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Candidate Vaccines against Tuberculosis and the Future of Novel TB Vaccine Research

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Abstract

Introduction: Tuberculosis (TB) continues to be a global health challenge and currently only one licensed vaccine is available. For nearly 100 years, the Bacillus Calmette-Guérin (BCG) vaccine has been in use. While it provides protection against disseminated TB in infants, its protection against adult and adolescent pulmonary tuberculosis (PTB) is variable. This literature review will provide an overview of the clinical status of candidate TB vaccines and discuss the challenges and future development trends of novel TB vaccine research, in combination with a general overview of the Tuberculosis (TB) disease and Mycobacterium tuberculosis itself. **Methods:** Bibliographic searches were carried out on medical journal databases, publishers, and aggregators. The most used databases were PubMed, NCBI and MDPI. Publications in English on these and other databases relating to novel TB vaccines were included in this review. **Results:** Currently, there are 12 main vaccine candidates in various phases of clinical trials, they include four protein or adjuvant vaccines, three viral-vectored vaccines, three mycobacterial whole cells or extract vaccines, and one each of the recombinant life and the attenuated Mycobacterium tuberculosis vaccine. Currently, the most likely candidate vaccines are the M72 + AS01E and Vaccae vaccines. M72 + AS01E is a recombinant fusion protein vaccine candidate, clinical trials showed that administering two doses of M72/AS01E was successful in reducing the development of active TB disease with 50% efficacy. Studies have also proven the efficacy of Vaccae (which is currently in phase III clinical trials) as an adjunctive therapy, with it being curative in conjunction with current therapy. **Conclusion:** Given the morbidity and mortality suffered globally by *M. tuberculosis*, it is time to realize the seriousness of the situation and accelerate our commitment and investment to the eradication of this infectious disease. With the number of vaccine candidates currently in clinical trials having promising results, it is imperative to continue these studies and accelerate towards phase III licensure trials if we are to achieve the milestone of

"End TB Strategy" by 2035. Today, we are witnessing immense progress in both preclinical and clinical TB vaccine research despite disappointing results from some of the clinical efficacy trials like that of MVA85A. We can revisit the design of vaccines and learn from them. It is important not only to recognize and give credit to those that have tested well in human trials, such as M72 + AS01E, but to expedite and improve its efficacy through funding of its research.

Keywords

Tuberculosis, Novel TB Vaccines, Clinical Trials, Bacillus Calmette-Guérin (BCG), Tuberculosis Prevention

1. Introduction

Tuberculosis (TB), a bacterial infection caused by the bacillus mycobacterium tuberculosis, is one of the leading causes of death worldwide. Until the coronavirus (covid-19) pandemic, tuberculosis was the leading cause of death from a single infectious agent [1]. The bacterium is spread when an infected individual expels it into the air (e.g., by coughing) which could then be inhaled by anyone within the contact range [2]. Tuberculosis usually affects the lungs (pulmonary TB), but it can attack any part of the body like the spine, kidney and brain [3]. Most people that develop the disease are adults, with more cases being reported among men than women. According to the WHO, about one-quarter of the world's population has been infected with TB [4] [5].

Tuberculosis is both a treatable and preventable disease, however, there is only one licensed TB vaccine worldwide, the Bacillus Calmette-Guérin (BCG) vaccine. This vaccine was developed over 13 years, from 1908 to 1921 by French bacteriologists Albert Calmette and Camille Guerin [6]. The vaccine has shown great efficacy in newborns and is in great use presently, it is given shortly after birth to prevent disseminated TB in infants, however, the results of the use of the BCG vaccine in adults to prevent pulmonary TB are variable [2].

The goal of the WHO and other global organizations is to end the TB epidemic by 2030 and to completely eradicate the disease by 2050. The target for 2030 is a 90% reduction in the number of deaths caused by TB and an 80% reduction in the incidence rate compared to 2015 [3]. To achieve this ambitious goal, there is an urgent need to develop novel tuberculosis vaccines. Currently, there are 12 main vaccine candidates in clinical trials, some with promising results, however, these are only expected to be ready in the coming years.

This overview article will outline the current situation of TB globally, and it will also delve into the causative agent, the course and the progression of the disease. It will further look at the risk factors and the populations affected, as well as an overview of the clinical status of the candidate vaccines and the future development trends/challenges of the novel TB vaccine research.

1.1. About Mycobacterium Tuberculosis

1.1.1. History

Heinrich Hermann Robert Koch was a German physician and microbiologist, he is regarded as one of the main founders of modern bacteriology, he is popularly nicknamed the father of microbiology. On March 24, 1882, Dr. Robert Koch announced the discovery of *Mycobacterium tuberculosis*, he successfully identified, isolated and cultured the bacillus in animal serum. During this time, one in seven deaths in the United States and Germany was caused by this infectious agent. March 24 is now designated as World TB Day, a day to educate the public around the world about the impact of TB [7] [8].

TB in humans can be traced back to 9000 years ago, in a city off the coast of Israel, where archaeologists found TB in the remains of a mother and child buried together. The earliest written records of TB were in India 3300 years ago and in China 2300 years ago, however, studies on Egyptian mummies (2400-3400 B.C) revealed the presence of skeletal deformities that is related to tuberculosis, such as characteristic Pott's deformities [7].

Mycobacterium tuberculosis was the cause of the "White Plague" of the 17th and 18th centuries in Europe, so called due to the paleness of the patients. During this period nearly 100 percent of the European population was infected with TB, and 25 percent of all deaths were caused by the bacterium. It is undeniably among some of the deadliest agents in history and till today it remains as one of the leading causes of death all around the world [3] [7] [9].

1.1.2. General Characteristics

Mycobacterium tuberculosis is a species of pathogenic bacteria belonging to the family Mycobacteriaceae. MTB is a nonmotile rod-shaped bacterium, measuring 2 - 4 micrometers in length and 0.2 - 0.5 micrometers in width. It is an obligate aerobe bacterium therefore in classical cases of tuberculosis, complexes of the bacteria can be found in the well-aerated upper lobes of the lungs. Being a facultative intracellular bacterium, it is usually found in macrophages, and has a slow generation time [10].

