Review article

CLINICAL AND EXPERIMENTAL VACCINE RESEARCH

Clin Exp Vaccine Res 2024;13:184-201
https://doi.org/10.7774/cevr.2024.13.3.184
pISSN 2287-3651 • eISSN 2287-366X

The BCG vaccine, advantages, and disadvantages of introducing new generation vaccines against *Mycobacterium tuberculosis*

Marzie Mahdizade Ari[1,*], Masoumeh Beig[2,*], Mohammad Sholeh[2], Majid Khoshmirsafa[1]

[1]Department of Microbiology, School of Medicine, Iran University of Medical Sciences, Tehran; [2]Department of Bacteriology, Pasteur Institute of Iran, Tehran, Iran

Received: October 29, 2023
Revised: March 22, 2024
Accepted: March 29, 2024

Corresponding author: Majid Khoshmirsafa, PhD
Department of Immunology, Faculty of Medicine, Iran University of Medical Sciences, Tehran, Iran
Tel: +98-21-8670-3282, Fax: +98-21-8670-3282
E-mail: khoshmirsafa@miums.ac.ir

*These two authors contributed equally to this work as the first authors.

No potential conflict of interest relevant to this article was reported.

We want to thank the personnel of the Department of Microbiology of Iran University of Medical Sciences and the Bacteriology Department of the Pasteur Institute of Iran for their assistance.

Tuberculosis (TB) is consistently ranked among the deadliest diseases worldwide, causing millions of deaths annually. *Mycobacterium tuberculosis* is the causative agent for this infection. Different antibiotics and vaccines have been discussed as potential treatments and prevention. Currently, there is only one licensed vaccine against TB, Bacillus Calmette-Guérin (BCG). Despite its protective efficacy against TB in children, BCG has failed to protect adults against pulmonary TB, lacks therapeutic value, and can cause complications in immunocompromised individuals. In this review, BCG, the most widely administered vaccine, is discussed, and the newest vaccines available in medicine are discussed. Based on the restrictions that prevent optimal BCG efficacy and the vaccines that are now being tested in various clinical studies, some criteria need to be considered in designing future vaccines.

Keywords: Tuberculosis, *Mycobacterium*, *Mycobacterium bovis*, Subunit vaccines, Synthetic vaccines

Introduction

Recent reports indicate that one-third of the world's population (approximately 1.5 billion people) is infected with *Mycobacterium tuberculosis* (Mtb). It is also one of the top 10 causes of mortality worldwide. This bacterium has been reported in all ages and geographical areas; however, most people with active disease are adults (90%), and it is more common in men than in women (2:1 ratio). The most recent statistical report in 2018 indicated that 57% of males, 32% of women, and 11% of children were infected with Mtb, whereas 8.6% of patients had human immunodeficiency virus (HIV). Drug therapy is associated with a poor response due to the complexity and long duration of treatment protocols. This poor response to treatment seems to be a significant cause of multidrug-resistant (MDR) strains. Approximately 50 million cases of MDR-type Mtb have been reported. Although many people are exposed to Mtb, only a few develop active disease. Among the people considered infected or with a history of exposure to Mtb, the disease progresses to the active form in only 10% of the population. According to the World Health Organization (WHO), in people who have suffered from both HIV and Mtb simultaneously, progression to active disease is more complicated than in people who are only affected by Mtb.

According to reports published in 2004, Mtb infection is most frequently reported in developing regions such as Asia (especially Russia, China, and India, with approxi-

mately 2 million people with active Mtb disease) and Africa [1]. Mtb has proliferative and active metabolic activities, and upon entry into the lung alveoli, Mtb is phagocytosed by alveolar macrophages and dendritic cells. Compared to most bacterial pathogens, Mtb can survive in phagocytes and inhibit phagosome-lysozyme maturation in the early stages (Fig. 1). Dendritic cells then transfer Mtb to the lymph nodes, where T lymphocyte stimulation occurs. Usually, the disease cannot be eradicated in patients infected with Mtb because of the restriction of Mtb to granulomas in the lungs. Granuloma is an immune response that prevents Mtb spread [2,3]. Although a protective immune response cannot wholly eliminate Mtb infection, it prevents the disease in approximately 90% of the infected individuals. This protective immune response causes granuloma lesions at the site of mycobacterial accumulation where Mtb is latent. Mycobacteria in the latent phase are characterized by inactivity, reduced metabolic levels, and proliferation. Mtb is resistant to immune responses [4]. Attenuation of the immune system leads to Mtb reactivation during the latent tuberculosis infection (LTBI) phase, leading to active tuberculosis (TB). Although the immune system destroys Mtb, it appears less resistant to LTBI. Granulomas occur with caseous necrosis. Host cell death and tissue destruction favor Mtb growth and expansion. Damage to lung tissue leads to symptoms such as circulation, sweat, fever, and severe cough [5].

The Immune Response in Patients with Tuberculosis

The immune response to Mtb is related to the activation of type 1 T helper (Th1) responses and the production of a range of cytokines such as tumor necrosis factor-alpha (TNF-α) and interferon-gamma (IFN-γ). Genetic defects, such as mutations in receptors for cytokines such as interleukin-12 (IL-12) or IFN-γ, enhance the differentiation of Th1 cells, resulting in

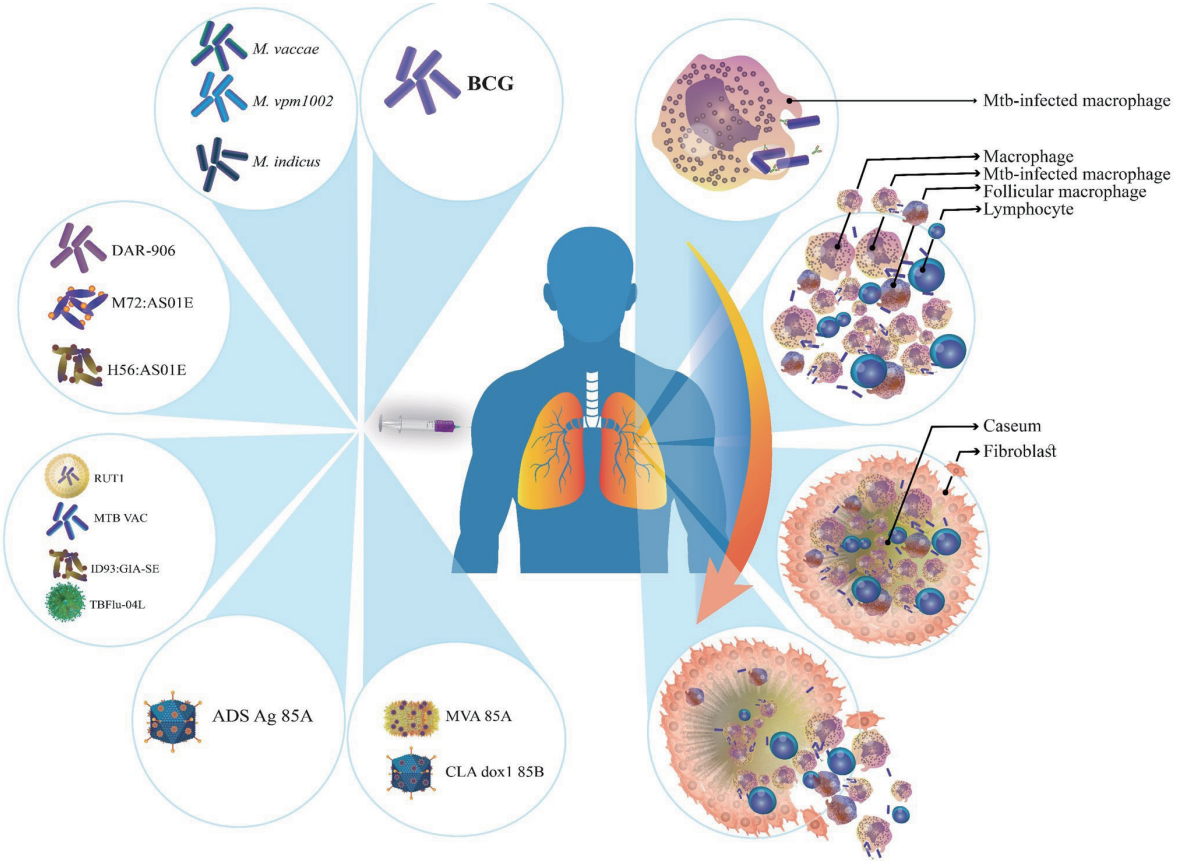

Fig. 1. *Mycobacterium tuberculosis* (Mtb) pathogenesis cycle. Mtb entrance in macrophage, granuloma formation, and Mtb release into the respiratory tract or airway (right); licensed and recruiting vaccines (left). Following the phagocytosis of Mtb by alveolar macrophage cells, granuloma formation has occurred with Mtb in infected macrophages surrounded by different immune cells (e.g., mononuclear phagocytes and lymphocytes). BCG, Bacillus Calmette-Guérin.

increased susceptibility to progressive mycobacterial disease. In addition, the neutralization of TNF-α by monoclonal antibodies (such as those used in treating rheumatoid arthritis) can prevent LTBI reactivation. Hence, it is reasonable to develop vaccines that enhance Th1 protective response. This has led to the production of vaccines against other infections, such as varicella virus infection, which causes chickenpox. In the last century, there has been a dramatic increase in the design of new vaccines against Mtb. Although vaccines can elicit an immune response that helps reduce bacterial load in the lungs, they do not entirely eradicate the disease.

Th1 cells are thought to be insufficient in patients with TB because peripheral T cells often respond weakly to Mtb-derived antigens. Recently, it was suggested that this problem may be due to regulatory T-cell activity [6].

Patients with TB and those who are anergic show an adverse reaction to tuberculin skin testing, T cells cytokines such as IL-10, and express small amounts of IL-2 or IFN-γ against Mtb. Many T cells in the lungs of TB patients secrete IL-10, which may be Treg cells. These cells also express both T-bet and forkhead box P3 (FOXP3) [7]. Although patients with TB are likely to develop IL-10-producing Tregs, the importance of these Tregs remains unknown. Most TB patients show significant amounts of IFN-γ, recruitment of T cells to the infection site, and their considerable destruction, leading to the formation of cavitation (referred to as the cavity in the lung, which contains large amounts of bacilli and releases them into the respiratory tract) and facilitates bacterial proliferation [8]. Thus, although the importance of Treg cells cannot be ignored, most TB cases do not appear to result from the suppression of Th1 response activity by over-regulation of regulatory T cells. Overexpression of regulatory T cells in the early stages of infection may lead to the delayed development of Th1 cell activity, as has been observed in malaria. Although malaria proliferates very quickly and has a delayed Th1 cell response, it appears to be less similar to TB, which proliferates more slowly, especially in developing countries, where the Th1 response is enhanced by other mycobacterial spp. [9].

