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Progress towards a better vaccine against tuberculosis

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The University of Toledo

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Table of Contents

Introduction....1

Background....2

Statement of Problem....3

Statement of purpose....3

Research Question....3

Definition of Terms....3

Methodology....11

Literature Review....12

Tuberculosis (TB) Bacteria....12

Mycobacterium bovis....13

Mycobacterium tuberculosis....14

Mechanism of Antibiotic Resistance....16

The Reaction of the Host....18

BCG Vaccine....20

Active TB Treatment....21

Preclinical and Clinical Testing of Vaccines....22

Preclinical Vaccine Studies....24

Phase I Clinical Trials....25

Phase II Clinical Trials....32

Phase III Clinical Trials....38

Phase IV Clinical Trials....40

Discussion....41

Conclusion 43

References..44

Abstract 57

Introduction

Clinically, socially, and economically, the global tuberculosis (TB) burden continues to be very high despite advances in drug and vaccine development (Principi & Esposito, 2015). In 2014 alone, an estimated 9.6 million people became ill due to TB and approximately 1.5 million people died from TB (World Health Organization, 2015). An estimated 95% of TB mortalities took place in countries considered to be low- to middle-income and TB is now the 5th leading cause of mortality in women between the ages of 15 to 44 (World Health Organization, 2015). In children, the number of pediatric illnesses due to TB were estimated to be 1 million and, of those, approximately 140,000 children died due to TB infections in 2014 (World Health Organization, 2015). TB is now the leading cause of death among Human Immunodeficiency Virus (HIV)-infected individuals, killing 1 out of every 3 persons with HIV-TB co-infection (World Health Organization, 2015). The prevalence of TB in the U.S. is substantially lower than other countries around the world, with 9,421 TB cases reported in 2014 and infection rates appear to be decreasing yearly (Salinas et al., 2016). Although antibiotic regimens are available to treat TB, there were an estimated 480,000 worldwide cases of Multidrug Resistant-TB (MDR-TB) in 2014 (World Health Organization, 2015) and the emergence of Extensively Drug-Resistant TB (XDR-TB) strains is especially concerning. Drug resistance by TB has been attributed to many factors, including lack of patient compliance, provider under-prescription, limited access to antibiotics, and high prices of antibiotic regimens that need to be taken for months (Orme, 2011). Despite the existence of the BCG vaccine, BCG use is limited and controversial. First, the effectiveness of BCG in children is approximately 80% (Mangtani et al., 2014) while its effectiveness in adults is estimated to be approximately 50% (Principi & Esposito, 2015). Second, BCG vaccination often interferes with TB diagnosis (Hawkridge &

Mahomed, 2011). Taken together, there is a clear need for improved or new vaccines that prevent TB infection or limit clinical disease severity. The purpose of this review is to highlight current efforts to develop new vaccines against TB, including descriptions of ongoing clinical trials (phase I to III), reported outcomes, and pros/cons of each new vaccine.

Background

TB is caused by a group of infectious bacteria referred to as the *Mycobacterium tuberculosis* complex (MTBC) (Sakamoto, 2012). These bacteria include *Mycobacterium tuberculosis*, *Mycobacterium bovis*, *Mycobacterium africanum, Mycobacterium microti, Mycobacterium canettii, Mycobacterium pinnipedii, Mycobacterium caprae, Mycobacterium orygis (oryx bacillus),* and *Mycobacterium mungi (dassie bacillus)* (Rodriguez-Campos, Smith, Boniotti, & Aranaz, 2014). Tuberculosis is primarily acquired by aerosolization from an infected person to an uninfected person (Dheda, Barry, & Maartens, 2015). Although there is some debate about the actual dose needed to infect humans, between 1 and 200 bacilli must be inhaled for a healthy person to become infected with TB (Sakamoto, 2012). Depending on the host immune status, MTBC infection can result in one of three scenarios: no infection, latent infection, or active disease (Ottenhoff & Kaufmann, 2012; Tye et al., 2015). Patients that have no infection or a latent infection will not transmit TB but individuals with an active TB infection can transmit the disease to other persons. The BCG vaccine is a live attenuated vaccine, derived from *M. bovis,* that has been used for over 90 years to prevent TB (Principi & Esposito, 2015). Despite the fact that BCG is given as a part of childhood immunizations in many countries around the world, its effectiveness against TB is limited (Principi & Esposito, 2015).

