A Overview of Vaccine with Focusing on the Tuberculosis Vaccine Development

Running title: Tuberculosis Vaccine Development

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Abstract

Prophylactic vaccines play a vital role in contemporary healthcare and have been effectively utilized to fight against bacterial and viral infections. Diseases such as tuberculosis, which were once feared and caused significant harm throughout history, are now uncommon. Unlike other types of medications that are typically given to treat sick individuals, vaccines are generally given as a preventive measure to normal people, although there are some exceptions to this approach. The history of infections and prophylactic vaccines is rich with significant scientific lessons, offering invaluable insights for future advancements. Vaccines have been utilized for various pathogens for a long time. Advances in basic immunology and recombinant DNA technology have primarily altered vaccine production, antigen optimization, and the selection of efficient vaccine delivery methods. Historically, tuberculosis (TB) has been a crucial cause of mortality all around the world. TB infection continues to pose a serious threat to human health because of its contagious nature, the potential for it to remain latent in the host extended periods, and its emergence as an active infection. A total of 1.23 million people died from TB in 2024 (including 150 000 among people with HIV). Globally, TB is the world's leading cause of death from a single infectious agent and among the top 10 causes of death. Those experiencing latent infection are only infected with *M. tuberculosis* without having any clinical symptoms. Therefore, being aware of effective vaccines against TB is of paramount importance. The Bacillus Calmette–Guérin (BCG) vaccine is mostly applied against TB. New TB vaccine development is a priority for world health organization (WHO) as it is an important unmet medical need. In this study, after a brief overview of vaccine types, several vaccines effective against tuberculosis were investigated.

Key words: Recombinant vaccines, Tuberculosis, Vaccines

1. Context

A vaccine is a biological product that induces active acquired immunity against a specific microbial disease. It is indeed a suspension of killed or attenuated microorganisms, such as viruses or bacteria, or their antigenic proteins, administered to prevent, improve, or treat infectious diseases. Nowadays, antigenic proteins are often produced using recombinant DNA technology, eliminating the need for attenuated or killed microbes [1].

According to the priorities of the World Health Organization (WHO), developing and designing new vaccines are needed more than ever to control the spread of this disease. 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 older vaccines. Therefore, to control TB, an effective preventive vaccine can play an essential role in averting the spread of this global epidemic. Dozens of candidate TB vaccines have been designed to enhance Bacillus Calmette–Guérin (BCG) or replace it while many vaccine candidates are in the stages of clinical trials. 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.

2. Data Acquisition

This study collected information from several published sources across four databases: Web of Science, PubMed, Scopus, and Google Scholar. Databases were searched for studies indexed without a time limit, using the terms: Recombinant vaccines, Tuberculosis, and Vaccines. In addition, data collection in this study was conducted using library research. The collected documents were screened for their titles and abstracts. No automation tools were used for screening and selection of the literature.

3. Result

3.1. Vaccine classification

Vaccines are offered in various categories, the most appropriate category being mentioned below (Figure 1):