Mtb latent disease

Following exposure to Mtb, the Th1 response increased. The ability of T lymphocytes to release IFN-γ in response to Mtb antigen has been used as an indicator of Mtb exposure. In 90% of infected people, this response causes the bacilli to enter the latent phase, resulting in no symptoms of the disease. A study was designed using *in situ* polymerase chain reaction (PCR) to find DNA of the Mtb organism in the tissues of people who were killed by accident or by heart attack or who had no signs of TB. In an investigation of tissues from endemic areas of TB patients, Mtb DNA was frequently found histologically in healthy tissues. In comparison, no DNA was found in samples from people like Norway, which is not endemic for TB [10].

Association of humoral response with cellular immune response

Specific Mtb antibodies and professional phagocytes must be treated to provide complete protection against Mtb entering alveolar lungs. Antibodies can eliminate Mtb by disrupting metabolism, such as inhibiting the absorption of essential nutrients. These antibodies can facilitate Mtb removal and induce mechanical mechanisms such as respiratory explosion and toxic oxygen and nitrogen species formation in professional phagocytes [11]. Antibodies prevent Mtb uptake by epithelial cells, thus making them a safe niche for Mtb uptake. It is believed that this initial attack could eradicate all organisms that have not yet established a primary infection. In such cases, the immune response can inhibit the Mtb infection. Although many Mtb organisms may escape the first line of immune defense through antibodies, they require T lymphocytes to eliminate Mtb from the macrophages. When Mtb can escape from the second line of defense of the immune system, it can stabilize LTBI and enter the latent phase. The coordinated performance of the adaptive immunity is essential for latent Mtb liquidation. Achieving this goal is challenging because latent Mtb is resistant to immune responses. This situation is further complicated by factors such as co-infection with worms. Infection with worms diverts the immune response to type 2 T helper (Th2), limiting and weakening Th1 protective reactions. HIV infection complicates this situation because it directly destroys TCD4 lymphocytes. Mtb organisms can remain in the latent phase for long periods. However, these bacteria may become reactive when the body has a weakened immune response [11].

Impact of IL-4 on TB Disease and Mortality

Although an investigation of Mtb *in vivo* has shown that IL-4 gene deletion has a low effect on bacterial growth pre-immunization of BALB/c mice, IL-4 responses induced before Mtb infection can lead to an increase in injury and mortality [12]. Following deletion of the IL-4 gene, significant decreases in

 https://doi.org/10.7774/cevr.2024.13.3.184

fibrosis and TNF-α toxicity were observed in BALB/c mice, both of which are essential in human TB but have been neglected in previous studies [13]. TNF-α is crucial for the induction of protection; however, in patients with progressive TB, it causes toxic effects, so the symptoms of the disease can be reduced by reducing TNF-α levels. Researchers have shown that the harmful effects of TNF-α depend on the presence of IL-4 [14]. The involvement of IL-4 in TNF-α toxicity has also been observed in inflammatory lesions caused by Th1 cells in other infectious diseases (such as schistosoma-induced fibrosis). This phenomenon may explain the high mortality rate during TB treatment in developing countries (where IL-4 is the dominant immune response) [15]. In addition to IL-4 role in toxicity and induction of fibrosis, it can also decrease inducible nitric oxide synthase expression and activate macrophages via an alternative pathway with minimal antimicrobial effects. Therefore, IL-4 regulates macrophage-retaining Mtb during the latent phase [16]. Several components of Mtb appear to modulate antigen presentation by enhancing the Th2 response. The ability of Mtb Beijing's highly infectious genotype strain to express IL-4 in human monocytes was observed.

Similarly, Mtb heat shock protein crystallineα contains epitopes detected by Th2 cells in patients with TB. These studies have demonstrated the involvement of specific lymphocytes, which, in addition to pharmacological bystander effects, can also identify antigens and epitopes that elicit Th2 responses. They may be used to design new adjuvant vaccines, inhibiting IL-4 responses by either enhancing specific regulatory cell proliferation, inhibiting Th2 cell proliferation, or diverting Th1 responses [17].

Robert Koch and Immunopathology

Koch [18] has shown animal and human TB in response to the several components of Mtb. Six weeks after infection in guinea pigs, intravascular injection of a live organism or supernatant of the organism cultured at different sites induces necrosis at the injection site and the primary lesion [18]. Koch [18] tried to use the supernatant as a substance to treat patients with TB. High doses of old tuberculin in the skin can lead to necrosis and spread to areas beyond the skin lesions, which can also induce necrosis in the spinal cord and lung lesions with more dangerous outcomes [19].

Vaccination

The most important antibiotics for TB are isoniazid, rifampicin, pyrazinamide, streptomycin, and ethambutol. However, the treatment duration is long (6 months to 1 year), and the probability of antibiotic resistance is high [20]. Approximately 50 million TB patients are infected with drug-resistant strains. However, this treatment is expensive. For this reason, some countries may not be able to treat TB patients; therefore, it is essential to develop effective and inexpensive vaccines. Historical efforts to create an effective TB vaccine have been undertaken within this timeline (Fig. 2). The development of vaccines can be divided into stages from discovery to implementation. The TB Vaccine Development Pathway proposes an assessment process to track progress and make decisions on advancing a TB vaccine candidate to its next stage of development (Fig. 3).

BCG vaccine

The vaccine commonly used in the world's health program, Bacillus Calmette-Guérin (BCG), is attenuated by *Mycobacterium bovis*. It is administered more than 3 billion times and has a total of 100 million newborns annually. Robert Koch, Albert Calmette, and Camille Guérin are research pioneers on Mtb. Robert Koch, who first described the etiological agent of Mtb in 1882, can be considered the father of subunit vaccines. He introduced the first subunit vaccine in 1890 and named it tuberculin. He believed that this vaccine protects guinea pigs against Mtb infection. Although this claim was rejected in an extensive study of more than 1,700 patients, it did not succeed in treating patients with cutaneous and pulmonary TB. Therefore, tuberculin has never been used as a prophylactic vaccine [4].

Albert Calmette and Camille Guérin introduced the first live vaccine against TB due to the culture of *M. bovis* on potato slices. Then they soaked in Ox Gall (the secretion from the gall bladder of one that was previously used to emulsify lipid–water mixtures, for example, to modify the lipid nature of mycobacteria). BCG was introduced in 1906 with continuous passages on *M. bovis*, which lasted 14 days. After the 30th passage in 1908, weakened *M. bovis* was examined to evaluate its function in an animal study. Owing to the positive feedback received in 1919, it was used on a larger scale for different species of animals. These experiments showed that *M. bovis* is completely weakened so that it does not lead to disease and induces a protective effect against infections caused by Mtb

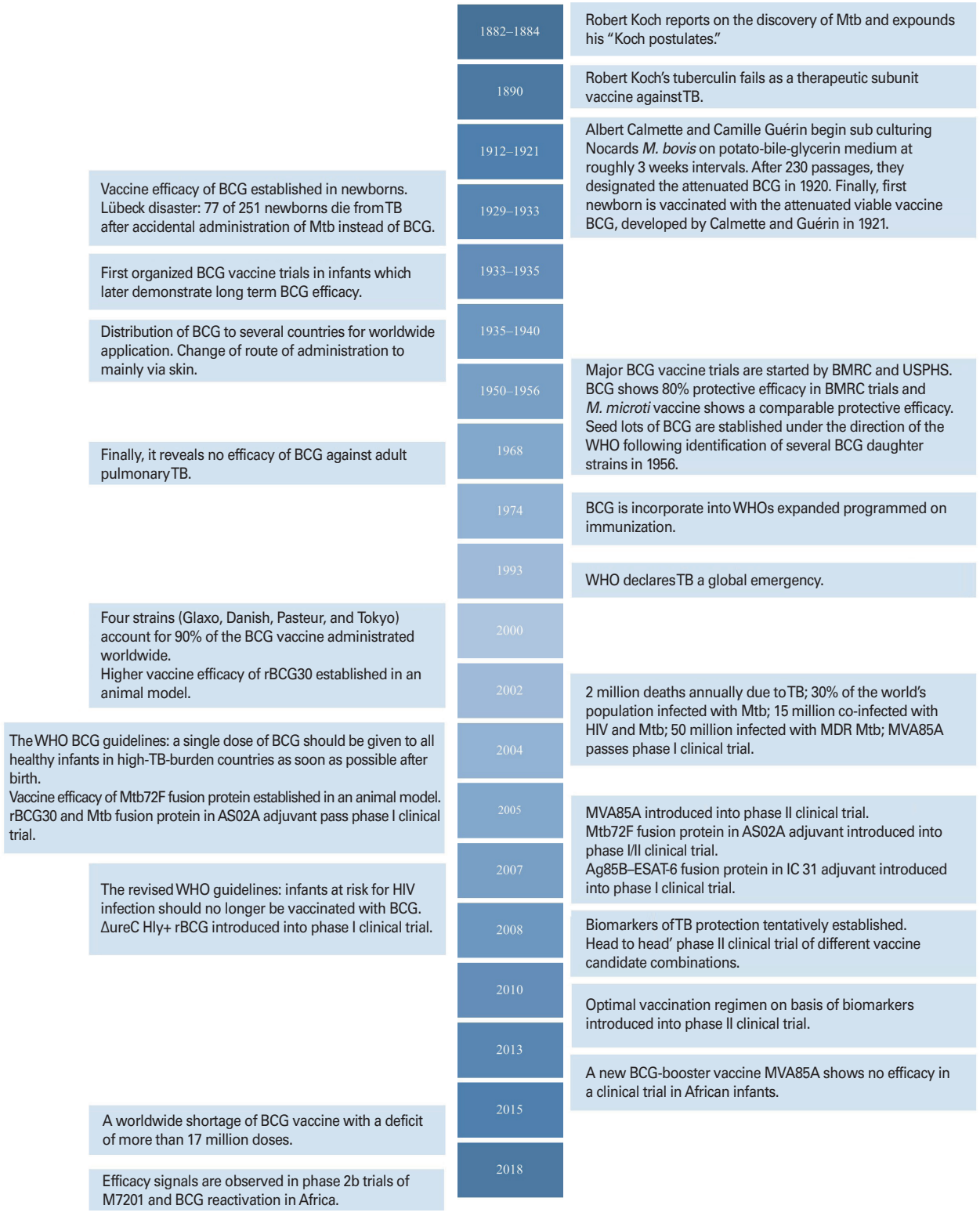

Fig. 2. The current global tuberculosis vaccine development pipeline was last updated in 2019 (https://www.tbvi.eu/what-we-do/pipeline-of-vaccines). Mtb, *Mycobacterium tuberculosis*; TB, tuberculosis; *M. bovis*, *Mycobacterium bovis*; BCG, Bacillus Calmette-Guérin; BMRC, British Medical Research Council; USPHS, U.S. Public Health Service; *M. microti*, *Mycobacterium microti*; WHO, World Health Organization; rBCG, recombinant BCG; MVA85A, modified vaccinia virus Ankara vector expressing antigen 85A of Mtb; HIV, human immunodeficiency virus; MDR, multidrug-resistant.

