

**DEVELOPMENT OF VACCINE CANDIDATES AGAINST MYCOBACTERIUM
TUBERCULOSIS IN 2019-2023****Jongga Adiyaksa^{1*}, Fithriyah Sjatha²**¹⁻²Faculty of Medicine, Universitas Indonesia

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Doi: <https://doi.org/10.33024/mnj.v6i5.14396>**ABSTRACT**

With extensive use of the Bacillus Calmette-Guérin (BCG) vaccine, the global prevalence of Mycobacterium tuberculosis (MTB) remains. A number of vaccines proposed to cure and prevent tuberculosis (TB) infection are undergoing various stages of clinical trials. Although vaccine production is progressing, more attention is needed. A number of TB vaccines are currently undergoing clinical trials, most of which rely on a combination of proteins and/or adjuvants or recombinant viral vectors specific for MTB antigens. We tried to cover the range of TB vaccines in this study by analyzing their composition, the immunological responses they elicit, and the stages of clinical trials. To find out the Development Of Prospective Vaccines Against Mycobacterium Tuberculosis. This research uses a literature review, between August 2023 and November 2023, the authors of this literature review checked PubMed, Science Direct, Google Scholar, and other databases containing research findings or scientific articles. Only studies that met the above search criteria were included in the systematic review. Many recently developed tuberculosis vaccines are reportedly in the final stages of clinical trials, where they have significantly strengthened the immune system and even produced protection against the host. Immunization produced by vaccines that have successfully passed the initial stages of clinical trials is safe and effective, and can even surpass BCG in terms of immunity. Based on the description above, it can be concluded that many recently developed tuberculosis vaccines are reported to be in the final stages of clinical trials, where they have significantly strengthened the immune system and even produced protection against the host. Immunization produced by vaccines that have successfully passed the initial stages of clinical trials is safe and effective, and can even surpass BCG in terms of immunity. With the development of new TB vaccines that strengthen the body's immunity and create effective delivery mechanisms, hopes for TB treatment and prevention are increasing. The development of vaccine effects can be facilitated, in part, through the use of effective delivery mechanisms, which have also been used in TB vaccines.

Keywords: Tuberculosis, Vaccine, Bacteria, Mycobacterium Tuberculosis, BCG**INTRODUCTION**

Mycobacterium tuberculosis (MTB) can be eradicated and an immune response can be elicited, preventing the spread of new

infections, thanks to recently developed TB (tuberculosis) vaccinations. The World Health Organization released the Global

Tuberculosis Report 2022 on October 27. This report uses information from 202 nations and territories to provide a thorough assessment of the global tuberculosis condition. The data includes TB cases and more than 99% of the world's population. (Bagcchi, 2023)(Schrager et al., 2020)

In 2021, 10.6 million people were estimated to have contracted tuberculosis (TB), which is a rise from the 10.1 million cases reported in 2020. Compared to 1.5 million in 2020, which included 214,000 HIV-positive patients, the number of TB-related deaths in 2021 was 1.6 million, including 187,000 HIV-positive patients (Tait, Hatherill, et al., 2019). These vaccines contain antigens expressed by MTB bacteria during latent-phase infection, which can prevent the risk of TB recurrence from latency, which can occur years after primary infection. There is hope for the creation and development of TB vaccines that are even more successful than Bacillus Calmette-Guérin (BCG) in light of some study findings. Furthermore, greater political, financial, and international assistance, as well as public awareness, are needed for more successful TB control (Mahasha et al., 2019). Furthermore, a number of cutting-edge tactics are presently being researched to enhance TB vaccinations. Albert Calmette and Camille Guerin created the BCG vaccine in 1921 by attenuating the *Mycobacterium bovis* strain against tuberculosis (Riccò et al., 2020). Because the BCG vaccination offers strong protection against the baby form of tuberculosis, it is widely utilized in places where tuberculosis is widespread. Newborns in these regions receive a single intradermal vaccination as soon as feasible after delivery (Ottenhoff, 2020)(Bekker et al., 2020) Currently, newborns receive roughly 120 million BCG