The cell wall structure of MTB is of great significance as it is unique among other prokaryotes and is a major determinant of virulence for the agent. The cell wall contains peptidoglycans, but the majority of the wall is made of complex lipids including Mycolic acids, Cord Factor and Wax-D, therefore *Mycobacterium tuberculosis* is not classified as either Gram-positive or Gram-negative, but if Gram staining is performed it stains very weakly Gram-positive or not at all [10]. *Mycobacterium* species are classified as acid-fast bacteria, their wall is impermeable to certain dyes and stains. One acid-fast staining method used to visualize MTB is the Ziehl-Neelsen stain.

Mycolic acids are strong hydrophobic molecules that form the lipid shield around the organism, and it significantly affects the permeability of the cell wall and determines the virulence of MTB. This serves as a protective mechanism for the bacterium as it prevents its destruction by cationic proteins, lysozymes and

oxygen radicals in the phagocytic vesicles of macrophages. In addition to the high lipid concentration in the cell wall causing impermeability to stains and evasion of destruction from macrophages, it is also a cause of resistance to many antibiotics, which is a major problem when it comes to the treatment of the disease.

1.1.3. Pathophysiology, Clinical Presentation and Course of the Disease

Tuberculosis occurs typically in 3 distinct stages, the so-called primary infection is the first, representing the actual infection by *M. tuberculosis* bacilli, these infections are usually asymptomatic (about 95%). Following this stage is the latent (dormant) stage [11]. Reactivation or reinfection causes progression to the final active infection stage, in which the symptoms become apparent, and the infection becomes transmissible.

Primary Infection:

To contract the infection caused by the microbe, it needs to be inhaled. The particles need to be small enough to pass through the defense systems of the upper respiratory tract. They deposit mainly deep in the subpleural airspaces of the middle and lower lobe. To initiate the infection, the rod-shaped microbe needs to be phagocytosed by alveolar macrophages. Those that survive the killing effects of macrophages replicate within them and ultimately kill them. Inflammatory cells, which are part of the hosts immune system, are attracted to the area of infection, here they induce focal pneumonitis that coalesces into the characteristic tubercles seen histologically.

Soon after the infection, some of the infected macrophages migrate to regional lymph nodes, usually the mediastinal and hilar lymph nodes, here they can infiltrate the bloodstream. Hematogenously, the infected macrophages disseminate to any part of the body, mainly to the apical-posterior portion of the lungs, epiphyses of long bones, vertebral bodies, kidneys and meninges.

Latent Infection:

This stage occurs after 95% of primary infections. Approximately after 3 weeks of uninhibited growth, the immune system manages to inhibit the replication of the bacilli, this is usually before signs and symptoms start to become apparent. Foci of the bacilli in the lungs and other sites become epithelioid granulomas which can have either caseous or necrotic centers. [11] The bacteria can survive in these granulomas for many years. Equilibrium between the hosts resistance and the microbial virulence determines whether the infections resolve spontaneously, remains dormant or becomes active. During this stage of the infection, the tuberculin skin test and interferon-gamma release assays (IGRA) are positive.

In rare cases, the primary infection can advance immediately without a latent (dormant) stage, causing an acute illness characterized by pneumonia, pleural effusion, mediastinal and hilar lymph node enlargement and other severe symptoms. This is seen more commonly among children, reinfected or recently infected immunosuppressed patients [11].

Active Infection:

Healthy people become infected have a 5% - 10% risk of developing the active disease during their lifetime. However, this percentage varies by age and other risk factors. Any organ that is seeded by the migrating macrophages may become a site of reactivation; it is usually in the lung apices. This typical localization is possibly due to the high oxygen concentration and other favorable local conditions.

Conditions that impair cellular immunity significantly facilitates the reactivation of the M. tuberculosis, therefore in patients coinfected with HIV the risk of developing the active disease is great. Other conditions facilitating reactivation but to a lesser extent includes diabetes, head and neck cancer, chronic kidney disease and drugs that suppress the immune system.

Through a type IV hypersensitivity (delayed type) reaction, TB damages the tissues, create granulomas that have a characteristic caseous histological appearance. In active pulmonary tuberculosis, patients may have no symptoms except "not feeling well", this is usually accompanied by anorexia, fatigue and weight loss. The most common symptom is coughing, it becomes productive as the disease progresses. Hemoptysis (coughing up blood) occurs only with cavitary TB. A low-grade fever is common, but variable. Night sweats are a classic symptom, however, it is not specific for TB. Another defining symptom is dyspnea, this is difficulty breathing which may be due to multiple etiologies, including parenchymal damage, spontaneous pneumothorax, and pleural effusion.

Depending on the organ affected, extra pulmonary TB causes many different systemic and localized symptoms. These include pyelonephritis, TB meningitis, peritonitis, pericarditis, lymphadenitis and TB of the bones and joints called Pott diseases. In addition to these, many other organs can be affected.

1.1.4. Current Preventative Measures and Treatment

Preventative measures

Prevention and an effective therapy are both imperative in the eradication of Tuberculosis. Currently the BCG (Bacille Calmette-Guérin) vaccine is the only licensed vaccine against TB and has been in use since 1921 [12]. Although it is one of the most widely used vaccines worldwide, we still see around 10 million new cases of TB every year—a testament of its limited effectiveness. The BCG vaccine is usually more effective against complex forms of TB in children and is of limited effectiveness in adults over the age of 35 and when given in equatorial regions (due to the high levels of naturally occurring environmental mycobacteria) [12] [13].

Early diagnosis and treatment are the most effective methods to prevent the spread of tuberculosis. A person with infectious TB can infect up to 10 - 15 other people per year, but once diagnosed and started on treatment, patients are no longer infectious after a few weeks [13]. Limiting the spread can also be accomplished by successfully finding and treating infected individuals, this can be done through raising awareness of TB, and by means of outreach workers and volun-

teers to work within communities with high rates of TB, to find people presenting with symptoms and refer them for testing.

As TB is an airborne infection, the risk of infection can be reduced using a few simple precautions such as having good ventilation (TB can remain suspended in unventilated air for several hours), natural light (UV light can kill TB) and good hygiene (covering of mouth and nose when coughing or sneezing).

Treatment

TB treatment is highly effective, worldwide nearly 90% of cases of TB and 48% of drug-resistant TB are cured. However, the treatment is neither quick nor easy, the length of the treatment and side effects from the drugs pose a huge problem for TB patients. Standard treatment of TB lasts 6 months, in the first 2 months patients are treated with a combination of 4 antibiotics (Rifampicin, Isoniazid, Pyrazinamide and Ethambutol), then for the following 4 month they are given only 2 antibiotics (Rifampicin and Isoniazid) [13].