 https://doi.org/10.7774/cevr.2024.13.3.184

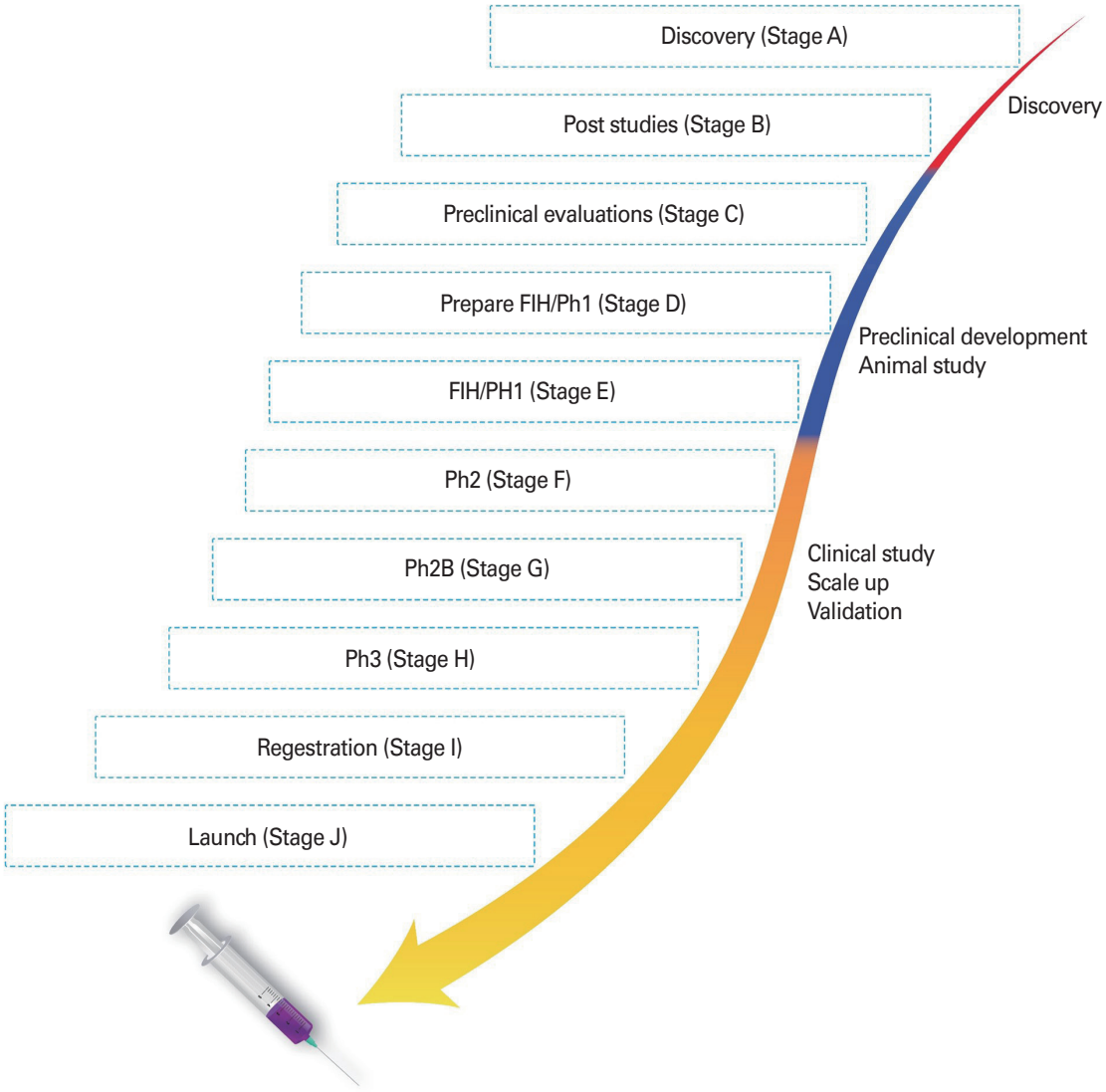

Fig. 3. TB Vaccine Development Pathway. The TB Vaccine Development Pathway serves as a guidance tool to help developers consider all the functions of development and advance a vaccine to its next stage of development. This chart was established by a team of scientific and technical experts from the Tuberculosis Vaccine Initiative (TBVI) and International AIDS Vaccine Initiative (IAVI) with the input of the tuberculosis vaccine community.

and virulent *M. bovis* [1]. Newborn infants were subsequently vaccinated with BCG between 1921 and 1924. In 1927, information on more than 20,000 newborns was collected and evaluated. Although BCG has been commonly administered orally in the past, topical administration to the skin is more important because it is more immunogenic and induces fewer severe side effects than oral administration. Most vaccines are injected before and after infection.

The first group of vaccines against Mtb includes BCG, recombinant Mtb, and other genetically modified mycobacteria associated with increased efficacy and immunity [1]. In the case of TB, a vaccine similar to polio, tetanus, diphtheria, measles, or rubella cannot prevent the disease. T lymphocytes that are activated following administration of BCG cannot prevent Mtb because they do not react directly with microbial antigens and only detect the presence of infected cells [21]. Although T lymphocytes can eradicate microorganisms, in the case of TB, pathogens escaping from T lymphocytes into cells such as macrophages are less frequent, resulting in the formation of a granulomatous structure surrounded by a wall of fibrotic cells that do not eradicate the disease [22]. The specific response of T lymphocytes to mycobacteria may also be unable to control the infection, which is associated with an increased Mtb reactivation risk in patients with HIV and Mtb [4].

Limitation of BCG and the need to develop new vaccines

According to several studies, although the side effects of BCG are tolerable and the only available vaccine against TB, they have generally been reported to have disadvantages. Although BCG can prevent severe forms of TB in young children and infants, some factors have led to the development of new vaccines. This vaccine cannot induce a protective response against advanced TB diseases, such as pulmonary TB, in young adults/elderly and Mtb-endemic countries. In addition, based on the results of Mahmoudi et al. [23], BCG vaccination-related adverse reactions and consequences could include supportive lymphadenitis, localized abscess, and, in rare instances, disseminated BCG, which is especially important for HIV-positive patients or infants who cannot be vaccinated with BCG. Generally, the BCG is (1) most effective in preventing severe forms of TB in children, such as TB meningitis; (2) of limited effectiveness against pulmonary TB in adults—the only form of infectious TB; (3) of limited effectiveness when given to people over the age of 35 years; and (4) less effective when administered to HIV-infected infants in equatorial regions.

For these reasons, fundamental advances in establishing safer and more effective new vaccines against MTB are urgently required in line with WHO's "End-TB Strategy." TB vaccine development is slow and laborious (Fig. 3). Although all age groups and patients with HIV can be targeted to develop the next generation of vaccines, epidemiologically, producing a vaccine that targets young adults and the elderly results in a more effective reduction in disease transmission. In contrast, other age groups did not induce such a significant effect. Many vaccines, such as BCG and attenuated Mtb, are currently being tested in clinical trials and are being designed as substitutes for conventional BCG vaccination (Fig. 4). Subunit vaccines have also been designed to boost the efficacy of neonatal BCG vaccinations.

BCG in developing countries versus developed country

Vaccination with BCG in developing countries may be associated with failure to protect humans against TB. TB-related mortality rates are unusually high during the first two months of standard treatment initiation with appropriate antibiotic administration in developing countries [24]. Increased mortality rates are associated with co-infection factors such as HIV, long-distance treatment centers, and diagnostic problems [8]. Healthy people in developing countries may have a predis-