injections annually throughout the world [2]. Some side effects are possible with the BCG vaccine, however they are rare and usually moderate. Serious side effects such bone inflammation, abscesses, and widespread tuberculosis are extremely uncommon. The BCG vaccine has a 70-80% efficacy rate against the most severe types of tuberculosis, including TB meningitis. Even though its effectiveness in avoiding pulmonary tuberculosis has been demonstrated, its ability to protect immunocompromised or immunocompetent persons remains uncertain. Although the BCG vaccine is about 80% effective in immunizing children against active tuberculosis, its protective effects are insufficient for adults. The BCG vaccination has not been able to considerably lower the global burden of tuberculosis because of its uneven efficacy in preventing adult pulmonary tuberculosis. Its effect on the tuberculin skin test renders it unfeasible for people with weakened cellular immunity, and its inability to elicit cytotoxic CD8⁺ responses presents further difficulties. As a result, different approaches to creating a fresh TB vaccination are required. There are two main strategies that can be taken into consideration: the first is switching out BCG for other vaccines that improve cellular immunity, and the second is using different vaccinations as boosters to increase BCG's efficacy (Hamiel et al., 2020). There are several TB vaccines in the trial phase, which includes three groups: therapeutic vaccines, priming, and prime boosters vaccines. The majority of TB vaccine development currently centers on the production of new vaccines instead of BCG or using them as the booster of BCG (Kaufmann, 2020). The majority of these vaccinations

are based on recombinant viral vectors that are employed for specific MTB antigens, or combinations of proteins and adjuvants. Researchers in this field can more effectively identify new vaccines and their limitations if they are aware of the current state of developing and existing choices for tuberculosis vaccination as well as vaccine delivery technologies. We tried to cover some of the most recent research on the many TB vaccine candidates, their effects on the immune system, the stages of their clinical trials, and the administration methods in this study. In order to accomplish this, information about TB vaccines was found in the PubMed, Scopus, and Google Scholar databases. To locate published articles using the keywords MTB, TB, and vaccination, internet searches were conducted (Darrah et al., 2020)(Messina et al., 2019)(Kashangura et al., 2019). This study formulated the problem of how the development of vaccines to date

LITERATURE REVIEW

The only known TB vaccine, Bacille Calmette-Guerin (BCG), is effective at shielding newborns and young children from the most severe, frequently fatal forms of the illness, but it does not consistently offer protection against pulmonary TB in adults and adolescents. We will require new therapies, such as better vaccinations that work in adult individuals who have not contracted *Mycobacterium tuberculosis* as well as in latently infected or immunocompromised subjects, if we are to meet the target of eliminating tuberculosis globally by the year 2050. Many novel vaccine candidates, such as adjuvanted proteins, vectored subunit vaccines, and whole cell

vaccines, have advanced to the point of clinical testing in recent decades. It is envisaged that these new TB vaccines will offer encouraging safety and immunogenicity under a variety of circumstances, including as the prevention of TB disease in adults and adolescents, as boosters or replacements for BCG, or as therapeutic vaccinations to shorten the course of TB therapy. (Khoshnood et al., 2019)

Many strains of the *Mycobacterium tuberculosis* complex (MTBC), which are grouped into seven evolutionary branches, are the cause of tuberculosis (TB) in humans. The bulk of TB cases globally are thought to be caused by lineages 2, 3, and 4, which are termed "modern" branches of the MTBC. New alternatives are being looked into since the present BCG vaccine offers inconsistent protection against pulmonary tuberculosis. (Pérez et al., 2020)

According to Khoshnood S et.all and Pérez I et.all the BCG vaccine is the only known vaccine to protect infants and children against tuberculosis, although to date BCG protection is still variable. The BCG vaccine also has disadvantages including the unproven effectiveness of BCG in adults to protect against *Mycobacterium tuberculosis*.

BCG as a vaccine used worldwide is still a polemic against the decline in tuberculosis rates. Therefore, in various countries, developments are still being carried out on the latest vaccines other than BCG. In this literature, various developments of tuberculosis vaccine candidates are presented, starting from the pre-clinical stage to the clinical stage.

The instruments used in this literature study are various information from various related journals consisting of google scholar, pubmed, sciencedirect for further

searches related to the latest tuberculosis vaccine developments, then the data is analyzed.