Patients usually begin to feel better within 2 weeks of beginning treatment, and those with pulmonary TB normally become non-infectious during this time. It is vital that patients complete their treatment so that the bacteria are completely killed off in the body and to prevent the risk of forming drug-resistant bacteria.

Drug-resistant TB requires a longer and more complex treatment, and this is usually patient specific. This involves the use of more drug combinations and use of stronger antibiotics which significantly increases the risk of drug-drug interactions and side effects.

1.2. TB Global Epidemiology

The annual TB Reports which are released by the World Health Organization (WHO) displays TB data reported to the WHO by most countries, these reports give an extensive and up to date assessment of the global TB epidemic [14]. The latest report was produced in 2021, it showed data relating to the TB epidemic for the year 2020, the data it contained were submitted to the WHO by 197 countries and territories covering more than 99 percent of the world population [14].

When considering all the United Nations General Assembly (UNGA) targets in 2020. The world fell off track and failed to meet the targets. [15] The normal small annual decline in TB incidence which were witnessed in previous years almost ground to a standstill, TB notifications declined by 18% compared to 2019. The number of estimated TB deaths increased for the first time in 9 years. The report also shows that there was a reduction in the funding for TB and BCG coverage for children. These results are mainly credited to the ongoing Covid-19 pandemic. Since 2020, the pandemic disrupted essential health care services including those for TB as well as severely affecting TB infected individuals who are more vulnerable to suffering severe complications due to this this new infectious disease [12] [14] [15].

While it is suitable to put the blame the Covid-19 pandemic for slowing progress towards achieving the targets set by the World Health Assembly in

2014 and those set by world leaders at the first ever United Nations High Level Meeting (UNHLM) on TB held in 2018, it is crucial to admit that some targets were already off track before the Covid-19 pandemic.

Progress according to the End TB Strategy:

In 2014 the World Health Assembly adopted the *End TB Strategy.* The objective of this strategy is to end TB as a global public health threat by 2035. If the strategy is followed to the extent that TB care and prevention progresses, in 2035 we would expect to see reductions in TB incidence and mortality by 95% and 90% respectively compared to 2015. If the world was on track to achieve the targets which were stated in Table 1, the TB incidence and mortality would have decreased by at least 20% and 35% respectively by now. However, due to sub-optimal performance, we only saw an 11% decrease in TB incidence and a 9.2% decrease in TB mortality by 2021.

If we consider the current course of these epidemiological indicators, it is not unreasonable to ask a few questions: Are these ambitious targets even achievable? Do we lack the necessary tools to prevent and manage TB? What can we do to get back on track?

1.3. The Current/Only Vaccine against TB: Bacillus Calmette-Guerin (BCG)

Bacillus Calmette-Guerin (BCG) is a live attenuated vaccine form of *Mycobacterium*

Table 1. End TB Strategy and UNHLM Targets and progress 2015-2022.

Parameter	Target	Achieved
Incidence decline (compared with 2015 baseline)	20%	11%
Deaths decline (compared with 2015 baseline)	35%	9.2%
Catastrophic costs incurred by individuals with TB/their families	0	47%
People treated for TB, 2018-2022	40 million	19.8 million (50%)
Children treated for TB, 2018-2022	3.5 million	1.4 million (41%)
People with MDR TB treated, 2018-2022	1.5 million	483,000 (32%0
Children with MDR TB treated, 2018-2022	115,000	12,200 (11%)
People treated for LTBI, 2018-2022	30 million	8.7 million (29%)
People living with HIV treated for LTBI, 2018-2022	6 million	7.2 million (>100%)
Household contacts < 5 years treated for LTBI, 2018-2022	4 million	1.2 million (29%)
Household contacts > 5 years treated for LTBI, 2018-2022	20 million	0.32 million (1.6%)
Annual funding needs	13 billion USD	5.3 billion USD
Annual Research funding for TB	2 billion USD	901 million USD

[Source: 5].

bovis used to prevent tuberculosis and other mycobacterial infections like leprosy and Buruli ulcers. It remains one of the oldest and most widely used vaccines worldwide, estimated to immunize 100 million newborns every year. Vaccination with BCG simulates natural infection with *M. tuberculosis* and results in cell-mediated immune reactions and immunity against tuberculosis [15].

Although BCG is protective against disseminated disease in young children, it has variable efficacy against pulmonary TB, particularly in adults. There are several factors that could explain the variable efficacy of BCG [15]. Over time hundreds of passages and differences in BCG growth protocols between laboratories have contributed to genetic variability among the strains, it is clear that in clinical use there are genetic differences between the strains however it is less clear how it impacts efficacy. Other factors influencing the efficacy of BGC may include differences in immunogenicity between strains as well as the culture medium used to grow BCG.

A more consistently effective vaccine than the BCG in both adolescents and adults is needed to achieve the "End TB Strategy" set by the World Health Organization. Different types of vaccines are currently being developed to better mimic the natural route of *M. Tuberculosis* infection in the lungs and thus induce a better immune response. In addition to new vaccines, it is important to design booster vaccines which are highly potent and capable of inducing a strong immune response that overcomes differences in genetic background, ethnicity, and prior mycobacterial exposure between individuals.

A greater understanding of the reasons for the variability of the BCG vaccine, together with a better understanding of the early, innate, and non-antigen specific mechanisms of protection would help facilitate the design and development of more effective vaccines.

1.4. The Indications and Target Populations for Novel TB Vaccines

The WHO has developed a Preferred Product Characteristics (PPC) and identified two target populations for new TB vaccines: 1) adolescents and adults; and 2) infants. The TB vaccine candidates according to the WHO PPCs include various vaccine platforms such as whole cell vaccines, adjuvanted proteins, and recombinant subunit vector vaccines [15]. Currently candidate vaccines are being developed for prevention of TB disease in adolescents and adults, for early life immunization as a replacement of the BCG, as BCG boosters, for vaccination of TB patients after treatment to prevent recurrence, or as immunotherapeutic adjuncts to drug therapy to reduce treatment duration.

2. Novel TB Vaccines in Clinical Trials

2.1. Phase 1

2.1.1. AdAg85A

AdAg85A is a recombinant adenovirus serotype 5 (expressing Ag85A) vector vaccine being developed by McMaster University (Canada) in collaboration with Tianjin-based Chinese biotechnologycompany CanSinoo (China) [16]. The results

Table 2. Developmental pipeline for new tuberculosis (TB) vaccines [JULY 2022].