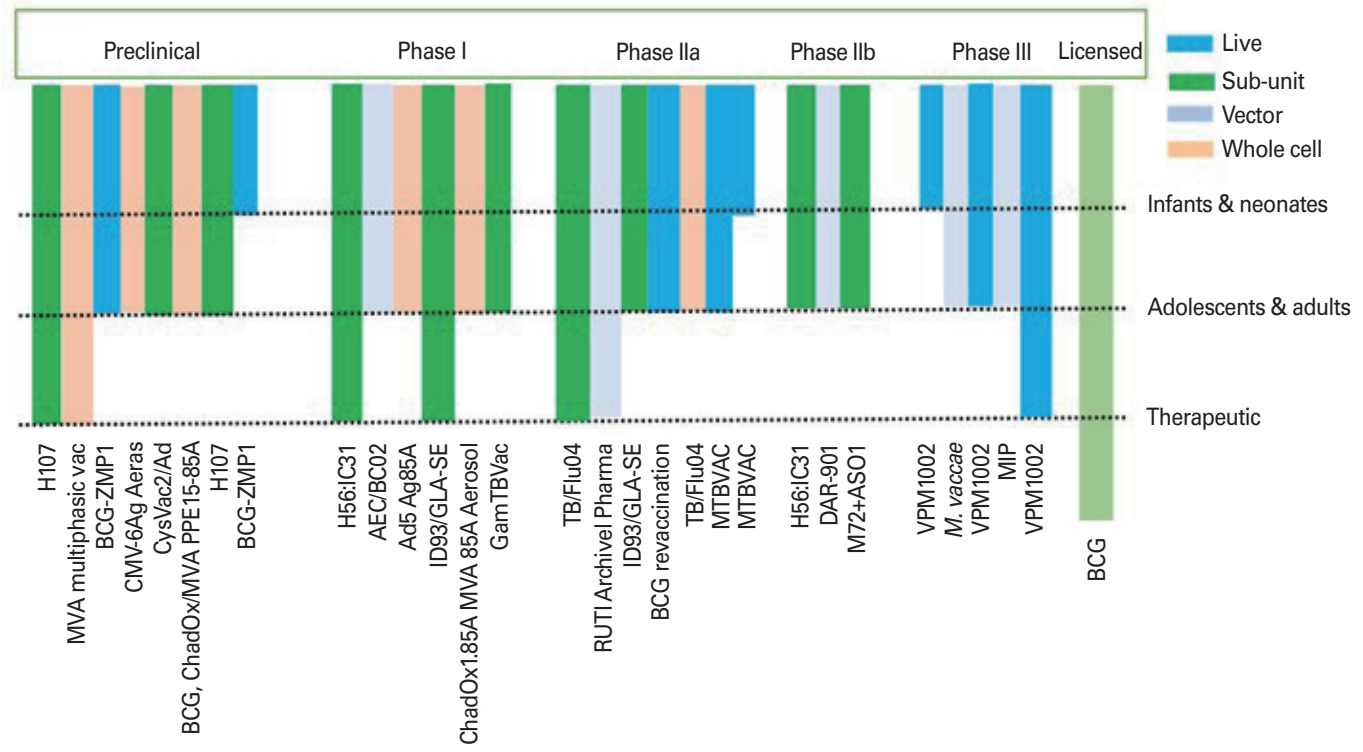

Fig. 4. Tuberculosis (TB) vaccination timeline in history. BCG, Bacilli Calmette-Guerin; MVA85A, modified vaccinia virus Ankara vector expressing antigen 85A of Mtb; M72:AS01E, a recombinant fusion protein of Mtb 39a (Rv1196) and Mtb 32a (Rv0125) in the AS01E adjuvant; Mtb, *Mycobacterium tuberculosis*.

 https://doi.org/10.7774/cevr.2024.13.3.184

posing response to mycobacteria and may cross-react with Mtb, resulting from exposure to environmental strains of mycobacteria. These strains contain one or more saprophytic strains that are abundant in the soil and water. In developing nations, most individuals have a positive skin test response to all mycobacterial antigens, according to research involving reagents produced by the same strains [25].

Interestingly, this is unusual in developed countries because of lifestyle differences and widespread chlorination of water resources. The predisposing response in developing countries is not Th1. This result was observed in African blood samples, in which purified protein derivatives induced IL-5 secretion via Th2 responses. However, very little IL-5 was observed in the UK samples. Vaccination with BCG in African countries cannot reduce the production in IL-5 responses to Mtb. The induction of Th2 response may be due to exposure to helminth infections, which typically induce a Th2 response. Administration of BCG to newborn infants exposed to Th2-inducing helminth antigens elicits a Th2 cell response, which is thought to result from congenital infection (e.g., *Schistosoma haematobium*) [25]. Helminth infections in these individuals can modulate their cross-reactivity with environmental mycobacteria. Therefore, a combination of Th1 and Th2 responses to mycobacteria is observed in developing countries.

This response might also be protective under various conditions. Initial studies failed to demonstrate an increase in IL-4 concentrations in patients with TB living in northern countries, and claimed to be absent in infected primates. Recent data suggest that these findings may result from technical errors during cytokine expression, resulting in deficient copy number and half-life of messenger RNA (mRNA). This is further complicated by stimulus variants, which have been ignored in previous studies. IL-4 mRNA copy numbers in unstimulated BAL cells from TB patients in the United Kingdom were evaluated using reverse transcription-PCR. They showed that IL-4 levels were twice as high as those in BAL cells in the control group [26]. Patients with TB in impoverished nations, where BCG is ineffective and helminth infections are prevalent, have high IL-4 levels. This cytokine can be easily detected in patient serum or monocyte supernatants cultured with Mtb-derived antigens [27]. The importance of IL-4 is derived from studies of the IL-4δ2 variant. This variant, which is not usually detected, lacks exon 2 and is 48-fold shorter than IL-4 [28]. In patients with TB, IL-4 and IL-4δ2 are increased simultaneously with the expression of IL-4δ2 coding mRNA in latent TB cells compared to that in non-TB patients [29]. This suggests that the inhibition of IL-4 through increased production of IL-4δ2 may have protective effects. Whether IL-4 levels are associated with the failure of BCG vaccination and treatment and increased mortality in developing countries requires further information.

Introducing a new vaccine candidate

Vaccination against Mtb can be given as prophylaxis (to prevent infection) or after exposure (for treatment and treatment clinical trials). TB vaccine candidates can be classified as either subunit vaccines (Table 1) [30-54] or mycobacterial whole-cell-derived vaccines (Table 2).

Immune response is essential for the protection and pathogenesis of Mtb [2]. Because the specificity of an antigen determines the quality and efficacy of the immune response, it helps to better understand the escape and survival strategies of the bacterium. For example, microorganisms can escape detection through their immune systems by modifying and inducing mutations in their epitopes. Therefore, these antigens can be considered as targets for inducing immune responses following vaccination. Accordingly, it is necessary to focus on protective epitopes that, while essential and not variable, are also protected among the genotypes of Mtb family members. However, a recent study reported that antigens with a protected sequence contribute to the survival of organisms. It has more benefits for the pathogen than for the host. These findings suggest that future studies on protective antigens should focus on proteins that differ among different Mtb species. As a result, different vaccines are needed in areas where Mtbs predominate [11]. According to some studies, no unique antigen can lead to complete Mtb sterilization and none of the vaccines can induce such an effect. Mtb activates the response of T-cells to a wide range of epitopes on the antigen, which does not lead to the induction of a protective state. Analysis of the relationship between T cells and specific antigens did not reveal the presence of dominant antigens similar to those observed in viral infections [55]. Immunogenic compounds and antigens can induce sterile immune responses. Strong stimulants of the immune response include adjuvants, recombinant vectors (such as recombinant mycobacterium or viral vectors), and the use of prime-boost heterologous compounds [56,57].

Multiple mutations in genes encoding copying factors or Mtb-related components, including sigH, rpoV, and whiB3, result in loss of control over mycobacterium regulatory genes.

Table 1. Composition, microbial and immunological characteristics of subunit vaccines

Vaccine/type	Name	Composition of vaccine		Immunological aspect	Reference
		Antigen	Adjuvant		
Subunit vaccines					
Adjuvant protein subunit vaccines (recombinant protein in combination with an adjuvant)	M72	Rv1196 (PPE family) and Rv0125 (peptidase) encode mycobacterial proteins PPE18 and PepA	Liposomal adjuvant, AS01 (liposome TLR4 agonist)	(1) Stimulate humoral and cellular immunity. (2) Adults vaccinated with BCG need to receive two doses of M72/AS01. (3) Protective properties and not many side effects.	[37,38]
	H56	ESAT-6 (Mtb), Ag85B (mycolyl transferase), and Rv2660c (dormancy antigen)	Antimicrobial peptide KLK+oligodeoxynucleotide ODN1a TLR9 agonist	(1) Act as booster vaccine before immunization with BCG. (2) Protection of primates against Mtb and reactivation of LTBI. (3) Long-term safety, immunity, and well sustainability against TB disease. (4) No side effects at phase I clinical trial in both non-infected and infected TB individuals.	[33,34]
	ID93	Rv2608 (PPE family), Rv3619 (virulence factor), and Rv3620, Rv1813	GLA in the emulsion of squalene oil-in-water TLR4 agonist	(1) Stimulate Th1 responses, which are characterized by IFN-α, IL-6, IL-2, and TNF-α. (2) Long-term immunity, which is induced by the presence of GLA-SE adjuvant.	[39]
	GaMtbvac	Ag85A (mycolyl transferase) and ESAT6-CFP10	Oligodeoxynucleotide, TLR9 agonists	-	
	H64: CAF01	Six protein antigen such as EspD, EspC, EspF, EspR, PE35, and ESAT6	CAF01 as a cationic adjuvant ensures the long-term stability of the liposomes as an immune booster compound	(1) Enhance BCG efficacy. (2) Protective in animal model study but immunogenicity and safety of H64 in humans still need more investigation. (3) CAF01 elicits both Th1 and Th17 responses by activation of C-type lectin receptor following administration.	[30-32]
	H1: IC31	ESAT6 fusion protein and Ag85B	IC31 adjuvant	(1) In animals, practical and stimulate cellular Immunity and humoral Immunity against Mtb. (2) In human induction of the IFN-γ, safety and memory cells. (3) In patients with latent TB and individuals vaccinated with BCG, strengthening the immune response. (4) No serious side effects. (5) Protective and well-tolerated in patients with HIV. (6) False-positive in people who be vaccinated with H1 in IGRAs are used to diagnose active Mtb infection.	[35,36,40]
	H4: IC31	Ag85B and TB10.4	IC31 as adjuvant	Immunological aspect: (1) ESAT6 was substituted with TB10.4 to prevent ESAT6 cross-reactivity in T lymphocyte stimulation with the IGRAs method. (2) Used as a booster vaccine to enhance the anti-mycobacterial effect of BCG in a prime-boost manner. Microbial aspect: ESAT6 was substituted with TB10.4 to prevent ESAT6 cross-reactivity in T lymphocyte stimulation with the IGRAs method.	[35]
	HBAB	HBAB antigen in *M. bovis*	No definitive adjuvant	Immunological aspect: Mutant strains in HBAB antigen have not been able to colonize in the lungs, so it can be concluded that the immune response through antibodies may be able to increase Immunity against Mtb. Microbial aspect: (1) The hemagglutinin-binding heparin (HBAB) vaccine has HBAB antigen, which possesses a specific and methylated, carboxyl-terminal, lysine-, alanine-, and proline-rich repeat region. It enables Mtb extrapulmonary dissemination from the lung to other organs. (2) Did not reach the clinical trial stage.	[41-43] [44-46]
Recombinant viral vectored vaccines (selected protective antigens delivered in a recombinant viral vector)	Ad5Ag85A	Ag85A (mycolyl transferase)	Recombinant replication-deficient human adenovirus five vector	Immunological aspect: (1) Known as a booster vaccine candidate because of its protection, good tolerance, and its significant effect on the cellular immune response. (2) In an animal model, receiving intranasally AdAg85A vaccination led to mucosal immunization with more robust and more effective responses compared to conventional BCG. (3) It's associated with releasing IFN-γ into the lungs and the proliferation of T cells. (4) Intranasal BCG-prime with AdAg85A has booster and greater CD4 and CD8 T cells response in the lungs in contrast to subcutaneous BCG priming or intramuscular AdAg85A. (5) AdAg85A may not be effective in developing countries cause of the neutralization of specific antibodies against the vaccine by previous exposure. Microbial aspect: Ad5Ag85A, as a viral vectored vaccine, contains defective-replicating Ad5 that carries Ag85A antigen.	[47-49]