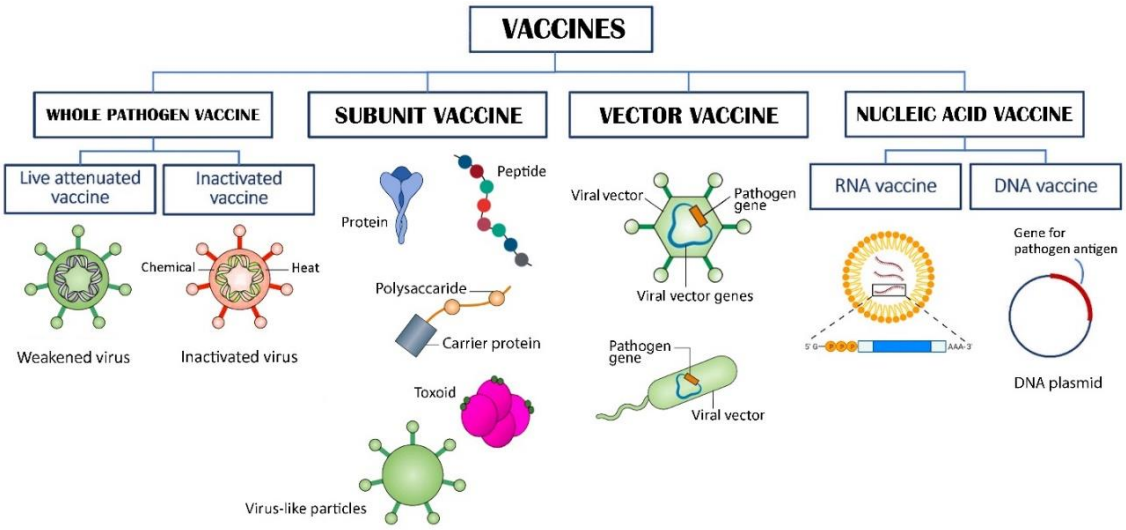

Figure 1. Types of vaccines

3.1.1. Whole-Pathogen Vaccines

Live vaccine. A vaccine produced by reducing the virulence and disabling the pathogen, resulting in a live but significantly attenuated pathogen. Vaccines against viral diseases, such as mumps, measles, rubella, influenza, chickenpox, oral poliomyelitis, yellow fever, and rabies, and bacterial diseases, such as tuberculosis, typhoid, and epidemic typhus, are of this type [2]. Inactivated vaccine (killed vaccine). A type of vaccine that involves pathogenic particles cultured under controlled laboratory conditions and then inactivated or killed to prevent them from causing disease. In this case, the pathogen is completely eliminated using heat, chemicals, or radiation, or its function is impaired by a solvent. Instances of inactivated viral vaccines encompass poliomyelitis, hepatitis A, rabies, tick-borne encephalitis, and most influenza vaccines. Instances of bacterial vaccines include injectable vaccines for typhoid, cholera, pertussis, and plague [3].

3.1.2. Vector vaccine

A type of vaccine that utilizes a viral (or bacterial) vector to deliver genetic compounds encoding antigens of a pathogen (such as a virus) into the host's body. With the entrance of designed genetic compounds into the host's body through these vaccines, the production of pathogen antigens begins, followed by the production of the desired antibodies in the host's body. Consequently, the body becomes resistant to potential future encounters with that pathogen, as seen in coronavirus disease 2019(COVID-19) and Ebola vaccines. Viral vector vaccines activate the expression of viral antigens in the body and also induce a cytotoxic T lymphocyte response. Viral vectors are generally designed to be replication-deficient, and the genes required for viral replication are removed from the vaccine content during vaccine design [4].

3.1.3. Subunit vaccine

A type of vaccine that, unlike inactivated vaccines, does not encompass cells of the disease-causing agent. Instead, these vaccines utilize pathogen antigens, making them inherently safer compared to inactivated vaccines. Subunit vaccines employ specific components or parts of the microbial cell to stimulate the immune system, rather than the entire microbe [5].

Protein-based viral vaccines: These vaccines consist of precisely identified viral protein fragments that are selected and purified for their ability to stimulate the immune system. Most of these vaccines are presented through the major histocompatibility complex (MHC) class II pathway, which primarily activates antibody production rather than a cytotoxic T lymphocyte response. Adjuvants and immune-stimulating molecules are added to protein-based vaccines to enhance their endogenous processing, thereby increasing their uptake and presentation by MHC class I molecules [6,7].

A conjugate vaccine: A type of vaccine that combines a weak antigen with a strong antigen as a vector so that the immune system has a stronger and more protective response to the weaker

antigen. In this context, “weak” and “strong” do not refer to the level of pathogenicity but rather to the power and capacity to stimulate the human immune system and the intensity of triggering immune system components to react (antibody production). Conjugate vaccines are specifically designed to immunize against a pathogen whose causative agent is a weaker antigen in terms of stimulating the immune system. Linking an antigen as an immune system stronger stimulant with the disease-causing antigen culminates in a vaccine’s efficacy in stimulating the immune system to fight the target disease [8].

Toxoid: Type of vaccine derived from toxins generated by harmful infectious organisms (such as the microbes responsible for botulism and diphtheria). A toxoid is an inactivated toxin [typically an exotoxin] that has been rendered harmless through chemical processes, without losing its antigenicity (immunogenicity). Consequently, when utilized in vaccination, it elicits an immune response and establishes immunological memory against the molecular markers of the toxin, without inducing toxin-mediated disease [9].

Virus-like particles (VLPs): VLPs are similar to viruses with multi-protein structures but are not infectious because they lack the virus’s genetic material. Viral expression of structural proteins, such as envelope or capsid, can lead to the self-assembly of VLPs. VLPs can be produced in a spectrum of cell culture systems, as well as under cell-free conditions [10].

3.1.4. Nucleic acid vaccines

DNA vaccines: A type of vaccine that, through transfection of the antigen-encoding segment of an infectious agent into cells, stimulate the host’s immune system against it. These vaccines consist of a plasmid designed and constructed using genetic engineering techniques. The plasmid is directly injected into cells, enabling them to produce the desired pathogen antigens. DNA vaccines share similarities with inactivated virus vaccines and recombinant vaccines.