Phase	Tb vaccine	Tb vaccine type
Phase I	AdAg85A	Viral vectored (Adenovirus serotype 5 expressing Ag85A)
	MTBVAC	Attenuated *Mycobacterium tuberculosis* strain (Attenuated M. tuberculosis clinical isolate with ESAT6 & CFP10 & independent stable genetic deletions of phoP & fadD26 genes)
	ID93 + GLA-SE	Protein/adjuvant (Fusion Rv1813, Rv2608, Rv3619, Rv3620 with GLA-SE adjuvant)
	DAR 901	Mycobacterial—whole cell or extract (Agar-grown SRL172 by scalable, broth-grown manufacturing technique)
	TB/FLU-04L	Viral vectored (Attenuated replication- deficient influenza virus vector expressing antigens Ag85A & ESAT-6)
Phase IIa	VPM 1002	Recombinant BCG (rBCG vaccine with listeriolysin O encoding gene)
	RUTI	Mycobacterial, whole cell or extract (Polyantigenic liposomal vaccine of detoxified, fragmented M. tuberculosis)
	H56: IC31	Protein/adjuvant (Fusion protein of Ag85B, ESAT-6, latent Rv2660c with IC31 adjuvant)
	H4: IC31	Protein/adjuvant (H4 antigen, IC31 adjuvant)
	MVA85A	Viral vectored (Recombinant replication-deficient modified Vaccinia virus Ankara expressing Ag85A)
	M72 + AS01E	Protein/adjuvant (Fusion protein Mtb32A & Mtb39A, AS01E adjuvant)
Phase III	*Vaccae*	Mycobacterial, whole cell or extract (Heat-killed M. vaccae)

from animal studies have shown safe, immunogenic and enhanced protection against virulent *Mycobacterium tuberculosis* in murine, bovine and guinea pig models [**Figure 1**]. Intranasal BCG boosted with AdAg85A is able to significantly enhance the survival of BCG-primed guinea pigs following pulmonary M. tuberculosis infection [17]. In another experiment conducted on goat kids, those vaccinated with BCG-AdAg85A exhibited reduced pathology in the lungs and pulmonary lymph nodes compared to those vaccinated with BCG [16]. After intramuscular vaccinations during this phase, the vaccine was seen to be safe and immunogenic as it stimulated a polyfunctional T-cell responses [18]. In November 2021, recruitment of healthy volunteers who have been previously

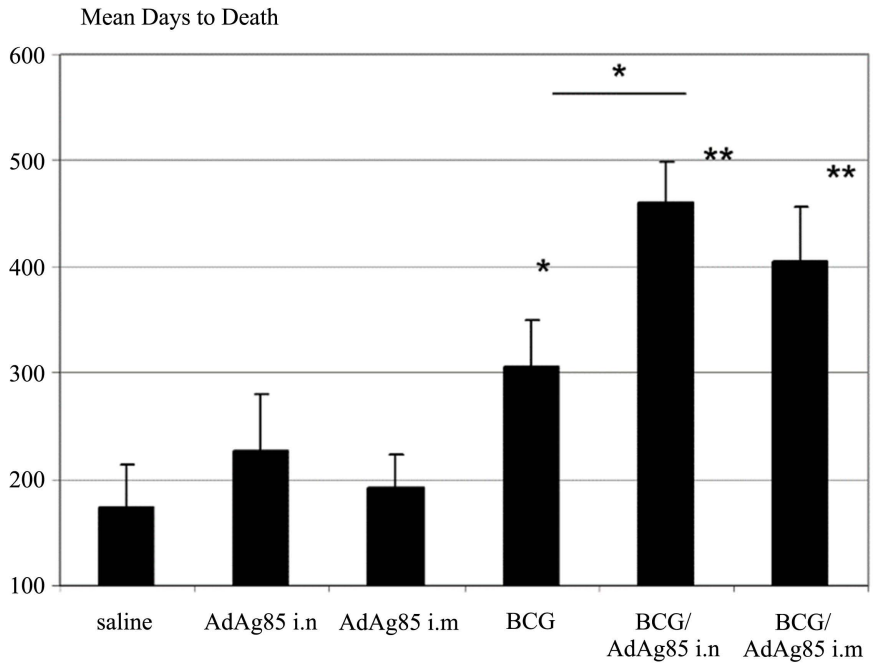

Figure 1. Comparison of mean days to death of various treatment groups of guinea pigs [Source: 11].

immunized with BCG was completed. The study will evaluate the safety and immune responses that develop in the blood and lungs following administration by aerosol drug dosage form [**ClinicalTrials.gov Identifier: NCT02337270**].

2.1.2. MTBVAC

MTBVAC is a live attenuated mycobacterial vaccine, with deleted *pho*P and *fad*D26 genes [16]. It is dependent on an attenuated clinical isolate of the mycobacterium that is associated with modern lineage 4. It maintains most of the original T-cell epitopes described for TB that includes the antigens ESAT6 and CFP10 of the RD1 [18]. The aim of this vaccine is to hopefully take the place of the BCG vaccine which is currently used in newborns as well as a preventative vaccine in adults and adolescents. It is being developed by TBVI (Netherlands), University of Zaragoza and Biofabri (Spain) [16].

Preclinical studies have shown that MTBVAC induces immunity to ESAT6 and CFP10, exhibits safety, biodistribution like the BCG and an improved efficacy than BCG. As a result, phase I human trials started—it was a dose escalation randomized controlled double-blind study in adults and neonates [ClinicalTrials.gov Identifier: NCT02729571]. Everyone who received the vaccines was subject to safety and immunogenicity analysis. As a result of the analysis, mild injection-site reactions were found, however the reactions were only in infants in the BCG and MTBVAC groups. Systemic AEs were seen to be distributed uniformly across both BCG and MTBVAC dose groups and were mostly mild in severity [18]. At the moment a phase II clinical trial is happening to assess the dose-defining safety and immunogenicity of the vaccine in

neonates (ClinicalTrials.gov: NCT03536117). A separate phase II clinical trial determining the same parameters in adults with and without latent TB, had been enlisting, however, the current status of the trial is unknown (ClinicalTrials.gov: NCT02933281).

So far this vaccine candidate looks highly promising and has the potential to be used as an alternative to the BCG vaccination in infants or for prevention of tuberculosis in adults and adolescents [19] [20].