(Continued on next page)

Table 1. Continued

Vaccine/type	Name	Composition of vaccine		Immunological aspect	Reference
		Antigen	Adjuvant		
	ChAdOx185A+ MVA85A boost	Ag85A (mycolyl transferase)	ChAd, MVA virus	Immunological aspect: (1) ChAd as a vector in a viral vaccine, made by a mutation in E1 and E3 genes. (2) Adequate protection, safe induction of cellular and humoral immune responses in the host, as the same adjuvant effect makes it an excellent candidate for the viral vectored vaccine. (3) ChAdOx1, along with Ag85B antigen, can provide a more robust immune response. Microbial aspect: Defect ChAd are carriers for the viral vector vaccine, made by a mutation in E1 and E3 genes, and safety. Also, ChAd can induce an adjuvant effect that stimulates host cellular and humoral immune responses. Therefore, this virus was selected as the best tool to deliver the vaccine against TB.	[50]
	TB/Flu-04L	Ad5 Ag85A (mycolyl transferase)	Attenuated replication-deficient influenza virus (H1N1)	Immunological aspect: It bears influenza virus strain A, which contains Ag85A and ESAT6 antigens of Mtb. The vaccine is protective and has no side effects. Microbial aspect: This recombinant viral vaccine has influenza virus strain A, which harbored Ag85A and ESAT6 antigens of Mtb.	[51]
	MVA85A/ Aeras-485		TPA gives a more robust immune response, which is why the virus, although defective in reproduction, seeks to provide a robust immune response.	Immunological aspect: (1) Protective and good safety against latent infections in all people and immunocompromised individuals/no side effect. (2) Prime-boost strategy along with TPA show an increase in cytokine and antibodies induction and T-cell activation. (3) Any administration stimulates both CTL, helper lymphocytes (Th1), and subgroups of Th1 and Th17 and also induces inflammatory cytokines such as TNF-α, IL-2, IL-17, and IFN-γ. (4) More cytokines induction and CD+4 cell are produced by nasal administration; therefore, mucosal administration. (5) Strengthens the immune system better. (6) MVA-85A-IMX313, as a variant of MVA-85A, composes a part of DNA that increases the immune system responses. Microbial aspect: MVA virus is an attenuated viral-vectored vaccine, a strain of the vaccinia virus that was under 156 passages on chicken embryo fibroblast cells.	[52-54]
Naked DNA	HspX PPE44-EsxV fusion DNA	Fusion antigen		Immunological aspect: (1) Some antigens like Hsp60, Hsp70, ESAT-6, PPE44, and HspX can stimulate cellular immune responses. (2) These immunogenic antigens could be used in the DNA vaccine and induce a more robust immune response than when only a single antigen is used. (3) DNA vaccines are safe, tolerable in HIV-suffering patients, and are long-lasting immunity inducers. (4) According to the prime-boost strategy, the DNA vaccine can enhance the BCG's effectiveness as a booster. (5) The immunogenic antigens can increase immunity (increase more cytokines such as IFN-γ, IL-12, and TGF-β levels, strong CD8+ T-cell responses) after BCG administration in the prime-boost strategy, which exacerbates intracellular activity of macrophages.	

TLR, Toll-like receptor; BCG, Bacillus Calmette-Guérin; Mtb, *Mycobacterium tuberculosis*; LTBI, latent tuberculosis infection; TB, tuberculosis; Th1, type 1 T helper; IFN-γ, interferon-gamma; IL, interleukin; TNF-α, tumor necrosis factor-alpha; GLA, glucopyranosyl lipid adjuvant; HIV, human immunodeficiency virus; IGRAs, interferon-gamma release assays; *M. bovis*, *Mycobacterium bovis*; Ad5, adenovirus serotype 5; ChAd, chimpanzee adenovirus; MVA, modified vaccinia Ankara; TPA, tissue plasminogen activator; CTL, cytotoxic T lymphocytes; TGF-β, transforming growth factor beta.

Table 2. The adjuvant used in TB vaccine candidate

Adjuvant/delivery system	Components	Antigen	Mechanism of action	Function
Advantix	Delta inulin particle	Ag85B, CysD (CysVac2)	Enhanced phagocytosis, immune cell recruitment and low reactogenicity	Th1, Th17
AS01	MPLA and QS21	Mtb32, Mtb 39 (M72)	TLR4 activation (MPLA), liposomal disruption and Syk activation, CD2 activation on T-cells, NLRP3 inflammasome (QS21)	Th1
CAF01	DDA and TDB	Ag85B, ESAT-6 (H1)	TDB activates Mincle, MyD88-dependent Th1/Th17 polarising cytokines. DDA forms cationic liposomes that TDB stabilizes.	Th1, Th17
Chitosan and derivatives		Ag85B, ESAT-6 (H1)	Activates cGAS-STING pathway, mucoadhesive and mucosal epithelial penetration properties, suitable for mucosal administration	Th1, low Th17
Cyclic dinucleotides	Synthetic dinucleotide analog of cyclic diguanylate	Ag85B, ESAT-6, Rv1733c, Rv2626c, RpfD (5Ag)	STING activation (IRF-3 type I IFN production, NF-kB, STAT-6 chemokine expression)	Th1, low Th17
Dextran		Ag85A, ESAT-6-CFP10	Activates DC-SIGN receptor family, mannose receptor, langerin	Th1/Th2
GLA-SE	GLA in squalene emulsion	Rv2608, Rv3619, Rv3620, Rv18183 (ID93)	GLA is a synthetic TLR4 agonist in squalene in water emulsion that activates NLRP3 inflammasome.	Th1
IC-31	KLK and ODN1a	Ag85V, ESAT-6 (H1); Ag85B, ESAT-6 and Rv2660c (H56) and Ag 85B, TB10.4 (H4)	ODN1a binds TLR9, KLK forms aggregates with ODN1a and enhances translocation into cells.	Th1
ISCOMs	Immune stimulatory complexes (saponin, cholesterol, and phospholipid)	Ag85B, ESAT-6 (H1); Ag85A	TLR independence may be inflammasome mediated (under investigation).	Th1/Th2
Nanoemulsion	Soybean oil phase mixed into the aqueous phase	ESAT-6, Ag85B	Mucoadhesive, highly tolerated, suitable for mucosal administration	Th1/Th17
PLGA (poly [lactic-co-glycolic acid])	Microsphere delivery system	Ag85B, ESAT-6 (H1); MPT83	Antigen protection, depot formation, controlled release, enhanced phagocytosis, biodegradable, suitable for mucosal administration	Th1/Th17
Polyl: C	dsRNA	BCG; Ag85B, HspX	TLR3 agonist	Th1/Th2

TB, tuberculosis; Th1, type 1 T helper; MPLA, 3-O-diacyl-40-monophosphoryl lipid A; TLR, Toll-like receptor; Mtb, *Mycobacterium tuberculosis*; DDA, dimethyl octadecyl-ammonium; TDB, trehalose 6,6-behenate; STING, stimulator of interferon genes; IRF-3, interferon regulatory factor 3; IFN, interferon; NF-kB, nuclear factor kappa B; STAT, signal transducer and activator of transcription factor; DC, dendritic cell; SIGN, specific intercellular adhesion molecule-3-grabbing non-integrin; GLA, glucopyranosyl lipid adjuvant; NLR, NOD-like receptor; KLK, KLKL5KLK; ODN1a, oligodeoxynucleotide; dsRNA, double-stranded RNA.

The genes essential for Mtb have not yet been identified. Although these mutated mutant strains usually reproduce, they have fewer immunopathological effects than wild species. Animals infected with the wild-type strain of Mtb died of severe lung damage. However, the mutated sigH strains survived despite having a similar number of bacilli in the lungs. Therefore, the pathology induced by wild-type strains of Mtb was not observed in the sigH mutant, which depends on the response of T cells. Studies have shown that only a relatively small number of Th1 cells are sufficient to control bacterial proliferation, and the remaining 90% are concerned about the occurrence of immunopathological properties [11].

