

A Global Overview of Tuberculosis Vaccine Development

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ARTICLE INFO

Review Article

VacRes, 2022

Vol. 9, No.2, 47 - 55

Received: May 23, 2023

Accepted: June 14, 2023

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KEYWORDS: Tuberculosis, BCG, vaccine development, immunogenicity

ABSTRACT

Tuberculosis (TB) is one of the deadliest infectious diseases in the world; therefore, controlling TB epidemics is one of the main priorities of global public health. According to the priorities of the World Health Organization, developing and designing new vaccines are needed more than ever to control the spread of this disease. Meanwhile, efforts to produce new drugs against the infection can be effective for the treatment of the disease. Today, the only approved vaccine for TB prevention is Bacille Calmette-Guerin (widely known as BCG) which cannot provide immunity in adults. As a result, researchers have done many investigations in clinical development and pre-clinical trials to prepare new vaccines and to examine new candidates to replace or enhance BCG vaccine. This review investigates the history of BCG vaccine, its limitations as well as recent advances to improve its immunogenicity while reducing its side effects. Moreover, other TB vaccines along with new TB vaccine candidates in clinical trials are presented in this article.

INTRODUCTION

Tuberculosis (TB) is one of the oldest diseases known to mankind. Despite the availability of antibiotics and a live attenuated vaccine, TB is still considered one of the most serious medical problems for the public health [1]. TB is caused by a bacterium called *Mycobacterium tuberculosis* (Mtb), an aerobic, rod-shaped bacterium with a cell wall riched in lipids and long-chain mycolic acid, which can survive against destructive agents [2]. TB is a major cause of death in endemic areas, especially in adolescents and adults [3]. One of the main reasons for the importance of the prevention of TB is the resistance of this microorganism to drugs and antibiotics, which has made the treatment of TB very complicated. Today, the use of new and optimized drugs with the help of nanotechnology with unique properties has partially eliminated the problem of drug resistance with respect to TB [4, 5]. However, the high costs and limitations of using these types of drugs have doubled the importance of preventing TB with the help of new vaccines. Approximately, one-third of the world's population is infected with Mtb. Diseases caused by multi-drug-resistant TB (MDR) and extensively drug-resistant TB (XDR) are also one of the main causes of this global epidemic [6]. After inhalation of Mtb, elements of innate immunity, including alveolar macrophages and granulocytes, are activated to fight the infection. In some people, the infection clears up, while in others, the infection

develops and remains [7]. People suffering from this disease can be divided into active and latent TB patients. Patients with latent TB infections include a range of people with different risks of developing active TB. Most people with latent TB do not develop active TB or show any symptoms. However, it may take months to years for symptoms to appear. The time required for the symptoms to appear depends on the individual's immune response [8]. Identifying the genes involved in creating drug resistance and investigating the involvement of these genes in the immune system can be effective for developing and designing new vaccines [9].

Therefore, to control TB, an effective preventive vaccine can play an essential role in averting the spread of this global epidemic [6]. Dozens of candidate TB vaccines have been designed to enhance BCG or replace it while many vaccine candidates are in the stages of clinical trials [10]. Vaccination of newborns with BCG can be effective against pulmonary TB and its spread in infants and children. In contrast, BCG vaccination for adults can only protect against pulmonary TB if the patient has not been infected with mycobacterium infection [11]. BCG vaccines, inactivated whole-cell mycobacterial vaccines derived from Mtb and non-tuberculous mycobacteria, were effective in preventing TB in experimental models and clinical trials; however, they have not been widely used [6].

Protective immunity against TB has yet to be fully understood. T-cell immunity, including CD4⁺ and CD8⁺ cells, is thought to be important for effective disease prevention after Mtb infection. Therefore, the induction of a long-lasting Mtb-specific T-cell response is one of the goals of new vaccine strategies. Several T cell effector molecules may play an essential role in Mtb control, including the T cell helper type 1 (Th1) cytokines, IFN- γ , tumor necrosis factor (TNF)- α , and IL-2. They promote secondary memory T cell proliferation and maintain a stable collection of these cells. In addition, vaccination can induce multifunctional T cells, which simultaneously express IFN- γ , TNF- α , and IL-2. In this review, the types of experimental vaccines produced for the prevention of TB are examined [12].

BCG

BCG is the only approved and most used vaccine against TB worldwide. Since 1974, the developed program of the World Health Organization (WHO) on immunization has recommended that BCG should be administered as soon as possible after birth in countries with a high prevalence of TB, with coverage of more than 80% of infants (<https://www.cdc.gov/mmwr/preview/mmwrhtml/00054407.htm>).

BCG vaccine stands for "Bacillus Calmette-Guerin", which refers to the bacterial species of this disease, and Calmette and Guerin are the names of the main discoverers of this vaccine. These two French scientists were Albert Calmette (physician and bacteriologist) and Camille Guerin (veterinarian) who had been researching on TB vaccine since 1905. The BCG vaccine was first tested on humans in 1921 in Paris. Finally, in 1927 and 1928, Dr. Calmette published reports on the results of experimental vaccinations between 1921 and 1927 [13] (Fig. 1).