2.1.3. ID93 + GLA-SE

ID93 + GLA-SE is classified as a subunit vaccine. It has four antigens which represent the different families of *M. tuberculosis* proteins (two outer membrane proteins: Rv1813 and Rv2608; and two secreted proteins: Rv3619 and Rv3620) [16] [18]. The vaccine is currently being developed by the Infectious Disease Research Institute (USA), in collaboration with Aeras. In animal models, the vaccine demonstrated prophylactic and therapeutic immunization potential. After the animal models proved successful, a randomized double-blind placebo-controlled phase I clinical trial began. The trial evaluated the safety and immunogenicity of the vaccine in healthy BCG-vaccinated adults, it was completed in 2015 (ClinicalTrials.gov: NCT01927159). The trial consisted of 66 healthy HIV-adults with a mean age of 25 years, they were randomly split into five separate groups: one placebo and four cohorts, with differing doses of the vaccine [18]. The trial showed mild-moderate AEs and no SAEs in the vaccine groups. When evaluating the vaccine-induced immunogenicity, the vaccine caused a rapid increase in the frequency of total cytokine-expressing CD4+ T cells specific to Rv1813, Rv2608, Rv3619, and Rv3620 in all four cohorts.

Another phase I trial followed this, a randomized double-blind clinal trial, to evaluate the safety, tolerability and immunogenicity of ID93 + GLA-SE (ClinicalTrials.gov: NCT02508376). Recently, another phase I clinical trial also evaluates the same parameters of the vaccine in adults. (ClinicalTrials.gov: NCT03722472) was completed, the results yet to be posted. A similar clinical trial in adolescents and in the phase II stage (ClinicalTrials.gov: NCT03806686), is underway. A phase IIa clinical trial to assess the safety, immunogenicity and efficacy of the vaccine in BCG-vaccinated healthcare workers is also underway (ClinicalTrials.gov: NCT03806686).

2.1.4. DAR 901

DAR 901 is a heat-inactivated *Mycobacterium obuense* strain of *M. tuberculosis* developed at Dartmouth University in collaboration with Aeras. It is made in the Master Cell Bank of agar-brown SRL172 using a new scalable broth-grown manufacturing technique. [16] [18]. DAR-901 booster in murine subjects conferred superior protection from TB challenge compared to the BCG booster.

A phase I clinical trial was concluded, it recorded the CD4+ T cell cytokine response to the booster vaccine in BCG-primed adults. The results showed that the receivers of the vaccine showed elevated DAR-901 antigen-specific polyspecific or bifunctional T cell responses compared to the baseline (Clinical-

Trials.gov: NCT02063555). [19] However, the currently used BCG vaccine caused a bigger CD4+ T cell response than the placebo, while the experimental vaccine sowed the same results as the placebo. Neither the DAR-901 nor the BCG vaccine induced substantial or sustained Th17/Th22 cytokine responses.

A phase II clinical trial has been concluded; however, the results are yet to be made available. The trial evaluated DAR-901 as a booster vaccine to prevent TB in BCG primed adolescents (ClinicalTrials.gov: NCT02712424) [16] [18] [19].

2.1.5. TB/FLU-04L

This is a recombinant influenza vaccine candidate. It is an attenuated replication-deficient influenza virus vector expressing the antigens Ag85A and ESAT-6 [16]. This vaccine, which is designed to be a prophylactic booster for all ages, is being developed by the Research Institute for Biological Safety Problems and the Research Institute on Influenza [18]. A phase I double-blind randomized placebo-controlled study was performed and concluded in 2015. It evaluated the safety and immunogenicity of two doses of this candidate vaccine versus the placebo in BCG-vaccinated healthy adults (18 - 50 years) (ClinicalTrials.gov: NCT02501421). The results have not yet been released to the public. Currently a phase II trial focusing on latent TB infection is being executed [18].

2.2. Phase IIa

2.2.1. VPM-1002

VPM-1002 is a recombinant BCG vaccine in which the *urease C* gene (responsible to the inhibition of phagolysosomal maturation) has been replaced by the *listeriolysin O*-encoding gene from *Listeria monocytogenes* (to improve immunogenicity) [16]. VPM-1002 has been made with the purpose of preventing pulmonary TB in newborns and TB recurrence in adults through post-exposure immunization [21].

Clinical Trial Phase I: Dose-Escalation Study on Safety and Immunogenicity of VPM1002 in Comparison with BCG in Healthy Male Volunteers (ClinicalTrials.gov: NCT00749034)—Completed.

Clinical Trial Phase II: Safety and Immunogenicity of VPM1002 in Comparison with BCG in HIV-exposed/-Unexposed Newborn Infants in South Africa (ClinicalTrials.gov: NCT02391415)—Completed.

Clinical Trial Phase III: Double-blind randomized clinical trial in which the efficacy, safety and immunogenicity of VPM-1002 is studied compared to BCG, in preventing TB infection in newborn infants in India (ClinicalTrials.gov: NCT04351685)—currently recruiting [16].

The phase II study found that VPM-1002 was not associated with SAEs [21].

2.2.2. RUTI

RUTI is a polyantigenic liposomal vaccine made of detoxified fragmented *M. tuberculosis* cells, it is hoped that this could be used as a therapeutic vaccine in combination with short-term anti-tuberculosis chemotherapy for prophylactic

treatment of patients with MDR-TB [2]. Murine models demonstrated that RUTI can be given as an adjuvant intervention to already therapeutic agents such as rifampicin and isoniazid, in which the results were shown to be markedly more effective [18]. Phase I studies involving healthy volunteers (ClinicalTrials.gov: NCT00546273) and phase II studies with LTBI (Latent TB Infection) (ClinicalTrials.gov: NCT01136161) have found that, at all study doses, the candidate vaccine is tolerable, safe and has good immunogenicity [2]. At present, a phase IIa clinical trial is being administered by the University Medical Center Groningen, its aim is to assess the safety of the vaccination in those with multidrug-resistant TB after successful treatment, however, its status is not known (ClinicalTrials.gov: NCT02711735). Another phase II clinical trial to assess the efficiency of the RUTI vaccination in drug sensitive and multidrug-resistant patients is organized, but not yet enlisting (ClinicalTrials.gov: NCT04919239) [2] [16] [18].