Subunit vaccine

Subunit vaccines target six or fewer antigens. The main challenge in the development of protein subunit vaccines is the identification of the optimal antigens. Recently, some antigens were selected that were expressed from nearly 4,000 genes related to Mtb that could induce a protective immune response against Mtb [58]. These antigens have been tested in animal studies for their ability to elicit a protective immune response against Mtb. Most of these induce the release of IFN-γ, and TNF-α activates Th1 responses [59]. Subunit vaccines can be classified into two general groups: adjuvant protein subunits, and recombinant viral vector vaccines. Nota-

 https://doi.org/10.7774/cevr.2024.13.3.184

Table 3. Composition, microbial, and immunological characteristics of whole-cell vaccines

Whole-cell vaccine/ type	Name	Modification	Microbial aspect	Immunological aspect	Reference
Live attenuated vaccine	VPM1002 (BCG ΔureC::hly)		The vaccine, an rBCG, integrates the listeriolysin encoding gene from *L. monocytogenes* and inserts *urease* gene deletion. Hemolysin has activity at pH 5.5 and harbors the PEST sequence, containing peptide sequence rich (glutamic acid, serine, and threonine). PEST limited hemolysin activity by degradation in the cytoplasm in the phagosome and preventing its activation in the extracellular environment to provide an optimal acidity for hemolysin activity; the urease gene was deleted in VPM1002. This enzyme neutralizes its surrounding environment by producing NH4+. The BCG vaccine inhibited the phagosome maturation process, which resulted in a neutral pH.	Currently undergoing mid-phase three clinical trials (significant progress) and may substitute with BCG. An rBCG with a deletion in the *urease* gene and insertion of the *listeriolysin* gene (derived from *L. monocytogenes*). Activate the immune responses of CD4 and CD8 lymphocytes as well as both Th1 cell and Th17 cell. In immunocompromised animals is safer than the BCG vaccine and provides high protection in mice against Mtb infection, including the H37RV laboratory strain and clinical isolate from the Beijing family.	[63-67]
	MtbVAC	Transcription factor phoP and fadD26 (phthiocerol dimycocerosate synthesis) gene deletions	A live attenuated Mtb vaccine with genetic deletions in phoP and fadD26 genes reduces the risk of becoming a wild-type strain. PhoP is a transcriptional regulator that controls the production of other pathogenic virulence factor genes of Mtb, and reported mutation in the *phoP* gene is necessary to transform the Mtb H37Rv pathogenic strain into Mtb H37Ra nonpathogenic strain. ESAT6 was encoded in the ESX-1 region and involved cell-to-cell movement by enhancing the apoptosis of the infected. Despite the presence of ESAT6 in Mtb, phoP mutants cannot export it and have a smaller size than the wild-type strain. fadD26 is related to synthesizing special lipids in the Mtb cell wall, phticeroldimycocerosates, which are essential in the organism's survival in a cell. Mtb deficient with this lipid has little resistance to the immune response and cannot inhibit phagolysosome formation. This results in the intracellular killing of the bacteria by phagolysosome acidity.	MtbVAC vaccine (Mtb ΔphoPΔfad D26) is currently in phase II clinical trials with both newborns and adults. The first administration of MtbVAC in-human phase I clinical trials began in 2013 in adults in Switzerland and continued in 2016 in South African newborns. The successful results allowed advancement into phase II trials in 2019 in South Africa, both in populations.	[68-71]
	rMsmegΔesx3 esx3 (Mtb)		A recombinant product of *M. smegmatis* was prepared by replacing ESX3 with a derivative of Mtb ESX3. A member of the ESX family encodes ESAT-6-like proteins and is involved in iron uptake in *M. smegmatis*, an unusual mycobacterium with no pathogenic activity.	Very effective protection in mice.	[72]
	rBCGΔais1 zmp1 vaccine candidate		An rBCG vaccine that eliminates the zmp1 gene encoded a zinc metalloprotease. This enzyme prevents phagosome maturation. Therefore, an organism without zmp1 cannot prevent phagolysosome formation. Defective strains of metalloprotease have better protective effects against Mtb in the lungs of guinea pigs than BCG.	In an animal model study, increasing immunogenicity and great immune response to conventional BCG and protective in immunocompromised.	[52,73]
Killed or extracted vaccine (whole [killed] organism or fragments of the whole organism)	*M. vaccae*	Heat-killed	MV, as nonpathogenic mycobacteria, has a wide broad effect on other mycobacterium species, like infected patients with Mtb. Also, can be used in cancer therapy and mental disorder. This species was the first inactive bacteria approved by WHO in 1991 against Mtb.	Protective in HIV-infected, BCG-vaccinated adult patients with Mtb that has not been vaccinated with BCG for a long time (enhanced Th1 response). Safe, good-tolerated in the body, immunogenic, and enable the treatment of drug-resistant cases following MV. No serious side effects. In an animal study, MV shows a reduction in Mtb load.	[33,74,75]
	MIP	Heat-killed		Inactivated mycobacterium whole-cell vaccine that shows protective effect in mouse model study. Induction of high immunogenicity (IFN-γ and antibody responses).	
	DAR-901	Heat-killed		Induce immune response and reduce the bacterial growth. An animal study was safe and high quality due to the induction of vigorous immune response against Mtb. Good protective effects so it can be given as prophylaxis or for infected people with Mtb.	[76-78]
	RUTI	Cell wall fragments of Mtb in the liposome suspension	RUT1 is a kind of extracted (or killed)—a vaccine that can potentiate immune response and reduce bacterial growth. These vaccines that compose of Mtb fragments.		[57,79]

BCG, Bacillus Calmette-Guérin; rBCG, recombinant BCG; *L. monocytogenes*, *Listeria monocytogenes*; PEST sequence, proline–glutamic acid–serine–threonine sequence; Th1, type 1 T helper; Mtb, *Mycobacterium tuberculosis*; *M. smegmatis*, *Mycobacterium smegmatis*; MV, *Mycobacterium vaccae*; WHO, World Health Organization; HIV, human immunodeficiency virus; MIP, *Mycobacterium indicus pranii*; IFN-γ, interferon-gamma.

bly, subunit vaccines with secretory antigens can further enhance the immune response after BCG administration. Although these vaccines have few side effects, they should be used as adjuvant [60].

TB vaccine adjuvants

Antigen prescriptions alone are insufficient for boosting the immune system. In contrast, T cells require co-stimulant substances, such as cytokines and T-cell receptor signaling, to become activated. Similarly, subunit vaccines require stimulants such as adjuvants to boost the immune response. Choosing a suitable adjuvant is vital for obtaining the best results from the vaccine. Every adjuvant is critical for triggering part of the immune response specific to the pathogen. Pattern recognition receptors (PRRs), including Toll-like receptors (TLRs), NOD-like receptors (NLRs), and RIG-I-like receptors, as host sensors play crucial roles in the proper functioning of the innate immune system, which detects molecules typical of pathogens. Adjuvants can activate these PRRs, and each of these receptors induces different cytokine signaling [61]. Alum is one of the most well-known adjuvants that activates inflammasomes and releases inflammatory cytokines, leading to dendritic cell activation [62]. The adjuvants used in the TB vaccines stimulated a more robust Th1 response (Table 3) [33,52,57,63-79]. For example, by releasing IL-12, adjuvants can alter the immune response to Th1 responses [80]. However, researchers have found that combining Th1 and Th17 responses is more effective in protecting individuals against Mtb than inducing Th1 response alone.

Interestingly, establishing this balance depends on the route of vaccine administration and type of adjuvant administered. To achieve this goal, some adjuvants enhance the Th17 response by activating TLR4, TLR7, and TLR8 and others by activating C-type lectin receptors [30,81,82]. Since mucosal stimulation provides better immunity, it is better to focus on designing a vaccine that strengthens mucosal immunity. For example, mucosa-specific cell types, known as M cells, are present in nasal-associated lymphoid tissue. Owing to their transcytosis properties, these cells rapidly transfer antigens to the dendritic cell submucosa to form an immune response as soon as possible. Therefore, these cells are important targets for TB vaccine development. In nasal vaccination, adjuvants such as chitosan can strengthen the immune system response [31]. The adjuvants used for TB vaccines are classified as nano-particulate, delivery system-based, and plant-or microbially derived adjuvants. Adjuvants should be designed to minimize damage to lung tissue, which is possible with fewer inflammatory mechanisms of action, to be safer in pulmonary administration [32]. Among the introduced adjuvants, Advax, in addition to inducing a better immune response and the absence of damage caused by excessive inflammation, can activate a wide range of T cell subtypes, such as Th1, Th2, Th17, and CD8 memory cells, and is considered a significant adjuvant [32].

Whole-cell vaccine

The whole-cell vaccine was provided by the Mtb, BCG, and nontuberculous mycobacteria species. For several reasons, these vaccines seem to be a good strategy. The main reason is the reliable efficacy of BCG, which provides a strong sense. In addition, these vaccines can stimulate a broader range of immune responses than subunit vaccines owing to their different protein and non-protein antigens (such as lipids and glycolipids in the Mtb membrane). These vaccines are divided into live-attenuated vaccines and killed or extracted vaccines. BCG variants exist in varying degrees of attenuation that induce severe effects, which differ between vaccines [60]. (1) Live attenuated vaccine: These live vaccines, such as VPM1002 (a genetically modified BCG) and MtbVAC27, have been attenuated through genetic modification [60]. (2) Killed or extracted vaccines: These vaccines contain the whole cell of an organism, derived from heat-killing modifications of *Mycobacterium vaccae* (*M. vaccae*), *Mycobacterium indicus pranii* (MIP), and DAR-901 or provided from Mtb fragments under stress conditions (RUT1) [60].

Prime-boost strategy

The prime-boost strategy is based on priming immunization with a vaccine such as naked DNA expressing the antigen, followed by vaccination with another effective vaccine such as a recombinant viral vector expressing the same antigen. Prime-boost vaccines are also known as heterologous boost vaccines. To increase the protective immunity of BCG, a prime-boost strategy involving priming with BCG, recombinant BCG, and other attenuated mycobacteria followed by a heterologous booster vaccination can improve the efficacy of the response [33]. High-level T cytotoxic cellular responses and the induction of long-lasting CD4+ T cell immune responses are the most consequential of the prime-boost strategy. In line with this strategy, subunit vaccines that include highly immunogenic Mtb antigens in formulations with adjuvants are under clinical trial investigation for the introduction of boost

 https://doi.org/10.7774/cevr.2024.13.3.184

BCG-primed vaccines. Hence, following administration of the recombinant BCG vaccine, the immune response was exacerbated by the subunit vaccine. This method is also known as the prime-boost vaccine. Before it becomes a satisfactory vaccine strategy against Mtb, several years of repeated vaccinations are required. Approximately 10 years of phase 3 clinical trials have described that protection due to the vaccine's long-timescale capability should also be examined. Biomarkers are excellent ways to achieve this goal. Biomarkers are small molecules that can be used to evaluate diseases, drug activity, and vaccine efficacy. Biomarkers, also known as biosignatures, are often found in samples of biological fluids, such as blood, plasma, or urine, in the form of proteins, small molecules, and mRNA. Different biomarkers have been described as protective against TB, the most widely used of which is IFN-γ. This cytokine, secreted by T lymphocytes, is one of the best indicators of protection against Mtb. However, they believe that IFN-γ alone is insufficient and that the protective immunity against TB depends on other factors, such as other cytokines, chemokines, different T cell subtypes, and migration patterns of lymphocytes.