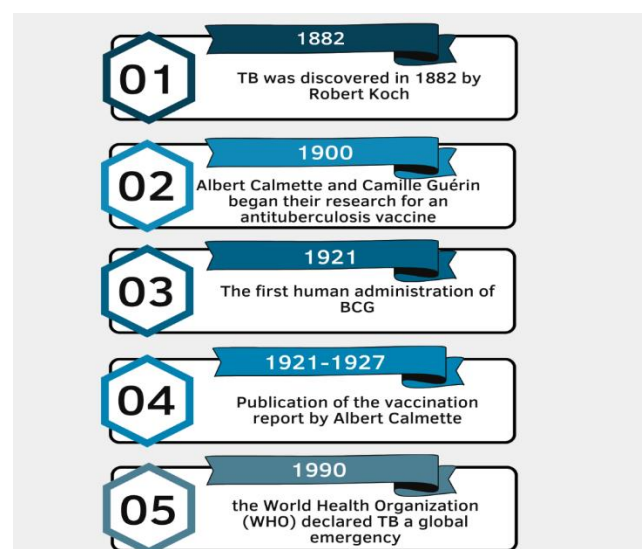


Fig. 1. Important milestones of BCG vaccine [13].

The effectiveness of BCG in preventing disease development into pulmonary TB in adults has always been controversial. At the same time, BCG vaccination confers immunity in infants and children against TB-meningitis and severe forms of disseminated TB. HIV/AIDS epidemics have increased the global prevalence of TB. A low number of CD4⁺ T cells predispose HIV-infected infants to TB. Therefore, according to the WHO recommendation, the BCG vaccine was prohibited for HIV-

infected children as a live attenuated *Mycobacterium Bovis*. Since vaccination is essential to reduce BCG-related diseases, an urgent need is to develop an alternative non-live bacterial vaccine suitable for preventing TB transmission, especially among children living in HIV epidemic areas [14]. Studies demonstrate that adding *Mycobacterium leprae* antigens to the BCG vaccine may improve its effectiveness against TB and leprosy diseases [15, 16]. This was despite the fact that, unlike other studies, BCG vaccine injection in infected monkeys with progressive disease with a high level of Mtb-specific IgG reactive protein secretion and extensive damage did not provide specific immunity [17]. In this regard, the use of the *Mycobacterium w* (Mw) vaccine, which was prepared from a cultivable and non-pathogenic mycobacterium, showed a practical protective effect against pulmonary TB [18]. Also, the protective effect of the vaccine against multidrug-resistant TB in 360 patients with TB in two groups (only with 2HRZ/4HR chemotherapy and chemotherapy with BCG) further enhanced the effects of chemotherapy. It reduced the incidence of multidrug-resistant TB [19]. Two groups of 70 male Holstein calves, including a placebo group and a vaccinated group, which were vaccinated in the second week of their age, were studied. All the PCR results in non-vaccinated animals were declared positive. The results showed that the BCG vaccine prevents the excretion and spread of the Mtb bacillus by the animal's nasal secretions, although it could not provide 100% protection [20].

NEW TB VACCINE

Protective immunity against TB has yet to be fully understood. We assume T-cell immunity, including CD4⁺ and CD8⁺ cells, are necessary for effective disease prevention after Mtb infection in adults. Therefore, induction of a long-lasting Mtb-specific T-cell response is one of the strategies of new vaccines. Several T cell effector molecules may play an essential role in Mtb control, including the T cell helper type 1 (Th1) cytokines such as IFN- γ , tumor necrosis factor (TNF)- α , and IL-2. They promote secondary memory T cell proliferation and maintain a stable collection of these cells. In addition, vaccination can induce multifunctional T cells, which simultaneously express IFN- γ , TNF- α , and IL-2 [21].

I- TB SUBUNIT VACCINES

A: *Mycobacterium vaccae*

M. vaccae, a saprophytic mycobacterium that contains multiple antigenic epitopes shared with Mtb, is an immunotherapy vaccine. Currently, three *M. vaccae* vaccine models have been produced, including a heat-killed vaccine in the UK, a model from Dartmouth, and a lysate vaccine from China [22]. Immunogenicity of the TB vaccine was observed among BCG-vaccinated HIV-infected adults with CD4 counts of less than 200 cells/ μ L per microliter. The effectiveness of the *M. vaccae* vaccines are shown by preventing TB infection [14] and their effect on cellular immunity and treatment of patients with pulmonary TB in a study in 1999 in which 70 people in two groups who received 2HRZS/4HR vaccine were examined. The obtained results demonstrated that this vaccine could stimulate the activation of cellular immunity and could cause the sputum test to be negative in patients while being a promising mean for immunotherapy [23]. Furthermore, a comparison of two methods of TB treatment on 342 people with pulmonary TB, treated initially with positive smear and culture, was made between the two groups. The first group's treatment included chemotherapy with a 2HRZE/2HR regimen and immunotherapy with the *M.*