2.2.3. H56: IC31

This vaccine candidate is especially targeted at the *M. tuberculosis*-infected population as a post exposure vaccine candidate. It has a fusion protein of three mycobacterial antigens (early secreted Ag85B and ESAT-6, and the latent Rv2660c) formulated in the Th1-stimulating IC31 adjuvant [16]. It is currently being developed by SSI in Copenhagen, in collaboration with Aeras and Intercell. In healthy HIV-adults an antigen-specific IgG response and Th1 cytokine-expressing CD4+ T-cells were seen. The trial also revealed no SAEs, but mild/transient AEs when using the vaccine [18]. A phase I trial to determine the safety and immunogenicity parameters of H4: IC31, H56: IC31, and BCG vaccination in previously BCG-vaccinated healthy adolescents **(ClinicalTrials.gov: NCT02378207)** was concluded, it was found that in the H56: IC31 group, 20 out of 24 participants exhibited AEs. In a separate phase I trial testing the same parameters of the vaccine in adults who had recently been successfully treated for drug-susceptible pulmonary TB **(ClinicalTrials.gov: NCT02375698)**, similar results were found (87.5 percent of participants in the H56: IC31 group reported AEs, however no SAEs were reported) [18]. Currently another phase 1 trial is being conducted to test the same parameters of H56: IC31 in conjunction with a COX-2 inhibitor. This is to test the hypothesis that this may enhance the response to the vaccine **(ClinicalTrials.gov: NCT02503839)**. Currently a phase II clinical trial to determine the effectiveness of the vaccine in preventing reinfection in adults who are successfully treated for drug-susceptible PTB and HIV-negative, is ongoing in South Africa and the United Republic of Tanzania **(ClinicalTrials.gov: NCT03512249)** [2].

2.2.4. H4: IC31

H4: IC31 is a protein subunit adjuvanted vaccine like H56: IC31, it contains two active components: H4 antigen (which is a fusion protein of *M. tuberculosis* antigen 85B and TB10.4), and an immunological adjuvant called IC31 (which is a combination of the antimicrobial peptide KLK and oligodeoxynucleotide 1a –

ODN1a) [16] [18]. It was made by SSI together with Sanofi-Pasteur (France), aeras and Intercell. Phase I trials demonstrated an acceptable safety profile and an immunogenicity which can trigger multifunctional CD4+ T cell responses in individuals previously vaccinated with BCG [22]. A separate phase II clinical trial was also carried out, it evaluated the safety, immunogenicity, and prevention of TB with the vaccine, in comparison to BCG revaccination (ClinicalTrials.gov: NCT02075203). [23] Out of the 989 healthy adolescents who were enlisted in the trial, 330 made up the H4: IC31 cohort. The results revealed that 35.76% of participants in this cohort had some AEs, compared to 99.7% of participants reporting AEs in the BCG revaccination group. The candidate vaccine cohort also had a higher percentage of participants with an immune response compared to the BCG revaccination group. Effectively H4: IC31 boosted and prolonged immunity induced by BCG, leading to increased protection against M. tuberculosis with immune responses dominated by IFN-γ, TNF-α, IL-2 or TNF-α, IL-17, CD4+ cells [1] [22] [23].

2.2.5. MVA85A

This vaccine candidate is. a vector vaccine, it utilizes a recombinant replication-deficient modified vaccina virus, Ankara (MVA), which holds the *M. tuberculosis* Antigen 85A (Ag85A) [18]. Phase I trials found that this candidate is well-tolerated and extremely immunogenic when given as a boosterintradermal and aerosol administration to BCG-primed individuals. It was also discovered that it is capable of intramuscular, A clinical trial was then carried out based on this information to determine which route of administration would have the best cellular immunity and have the least AEs **(ClinicalTrials.gov: NCT01954563)**. Most of the AEs reported during the trial were mild injection site reactions following intradermal vaccination. Mild temporary systemic AEs were seen after vaccination by both intradermal and intramuscular, as well as respiratory AEs after primary aerosol administration. The most significant AEs were observed after boosting an intradermal MVA85A prime with an aerosolized MVA85A boost one month later, this led to transient moderate, or severe respiratory, and systemic AEs. However, no SAEs were reported. Phase IIb studies in 2797 infants showed MVA85A was well tolerated and induced modest cell-mediated immune responses but showed poor protection against Tb infection [24] [**Figure 2**]. Reasons for the absence of MVA85A efficacy against *M. tuberculosis* infection in infants needs more exploration. A phase IIa randomized clinical trial in adolescents and adults is currently enlisting. This trial aims to determine the vaccines immunogenicity in these populations.

2.3. Phase IIb

M72 + AS01E

M72 + AS01E is recombinant fusion protein vaccine candidate, incorporating the *M. tuberculosis* antigens Mtb32A and Mtb39A, in combination with the AS01E adjuvant system [18]. It was developed by Glaxo-SmithKline (United

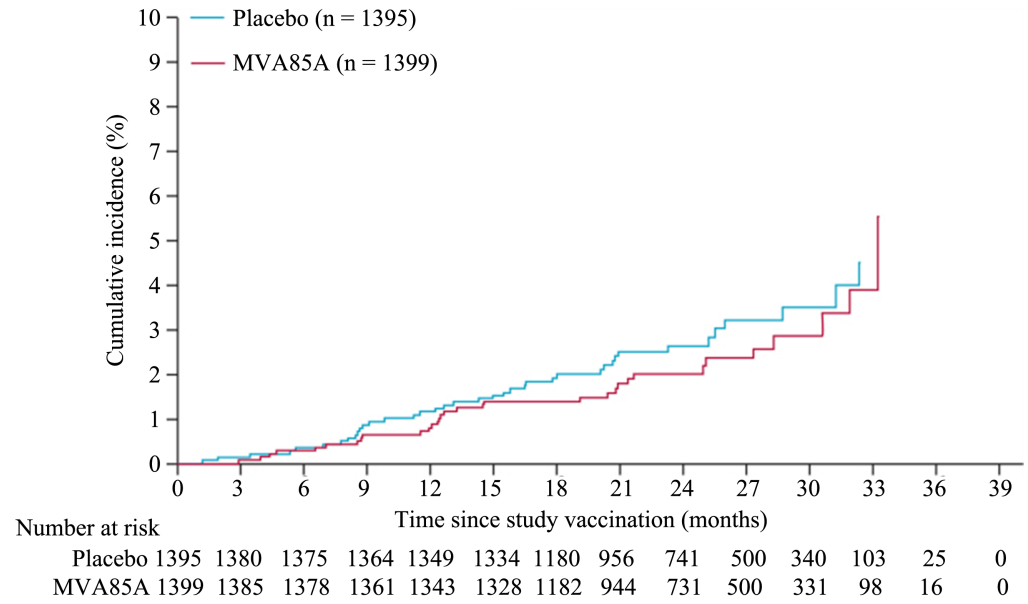

Figure 2. Interpretation/results: MVA85A was shown to be well tolerated and immunogenic in healthy infants who had previously been vaccinated with BCG, with a safety and immunogenicity profile consistent with that reported in other studies of infants. However, there was no significant efficacy against tuberculosis or *M. tuberculosis* infection [9].