RESEARCH METHODOLOGY

This research uses a literature review, between August 2023 and November 2023, the authors of this literature review checked PubMed, Science Direct, Google Scholar, and other databases containing research findings or scientific articles. Only studies that met the above search criteria were included in the systematic review. Among the search terms used in this case were tuberculosis, vaccine, Mycobacterium tuberculosis, tuberculosis. There were no

restrictions on the year of research or publication; the only exclusion criterion was duplicate publications. We look at the latest research in an effort to learn more about latent tuberculosis. 110 articles were found when these keywords were searched in various databases. Of the 110 articles that need to be removed at this time, twenty are duplicates. Some publications—from abstracts to full-text articles—may undergo screening. Due to its inability to meet requirements, 52 of the 90 products intended for use were removed. As a result, 38 articles met the requirements for inclusion and are currently under review.

RESULT AND DISCUSSION

a. vaccine candidate including an adjuvant or protein M72/AS01E

M72/AS01E is a recombinant vaccination that was developed to improve the immune response elicited by BCG or MTB. There is a close link between the M72 antigen and Mtb72F, a fusion protein consisting of the Mtb32a and Mtb39a antigens. A point mutation in the Mtb32a antigen of M72 was created in order to improve the long-term sustainability of Mtb72F. The AS01E liposome system combines adjuvant with Quillaja saponaria fraction 1 (QS21) and immunostimulants monophosphoryl lipid (MPL). It elicits humoral and cellular TH1 responses (Tait, Meeren, et al., 2019)(Idoko et al., 2019).

The M72/AS01E vaccination has been shown to produce an acceptable immunity and reactogenicity profile in both MTB-infected and uninfected healthy individuals from a TB

endemic area. It has also been shown to produce potent and sustained CD4 and CD8 T cell responses, as well as CD4⁺ T cell dependent IFN- recall responses in NK cells. Furthermore, two M72/AS01 doses were shown to produce higher immune responses than a single dose.(Meeren et al., 2019)(Kwon et al., 2019).

M72/AS01E offered 54.0% protection against MTB infection in adults, according to a phase 2b controlled trial, with no obvious safety issues. Furthermore, studies have shown that M72/AS01E significantly reduced pulmonary TB cases among healthy MTB-infected, BCG-vaccinated, and HIV-negative adults compared to placebo[20], that MTB-infected, HIV-negative adults had approximately 50% protection against the development of active pulmonary TB for three years, and that bacteriologically confirmed pulmonary TB cases significantly

decreased (Tafaghodi et al., 2020).

According to Kumarasamy et al., there were no safety issues when the M72/AS01E vaccine elicited 3-year humoral and cellular immune responses in adults living with HIV and those living with HIV. As a result, M72/AS01E is the first TB vaccination with significant efficacy against TB in a century (Kumarasamy et al., 2019).

b. TB vaccine candidate containing intact or extracted mycobacterial cells

M. vaccae

Mycobacterium vaccae (MV), a nonpathogenic species typically found in soil, is a member of the MTB genus. Its antigens are similar to those of MTB and non-TB mycobacteria. In numerous assessments, the efficacy of injectable and oral *M. vaccae* products has been investigated for the treatment of leprosy, tuberculosis, and other illnesses like depression and cancer. It has been demonstrated during the third stage of a clinical trial that *M. vaccae* elicits humoral responses, but that the amount of INF- γ varies according on the HIV viral load, CD4 T cell count, and history of TB medication. By enhancing Th1/Th2, inducing the immunological response mediated by the cytokine Th1, and inducing MTB macrophage phagocytosis, MV can eradicate and suppress MTB. Pharmaco-economically speaking, even though treating MDR-TB with MV and chemotherapy became more expensive, the incremental cost decreased because the cure rate was significantly higher. MV is well tolerated and has not been known to produce any major

negative effects. Additionally, research has demonstrated that MV is immunogenic and safe for HIV-positive individuals. It has also been proven to activate CD4⁺ T-cells to express IL-10 and IFN- γ in the culture of mice treated with MV as opposed to mice not treated with MV. It is important to emphasize that MV can increase the rate of sputum smear conversion in patients with pulmonary tuberculosis in the first two to six months after treatment.

TB-based immunotherapy treatments are more necessary because host immune system interventions have been largely overlooked and the majority of studies have focused on the inhibition of mycobacterium.