vaccae vaccine for six months, and the second group only received chemotherapy with a 2HRZE/4HR regimen. The results revealed that the simultaneous use of the *M. vaccae* vaccine as a complementary drug with chemotherapy in patients with TB is effective and can shorten the treatment period [24]. The amount of lymphocyte proliferation assay (LPA) and IFN- γ response to mycobacterial antigens during the first year of inactivated *M. vaccae* vaccine injection was measured along with a random control in which five doses of BCG vaccine were immunized in 39 HIV+ patients. The outcome revealed that, among HIV+ patients, 19 recipients of the *M. vaccae* vaccine had higher LPA and IFN- γ responses than 20 control vaccine recipients after three to five doses of vaccine and more than one year after the vaccine injection. Also, immunization with the *M. vaccae* vaccine was reported to be safe, and there was no adverse effect on HIV viral load or CD4 cell counts [25]. Researchers have conducted a randomized controlled trial of placebo and vaccine in Tanzania on HIV-infected patients with CD4 counts of at least 200 cells/ μ L in 5 intradermal doses of *M. vaccae* vaccine. Also, a tuberculin skin test with a 5 mm reaction cut-off was performed for six months with isoniazid. According to the obtained results, the injection of cells/ μ L vaccine with the help of BCG injection in childhood was safe. Moreover, it provided immunity against TB without any adverse effects on the number of CD4 cells or HIV viral load [6].

B: AEC/BC02

Latent TB infection (LTBI) is one of the main causes of active TB. Therefore, to eliminate TB, the treatment of LTBI is very important. To treat TB, a six-month course of multi-drug therapy is often required while drug-resistant TB requires a longer course that can cause many complications. Currently, vaccines have been proposed as adjuncts to treat populations with LTBI and can delay its recurrence. After examining the dose and the treatment time, it was found that taking isoniazid-rifampin tablets for four weeks along with three or six vaccine injections can significantly reduce the organ's bacterial load and improve the pathological lesions [26]. In a study, the therapeutic effect of a new AEC/BC02 subunit vaccine was evaluated after treatment in a spontaneous relapse model of *Mtb*. The results showed that the bacterial load increased slowly in the spleen and lungs of mice inoculated with AEC/BC02, compared to a control group. In addition, it was found that the AEC/BC02 vaccine increased the induction of IFN- γ or IL-2-secreting cellular immune responses, which decreased with the number of vaccinations. Also, an increase in Ag85b-specific IgG was reported in mice after treatment with AEC/BC02 [27]. The *Mtb* AEC antigen proteins consisting of Ag85b and ESAT6-CFP10 proteins was investigated as a new vaccine for TB treatment in association with aluminum (Al) and polyribinosinic-polyribocytidylic acid (poly-IC). The results showed an increase in specific humoral immune responses and cellular immunity in mice vaccinated with AEC/Al/poly-IC and the protective effect of the vaccine in a latent TB infection guinea pig model [28].

C: Hybrid: IC31

H4:IC31 is an investigational vaccine containing two active components of H4 antigen. It is a fusion protein designed from two *Mtb* antigens, namely 85B (Ag85B) and TB10.4. The second component of the vaccine is an immunological adjuvant called IC31. The logic behind designating H4:IC31 vaccine is to provide *Mtb*-specific antigens to enhance BCG-induced T-cell immunity and thus improve the protection against TB. Ag85B is a mycolyl transferase protein, and TB10.4 is one of three similar

proteins with the antigenic purpose of (ESAT)-6 secretory found in *Mtb* cultures. These two proteins cause a significant protective and synergistic effect against *Mtb* aerosols [29]. In a study, H4:IC31 vaccine was administered, to healthy and HIV- adults with a history of BCG vaccine injection in childhood, in doses of 5, 15, 50, or 150 micrograms of H4 formulated with 500 nmol IC31 with an interval of two months between each injection. Mild side effects were reported, such as fatigue and pain in the injection area. Antigen-specific CD4 T cells were induced by all doses of H4:IC31, whereas doses below 50 μ g elicited the highest frequency of CD4 T cells, which was predominantly caused by IFN- γ +TNF- α +IL-2 or TNF- α +IL-2 [30]. The difference between BCG and H4:IC31 vaccines was investigated in a study with 990 teenagers vaccinated in childhood which proved that both BCG and H4:IC31 vaccines can effectively create immunity. However, based on QuantiFERON-TB (QFT) method which is the latest generation of IFN- γ release assays (IGRAs), BCG vaccine effectiveness was reported at 45.4% ($P = 0.03$). In comparison, the effectiveness of the H4:IC31 vaccine was 30.5% ($P = 0.16$) [31]. IC31 is a TB subunit vaccine containing Ag85B fusion protein in addition to ESAT-6 (H1). A study conducted on H1 vaccine alone and H1 formulated with IC31 adjuvant in 5 different groups indicated a better response for *Mtb*-negative group who were vaccinated with IC31 adjuvant [11]. H56:IC31 is a TB vaccine candidate that includes Ag85B, ESAT-6, and Rv2660c fusion proteins formulated with IC31 adjuvant. Intramuscular injection of 25 people who had participated in the experiment revealed that H56:IC31 vaccine causes antigen-specific IgG responses and Th1 cytokine-expressing CD4+ T cells. Compared to high-dose vaccination, low-dose vaccination induced H56-specific CD4+ T cells (IFN- γ +TNF- α +IL-2+). After the injection of the second dose of a high-dose vaccine, there was a significant increase in CD4+ T cells expressing IFN- γ [32].