144 However, they differ from live virus vaccines because DNA vaccines do not cause infection in
145 organisms [11].
146 Messenger RNA (mRNA) vaccines: A type of vaccine that employs a copy of mRNA to
147 stimulate an immune response. This vaccine transfers synthetic RNA molecules (i.e., artificial
148 RNA) to immune cells. Once inside the immune cells, the RNA vaccine functions as mRNA,
149 promoting the cell to produce the surface protein typically made by a pathogen or cancer cell.
150 These protein molecules trigger an adaptive immune response that teaches the body how to
151 recognize and eliminate the relevant pathogen or cancer cells [12].
152 Recombinant Vaccine: These vaccines are typically produced using bacterial, yeast,
153 mammalian, and insect cells. Recombinant vaccines require the attachment and transfer of the
154 DNA segment responsible for encoding the antigen. Among the mentioned cell types, bacterial
155 expression systems are the most commonly used, as they do not require the modifications
156 associated with mammalian and insect cells. A major drawback of recombinant protein
157 vaccines is their high cost and limited accessibility. However, their safety profile is
158 considerably better than other types. In some recombinant live vaccines, an attenuated virus or
159 bacterium is used as a vector. These vectors elicit an immune response very similar to that
160 induced by natural infectious microorganisms. The DNA of the microorganism is attached to
161 the vector, stimulating the immune system. Another type of recombinant vaccine is the DNA
162 vaccine, also known as “a genetic vaccine” [13].
163
164 **3.2. Tuberculosis vaccines**
165 TB is a frequent infectious and, in several cases, deadly disease. This disease can be caused by
166 different species of mycobacteria, most commonly *M. tuberculosis*. Tuberculosis mainly
167 affects the lungs; however, other parts of the body can be affected. Individuals with active
168 tuberculosis infections transmit their cough, sneeze, or saliva through the air, and this disease

is spread through the air. Often, infections have no symptoms and are latent, but typically, one out of every 10 latent infections can lead to active disease. Without treatment, TB can kill over 50% of individuals infected. Active tuberculosis is diagnosed using radiology, microscopic tests, and microbiological cultures of body fluids [14]. Latent TB is diagnosed by blood tests and the tuberculin skin test. Treatment of the disease is challenging and needs multiple antibiotics for a long time. However, antibiotic resistance is an increasing challenge in multidrug-resistant TB infections. The emergence of strains resistant to drugs and the co-infection of TB and human immunodeficiency virus [HIV] have transformed it into a serious problem, placing a heavy burden on society for its treatment and control. Tuberculosis screening and subsequent vaccination should be carried out to prevent this disease [15].

3.2.1. BCG vaccine

Bacillus Calmette-Guérin (BCG), as a live attenuated *Mycobacterium bovis* strain, was first used to induce immunity against tuberculosis infection. Albert Calmette and Camille Guérin began bovine malignant strain culturing on potato slices impregnated with glycerin and ox bile in 1908. Following 3 to 4 weeks, colonies typically appeared as dried, brownish-green structures. From 1908 to 1920, Calmette and Guérin conducted approximately 230 passages, ultimately achieving a *M. bovis* strain causing no progressive disease in cows, rabbits, guinea pigs, or horses. It is now called BCG and was initially administered orally to infants. Over the next three years, BCG was given orally immediately following birth to 317 infants from families without / with obvious cases of tuberculosis. As no serious adverse effects were observed until July 1924, it was decided to distribute BCG to other laboratories needed BCG (Figure 2) [16,17].

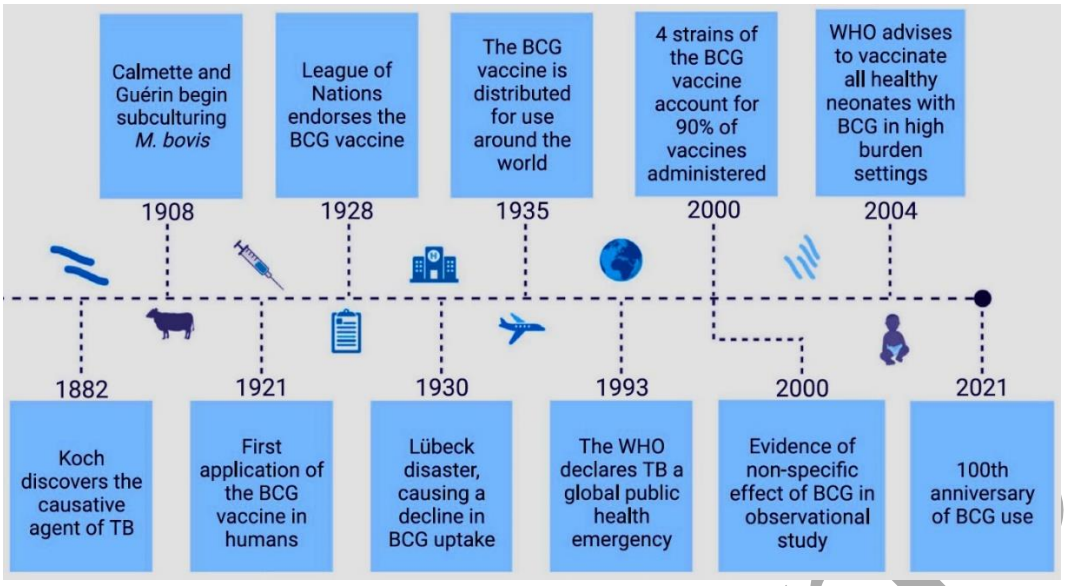

Figure 2. Historical timeline of the discovery and distribution of BCG. This figure shows a timeline with the most influential events in the history of BCG [17].