Future vaccination strategy

Alternative strategies to eradicate the disease should be considered if vaccines cannot eliminate infection in patients with TB. One strategy is to produce a specific generation of antibodies that can prevent Mtb [1]. This third generation of vaccines focuses on the site of Mtb entry into the lungs. This vaccine, administered intranasally, neutralizes and traps Mtb in the alveolar space by stimulating local secretion of specific antibodies, such as immunoglobulin A (IgA). Because Mtb enters the lungs in small numbers, IgA antibodies, the first defense barrier in the pulmonary areas, can help the immune system by inactivating pathogens. Presently, the mechanism by which the immune system helps maintain the Mtb in a latent state is abolished. New drugs have been designed to facilitate the intracellular elimination of microorganisms. These drugs can potentiate signaling pathways within cells and contribute to the increased activation of phagocytic cells, which are typically impaired by Mtb [34]. The lack of an optimal BCG quality in humans has raised concerns. Thus, it may increase the protective output response of BCG by priming the Th1 cell response. The protective output of ineffective vaccines can be increased by injecting a booster vaccine, which increases IFN-γ levels. Studies on animal models have shown that injection of the naked DNA vaccine before receiving BCG increased the Th1 response compared to the group receiving BCG alone, but did not protect against Mtb infection.

Similarly, immunization with recombinant BCG strain-induced IL-8 expression synergistically with IL-12 resulted in the release of IFN-γ from lymphocytes and the Th1 response, which did not induce protection following vaccination. These findings suggest that it may not be necessary to enhance Th1 cell responses alone [28]. Behatt et al. [83] believe that the proliferation of multifunctional Th1 cells with the ability to release inflammatory cytokines, such as IFN, TNF, and IL-2, is associated with significant protection and immunity against TB. However, some believe they may not provide substantial protection [35,83]. Moreover, some studies have shown that excessive Th1 cell differentiation may interfere with the activation of other T cell subsets, leading to a lack of immune formation. In parallel, Aagaard et al. proved that lower antigen doses induce more immunity and suitable long-term protection against TB than higher exposure to the same antigen [36].

Conclusion

The vaccine used today, BCG, is an attenuated live vaccine derived from consecutive passages of *M. bovis*. Although the only vaccine used against Mtb is BCG, it is not protective against adult TB or endemic areas. New vaccines include a variety of recombinant and subunit vaccines introduced to resolve conventional BCG complications. The most significant advantage of these vaccines is that they are suitable immunogenic compounds with antigens that can induce a sterile immune response. Immune solid response stimulants include adjuvants, recombinant carriers (such as recombinant mycobacterium or viral carriers), and prime-boost heterologous compounds. Among these vaccines, *M. vaccae*, MIP, and VPM1002, which are currently under investigation in phase 3 clinical trials, can overcome BCG failures in HIV patients. The shared features of these vaccines are safety and good tolerance in immunocompromised individuals such as HIV patients and in adults who have not been vaccinated with BCG for a long time. Despite the development of various new vaccines, TB remains one of the most threatening infectious diseases worldwide.

ORCID

Marzie Mahdizade Ari *https://orcid.org/0000-0002-6285-5886*
Masoumeh Beig *https://orcid.org/0000-0003-1243-3164*

Mohammad Sholeh *https://orcid.org/0000-0003-1748-7225*
Majid Khoshmirsafa *https://orcid.org/0000-0002-1843-8372*

References

1. Kaufmann SH. Envisioning future strategies for vaccination against tuberculosis. Nat Rev Immunol 2006;6:699-704.
2. Torrado E, Robinson RT, Cooper AM. Cellular response to mycobacteria: balancing protection and pathology. Trends Immunol 2011;32:66-72.
3. Rohde K, Yates RM, Purdy GE, Russell DG. Mycobacterium tuberculosis and the environment within the phagosome. Immunol Rev 2007;219:37-54.
4. Jouanguy E, Lamhamedi-Cherradi S, Altare F, et al. Partial interferon-gamma receptor 1 deficiency in a child with tuberculoid bacillus Calmette-Guerin infection and a sibling with clinical tuberculosis. J Clin Invest 1997;100:2658-64.
5. Gengenbacher M, Kaufmann SH. Mycobacterium tuberculosis: success through dormancy. FEMS Microbiol Rev 2012;36:514-32.
6. Mills KH. Regulatory T cells: friend or foe in immunity to infection? Nat Rev Immunol 2004;4:841-55.
7. Stock P, Akbari O, Berry G, Freeman GJ, Dekruyff RH, Umetsu DT. Induction of T helper type 1-like regulatory cells that express Foxp3 and protect against airway hyper-reactivity. Nat Immunol 2004;5:1149-56.
8. Wilkinson KA, Wilkinson RJ, Pathan A, et al. Ex vivo characterization of early secretory antigenic target 6-specific T cells at sites of active disease in pleural tuberculosis. Clin Infect Dis 2005;40:184-7.
9. Hisaeda H, Maekawa Y, Iwakawa D, et al. Escape of malaria parasites from host immunity requires CD4+ CD25+ regulatory T cells. Nat Med 2004;10:29-30.
10. Richeldi L, Ewer K, Losi M, et al. T cell-based tracking of multidrug resistant tuberculosis infection after brief exposure. Am J Respir Crit Care Med 2004;170:288-95.
11. Kaufmann SH. Tuberculosis vaccine development: strength lies in tenacity. Trends Immunol 2012;33:373-9.
12. Wangoo A, Sparer T, Brown IN, et al. Contribution of Th1 and Th2 cells to protection and pathology in experimental models of granulomatous lung disease. J Immunol 2001;166:3432-9.
13. Tramontana JM, Utaipat U, Molloy A, et al. Thalidomide treatment reduces tumor necrosis factor alpha production and enhances weight gain in patients with pulmonary tuberculosis. Mol Med 1995;1:384-97.
14. Hernandez-Pando R, Aguilar D, Hernandez ML, Orozco H, Rook G. Pulmonary tuberculosis in BALB/c mice with non-functional IL-4 genes: changes in the inflammatory effects of TNF-alpha and in the regulation of fibrosis. Eur J Immunol 2004;34:174-83.
15. Pina A, Valente-Ferreira RC, Molinari-Madlum EE, Vaz CA, Keller AC, Calich VL. Absence of interleukin-4 determines less severe pulmonary paracoccidioidomycosis associated with impaired Th2 response. Infect Immun 2004;72:2369-78.
16. Voskuil MI, Schnappinger D, Visconti KC, et al. Inhibition of respiration by nitric oxide induces a Mycobacterium tuberculosis dormancy program. J Exp Med 2003;198:705-13.
17. Zuany-Amorim C, Sawicka E, Manlius C, et al. Suppression of airway eosinophilia by killed Mycobacterium vaccae-induced allergen-specific regulatory T-cells. Nat Med 2002;8:625-9.
18. Koch R. Fortsetzung der Mittheilungen über ein Heilmittel gegen Tuberculose [Continuation of the reports on a remedy for tuberculosis]. Dtsch Med Wochenschr 1891;17:101-2. https://doi.org/10.1055/s-0029-1206198
19. Anderson M. On Koch's treatment. Lancet 1891;137:P651-2. https://doi.org/10.1016/S0140-6736(02)18484-4
20. World Health Organization. Global tuberculosis report 2013. Geneva: World Health Organization; 2013.
21. Kaufmann SH. Recent findings in immunology give tuberculosis vaccines a new boost. Trends Immunol 2005;26:660-7.
22. Ulrichs T, Kaufmann SH. New insights into the function of granulomas in human tuberculosis. J Pathol 2006;208:261-9.
23. Mahmoudi S, Khaheshi S, Pourakbari B, et al. Adverse reactions to Mycobacterium bovis bacille Calmette-Guerin vaccination against tuberculosis in Iranian children. Clin Exp Vaccine Res 2015;4:195-9.
24. Fine PE. Variation in protection by BCG: implications of and for heterologous immunity. Lancet 1995;346:1339-45.
25. Fine PE, Floyd S, Stanford JL, et al. Environmental mycobacteria in northern Malawi: implications for the epidemiology of tuberculosis and leprosy. Epidemiol Infect 2001;126:379-87.
26. Dheda K, Chang JS, Breen RA, et al. In vivo and in vitro studies of a novel cytokine, interleukin 4delta2, in pulmonary tuberculosis. Am J Respir Crit Care Med 2005;172:501-8.
27. Rook GA, Dheda K, Zumla A. Do successful tuberculosis