Kingdom) in collaboration with Aeras with the aim of promoting BCG-induced immune responses [2]. In preclinical trials, M72 + AS01E proved to be more efficient in inducing immune responses in animal models than either Mtb32 or Mtb39 alone, as well as more efficient protection than BCG alone in mice, guinea pigs, and non-human primates. After completion of a phase II clinical trial in infants to assess the safety and immunogenicity of M72 + AS01E in 2010, it was shown to have an acceptable safety profile with no safety concerns identified. A phase II clinical trial, completed in 2015, assessing the safety and immunogenicity of M72/AS01E in HIV-positive (HIV+) adults, aged 18 - 59 years, living in a TB-endemic region **(ClinicalTrials.gov: NCT01262976)** proved to have a clinically acceptable safety profile and the ability to induce a strong humoral and cellular immunity [2] [18]. Similar results were observed in TB-infected adults **(ClinicalTrials.gov: NCT01424501)**, and adolescents with PPD negative or positive skin reactivity **(ClinicalTrials.gov: NCT00397943, NCT00621322 and NCT00950612)**.

Most recently a phase IIb trial was completed, the aim of the study was to evaluate the safety, immunogenicity and protective efficacy of M72/AS01E vaccine against pulmonary TB, as compared to placebo in HIV negative adults with latent TB infection living in high TB burden countries (South Africa, Kenya and Zambia) and aged 18 - 50 years **(ClinicalTrials.gov: NCT01755598)**. Participants vaccinated with M72 + AS01E experienced more local and flu-like general reactions than placebo recipients. The results showed that administering two-doses of M72/AS01E was successful in reducing the development of active TB disease with 50% efficacy. Within the M72/AS01E group, the concentrations

of M72-specific antibodies and the frequencies of M72-specific CD4+ T cells increased after the first dose and were sustained throughout the follow-up period. SAEs, potential immune-mediated diseases, and deaths occurred with similar frequencies in the two groups [22].

In 2020, the Bill & Melinda Gates Foundation (Gates Foundation) and the Bill & Melinda Gates Medical Research Institute (Gates MRI) announced that GSK has out licensed M72 + AS01E to the Gates MRI, "Paving the way for continued development and potential use of the vaccine candidate in countries with high TB burdens"

The WHO is encouraging the planning of accelerated progress towards a well-designed phase III trial. Various priorities for future trials can be highlighted, including the need for a more precise estimation of vaccine efficacy, use in different geographical settings, and further evaluation of safety. The effect of vaccination should also be characterized in people who do not have TB infection, in children, and in specific risk groups such as persons infected with HIV. This will require adequate vaccine production and financing, therefore the WHO calls on "all relevant stakeholders including pharma, funders, governments, civil society, health care practitioners, policy makers and international agencies to work with a sense of urgency, in spirit of collaboration and a sense of responsibility towards public health, to bring forward the expedited validation of this product in the fight against tuberculosis". [3]

2.4. Phase III

Vaccae

Vaccae is a vaccine candidate composed of heat-killed *Mycobacterium vaccae*, which was found to enhance anti-TB mycobacterial infections in patients with cellular immunity function and when combined with chemotherapy, it can enhance the efficacy of therapy in the adjunctive treatment of TB [18]. It was jointly developed by National Institutes for Food and Drug Control (China) and the 309th Hospital of the People's Liberation Army (Beijing, China) and is manufactured by Anhui Zhifei Longcom Biologic Pharmacy Co. (Anhui, China).

Studies have proven the efficacy of Vaccae as an adjunctive therapy, with it being curative in conjunction with current therapy. In addition to this, there was also improvement in symptoms when used as an adjunctive therapy, with an associated increase in CD4+ counts [18]. Currently a phase III clinical trial is being completed, assessing the safety and efficacy of Vaccae in TB prevention, enrolling 10000 participants, although results have not been made available **(ClinicalTrials.gov: NCT01979900)**.

3. The Challenges of TB Research and Its Future

3.1. Challenges Faced in the Research and Development of TB Vaccines

An ideal TB vaccine is one that will have a strong protective efficacy against *M. tuberculosis*, a strong and long-lasting immunogenicity and have no adverse

reactions [2]. However, currently there is no vaccine that is more protective or sustainable than BCG. MVA85A, one of the many vaccines to receive attention in the past two decades, was found to significantly enhance BCG immunogenicity priming in guinea pigs, rhesus monkeys, and cattle. Its safety and tolerability have also been validated in phase I and phase IIa clinical trials in healthy adults and TB- and HIV-infected infants, children, and adolescents. However, as seen above in **Figure 3** demonstrating the results of a phase IIb trial to assess the protective effects of the vaccine in healthy infants, there was no significant difference when compared to BCG. This was a great disappointment for the TB vaccine research community, it was also a lesson suggesting that some of the results obtained from animal models do not align with clinical trials. This is one of the many examples of challenges that hinder the development of TB vaccines.

The first challenge is the current epidemiology and the challenge of TB itself. As discussed above, the problem of TB incidence and prevalence around the world continues to be a major contributor to the global health crisis. While data has shown a slight decrease in these parameters over the past few years, if we are achieve the goals set by the World Health Assembly in the "End TB Strategy" by 2035 a new and more effective vaccine is needed. As treatment and therapy differs among nations, more drug-resistant strains of *M. tuberculosis* are being formed, which poses a very significant public health threat. As the world is highly connected, people are mobile and as this is an infection that is spread by coughing, sneezing or exposure to an infectious case the development of MDR-TB is rapid. Therefore, the challenge for TB vaccine developers is to develop a new TB vaccine in time to prevent spread of these drug-resistant strains of TB.