D: M72/AS01

The M72/AS01 vaccine is a recombinant vaccine containing *Mtb*39a and *Mtb*32a antigens that can only be expressed in *Mtb* and BCG. A clinical trial in 110 volunteers in Belgium demonstrated that M72/AS01 was clinically well tolerated and induced acceptable cell-mediated and humoral immune responses [14]. Three doses of M72/AS01 vaccine were administered to adults, aged 18-40 in a clinical trial based on a 0-1-2-month injection schedule. No severe side effects were reported, and *Mtb*72F-antigen induced IL-2 and IFN- γ production based on ELISPOT method, and the expressed CD4+ T cells were observed with ICS [33]. Similar to this study, three doses of the vaccine were injected at an interval of one month into 50 adults. After the second and the third doses, similar results were reported [34]. The new M72/AS01 vaccine was investigated in a phase 2 clinical trial on healthy adults in South Africa, which increased the frequency of T cell induction [35]. The immunogenicity of a new TB vaccine candidate containing M72 antigen and the liposome-based AS01 adjuvant system were evaluated randomly on healthy adults in the Philippines at doses of 10/20/40 μ g. The M72-Adjuvanted vaccine has induced CD4+ T cells and an M72-specific humoral response that persisted six months after the vaccination. However, injection of higher doses increased the rate and stability of CD4+ T cell responses [36]. Twenty adults vaccinated with BCG and 18 adults infected with TB were randomly evaluated by receiving three doses of *Mtb*72F/AS02 and AS02 vaccines at one-month intervals. The group vaccinated with *Mtb*72F/AS02 vaccine exhibited the induction of multifunctional *Mtb*72F-specific

CD4+ T cell responses and anti-Mtb72F humoral responses [37]. Considering the immunogenicity of M72/AS01 vaccine, the second phase study was conducted in two groups, including 150 formerly-vaccinated infants with BCG. The results indicated that after the injection of two doses, humoral T cells, and CD4+ responses were higher compared to one dose in all groups [38]. Researchers randomly evaluated the immunization and immunogenicity of M72/AS01 vaccine in HIV+ adults in Switzerland in two doses of M72/AS01 and AS01. The researchers found that M72/AS01 vaccine induces M72-specific CD4+ T cell responses, increases M72 IgG in the studied groups, and is monogenic [39]. In another second phase study, three groups composed of TB-free adults, previously treated TB patients, and adults undergoing intensive therapy, were screened for M72/AS01 vaccine. The study was stopped in people undergoing simultaneous treatment due to swelling and inflammation in the early stages. However, in the other two groups, humoral and CD4+ immune responses caused by T cells were observed after vaccination with M72/AS01 [8]. In another study, 240 HIV- and HIV+ adults on stable antiretroviral therapy (ART) participated. The study included 0 to 12 months for receiving M72/AS01 or placebo to evaluate humoral responses, adverse events and M72-specific T cells. The obtained results indicated immunogenicity and persistence in both HIV+ and HIV- groups [40]. Long-term immunization evaluation of M72 vaccine has been done in a second phase study for three years after the injection of three doses in HIV+ and HIV- Indian adults. The researchers found that despite creating a humoral immune response, the vaccine could cause serious side effects [41]. The same results were reported in a similar study conducted in South Africa and Zambia on adults aged 18 to 50 [42]. A study aimed to identify the optimal time points for M72/AS01 vaccine injection and to evaluate the RNA expression levels of whole blood and PBMCs along with vaccine immunogenicity, using a microarray technology. The results included IFN- γ serum responses and M72-specific CD4+ T cells, similar to the previous studies [3]. Furthermore, the immunization and immunogenicity study of Mtb72F/AS02 vaccine was conducted in Mtb-free adults containing 40 micrograms of M72 antigen with AS02 or AS01. The results were compared with Mtb72F/AS02 vaccine (40 μ g dose), M72 in saline (40 μ g dose), and AS01. This study exhibited that both vaccines induce immunogenicity while Mtb72F/AS02 and M72/AS02 vaccines produce much higher immunity than a M72/saline control [43].

E: ID93/GLA-SE

ID93 is one of the TB subunit vaccines that includes four antigens while each of these antigens represents different families of Mtb proteins. One of these proteins is *Rv1813*, regulated under hypoxic growth and is probably located in the outer membrane. The other putative protein is *Rv2608* (*PPE42*) which contains a Pro-Pro-Glu (PPE) motif, associated with the outer membrane. The rest are *14Rv3619* (*EsxV*) and *Rv3620* (*EsxW*) proteins that belong to the family of ESAT-6 virulence factors [44].

