style="list-style-type: none"> Percentage of overall TB recurrence either bacteriologically confirmed or clinically diagnosed. Safety assessed by solicited local and regional reactogenicity.

IM intramuscular, *Inhal* inhaled, *ID* intradermal, *AEs* adverse events, *SAEs* serious adverse events, *BAL* bronchoalveolar lavage, *WB* whole blood, *PBMCs* peripheral blood mononuclear cells, *MHC* major histocompatibility complex, *PTB* pulmonary tuberculosis, *LTBI* latent tuberculosis infection, *HIV* human immunodeficiency virus, *ELISA* enzyme-linked immunosorbent assay, *ELISPOT* enzyme-linked immunosorbent assay, *ICS* intracellular cytokine staining, *QFT* QuantiferON[®]-TB Gold Plus, *IGRA* interferon- γ release assays

- mechanism of MTB infection and so induce more effective protective immunity [159].
- (2) Lack of credible preclinical evaluation indicators: In early evaluation of immunogenicity, the novel TB vaccine mainly detects specific antibody titers induced by the vaccine, different types of cytokines produced by CD4⁺ and CD8⁺, and other T cells following immunization [160]. However, the correlation between these specific immune cells or cytokines in the host's immune protection against natural TB infection needs further study [161].
 - (3) Lack of suitable vaccine evaluation animal model: Animal experiments have provided evaluation of vaccines in terms of immunogenicity, safety, and efficacy. Mouse models have the advantages of low price, strong viability, and easy access to detection reagents, meaning they are the first choice for the evaluating the preliminary effects of novel vaccines [162]. However, mouse models do not accurately reflect the authenticity of vaccine protection, especially when studying the long-acting immune protection response. Therefore, the use of mouse models to evaluate the effect of novel TB vaccines may result in a deviation from the actual clinical application effect [163]. In addition, evaluation results from the same vaccines in different animal models were inconsistent, with mice and guinea pigs achieving good immunoprotective effects, which failed in non-human primates [164]. Infection and disease manifestations of MTB vary from species to species, and immune response following vaccination is more variable in humans [23]. Therefore, differences between humans and animals may be one of the main factors affecting the predictive ability of animal models.
 - (4) Lack of emulated exposure methods: A significant difference exists in the nature of exposure between animal models and natural human infections. In preclinical animal models, MTB are initially infected by high-dose, single-strain, single exposure through various routes of attack (such as aerosol, tail vein, or intraperitoneal injection). However, in natural conditions, humans often experience multiple low doses, and infections are established following exposure to different strains [165].
 - (5) Lack of a unified clinical trial endpoint criteria: Reducing the incidence of TB and improving the disease status of patients are the main criteria for evaluating TB vaccines. In preclinical animal models, the effectiveness of vaccines tends to be assessed by improvements in organ bacterial counts, histopathological damage, and time of animal death. In contrast, scientists use TB incidence as an end point of human clinical trials. Obviously, there is a fundamental difference and lack of relevance between preclinical trials and clinical trials in determining the endpoint of vaccine effectiveness.
 - (6) Lack of consistent evaluation environment: Unlike in a single laboratory environment, there may be multiple uncertainties in a human clinical test environment, such as diet, nutritional status, NTM, worm co-infection, and host genetic heterogeneity on susceptibility to TB. In contrast, preclinical animal models tend to use specific pathogen-free animals with a consistent genetic background.
 - (7) Immunization population is complicated: When compared with other infectious disease vaccines, a difficulty in researching novel TB vaccines is that the immune status of the vaccination population or infection background is complicated. The widespread vaccination of BCG and NTM infections in countries that have high rates of TB further exacerbates the complexity of vaccination populations. In particular, whether a vaccination with a strong immunogenic vaccine in the LTBI population leads to a Koch response is a safety issue that cannot be ignored. Therefore, those who are BCG naïve, negative, or maintain positive after BCG vaccination and LTBI need to be accurately differentiated and accurately immunized to optimize vaccination effectiveness.

The future of TB vaccine research

Since the first use of BCG, there have been controversies about its advantages and disadvantages, including its safety, sensitivity to the diagnostic reagent tuberculin, and especially the failure of some BCG vaccination trials. Novel TB vaccines, which provide effective, sustained protection, have always been an international priority. Especially in recent years, given the advantages of novel vaccine research technology, new methods and ideas for various vaccine research have been established. A large number of different vaccine candidates and their advanced stages in clinical development denote a unique and exciting phase in TB vaccine research. There are also a large number of novel vaccine candidates in preclinical development, including more recently developed vaccine formats such as DNA vaccines, new adjuvants and delivery systems, and combination vaccines. In summary, the development of novel TB vaccines remains mostly focused on the following regimens:

- (1) Priming vaccine: Developing a new vaccine with longer lasting immunoprotective properties and better protection than BCG to replace the primary vaccination of BCG.
- (2) Booster vaccine following BCG vaccination: Performing short- or long-term immunization and heterologous or homologous booster to enhance or prolong BCG priming protective effects, improving immunogenicity of BCG,

and making up for the shortcomings of BCG long-term effects.

- (3) Latent infection preventive vaccine: Used to inhibit endogenous re-ignition of infected individuals with MTB while preventing exogenous re-infection.
- (4) Therapeutic vaccine: As an effective supplement to conventional chemotherapy, which can reduce lesions, promote sputum MTB negative and cavity closure, shorten the course of treatment, consolidate the effect of chemotherapy, and promote the outcome of the disease, effectively treating drug-resistant TB.

Conclusion

TB remains one of the greatest threats to global health, meaning the importance of effective and safer vaccines for controlling this epidemic is undisputed, particularly for MDR-TB and TB-HIV co-infection. In the past 30 years, TB vaccines have developed greatly, and vaccine candidates for different age groups and different forms of TB have emerged. Despite disappointing results from some of the clinical efficacy trials, these experiences are sufficient to remind us to revisit the design and evaluation of TB vaccines. Today, we are witnessing immense progress in both preclinical and clinical TB vaccine research. With the continuous coordination and cooperation of the TB vaccine community, building a world free of TB may be just around the corner.

Author contribution Junli Li, Chuan Qin, and Lingjun Zhan conceived the project direction; Junli Li, Jun Tang, and Yanan Shi researched and collated all the relevant literature; Aihua Zhao and Guozhi Wang revised and edited the manuscript; Junli Li wrote the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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