The administration of the oral BCG vaccine to over 114,000 infants in 1928, with no reports of serious adverse events, marked the beginning of the vaccine's acceptance in Europe. However, a catastrophic incident occurred in 1930. Out of 251 German infants vaccinated in Lübeck, 173 exhibited radiological or clinical symptoms of tuberculosis, and 72 of them died. Next research confirmed that this rate stemmed from BCG vaccine contamination with a dangerous *M. tuberculosis* strain in the same laboratory, but this phenomenon led to a significant decline in public confidence in the vaccine's safety [17].

The BCG vaccine should only be administered to individuals with a healthy immune system and should be avoided in those with a compromised immune system. Additionally, this vaccine is ineffective in cases previously infected with TB or who have been infected with environmental mycobacteria. According to past meta-analyses, the effectiveness of this vaccine against respiratory infections varies significantly, ranging from 0% to 80%. However, it has been shown to be highly effective against serious disseminated types of TB, such as meningitis and miliary tuberculosis. Despite numerous efforts and the development of new generations of

tuberculosis vaccines, the BCG vaccine remains the only effective vaccine against tuberculosis infection, as attempts to replace it with a more effective alternative have been unsuccessful [17].

Following World War II, there was a resurgence of TB, leading to the widespread BCG vaccine adoption after it was endorsed by the World Health Organization. After that, several countries have integrated BCG vaccination into their national immunization plans. Of the 223 countries for which data is available, more than 85% advocate for universal BCG vaccination. Other countries have either stopped BCG vaccination because of a decline in tuberculosis cases or have never suggested widespread BCG immunization, choosing instead to focus on selective vaccination for populations at risk [17].

In countries where TB is prevalent, BCG vaccination methods are typically incorporated into childhood immunization pans, with the vaccine primarily given to infants. Currently, BCG is the merely licensed vaccine for tuberculosis and is the most widely distributed vaccine globally. Multiple BCG vaccines are currently available, each produced using different methods and demonstrating variations in quality, especially in terms of the number of viable cells for each dose. BCG strains that have been obtained from the original Paris strain since 1925 are missing a genomic segment known as region of deletion 2 (RD2), which can be detected in strains developed before that year [18].

Phenotypic variations among these BCG vaccine strains were initially found in the 1920s, and recent molecular research has elucidated their genomic distinctions. The particular strain of BCG vaccine affects administered for immunization the mycobacterial-specific immune reaction, but there is limited information to endorse or suggest a specific BCG vaccine strain. Nonetheless, most individuals are vaccinated with the BCG vaccine, provided by the United Nations Children's Fund (UNICEF) on behalf of the Global Alliance for Vaccines and Immunization. UNICEF utilizes merely four BCG vaccine suppliers producing merely three

distinct BCG vaccine strains: BCG-Russia (genetically similar to BCG-Bulgaria) generated by Bulbio in Bulgaria, BCG-Denmark generated by the Statens Serum Institute, Denmark, BCG-Japan produced by BCG Laboratory in Japan [16].

Three studies have assessed the protective effect of various BCG vaccine strains in human samples. In two of these research, which had follow-up periods ranging from 4 to 50 years, BCG-Pasteur was found to be statistically associated with greater protective efficacy compared to BCG-Glaxo or BCG-Phipps. In the third research, which followed participants for 15 years, BCG-Denmark demonstrated greater protective effect than BCG-Pasteur, with rates of respectively 25% and 17%. Such research provides limited insights into the protective effectiveness of the most widely used BCG vaccine strains, as BCG-Glaxo and BCG-Phipps are not used anymore, and BCG-Pasteur is administered in limited countries. Proper identification of the BCG vaccine strain is crucial, as it has significant implications for vaccine effectiveness [16].

Considering the vast number of infants vaccinated with BCG annually, even a slight enhancement in the protective immunity provided by a specific BCG strain could lead to better TB protection for a significant number of children. Numerous new tuberculosis vaccines are in development, including those aimed at replacing BCG and others meant to enhance its effects. The most advanced booster vaccines utilize live, subunit, or recombinant vectors intended for administration following the initial BCG vaccine dose. Therefore, we should identify which BCG vaccine strain generates the most effective primary immune reaction to deal with TB to enable effective boosting [19].