Statement of Problem

Infectious TB causes about one-third of global deaths and although antibiotic treatment regimens used to be extremely effective against TB, the increase of HIV-TB co-infections and emergence of MDR-TB and XDR-TB have limited treatment options for physicians (Dheda et al., 2015). To address these concerns, there are several promising vaccine candidates that are currently undergoing preclinical or clinical testing, but the side effects of these vaccines have raised questions about their future use (Dheda et al., 2015).

Statement of Purpose

The purpose of this review is to highlight clinical testing of TB vaccines over the past five years. Throughout this review, new vaccines will be compared to the current BCG vaccine. This clinical review aims to highlight the progress that has been made thus far in TB vaccine discovery and development and examine which new vaccines may offer alternatives to the BCG vaccine.

Research Question

Are there new vaccines currently in clinical trials that can be used to prevent future TB infections?

Definition of Terms

MTBC

The MTBC is a group of infectious pathogens in the *Mycobacterium* genera (Sakamoto, 2012). The MTBC currently includes 10 species, including *M. tuberculosis*, *M. bovis*, *M.*

africanum, *M. microti*, *M. canettii*, *M. pinnipedii*, *M. caprae*, *M. orygis* (oryx bacillus), and *M. mungi* (dassie bacillus) (Rodriguez-Campos et al., 2014). All members of the MTBC are known to cause human TB, have 99.9% nucleotide sequence identity, and are virtually identical in their 16S rRNA sequences (Boddinghaus, Rogall, Flohr, Blocker, & Bottger, 1990; Huard et al., 2006; Sreevatsan et al., 1996).

M. bovis

M. bovis is a bacterium that causes TB in cattle and humans. Human TB can occur following ingestion of unpasteurized milk and dairy products containing *M. bovis* (Wilkins et al., 2008).

M. tuberculosis

M. tuberculosis is a bacterium that causes TB in humans via aerosolization from one infected individual and infection of the lungs of an uninfected individual. Inside the lungs, *M. tuberculosis* is phagocytosed by immune cells such as macrophages, where the bacterium either can be killed or can survive and replicate in these host cells (Yuk & Jo, 2014). *M. tuberculosis* is known to infect organs other than the lungs, including kidney, spine, and brain (Dheda et al., 2015).

Host

A human or animal that has been infected by a pathogenic strain of *M. tuberculosis* or any member of the MTBC can transmit the bacterium to an uninfected human or animal (Dheda et al., 2015).

Contact

Someone who has spent considerable amount of time with a TB-infected host (Dheda et al., 2015). As noted above, TB transmission occurs when a person with active TB coughs or sneezes, which aerosolizes the bacteria into tiny water droplets in the air. These small droplets can remain airborne for several hours and can be inhaled by uninfected persons. After inhalation, the bacteria accumulate in the alveoli sacs, infect macrophages, and multiply, leading to infection. Although TB infections typically result in latency, active TB can result in a matter of days, weeks, months, or years depending on the individual's immune status.

BCG

The BCG vaccine is administered to infants and adults to protect against TB infection. However, the reported efficacy of BCG is 80% in children (Mangtani et al., 2014) and 50% in adults (Principi & Esposito, 2015). In contrast, some studies have noted no real protection for adults (P. E. Fine, 1988). BCG was named after French scientists Albert Calmette and Camille Guérin, who discovered that repeated laboratory passage of *M. bovis* resulted in a strain that was less virulent.