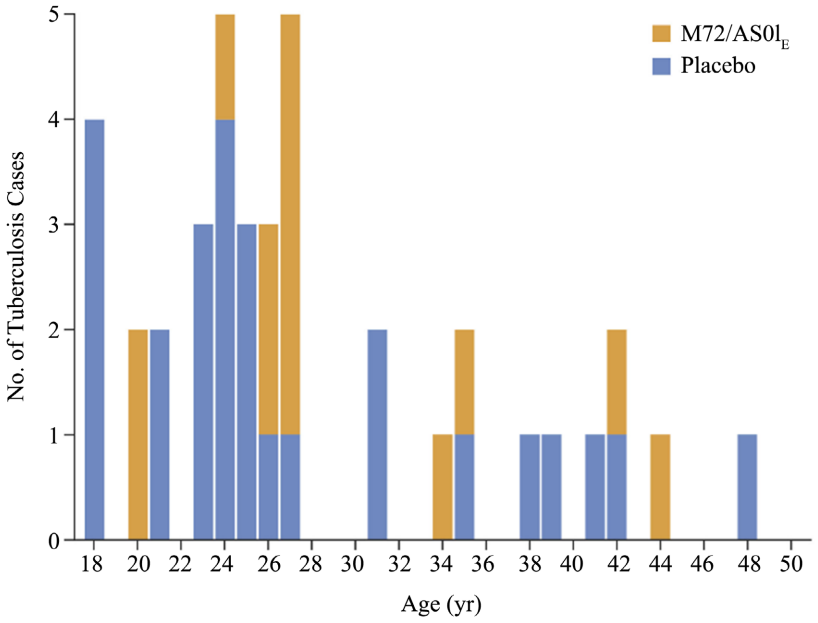

Figure 3. Tuberculosis cases according to age at enrollment (According-to-Protocol Efficacy Cohort). [Source: 20].

The second challenge that vaccine developers face is those included within the actual development of the vaccine, examples of these challenges are the lack of:

- Clear protective antigens: due to the fact that there is little clinical trial data, it is hard to identify specific antigens that can be used to produce vaccines against TB. Currently, most subunit vaccines contain proliferation-associated antigens, but the question remains, which is the best antigen to select and how many should be included in the vaccine. Therefore, it is important to choose more effective antigens to induce a more potent protection against *M. tuberculosis*.
- Credible preclinical evaluation indicators: the most commonly used assessment methods to determine the effectiveness of TB vaccines are to detect specific antibody titers caused by the vaccine, various types of cytokines made by CD4+ and CD8+, and other T cells following immunization. Further studies are required to find a direct link between these specific immune cells and cytokine to natural TB infection.
- Suitable vaccine evaluation animal model: as stated above with MVA85A, the correlation between results obtained from animal models and those of clinical trials did not align. The disease manifestations caused by the mycobacterium varies in different species, and immune response following vaccination is more variable in humans. Therefore, the predictive ability of animal models is limited by these interspecies differences.
- Emulated exposure methods: the conditions of exposure between animal models and humans to induce infection differs, for example animal models are infected by a single strain in a high-dose, and by single exposure by different routes of administration, while in natural conditions humans experience multiple low-doses with different strains by inhalation.
- Unified clinical trial endpoint criteria: there are fundamental differences and the absence of significance between preclinical trials and clinical trials in establishing the endpoint of vaccine effectiveness. For example, the use of bacterial counts and histopathological damage in animal models as the endpoints compared to TB incidence in human trials.
- Consistent evaluation environment: when comparing clinical trials to preclinical trials, the many differences in the environment in which they are evaluated drastically affects the results. In humans, factors such as diet, metabolic and nutritional status, co-infections with other microbes, genetic heterogenicity and many others affects vaccine effects in vivo, while animal models are specific pathogen-free and have a consistent genetic background which is strictly controlled.

Finally, a major obstacle for tuberculosis vaccine research and development is the lack of funding and resources. As shown in **Table 1**, funding for tuberculosis research is only halfway to the 2 billion USD per year required according to 2018 UN figures. This is in stark contrast to the large amounts of funding for the Covid-19 vaccines that was received from private reserves and the governments of

high-income nations such as the UK and the USA who pre-bought the vaccines, alleviating the usual financial concerns faced by pharmaceutical companies when developing a vaccine. The WHO is calling on all relevant stockholders, governments, pharmaceutical companies and international agencies to help in the funding of TB vaccine research to expedite its development.

3.2. The Future of TB Vaccine Research

In recent years, with advancement of novel vaccine research technology, new methods and ideas for various vaccines research has been established. Many different candidates and their advanced stages in clinical trials such as M72 + AS01E denote a unique and exciting phase in TB vaccine research. Some candidates appear to be more promising than others, and with discouraging trials like that of MVA85A it can be hard to stay optimistic, however when looking at TB vaccine research, we have discovered new and exciting ways to design vaccines in recent years, that would hopefully soon prove to a have a higher efficacy and immunogenicity in adults and adolescents than the current BCG vaccine [25]. There are also many novel vaccine candidates in preclinical development, including more recently developed vaccine formats such as DNA vaccines, new adjuvants and delivery systems, and combination vaccines.

The main regimens upon which novel TB development will be based on includes: 1) Priming vaccines, 2) Booster vaccines following BCG vaccination, 3) Latent infection preventative vaccines and 4) Therapeutic vaccines [2] [25].

4. Conclusion

TB remains one of the greatest threats to global health, therefore the importance for development of effective and safer vaccines to control this epidemic is critical, particularly for MDR-TB and TB-HIV co-infection. Over the past few decades, TB vaccines and vaccine candidates have developed greatly, for all age groups and different forms of TB. With the number of vaccine candidates currently in clinical trials having promising results, it is imperative to continue these studies and accelerate towards phase III licensure trials if we are to achieve the milestone of "End TB Strategy" by 2035. Today, we are witnessing immense progress in both preclinical and clinical TB vaccine research despite disappointing results from some of the clinical efficacy trials like that of MVA85A, as we can revisit the design of vaccines and learn from them. It is important not only to recognize and give credit to those that have tested well in human trials, such as M72 + AS01E, but to expedite and improve its efficacy through funding of its research. The remarkable speed at which Covid-19 vaccines have been developed demonstrates what real urgency can achieve and serves as a benchmark for the TB vaccine development. Given the morbidity and mortality suffered globally by *M. tuberculosis*, it is time to realize the seriousness of the situation and accelerate our commitment and investment to the eradication of this infectious disease.

Authors' Contribution

All the authors contributed in the achievement of this work and to the drafting of this manuscript. All authors have read and approved the final version of this manuscript.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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