Studies have indeed demonstrated that the type of vaccine strain is a substantial factor in inducing adverse reactions following BCG vaccination. For instance, a comparison of the genetic sequences of the BCG-Tokyo and BCG-Pasteur strains revealed that BCG-Tokyo varies in 18 genetic regions, each longer than 20 bp. Moreover, this strain harbors 20

insertion/deletion mutations with a genetic length of less than 20 bp and 68 single nucleotide polymorphisms compared to the Pasteur-BCG strain. These all indicate the evolution of BCG under laboratory conditions. The most critical reason for the attenuation of BCG is the loss of the Esx-1 protein secretion system, which all BCG strains have lost due to the deletion of the RD1 genetic locus. The RD1 genomic area exists in pathogenic *M. tuberculosis* strains but is absent in BCG vaccine strains. This genomic region plays an extremely substantial role in bacterial pathogenesis [20,21].

Research has shown that in this group of attenuated strains, the secretion of proteins like 10-kDa culture filtrate protein (CFP-10) and early secreted antigenic target 6 kDa (ESAT-6) is significantly reduced or completely abolished. This secretion is crucial in bacterial pathogenesis. Strains lacking this genomic region can proliferate well in tryptophan hydroxylase 1 (TPH-1) cells but are incapable of infecting macrophages [22].

Today, *M. bovis* and *Mycobacterium microti* vaccine strains are used for immunization against tuberculosis. Both vaccines are considered very safe and efficient, but for unknown reasons, they do not impact pulmonary infections in adults residing in highly endemic areas. However, prolonged passage of highly pathogenic BCG has rendered it non-pathogenic, while *M. microti* is naturally attenuated in humans and typically does not cause severe disease, exhibiting only mild pathogenicity. *M. microti* is a host-restricted mycobacterium that is naturally attenuated in humans, is unable to cause disease, and the immunity generated by it against tuberculosis is comparable to that provided by BCG [23].

The BCG vaccine is typically administered once and additional doses are not given. The highest incidence of tuberculosis occurs in young adults and adolescents, and the effectiveness of the BCG vaccine can last for 15 years. BCG vaccination of newborns protects them against non-infectious types of TB, like miliary TB, but it is ineffective against pulmonary T, which predominantly occurs in adults [24].

The most debated issue regarding the BCG vaccine is its inconsistent efficacy. A study conducted in Britain reported a 60-80% protective efficacy of the BCG vaccine, while another study reported no efficacy at all. Fine et al.'s research has shown that BCG can mitigate the incidence of tuberculosis by up to 50%. Studies have also demonstrated that the vaccine's efficacy diminishes to zero 20 years after immunization. However, some research has provided evidence that the vaccine's efficacy may persist for up to 60 years following the initial immunization [21, 25]. The BCG vaccine is highly successful in preventing miliary TB and meningitis; thus, it is still administered to deal with miliary tuberculosis and meningitis even when its efficacy in inducing immunity against pulmonary tuberculosis is negligible [26].

The utilization of BCG as a post-infection vaccine for tuberculosis does not provide adequate immunity against the recurrence of pulmonary infections in adults and may even exacerbate the existing infection. The exacerbation of the disease following BCG vaccination after a primary infection has also been observed in animal models, which may contribute to the failure of BCG vaccination in endemic areas. In highly endemic areas with a high prevalence of the bacteria, BCG revaccination can enhance the risk of developing active tuberculosis [27,28].

It has been determined that non-tuberculous mycobacteria, particularly environmental mycobacteria, like *M. avium* and *M. intracellulare*, induce non-specific immune responses against mycobacteria. The BCG administration to individuals with pre-existing non-specific immune responses against mycobacteria does not lead to the enhancement or augmentation of immunity against tuberculosis. Consequently, BCG is ineffective in these individuals, a

phenomenon known as “masking.” It occurs because the effects of the BCG vaccine are obscured by environmental mycobacteria. On the other hand, the immunity induced by these environmental mycobacteria culminates in the suppression of BCG replication and blocks the development of effective immunity against the vaccine. This hypothesis is termed the “blocking hypothesis” [29].

Tuberculosis has played a significant role in natural selection throughout history, with susceptible individuals being naturally eliminated and more resistant individuals surviving. For instance, Europeans exhibit greater resistance to this disease compared to Africans. This disparity is believed to be attributed to variations in genes related to immune responses, including mutations in receptors and chemokines [30].

BCG vaccination typically culminates in minor adverse effects, such as pain and scarring at the injection site. The vaccine must be administered intradermally; if it is administrated subcutaneously, it may give rise to local infection or the infection spread to the lymph nodes. Consequently, lymph node involvement may lead to lymphadenitis. Osteomyelitis and disseminated infection are among the infections that may occur following BCG vaccination. Although rare, these complications are very serious and life-threatening [31]. In cases where BCG is accidentally administered to individuals having a compromised immune system, namely cases having acquired immunodeficiency syndrome (AIDS), it can culminate in disseminated infection, which is extremely dangerous. The risk of disseminated BCG infection is about one in a million vaccinations [32]. It is also hypothesized that co-infection with parasites alters the immune reaction against BCG, resulting in a decrease in its efficacy. An effective immune response against TB triggers T helper (Th1) responses, while co-infection with parasites triggers Th2 responses, diminishing the BCG efficacy. In fact, it skews the immune response away from a Th1 toward a Th2 profile [33].