Clinical investigations have indicated that the TB vaccine in tableted form (V7), as an oral pill containing heat-killed MV (NCTC11659), has the potential to shorten and enhance standard TB treatment regimens by generating a safe and effective immunological supplement. (Khoshnood et al., 2019)(Usman et al., 2019)(Huang & Hsieh, 2019)(Bourinbaiar et al., 2020)

DAR-901

Mycobacterium obuense, DAR 901, has been rendered inactive by heat. A stage I trial was initiated in 77 adults who were HIV positive or HIV negative and had previously received BCG in order to evaluate the DAR 901 safety, immunogenicity, and tolerance to multiple vaccination treatments at different dosage levels, ranging from 0.1 to 1 mg (ClinicalTrials.gov identifier: NCT02063555). DAR 901 as a BCG booster has been shown to have a suitable safety profile and to be well tolerated at three doses.

Furthermore, DAR-901 promotes humoral and cellular immunity to mycobacterial lysates that are poly-antigenic. DAR-901 stimulated IFN and antibody responses in mice. Increased susceptibility to mycobacterial infection has been linked to deficiencies in IFN expression, and protective immunity against multiple mycobacterial antigens is predicted by IFN responses, even in cases where the immunological correlates of vaccination against tuberculosis are unclear. Increased antibody responses to DAR-901 were observed after immunization, but not to MTB lysate or pure protein derivative. In mice primed with BCG, DAR-901 at 1 mg boost provided greater protection against aerosol challenge compared to homologous BCG boost. Compared to the baseline, DAR-901 recipients showed an increase in T cell responses that were polyfunctional or bifunctional against the DAR-901 antigen. Seven days following the third dosage, higher levels of vaccine-specific CD4⁺ IFN, IL2, TNF-, and any other cytokine have been found to be generated. The Th1 response was predominant, and the majority of responder cells had a polyfunctional

effector memory pattern. BCG produced greater CD4⁺ T cell responses than the control, but the more muted DAR-901 response remained the same. BCG and DAR-901 were unable to sustainably or significantly raise Th17/Th22 cytokine responses. These results suggest that higher levels of CD4⁺ cytokine activation may not be a necessary or essential characteristic for future TB vaccine booster doses (Sweeney et al., 2019)(Lahey et al., 2019)(Masonou et al., 2019).

c. An attenuated Mycobacterium tuberculosis vaccine candidate MTBVAC

Two separate, stable deletions in the *phoP* and *fadD26* genes were used to build MTBVAC, allowing it to advance into clinical development. Since then, research on mice, guinea pigs, and rhesus macaques has shown that MTBVAC is as safe, protective, and immunogenic as prototype SO2. Furthermore, in guinea pigs and adult and newborn mice models³⁸, MTBVAC offers BCG better protection (Table 1).(Verreck et al., 2019)(White et al., 2021). The MTBVAC vaccine candidate received approval in 2012 to begin human clinical studies.

Table 1. preclinical research using the MTBVAC vaccination. (White et al., 2021)