The injection of ID93+GLA-SE research vaccine into 54 HIV- people did not cause severe side effects and induced antigen-specific IgG and Th1 cell responses. The number of antigens reached its peak with the injection of the second dose [45]. Similarly, a study was conducted in Africa, where the participants in this experiment were suffering from drug-resistant TB. Vaccination of 2 μ g ID93 + 5 μ g GLA-SE induced T cell responses of specific IgG and CD4 antigens. Although mild to

moderate pain was reported in the injection area, no other side effects were observed in the participants [46].

II-RECOMBINANT LIVE VACCINES

A: rBCG

The accepted policy to produce a vaccine to replace BCG is to add specific genes to BCG or remove certain genes from the natural mycobacterium genome. The first recombinant vaccine made was rBCG vaccine. This vaccine creates a robust immune response by overexpressing Mtb Ag85B protein [14]. A study conducted on 35 adults evaluated Ag85b-specific immune responses caused by a vaccine injection. The study's results exhibited that rBCG30 vaccine increased Ag85b-specific T cell lymphoid proliferation, IFN- γ secretion, IFN- γ enzyme-related immunoreceptor responses, and IFN- γ intracellular responses under *in vivo* condition [47].

B: TB/FLU-01L

During a phase I clinical trial in 2015, TB/FLU-01L vaccine was investigated. This vaccine contains ESAT-6 antigen of a recombinant influenza A virus. In this 42-day trial with 36 male volunteers, the immunogenicity analysis demonstrated that 72.2% of people from a sublingual injection group and 77.8% of people from an intranasal administration exhibited detectable immune responses [48].

C: TB/FLU-04L

Another mucosal vector vaccine that is in clinical trials is TB/FLU-04L vaccine. This vaccine designed based on an influenza virus vector, expresses Ag85A and ESAT-6 antigens. This vaccine was designed as a preventive booster vaccine to prevent the development of TB in infants, adolescents, and adults. A randomized, double-blind, placebo-controlled phase I trial investigated the safety and immunogenicity of two doses (day 1 and day 21) of TB/FLU-04L in healthy BCG-vaccinated adults. This study was conducted in 2015 on people aged 18 to 50 years (Clinical Trials: NCT02501421). Currently, the second phase of vaccine testing on latent TB infection is being implemented [49].

D: MVA85A (AERAS-485)

One of the boosters of the BCG vaccine is MVA85A which is a modified vaccinia Ankara (MVA) that expresses the secreted major antigen Ag85A (*MVA85A*, *AERAS-485*) of Mtb. This novel vaccine moderately improved the protective effect induced by BCG vaccination against Mtb in animal models, mainly due to better induction of CD4, CD8 T cell responses and Th1 and antigen-specific responses. This vaccine was investigated in several trials, and the efficacy of Th17 cells against Mtb53 and Mtb55 in infants in South Africa showed acceptable sequel. In this study, healthy infants aged 4 to 6 months who were vaccinated with BCG at birth received a dose of MVA85A or placebo between 4 and 6 months of age [14]. The first phase 2 clinical trial of the vaccine (MVA85A) was conducted on South African infants, vaccinated previously with BCG. The number of cells and ELISpot responses decreased significantly after one night, and surface flow-cytometry tests defined a significant decrease in CD4+ and CD8+ T cells [50]. A vital component that protects against Mtb is IFN- γ that causes a pro-inflammatory immune response. In the absence of a protective immunity, the production of IFN- γ -specific antigens is a common way for the induction of protective immune responses. In a phase I clinical

trial which was conducted using MVA85A vaccine candidate, higher baseline IFN- γ responses were detected by a whole blood culture ELISA test [51]. Acceptable immunization and immunogenicity were observed in UK trials of MVA85A-boostered BCG vaccine against non-infectious Mtb. BCG-specific CD4 $^{+}$ T cells amplified by MVA85A vaccine from different populations expressed IFN- γ , TNF- α , IL-2, and IL-17 [52]. One of the ways to enhance the BCG vaccine is to use modified Ag85A, called MVA85A. Meanwhile, the induction of new Th1 cell populations is required for new TB vaccines [53]. This new vaccine was tested on infants. The vaccine was well-tolerated and induced a strong cellular immune response [10, 54]. The same study was also conducted on 12 teenagers, and 24 children vaccinated for 12 and 6 months, respectively and similar results were obtained [55]. The assessment of immunization and immunogenicity of MVA85A vaccine in groups infected with MTB and HIV in a second phase clinical trial on 48 adults in 52 weeks determined this vaccine causes a strong and long-lasting response of CD4 $^{+}$ T cells which express IFN- γ , TNF- α and IL-2 [56]. MVA85A vaccine was well-tolerated and was immunogenic in HIV-1 infected adults [57]. Evaluating MVA85A vaccine in South Africa on infants found encouraging results [58]. Manufacturing modified recombinant MVA85A vaccine with increased efficiency and examining this vaccine on people with LTBI has become a priority for the global health authorities. The results of most of these studies indicate the safety and immunogenicity of this vaccine [59]. Intradermal injection of MVA85A vaccine induced Ag85A-specific CD4 T cells in adults who had previously received BCG vaccine [50, 60]. Another vaccine was designed to enhance BCG vaccine based on IMX313 carrier protein which improves cellular and humoral immunity. MVA85A-IMX313 vaccine was designed to enhance the immunity created by BCG and its immunogenicity was investigated in clinical studies. Examination of MVA85A-IMX313 and MVA85A vaccines showed that both vaccines cause a significant increase in ELISpot IFN- γ response [61].