The extension of modern molecular techniques and the extensive breakthroughs in immunology, coupled with identifying the whole genome sequence of the wild-type H37Rv strain, completed in 1990, have made it possible to develop a new tuberculosis vaccine with higher efficacy. Over the past 10 years, two strategies have been explored for developing a new tuberculosis vaccine [34]. The first strategy is to prepare a vaccine that could offer better and higher efficacy than BCG and could eventually replace BCG, such as preparing a better version of BCG or attenuated live *M. tuberculosis*. The second strategic is the idea a prime-boost vaccination regimen. In this strategy, the BCG vaccine is administered to children as before to protect against dangerous forms of tuberculosis infection. A new vaccine is then administered as an adjuvant to increase the BCG vaccine efficacy and prolong the duration of immunity [35]. Various studies have estimated that if this strategy is implemented, the prevalence and incidence of tuberculosis will be remarkably reduced by 2050 [18,36].

3.2.2. Recombinant tuberculosis vaccines

Considering the variable efficacy of BCG, there is a necessity for greater immunity and the introduction of more efficient vaccines for the control of tuberculosis in the future. Due to this problem, many researchers are investigating immunogenic antigens to develop more efficient vaccines. These antigens include Ag85, TB10.4, ESAT-6, CFP10, MPT64, HSP65, P450 Cyp141, etc., which have been used to design recombinant vaccines [37,38].

TB10.4, a 10-kDa secreted protein belonging to the ESAT-6 family, is encoded by the Rv0288 region, termed *esxH*, and appears to be essential for the pathogenicity of the tuberculosis-causing bacillus. This antigen is regarded as a target for antimicrobial immune reactions and is strongly identified by the immune system in tuberculosis patients, inducing a high level of interferon-gamma (IFN-γ) secretion in such individuals [39].

Ag85B (a 30 kDa fibronectin-binding protein) has mycolyltransferase activity, and is one of the most abundant secreted proteins of tuberculosis bacillus that is secreted in the early phase of the disease and found in infected macrophages. Evaluations conducted on patients with active and latent tuberculosis have demonstrated that this antigen serves as a potent immunogen, inducing both cellular and humoral immune responses [40].

ESAT-6, a potent T cell antigen and secretory protein generated by *M. tuberculosis*, plays a key role in TB diagnosis through the QuantiFERON-TB Gold, whole blood interferon γ test, alongside CFP-10. It can directly bind to the Toll-like receptor 2 (TLR2), which inhibits downstream signal transduction. Inactivating ESAT-6 reduces the *M. tuberculosis*' virulence, making its secretion a critical factor in the bacterium's virulence. ESAT-6 is a marker for tuberculosis and its treatment. Furthermore, the presence of ESAT-6 enhances the generation of virulent factors, contributing to the increased TB pathogenicity. ESAT-6 is an important protein that is suppressed in the development of vaccines for *M. tuberculosis*, alongside the enhanced antigenic factors agβ5-C and agβ5-A. Current research is exploring the relationship between ESAT-6 and the lung epithelial cells, revealing its reliance on the IL-8 promoter induction [37,41].

CFP-10 is an antigen involved in the virulence of *M. tuberculosis*. It forms a stable 1:1 heterodimeric complex with ESAT-6, and the two proteins rely on each other for stability within the mycobacterial cell. The ESAT-6/CFP-10 complex is released via the ESX-1 secretion system, and referred to as the RD1 region. *M. tuberculosis* utilizes this ESX-1 system to transfer virulence factors into host monocytes and macrophages thorough infection. The ESX-1 secretion system essential components in *M. tuberculosis* are Rv3877 and two AAA ATPases, Rv3871 and Rv3870, which is a cytosolic protein. A C-terminal signal sequence on CFP-10 facilitates the ESAT-6/CFP-10 heterodimer complex secretion identified by the cytosolic Rv3871 protein. Rv3871 can interact with the CFP-10 C-terminal and guides the

ESAT-6/CFP-10 complex to Rv3877 and Rv3870, a multi-transmembrane protein forming the cytosolic membrane pore spanning of the infected host cell. When ESAT-6/CFP-10 is near the virulent host cell's membrane, the CFP-10 C-terminal attaches itself to the cells surface. The secretion and binding of the ESAT-6/CFP-10 complex to the virulent host cell demonstrate its role in the *M. tuberculosis* pathogenicity [37,42].

MPT-64 is an immune system-stimulating antigen encoded by the RD-2 region [43]. A growing body of evidence confirms the effectiveness of genetically changed BCG strains, referred to as "recombinant BCG vaccines." Multiple recombinant BCG vaccines are now under investigation as potential replacements for the parental BCG strain.