Latent TB

State in which the MTBC organism survives within the host without causing any symptoms or TB disease (described in 'Active TB/Pulmonary TB' below) (Dheda et al., 2015). A latent infection could be simply defined as a 'stalemate' between the bacterium and the host immune response, where bacteria are not replicating and the immune system is unable to

completely clear the host of infection (Sanduzzi, Ponticiello, Bocchino, Perna, & Vatrella, 2016). Individuals with latent TB cannot spread the bacterium to others (Sanduzzi et al., 2016). These individuals react positively to the TB skin test (tuberculin) and have the potential to develop active TB if they do not seek TB treatment (Dheda et al., 2015).

Active TB/Pulmonary TB

An infection in which *M. tuberculosis* or any member of the MTBC are not cleared by immune cells but, instead, replicate in the lung or other tissues and cause active disease (Dheda et al., 2015). Active TB is characterized by symptoms such as chronic cough (lasting three or more weeks), chest pain, bloody sputum, fatigue, weight loss, fever, chills, night sweats, and diminished appetite (Dheda et al., 2015). For individuals with latent TB (described above), disease can develop when the immune system is compromised, including advanced age, HIV co-infection, or other infections (Dheda et al., 2015). Only persons with active TB can spread TB to others and active TB typically presents with the characteristic signs and symptoms described above.

Extra-pulmonary TB

Presence of *M. tuberculosis* or any member of the MTBC in tissues other than the lungs, including lymph nodes, kidney, spine, and the brain (Dheda et al., 2015). Extra-pulmonary symptoms of active TB vary depending on the affected organ and numbers of bacteria in the affected organ (Dheda et al., 2015).

MDR-TB

A well-established term and acronym that refers to any member of the MTBC that is resistant to Isoniazid (INH) and Rifampin (RIF), which are antibiotics commonly used to treat TB disease (Dheda et al., 2015). As of 2014, 480,000 (5%) of TB cases were estimated to be MDR-TB (World Health Organization).

XDR-TB

A well-established term and acronym that refers to any member of the MTBC that is resistant to Isoniazid (INH), Rifampin (RIF), any Fluoroquinolone, and at least one second-line antibiotic [e.g. amikacin, kanamycin, or capreomycin] (http://www.cdc.gov/tb/publications/factsheets/drtb/xdrtb.htm). As of 2014, an estimated 9.7% of MDR-TB strains are estimated to be XDR-TB (World Health Organization) (Dheda et al., 2015).

HIV-TB

Refers to individuals who have been co-infected with both HIV and TB. Because HIV infection is known to suppress the immune system and TB latency or infection susceptibility is known to depend on a fully-functional immune system, active TB can develop very quickly in HIV-TB co-infected individuals, which can result in severe disease, and can be difficult to treat (Dheda et al., 2015).

Preclinical Vaccine Testing

The experimental or new vaccine is tested in animals in a laboratory setting. After a series of successful animal studies, a summary of research findings is presented to the U.S. Food and Drug Administration (FDA) for evaluation. If deemed reasonably safe and efficacious, the FDA may approve a series of clinical trials in humans. Clinical trials are organized into 4 phases (described below), which are meant to answer distinct research questions at each phase (http://www.fda.gov/drugs/resourcesforyou/consumers/ucm143534.htm).

Phase I Clinical Trial

The experimental vaccine is tested in a relatively small group (20 to 80) of healthy people for the first time to assess the safety, dosage range, and identify any adverse reactions of the vaccine.

Clinical Phase II Trial

The experimental vaccine is given to a larger group (100 to 300) of people to assess its effectiveness and safety in the target population – people who the prospective vaccine is most likely to help.

Clinical Phase III Trial

The experimental vaccine is given to an even larger group (1000 to 3000) of people to confirm its effectiveness, closely monitor adverse reactions, compare the experimental drug to similar drugs on the market, and collect data so that the experimental vaccine can be safely used in the general population.