vaccines need to be immunoregulatory rather than merely Th1-boosting? Vaccine 2005;23:2115-20.
28. Rook GA, Dheda K, Zumla A. Immune responses to tuberculosis in developing countries: implications for new vaccines. Nat Rev Immunol 2005;5:661-7.
29. Vasiliev AM, Vasilenko RN, Kulikova NL, et al. Structural and functional properties of IL-4delta2, an alternative splice variant of human IL-4. J Proteome Res 2003;2:273-81.
30. Schoenen H, Bodendorfer B, Hitchens K, et al. Cutting edge: Mincle is essential for recognition and adjuvanticity of the mycobacterial cord factor and its synthetic analog trehalose-dibehenate. J Immunol 2010;184:2756-60.
31. Kim SH, Jang YS. Antigen targeting to M cells for enhancing the efficacy of mucosal vaccines. Exp Mol Med 2014;46:e85.
32. Stewart E, Triccas JA, Petrovsky N. Adjuvant strategies for more effective tuberculosis vaccine immunity. Microorganisms 2019;7:255.
33. Khoshnood S, Heidary M, Haeili M, et al. Novel vaccine candidates against Mycobacterium tuberculosis. Int J Biol Macromol 2018;120:180-8.
34. Walburger A, Koul A, Ferrari G, et al. Protein kinase G from pathogenic mycobacteria promotes survival within macrophages. Science 2004;304:1800-4.
35. Caccamo N, Guggino G, Joosten SA, et al. Multifunctional CD4(+) T cells correlate with active Mycobacterium tuberculosis infection. Eur J Immunol 2010;40:2211-20.
36. Aagaard C, Hoang TT, Izzo A, et al. Protection and polyfunctional T cells induced by Ag85B-TB10.4/IC31 against Mycobacterium tuberculosis is highly dependent on the antigen dose. PLoS One 2009;4:e5930.
37. Wang C, Chen Z, Fu R, et al. A DNA vaccine expressing CFP21 and MPT64 fusion protein enhances BCG-induced protective immunity against Mycobacterium tuberculosis infection in mice. Med Microbiol Immunol 2011;200:165-75.
38. Kaufmann SH, Dockrell HM, Drager N, et al. TBVAC2020: advancing tuberculosis vaccines from discovery to clinical development. Front Immunol 2017;8:1203.
39. Penn-Nicholson A, Tameris M, Smit E, et al. Safety and immunogenicity of the novel tuberculosis vaccine ID93+GLA-SE in BCG-vaccinated healthy adults in South Africa: a randomised, double-blind, placebo-controlled phase 1 trial. Lancet Respir Med 2018;6:287-98.
40. Penn-Nicholson A, Geldenhuys H, Burny W, et al. Safety and immunogenicity of candidate vaccine M72/AS01E in adolescents in a TB endemic setting. Vaccine 2015;33:4025-34.
41. Davidsen J, Rosenkrands I, Christensen D, et al. Characterization of cationic liposomes based on dimethyldioctadecylammonium and synthetic cord factor from M. tuberculosis (trehalose 6,6'-dibehenate): a novel adjuvant inducing both strong CMI and antibody responses. Biochim Biophys Acta 2005;1718:22-31.
42. Kamath AT, Valenti MP, Rochat AF, et al. Protective antimycobacterial T cell responses through exquisite in vivo activation of vaccine-targeted dendritic cells. Eur J Immunol 2008;38:1247-56.
43. van Dissel JT, Arend SM, Prins C, et al. Ag85B-ESAT-6 adjuvanted with IC31 promotes strong and long-lived Mycobacterium tuberculosis specific T cell responses in naïve human volunteers. Vaccine 2010;28:3571-81.
44. Homolka S, Ubben T, Niemann S. High sequence variability of the ppE18 gene of clinical Mycobacterium tuberculosis complex strains potentially impacts effectivity of vaccine candidate M72/AS01E. PLoS One 2016;11:e0152200.
45. Luabeya AK, Kagina BM, Tameris MD, et al. First-in-human trial of the post-exposure tuberculosis vaccine H56:IC31 in Mycobacterium tuberculosis infected and non-infected healthy adults. Vaccine 2015;33:4130-40.
46. Lin PL, Dietrich J, Tan E, et al. The multistage vaccine H56 boosts the effects of BCG to protect cynomolgus macaques against active tuberculosis and reactivation of latent Mycobacterium tuberculosis infection. J Clin Invest 2012;122:303-14.
47. Santosuosso M, McCormick S, Zhang X, Zganiacz A, Xing Z. Intranasal boosting with an adenovirus-vectored vaccine markedly enhances protection by parenteral Mycobacterium bovis BCG immunization against pulmonary tuberculosis. Infect Immun 2006;74:4634-43.
48. Usman MM, Ismail S, Teoh TC. Vaccine research and development: tuberculosis as a global health threat. Cent Eur J Immunol 2017;42:196-204.
49. Stylianou E, Griffiths KL, Poyntz HC, et al. Improvement of BCG protective efficacy with a novel chimpanzee adenovirus and a modified vaccinia Ankara virus both expressing Ag85A. Vaccine 2015;33:6800-8.
50. Wang J, Thorson L, Stokes RW, et al. Single mucosal, but not parenteral, immunization with recombinant adenoviral-based vaccine provides potent protection from pulmonary tuberculosis. J Immunol 2004;173:6357-65.

51. Reither K, Katsoulis L, Beattie T, et al. Safety and immunogenicity of H1/IC31(R), an adjuvanted TB subunit vaccine, in HIV-infected adults with CD4+ lymphocyte counts greater than 350 cells/mm3: a phase II, multi-centre, double-blind, randomized, placebo-controlled trial. PLoS One 2014;9:e114602.
52. Pu F, Feng J, Xia P. Association between heparin-binding hemagglutinin and tuberculosis. Adv Clin Exp Med 2020;29:893-7.
53. Bertholet S, Ireton GC, Ordway DJ, et al. A defined tuberculosis vaccine candidate boosts BCG and protects against multidrug-resistant Mycobacterium tuberculosis. Sci Transl Med 2010;2:53ra74.
54. Parra M, Pickett T, Delogu G, et al. The mycobacterial heparin-binding hemagglutinin is a protective antigen in the mouse aerosol challenge model of tuberculosis. Infect Immun 2004;72:6799-805.
55. Comas I, Chakravartti J, Small PM, et al. Human T cell epitopes of Mycobacterium tuberculosis are evolutionarily hyperconserved. Nat Genet 2010;42:498-503.
56. Aagaard CS, Hoang TT, Vingsbo-Lundberg C, Dietrich J, Andersen P. Quality and vaccine efficacy of CD4+ T cell responses directed to dominant and subdominant epitopes in ESAT-6 from Mycobacterium tuberculosis. J Immunol 2009;183:2659-68.
57. Sweeney KA, Dao DN, Goldberg MF, et al. A recombinant Mycobacterium smegmatis induces potent bactericidal immunity against Mycobacterium tuberculosis. Nat Med 2011;17:1261-8.
58. Commandeur S, van Meijgaarden KE, Prins C, et al. An unbiased genome-wide Mycobacterium tuberculosis gene expression approach to discover antigens targeted by human T cells expressed during pulmonary infection. J Immunol 2013;190:1659-71.
59. Boisson-Dupuis S, Bustamante J, El-Baghdadi J, et al. Inherited and acquired immunodeficiencies underlying tuberculosis in childhood. Immunol Rev 2015;264:103-20.
60. Schrager LK, Vekemens J, Drager N, Lewinsohn DM, Olesen OF. The status of tuberculosis vaccine development. Lancet Infect Dis 2020;20:e28-37.
61. Reed SG, Orr MT, Fox CB. Key roles of adjuvants in modern vaccines. Nat Med 2013;19:1597-608.
62. Hutchison S, Benson RA, Gibson VB, Pollock AH, Garside P, Brewer JM. Antigen depot is not required for alum adjuvanticity. FASEB J 2012;26:1272-9.
63. Mustafa AS. Biotechnology in the development of new vaccines and diagnostic reagents against tuberculosis. Curr Pharm Biotechnol 2001;2:157-73.
64. Dong Y, Gong JY, Liu X, Li JW. Enhanced immune response of a bicistronic DNA vaccine expressing fusion antigen Hsp65-Esat-6 of Mycobacterium tuberculosis with GM-CSF as a molecular adjuvant. Braz Arch Biol Technol 2013;56:757-65.
65. Moradi B, Sankian M, Amini Y, Meshkat Z. Construction of a novel DNA vaccine candidate encoding an HspX-PPE44-EsxV fusion antigen of Mycobacterium tuberculosis. Rep Biochem Mol Biol 2016;4:89-97.
66. Decatur AL, Portnoy DA. A PEST-like sequence in listeriolysin O essential for Listeria monocytogenes pathogenicity. Science 2000;290:992-5.
67. Nieuwenhuizen NE, Kulkarni PS, Shaligram U, et al. The recombinant bacille Calmette-Guerin vaccine VPM1002: ready for clinical efficacy testing. Front Immunol 2017;8:1147.
68. Hoft DF, Blazevic A, Abate G, et al. A new recombinant bacille Calmette-Guerin vaccine safely induces significantly enhanced tuberculosis-specific immunity in human volunteers. J Infect Dis 2008;198:1491-501.
69. Gonzalo-Asensio J, Marinova D, Martin C, Aguilo N. MTBVAC: attenuating the human pathogen of tuberculosis (TB) toward a promising vaccine against the TB epidemic. Front Immunol 2017;8:1803.
70. Lee JS, Krause R, Schreiber J, et al. Mutation in the transcriptional regulator PhoP contributes to avirulence of Mycobacterium tuberculosis H37Ra strain. Cell Host Microbe 2008;3:97-103.
71. Haile M, Kallenius G. Recent developments in tuberculosis vaccines. Curr Opin Infect Dis 2005;18:211-5.
72. Grode L, Seiler P, Baumann S, et al. Increased vaccine efficacy against tuberculosis of recombinant Mycobacterium bovis bacille Calmette-Guerin mutants that secrete listeriolysin. J Clin Invest 2005;115:2472-9.
73. Kupferschmidt K. Infectious disease: taking a new shot at a TB vaccine. Science 2011;334:1488-90.
74. Zhu B, Dockrell HM, Ottenhoff TH, Evans TG, Zhang Y. Tuberculosis vaccines: opportunities and challenges. Respirology 2018;23:359-68.
75. von Reyn CF, Lahey T, Arbeit RD, et al. Safety and immunogenicity of an inactivated whole cell tuberculosis vaccine booster in adults primed with BCG: a randomized, controlled trial of DAR-901. PLoS One 2017;12:e0175215.
76. Master SS, Rampini SK, Davis AS, et al. Mycobacterium

tuberculosis prevents inflammasome activation. Cell Host Microbe 2008;3:224-32.
77. Reber SO, Siebler PH, Donner NC, et al. Immunization with a heat-killed preparation of the environmental bacterium Mycobacterium vaccae promotes stress resilience in mice. Proc Natl Acad Sci U S A 2016;113:E3130-9.
78. Hernandez-Pando R, Pavon L, Arriaga K, Orozco H, Madrid-Marina V, Rook G. Pathogenesis of tuberculosis in mice exposed to low and high doses of an environmental mycobacterial saprophyte before infection. Infect Immun 1997;65:3317-27.
79. Diaz C, Perez Del Palacio J, Valero-Guillen PL, et al. Comparative Metabolomics between Mycobacterium tuberculosis and the MTBVAC vaccine candidate. ACS Infect Dis 2019;5:1317-26.
80. Moreno-Mendieta SA, Rocha-Zavaleta L, Rodriguez-Sanoja R. Adjuvants in tuberculosis vaccine development. FEMS Immunol Med Microbiol 2010;58:75-84.
81. Lyadova IV, Panteleev AV. Th1 and Th17 cells in tuberculosis: protection, pathology, and biomarkers. Mediators Inflamm 2015;2015:854507.
82. Zygmunt BM, Rharbaoui F, Groebe L, Guzman CA. Intranasal immunization promotes th17 immune responses. J Immunol 2009;183:6933-8.
83. Bhatt K, Verma S, Ellner JJ, Salgame P. Quest for correlates of protection against tuberculosis. Clin Vaccine Immunol 2015;22:258-66.