BCG-Zmp1. The initial example is the recombinant BCG-Zmp1 vaccine, which is still in the clinical phase. It is an attenuated form of the BCG vaccine that features a deletion mutation in the Zmp1 gene, encoding the zinc metalloprotease Zmp1. According to Waeckerle-Men et al., Zmp1-deficient BCG strain-immunized mice exhibited a more robust immune reaction, characterized by increased proliferation of antigen-specific T cells and higher levels of cytokine secretion, especially IFN-γ, in comparison to wild-type BCG-vaccinated cases. Furthermore, a study assessing vaccine efficacy and safety against BCG-vaccinated and BCG-unvaccinated controls, assessed through bacterial load in the lungs and spleen, indicated that immunodeficient mice receiving the BCG-Zmp1 vaccine had significantly improved survival times in comparison to standard BCG-vaccinated cases. Consequently, the BCG-Zmp1 vaccination offers enhanced protection against *M. tuberculosis* in mouse models due to its superior immunogenicity and immune reaction profile in comparison to traditional BCG vaccination [44,45].

SapM:TnBCG is the second vaccine option currently undergoing clinical trials. It is derived from the parental *M. bovis* BCG strain, featuring a deletion in the SapM gene. This gene encodes a secreted acid phosphatase that mainly interferes with the maturation of host

macrophages and the fusion of lysosomes with phagosomes, thus being involved in the pathogenesis of *M. tuberculosis*. SapM:TnBCG-vaccinated mice showed a more robust Th1 immune reaction, along with a reduced bacterial load and improved long-term survival in comparison to those vaccinated with the parent BCG strain. Notably, while there were no significant differences in autophagy or lysosome-phagosome maturation and fusion between the two strains, mice receiving the SapM:TnBCG vaccine displayed a greater degree of dendritic cell migration and activation in their lymph nodes [43].

CysVac2. The third pre-clinical vaccine option is a recombinant BCG vaccine CysVac2 that expresses a fusion protein including CysD protein expressed in persistent *M. tuberculosis* infection and the Ag85B antigen. CysVac2 vaccination in a mouse model induced a notable influx of innate immune cells, especially macrophages, DCs and neutrophils at the injection area. The number of Ag85B-specific CD4+ T cells in draining lymph nodes and spleen increased. Additionally, a many IFN-γ-secreting cells were observed in mice vaccinated with CysVac2 in comparison to BCG-unvaccinated and BCG-vaccinated controls. Therefore, CysVac2 vaccine provided more resistance to *M. tuberculosis* infection and also remarkably reduced pulmonary bacterial burden. Boosting BCG-vaccinated mice with CysVac2 resulted in a prolonged decrease in bacterial burden when in comparison to the BCG and unvaccinated groups that received only an adjuvant boost. This effect is believed is due to a greater population of CysD-specific CD4+ T cells producing IFN-γ and TNF-α against CysD expression during the infection later phases. The CysVac2 priming and boosting regimen generates lasting protective immunity before and following exposure to *M. tuberculosis* [43,44].

A recombinant BCG vaccine, VPM1002, includes the gene for listeriolysin O (hly) from Listeria monocytogenes, which can replace the urease C gene in BCG. The introduction of the hly gene into BCG promotes the cytosolic release of DNA and mycobacterial antigens, along

with activating autophagy, immune system activation, apoptosis, and antigen presentation. In animals, such as healthy mice, severe combined immunodeficiency (SCID) mice, guinea pigs, and newborn rabbits, VPM1002 exhibited a safety profile comparable to that of BCG. In fact, the VPM1002 strain is more virulent and is never found in the lungs of VPM1002-vaccinated mice. Also, VPM1002 vaccination conferred significant protection, eliciting a notable Th1 response and reducing bacterial burden in comparison to the BCG control group. In a phase I trial, three- and single-dose VPM1002 regimens were well-tolerated and triggered certain levels of multifunctional T cells expressing interleukin-2 (IL-2), IFN-γ, and TNF-α against *M. tuberculosis* [45].

Likewise, a phase II trial found that VPM1002 has safety and efficacy comparable to BCG in newborns. Given that VPM1002 is successful at eliminating *M. tuberculosis* in mice exposed to the bacteria, a phase III trial for post-exposure vaccination with VPM1002 is now in progress in India [45].

Animals immunized with rBCG exhibited smaller and fewer lesions in their lungs and livers compared to animals immunized with BCG. Compared to organs of tuberculosis animals, which have often developed into large arrowhead-shaped lesions, organs of rBCG-vaccinated animals exhibit progressively smaller and fewer lesions (Figure 3) [46].