E: ChadOx1-85A

ChAdOx1-85A is a new chimpanzee adenovirus vaccine expressing Ag85A, under investigation [62]. A first-in-human phase I trial was conducted to evaluate the safety and immunogenicity of ChAdOx1-85A as a prime booster with MVA85A in healthy BCG-vaccinated adults (ClinicTrial.gov: NTC01829490). In a related study, a total of 42 healthy adults vaccinated with BCG were enrolled. By evaluating the immunogenicity, it was found that with the injection of this vaccine, the level of Ag85A-specific ELISpot and intracellular CD4 $^{+}$ and CD8 $^{+}$ cytokine cell responses increased. Meanwhile, multifunctional CD4 $^{+}$ T cells and IFN- γ , TNF- α , and CD8 $^{+}$ T cells were induced by ChAdOx1-85A [63]. In another clinical trial conducted to investigate ChAdOx1-85A vaccine and MVA85A as a supplement, 42 healthy adults vaccinated with BCG were examined in 4 groups with low and high doses. ChAdOx1 85A induced Ag85A-specific ELISpot response and intracellular CD4 $^{+}$ and CD8 $^{+}$ T cell cytokines. While the level of immunogenicity was not enhanced by the injection of a second dose, it was enhanced by MVA85A. The results showed that multifunctional CD4 $^{+}$ T cells (IFN- γ , TNF- α , and IL-2) and IFN- γ , TNF- α CD8 $^{+}$ T cells were induced by ChAdOx1 85A and enhanced by MVA85A [63]. Another study was conducted to compare ChAdOx1 85A vaccine by intramuscular injection mode versus aerosol administration, in terms of immunity and immunogenicity on 29 participants formerly vaccinated with BCG as well as 10 non-vaccinated participants. The results

showed that the use of inhaled vaccine causes higher cellular responses, especially in IFN- γ /IL-17+ CD4 $^{+}$ T cells, which leads to an increase in systemic humoral and cellular responses [64].

F: VPM1002

VPM1002 is a recombinant BCG vaccine in which the urease C gene (responsible for inhibiting phagolysosomal maturation) has been replaced with an O-listeriolysin from *Listeria monocytogenes* encoded gene [62]. In preclinical studies, as well as in phase I and II clinical trials in infants and adults, the immunogenicity, efficacy, and immunogenicity of this vaccine have been evaluated. An additional Phase II clinical trial was completed in 2017 which examined the safety and immunogenicity of VPM1002, compared to BCG in HIV-exposed and unexposed newborns [65, 66]. A comparison of the safety and immunogenicity of VPM1002 (a BCG recombinant vaccine) with BCG was performed in South Africa on 416 infants who were exposed to HIV as well as infants who were not exposed to HIV. The results of the study showed that the recombinant VPM1002 vaccine was less reactive than BCG and did not have serious side effects. Both vaccines were immunogenic, although responses were greater with BCG vaccine [67]. In a phase II study, conducted by Dockrell [68], the results of a double-blind randomized controlled study on the safety and efficacy of a genetically modified BCG vaccine (VPM1002), compared with BCG in newborns in South Africa were reviewed. The results illustrated that VPM1002 vaccine which is designed to provide more immunity and better efficacy in immunocompromised children than BCG, can enable the bacilli to access the cytoplasm of the host cell by expressing listeriolysin.

G: Crucell Ad35 (AERAS-402)

AERAS-402 is a replication-deficient serotype 35 (Ad35) that contains DNAs and can express combinatorial proteins created from the sequences of mycobacterial antigens Mtb 85A (Ag85A), 85B (Ag85B), and TB104 (21, 22). Therefore, by enhancing the BCG vaccine with the help of AERAS-402, it is possible to increase multifunctional T cells in infants and adults [69]. The antigens are continuously fused as a single-piece fusion polypeptide which is ultimately expressed upon immunization with Ad35 vaccine (AERAS-402). A study on healthy, non-Mtb-infected adults vaccinated with BCG in South Africa evaluated the immunization and immunogenicity of AERAS-402. The outcomes confirmed the BCG vaccine's immunogenicity with increasing doses of AERAS-402, administered intramuscularly in three groups [70]. A booster dose of AERAS-402 in infants immunized at birth with BCG vaccine also induced a specific T-cell response without side effects. Injection of a single dose of AERAS-402 induced CD4 T cells that predominantly expressed just IFN- γ . In contrast, administering two booster doses induced CD4 T cells that predominantly expressed IFN- γ , TNF- α , and IL-2 together [71]. In another study conducted on 16 to 26 weeks-old infants, AERAS-402 injection showed an acceptable immunization profile and was well-tolerated at all doses [72]. An evaluation study of AERAS-402 vaccine immunogenicity conducted on Kenyan adults with and without latent Mtb infection demonstrates that the second dose increases the antibody in people. Besides, Walsh and van Zyl-Smit (71, 72) also expressed the same results in their studies.