Author (year)	Study	Aim	Results
Arbues A et al. (2013) ²⁵	Construction, characterisation, and preclinical evaluation of MTBVAC, the first live-attenuated <i>M. tuberculosis</i> -based vaccine to enter clinical trials	To describe the construction of MTBVAC from the SO2 prototype and preclinical evaluation following Geneva consensus: two independent deletions and non-antibiotic markers.	MTBVAC is functionally and phenotypically comparable to SO2 in several animal models studies prior to its entry into human clinical trials.
Aguilo N et al. (2016) ³⁸	Study of safety, immunogenicity, and protective efficacy of MTBVAC against <i>M. tuberculosis</i> in newborn mice.	To assess the safety and immunogenicity of MTBVAC in newborn mice to support Phase 1b trials in human neonates.	MTBVAC is safe, immunogenic and protects against <i>M. tuberculosis</i> and does not affect developing organs in newborn mice.
Clark S et al. (2017) ²²	Study of revaccination BCG and MTBVAC in Guinea Pigs.	To study MTBVAC and BCG, administered individually or in combination.	MTBVAC improves BCG's protection against <i>M. tuberculosis</i> supporting strategy to boost the effects of BCG vaccinated at birth in adolescents and adults in TB-endemic countries.
Diaz C et al. (2019) ²⁹	Comparative Metabolomics between <i>M. tuberculosis</i> and the MTBVAC	To compare the differences between metabolites produced by MTBVAC and <i>M. tuberculosis</i> .	34 metabolites were identified, of which 25 were increased in MTBVAC and 9 in <i>M. tuberculosis</i> .
Pérez I et al. (2020) ²³	Study MTBVAC representing the three modern <i>M. tuberculosis</i> lineages reveal that the Euro-American genetic background.	To evaluate the efficacy of the new variants of MTBVAC (L2 and L3 strains) compared to the original (L4 strain) and determine whether MTBVAC can protect against three modern strains.	All three variants of MTBVAC confer protection against the disease irrespective of the phylogenetic lineage used as the basis for their construction. Greater protection is observed for the variant based on the L4 lineage.
White AD et al. (2021) ³⁷	Protection study of intradermal vaccination with MTBVAC in rhesus macaques against aerosol challenge with <i>M. tuberculosis</i>	To determine the protection of a single intradermal dose of MTBVAC in rhesus macaques and to characterise the immune response for comparison with that defined in both preclinical studies in macaques and in human clinical trials.	MTBVAC vaccination conferred significantly improved protection against the BCG vaccinated group and the non-vaccinated group. Immunological profiles demonstrated a predominantly Th1-type response that correlated with results in clinical and preclinical trials.
Pérez I et al. (2022) ³⁰	Metabolomic study of MTBVAC	To analyse the expression of the second messenger c-di-AMP in MTBVAC and its impact on vaccine efficacy.	Deletion of <i>phoP</i> in MTBVAC induces increased production of c-di-AMP, which influences attenuation and efficacy, leading to an increased IL-1 β -mediated immune response to BCG.

As of September 2022, MTBVAC was partially funded to carry out a multi-center Phase 3 efficacious trial in neonates by the European & Developing Countries Clinical Trials Partnership (EDCTP2). This multi-center clinical trial is randomised, double-blind, and BGG controlled. Its evaluation is being carried out at six separate sites: four in South Africa for the efficacy research, one in Senegal, and one in Madagascar for safety and immunogenicity in comparison to BCG. Examining the safety, immunogenicity, and—above all—effectiveness of MTBVAC in healthy children delivered to women living with HIV/AIDS is the main goal of this research. It is anticipated that 7120 volunteers will be gathered and assigned 1:1 to the BCG or MTBVAC groups. Enrollment is presently in progress, with the trial scheduled to conclude in September 2029 (Bethesda (MD): National Library of Medicine, 2021).

Following over 20 years of research and development, MTBVAC

is now undergoing a Phase 3 efficacious trial to treat tuberculosis in neonates. Its effects, however, go beyond preventing tuberculosis.²⁶ Pre-clinical research is looking into additional applications for MTBVAC that capitalize on the non-specific effect of live attenuated vaccines in addition to TB protection. It has been shown to have the ability to treat BCG-refractory non-muscle-invasive bladder cancer by inducing an immune response specific to the tumor that is mediated by CD4+ and CD8+ T cells.⁶⁰ Similar to this, MTBVAC has been shown to be able to reverse established asthma in mice by inducing a Th1-type immune response at the expense of an IgE-producing Th2 response.⁶¹ Its ability to produce epigenetic modifications in primary myeloid cells, or "trained immunity", has also been investigated. This enhances the immune response against non-bacterial stimuli and offers heterologous protection against *Streptococcus pneumoniae* (Table

2).(Tarancón et al., 2020)(Vierboom et al., 2021)(Dijkman et al., 2021). However, in contrast to the intradermal inoculation that has been the subject of most investigations, its respiratory delivery has been investigated. In

these instances, MTBVAC elicited a localized reaction specific to the inoculant that spread beyond the site of inoculation and has been linked in the past to protection against TB.

Table 2. investigations into additional MTBVC vaccination indications and delivery methods.(Tarancón et al., 2020)