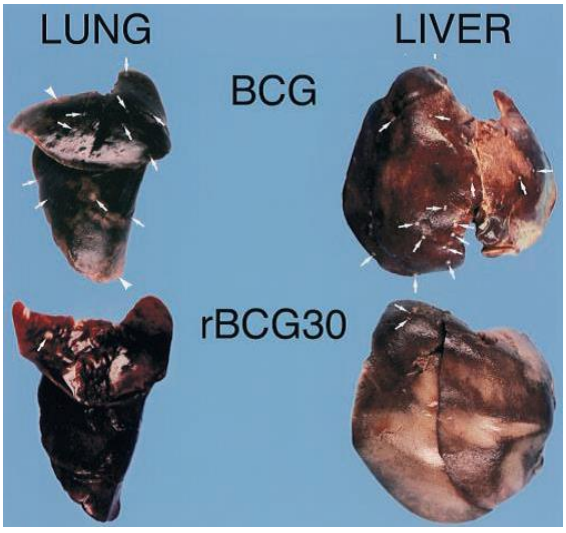

Figure 3. Animals immunized with rBCG and BCG [46].

AFRO-1. A recombinant BCG vaccine expressing the antigens TB10.4, Ag85B, Ag85A, and perfringolysin O derived from *Clostridium perfringens*. It well-tolerated and safe in immunodeficient SCID mice. rBCG-vaccinated mice exhibited a cellular immune reaction against *M. tuberculosis* (Strain HN878) infection. Mice vaccinated with AFRO-1 showed a higher survival rate compared to those receiving BCG [47].

BCG + H107 refers to a combination of the BCG vaccine and the H107 fusion protein, which includes MPT64, Rv3863, EspA, EspI, EspC, MPT70, ESAT-6, and MPT83. It triggered a Th17 response, promoted the formation of multifunctional T cell populations, and offered strong protection against pulmonary *M. tuberculosis* infection in mice [44].

The recombinant protein subunit GamTBvac vaccine includes Ag85A and ESAT-6/CFP-10 conjugated to a dextran-binding domain from Leuconostoc mesenteroides, mixed with an adjuvant and immobilized on dextran. The adjuvant consists of a diethylaminoethyl (DEAE)-dextran core and CpG oligodeoxynucleotides (CpG ODN). Phase II clinical trials in *M. tuberculosis*-uninfected adults vaccinated with BCG demonstrated that the GamTBvac vaccine induced a robust immunization, multifunctional $CD4^+$ T cells, and elevated IFN-γ release. There were specific vaccine immunoglobulin G (IgG) antibodies, exhibiting a humoral immune reaction. Phase III clinical trials currently assess GamTBvac [48].

hCMV/TB is a recombinant RhCMV/TB vaccine, derived from the *Rhesus cytomegalovirus* vector that express *M. tuberculosis* antigens. In cases of rhesus macaques' infection with *M. tuberculosis* one year after vaccination, the vaccinated macaques exhibited a stronger response of differentiated T cells compared to the unvaccinated controls, and the *M. tuberculosis* (Erdman strain) infection was *reduced* in them [49].

PE3 protein. Proteins of the proline-glutamic acid/proline-proline-glutamic acid (PE/PPE) family are predominantly found in pathogenic species of the *M. tuberculosis* complex, and a

scattered presence of PE/PPE proteins can be observed in environmental or non-pathogenic *Mycobacterium sp*. [50].

The PE/PPE family accounts for around 10% of the *M. tuberculosis* genome, and PE/PPE proteins play a role in evading host immune reactions to facilitate effective colonization within the host. Immunizing mice with the recombinant PE3 protein can generate a strong protective immune reaction against live mycobacterial infections and can be considered a subunit vaccine [50].

4. Conclusion

Despite its drawbacks, the BCG vaccine is a safe and efficient vaccine, and no better vaccine against tuberculosis infection has yet been developed. It is important to recognize that the BCG vaccine is only one aspect of the tuberculosis control process. Other critical factors include the identification of infections and new cases in children and adults, the prompt and appropriate treatment of infected individuals, and the education of medical staff and the public, all of which play a substantial role in managing and curbing the disease. On the other hand, researchers have been striving to develop a new and more effective vaccine. Recombinant vaccines are appropriate candidates to replace the BCG vaccine. Various studies have supported the efficacy of recombinant BCG antigens. A recombinant BCG vaccine against tuberculosis, similar to those described here, if shown to be more potent than conventional BCG vaccines in humans, could have a profound impact on human health. A modest improvement in the efficacy of the BCG vaccine could save hundreds of thousands of lives.

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Authors' contribution

MMF and NM developed the concept and designed the study. SB wrote the manuscript and created the figures/tables. MMF, NM and SB also contributed to the figures/tables and revised the manuscript. MMF edited and revised the manuscript. All authors have read and approved the final manuscript.

Ethics

Not applicable.

Conflict of Interest

The authors declare no conflict of interest.

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Data Availability

The data that support the finding of this study are available on request from the corresponding author.

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