H: AdAg85A

Adenovirus (Ad) vectors were first used to treat genetic diseases. They were used for permanent gene replacement due to

their high immunogenicity through the induction of T and B cells to Ad antigens. This feature made them a suitable choice for permanently treating genetic diseases and they were recommended as a suitable vaccine carrier. Examinations compared Ad vector from C families (human serotype HAdV-5; here called AdHu5) [73]. The induction rate of T cells by intramuscular injection of a new Ad-based TB vaccine (AdHu5Ag85A) was investigated using frozen PBMCs obtained from BCG-vaccinated healthy adults. The results showed that this vaccine could detect clinically-relevant T cell epitopes in addition to inducing T cells. The critical point in this study was the ability of T cells to effectively inhibit mycobacterial growth in the infected autologous cells [74]. The increasing incidence of TB, especially in communities with a high prevalence of HIV, requires new vaccines to confront BCG and enhance the immune responses. One of the ways to produce and develop better vaccines is to create a better primary immune response by delivering specific pathogen target antigens using adenoviral vectors. Therefore, using recombinant human adenovirus type 5 (AdHu5) with strong immunogenicity and high efficacy in BCG- and BCG+ adults can prevent the spread of TB. In this study, AdHu5Ag85A vaccine which is a human recombinant type 5 vaccine based on TB adenovirus (AdHu5), was tested in healthy adults who had previously been vaccinated with BCG. The results exhibited that the trial could cause immunogenicity in patients; however, it enhanced CD4+ and CD8+ polyfunctional T-cell immunity much more potently in volunteers that were previously vaccinated with BCG [75].

III- ATTENUATED LIVE VACCINES

A: MTBVAC

MTBVAC is the first live attenuated strain of Mtb developed at the University of Zaragoza (Spain) and is now in phase I clinical trials. To produce a safer and more effective vaccine, two factors were deleted; namely, *phoP* a transcriptional regulator involved in regulating TB virulence, and *fadD26*, crucial for significant mycobacterial surface virulence factors (PDIMs) [14]. The BCG and MTBVAC vaccine immunogenicity was compared by increasing the dose in three groups of healthy adult volunteers (HIV- and without TB). Measurement of IFN- γ release rate (IGRA) of PBMCs was done and the primary outcome was immunity in all vaccinated participants. The Secondary outcome of the vaccine inoculation included blood cell-mediated immune response to live MTBVAC and BCG. With these interpretations, it seems that MTBVAC is the first live attenuated TB vaccine whose immunogenicity was at least as high as BCG [76]. A study was conducted to investigate the efficacy of MTBVAC vaccine in adults and children living in a highly-infected environment. According to the results, the MTBVAC vaccine showed acceptable immunogenicity and induced a durable CD4 cell response in infants. Evidence of immunogenicity demonstrates the progress of MTBVAC in safety and efficacy trials of the vaccine against TB infection [77].

IV- INACTIVATED TB VACCINES

A: DAR-901

DAR-901 is an inactivated poly-antigenic whole-cell mycobacterium vaccine developed at Dartmouth University (USA) which contains inactivated *Mycobacterium Obuense*. SRL 172 (an earlier form of DAR-901) was used in a seven-year

phase in Tanzania. DAR-901 is produced and grown by different production methods instead of agar [14]. Early-phase DAR-901 trials are currently underway in BCG-vaccinated HIV- and HIV+ adults to assess its immunization, tolerability, and safety [78]. DAR-901 vaccine was evaluated to prevent Mtb infection among adolescents, aged 13 to 15 years in Tanzania with primary BCG injection. Randomly-performed T-SPOT.TBR negative test was used to measure IFN- γ release assay (IGRA) with the method of one to three intradermal injections of DAR-901 and placebo in months 0, 2, and 4 with repetition of IGRA at two months and 1, 2, and 3 years after the injection. The obtained results exhibited adequate immunogenicity in DAR-901 recipients with increased immune responses [79].

B: RUTI

RUTI is a poly-antigen liposomal vaccine developed by Archivel Farma (Badalona, Spain). This vaccine is prepared from detoxified and fragmented cells of Mtb (FCMtb) to prevent active TB in people with LTBI. In 2007, a phase 1 clinical trial on healthy volunteers was designed to test the tolerance and immunogenicity of 4 escalating doses of RUTI vaccine (5, 25, 100, and 200 μ g of FCMb). The results revealed that according to local and systemic clinical evaluations, vaccination had high immunogenicity, although few local side effects were observed according to the injection dose [80]. In another study, a placebo-controlled randomized trial was conducted to measure the vaccine's immunogenicity, tolerance, and efficacy in phase 2, including three doses of RUTI vaccine (5, 25, and 50 μ g of FCMb) on both HIV+ and HIV- individuals. Results exhibited that the immunogenicity of RUTI vaccine in LTBI subjects was variable, even among groups [81].