Author (year)	Study	Aim	Results
Tarancón R et al. (2020) ⁶²	To study MTBVAC trained immunity and protection against experimental lethal pneumonia compared to BCG in mouse model.	To determine the ability of MTBVAC to induce "trained immunity" and offer protection against a heterologous model of pneumococcal pneumonia in mice.	MTBVAC is able to generate metabolic and immunomodulatory effects and epigenomic modifications through mechanisms similar to BCG, providing protection against heterologous infections unrelated to TB, such as <i>Streptococcus pneumoniae</i> .
Dijkman K et al. (2021) ⁶⁴	Pulmonary MTBVAC vaccination in non-human primates	To assess the impact of using MTBVAC inoculated through the respiratory mucosa in primates to determine their ability to induce adaptive immunity.	Pulmonary vaccination with MTBVAC resulted in a local antigen-specific response that has previously been correlated with protection against TB. Both T lymphocytes and antibodies generated by the vaccine showed an enhanced capacity to respond to <i>M. tuberculosis</i> .
Tarancón R et al. (2021) ⁶¹	Therapeutic efficacy of pulmonary live tuberculosis vaccines against established asthma by subverting local immune environment in mouse model	To evaluate the therapeutic efficacy of intranasal administration of vaccines based on live attenuated mycobacteria in different models of bronchial hyperresponsiveness.	Both BCG and MTBVAC can reverse bronchial hyperresponsiveness in established asthma, where there is high pre-vaccination eosinophilia, by inducing a potent Th1-type immune response.
Vierboom MPM et al. (2021) ⁶³	Comparison standard intradermal vaccination to pulmonary router in non-human primates	To assess the impact of BCG and MTBVAC inoculated through the respiratory mucosa in primates to determine their ability to induce "trained immunity".	The study showed stronger induction of trained immunity by mucosal BCG or MTBVAC administration compared with standard intradermal route in monocytes present in peripheral blood and bone marrow, with inoculation.
Moreo E et al. (2022) ⁶⁰	Study of therapeutic MTBVAC immunotherapy in a mouse model of orthotopic bladder cancer.	To compare the efficacy of anti-tumour treatment with intravesical BCG versus intravesical MTBVAC.	MTBVAC acts as an antitumour treatment in non-muscle-invasive bladder cancer refractory to BCG treatment, and induce eradication of fully established bladder tumours, largely due to the expression of ESAT6 and CFP10.

d. Recombinant candidate vaccine TB VPM1002

The VPM1002 vaccine is a recombinant strain of BCG that generates membrane-penetrating listeriolysin. *Listeria monocytogenes*, the bacteria that produces listeriolysin, is hygromycin resistant and does not possess the urease C gene. It has been demonstrated that by inducing type 1 and type 17 cytokines in mice, it offers superior protection against tuberculosis (TB) than the parental BCG (pBCG) (Darrah et al., 2020). The rBCG vaccine candidate VPM1002 enhances antigen presentation by enabling mycobacterial antigens to reach the host cell's cytoplasm through the secretion of Hly, which breaks down phagosome membranes. A BCG expressing Hly was created in

the first stage. But Hly's biological activity in this recombinant mutant is not optimal. Only at an acidic pH is hly active. The urease C gene was removed in order to prevent BCG's neutralizing impact on phagosomes. Actually, the ensuing rBCG-ureC-Hly mutant set up the ideal pH in the phagosome for Hly. Through phagosome membrane penetration, mycobacterial antigens can reach the cytosol and be processed via the major histocompatibility complex pathway, which primes CD8 T cells. It has been revealed that the cytokine-producing cell profiles in the VPM1002 and BCG groups are similar. The description of CD4⁺ and CD8⁺ T cell cytokine production (for IFN- γ , IL-2, and TNF- α) is in line with phase I study results. In South African newborns who had not been exposed to HIV, a phase II

research verified the immunogenicity and safety of VPM1002. A dosage of the VPM1002 vaccine induced BCG-like polyfunctional CD4⁺ and CD8⁺ T cell profiles. It's interesting to note that, in a particular group, the ratio of CD8⁺IL-17 T cells was increased six months after vaccination. In a Phase II clinical study, healthy individuals who had not been exposed to HIV were randomly assigned to receive either the BCG vaccine or VPM1002. Compared to BCG, VPM1002 was well-tolerated and safe. Based on preliminary data analysis, VPM1002 was found to be both safer and more well-tolerated than a single dose of BCG in neonates. Like with the other Phase I investigations, VPM1002 also generated a robust

T-cell response that was skewed toward Th1-type immunity. In a different investigation, VPM1002 revealed a better safety profile than BCG in immunocompromised mice. VPM1002 is most likely a more potent vaccination than BCG, according to the results of the Phase II study in neonates and the Phase I trial in adults. The Friedrich Loeffler Institute in Germany has vaccinated goats against *M. caprae* infection using VPM1002. Table 3 shows that *M. bovis* and *M. caprae* infections in goats and cattle are significant in animal husbandry since they can also infect people (See table 3) (L. Grode, C.A. Ganoza, C. Brohm, J. Weiner 3rd, B. Eisele, 2019)(Loxton et al., 2019)(Minhas et al., 2019)(Nieuwenhuizen et al., 2019).