V- DNA TB VACCINES

DNA vaccines protect against diseases by injecting genetically-engineered DNA which results in production of the target antigen. Accordingly, DNA vaccines can induce a comprehensive immune response to recognize and kill the infected host cells and eliminate the intracellular Mtb pathogen. Currently, GX-70 is the only DNA vaccine in clinical trials [82]. GX-70 vaccine consists of four Mtb antigen plasmids together with a recombinant Flt3 ligand [62]. In its phase 1 clinical trial conducted by Yonsei University (South Korea), the immunity, and immunogenicity of GX-70 were evaluated in pulmonary TB patients. This study was conducted to detect specific antigen IFN- γ ELISPOT responses and Flt3L concentration within 8 to 24 weeks [82].

CONCLUSION

Fighting TB has always been a main priority for the global health authorities. Most vaccines studied so far have been conducted in clinical trials on infants [83]. In recent years, considerable progress has been made in making new vaccines and enhancing the old BCG vaccine, which promises an effective vaccine for adults in the future. One of the goals of such studies is the development of the vaccine towards the third phase trials using innovative designs [84]. However, the results obtained between the clinical trials have shown that our knowledge about the relationship between the host and the pathogen is limited. Here, the types of experimental vaccines against TB and their clinical phases were reviewed (Fig.2).

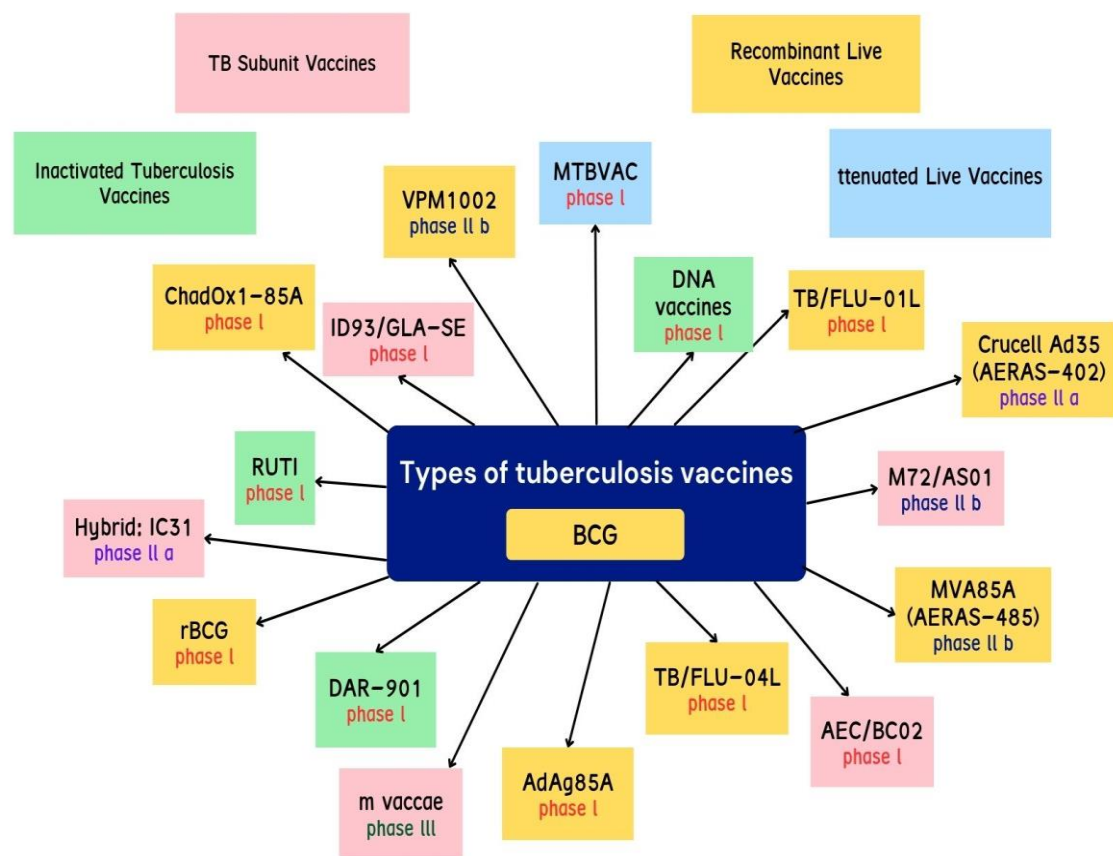


Fig. 2. Different types of TB vaccines produced so far.

Among the, M72/AS01 and MVA85A (AERAS-485) vaccines have attracted the most attention in recent years. The previous vaccine investigations were focused to achieve cellular immunity and induction of Th1 cytokines by CD4 or CD8 T cells [85]; nevertheless, the studies did not prove a specific relationship between the expression of Th1 cytokines and the development of immunity against TB. As a result, enhancing or improving the use of BCG vaccine in clinical trials should be further studied and investigated, in order to achieve a fully effective vaccine [86]. Meanwhile, the use of new technologies in drug and vaccine designs, such as nanotechnology, can create a revolution in improving the performance of such vaccines.

ACKNOWLEDGEMENT

None.

CONFLICT OF INTEREST

The authors declare they have no conflict of interests.

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