Table 3. Features of vaccinations against tuberculosis that are recombinant.(Nieuwenhuizen et al., 2019)

Candidate	Vaccine composition	Immune response	Clinical trial phases	Goal	Route of administration
VPM1002	Recombinant BCG strain	CD4 ⁺ and CD8 ⁺ T cell, IL-17	Phase 3	Prime, boost	Intradermal

Candidate Vaccine pCDNA 3.1-rpfB

Among the proteins in the RpfB family, the protein derived from the resuscitation promoting factors B (rpfB) gene of *Mycobacterium TB* possesses the most advanced biological and immunological properties. In latent tuberculosis infections, this protein can stimulate the growth of dormant tuberculosis germs. As a result, this protein has been developed as a potential TB vaccine. In this work, the

Beijing strain of *M. tuberculosis* was amplified using PCR and its rpfB gene cloned onto the pCDNA3.1 plasmid. Male Balb/C mice were immunized with the pCDNA3.1-rpfB recombinant plasmid to evaluate its capacity to elicit a humoral immune response. Transfection of recombinant plasmids in CHO-K1 cells demonstrated that RpfB protein was successfully expressed in mammalian cells based on immunostaining tests, while Western blot results using

immunized mice serum showed that the *rpfB* gene successfully induced the humoral immune response of mice with a specific band in the range of 66 kDa. The RpfB protein of *M. tuberculosis* strain Beijing can therefore be produced in mammalian cells and has been shown in this study to be an antigen that can trigger humoral immune responses in mice. (Saraswati et al., 2018)

Candidate Vaccine pCDNA 3.1-*rpfD*

Researchers at the University of Indonesia's Faculty of Medicine, Department of Microbiology, have determined that a new vaccine is desperately needed to combat tuberculosis. It is well known that *M. tuberculosis* secretes proteins that stimulate protective immunity. Resuscitation promoting factor Rpf is a protein found in the *M. tuberculosis* genome that contributes to the reactivation of the disease. Since RpfD belongs to the Rpf family and has been demonstrated to be immunogenic, it can be used as a TB vaccine. The objective of this research was to create a DNA vaccine that encodes the *rpfD* gene and examine the vaccination's immunogenicity in mice. Using PCR technology, the *rpfD* gene of *M. tuberculosis* was amplified. Following cuts with the restriction enzymes EcoRI and HindIII, the *rpfD* gene and pCDNA3.1 plasmid were ligated and transformed into *E. coli* DH5. CHO-K1 cells were transfected with the recombinant plasmid pCDNA3.1-*rpfD* after it had been verified to have the correct orientation and amino acid sequence. Balb/c mice were subsequently given an intramuscular pCDNA3.1-*rpfD*

immunization every two weeks. By using a Western blot, specific antibodies found in mouse serum were identified. The levels of IL-12, IFN-gamma, IL-4, and IL-10 were measured using ELISA. The study's findings demonstrated the presence of antibodies specific to pCDNA3.1-*rpfD*. Furthermore, but not IL-4 or IL-10, this DNA vaccine can stimulate the production of IL-12 and IFN-gamma. (Rakhmawati et al., 2018)(Pratama, 2019).

CONCLUSION

Based on the description above, it can be concluded that many recently developed tuberculosis vaccines are reported to be in the final stages of clinical trials, where they have significantly strengthened the immune system and even produced protection against the host. Immunization produced by vaccines that have successfully passed the initial stages of clinical trials is safe and effective, and can even surpass BCG in terms of immunity. With the development of new TB vaccines that strengthen the body's immunity and create effective delivery mechanisms, hopes for TB treatment and prevention are increasing. The development of vaccine effects can be facilitated, in part, through the use of effective delivery mechanisms, which have also been used in TB vaccines. It is hoped that in the future, new tuberculosis vaccines can be developed that have good effectiveness and efficacy in addition to the BCG vaccine, which is the only recommended vaccine for tuberculosis.

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