Clinical Phase IV Trial

Studies performed after the experimental vaccine is currently on the market to gather additional information about risks and benefits of long-term drug use and optimal drug use.

Limitations

This literature review has certain limitations, as is true for all reviews. Below is a list of limitations that I have identified in this review. These limitations are presented in the interest of full disclosure but should not distract the reader from the information presented in this review.

1. Levels of evidence. The levels of evidence used in this review were randomized control trials, which is third in the 'evidence hierarchy' for scientific experiments or categorizing healthcare information. 'Evidence hierarchy' is organized into a prioritized list of five categories as follows: First, meta-analyses and systematic reviews are the highest level of evidence; Second, evidence-based reviews are the next highest level of evidence; Third, randomized control trials are the next level of evidence; Fourth, case-control studies, case series, and case reports are the next level of evidence; Fifth, textbooks, review articles, expert opinions, and laboratory research are the lowest level of evidence. Although I have listed randomized control trials as a limitation of this review due of its relative 'evidence hierarchy' ranking, this may not be a limitation. Indeed, randomized control trials are often deemed the gold standard for clinical trials because this level of evidence examines both the expected difference between the control and experimental groups and the outcome variable that is under investigation.

2. Time constraints. This review was written as the scholarly project component of my physician assistant training. Thus, this review was completed in a limited time frame and only focuses on articles that were written in English and described progress on phase I to phase IV clinical trials.
3. Cost. This review only contains information obtained from publicly-available research studies, including publications available through interlibrary loan. Publications or databases that required pay-for-use were excluded from this review.

Methodology

A PubMed search was performed for new tuberculosis vaccines in articles written in English between January 01, 2010 and November 31, 2016. Articles from the U.S. and outside of the U.S. were included in this review. Articles were excluded if the research was not a phase I, phase II, phase III, or phase IV clinical trial for TB prevention. Although preclinical vaccine development articles generally were excluded from this review, there are two preclinical vaccines (BCGΔ*zmp1* and MtbΔ*secA2*) included below because of extensive testing in animals. Articles that reported phase I, II, III, or IV clinical trials were included because these trials were performed in human subjects. Search terms included: 'new TB vaccines,' 'current TB vaccines,' 'TB BCG vaccine,' 'MTBC,' and 'TB phase I to IV clinical trials.'

Literature Review

Tuberculosis (TB) Bacteria

The genus *Mycobacterium* is made up of more than 100 different species that are either saprophytes or pathogens (Whitman, 2015; Gao & Gupta, 2012). Mycobacteria are characterized as non-motile, non-spore forming, Gram-positive bacilli, although they do not stain well with the Gram stain due to the high lipid content in their cell wall. As such, Mycobacteria typically are characterized as acid-fast organisms because of their ability to be stained by acid-fast staining (Sakamoto, 2012). When viewed under the microscope, Mycobacteria are rod-shaped organisms that are either straight or with slight curvature (Sakamoto, 2012). The length and width of Mycobacteria range from 1 to 4 micrometers and 0.3 to 0.6 micrometers, respectively (Sakamoto, 2012). Mycobacteria, *Corynebacterium*, *Nocardia*, and *Rhodococcus* are all members of the *Actinomycetales* order due to the presence of mycolic acids in their cell walls, which are vital both structurally and functionally (Sakamoto, 2012). Because of their waxy cell walls, Mycobacteria are able to resist dessication, survive in acidic or alkaline environments, resist many antibiotics, and are capable of evading the host immune system (Sakamoto, 2012).

Of the 100 different *Mycobacterium* species, various members of the MTBC are known to cause disease in humans and animals, including *M. tuberculosis (Mtb), M. bovis, M. canettii, M. africanum (subtypes I and II), M. caprae, M. microti, M. pinnipedii, M. orygis* (van Ingen et al., 2012), *M. mungi* (K. A. Alexander et al., 2010), *M. suricattae* (Coscolla et al., 2013; El-Sayed, El-Shannat, Kamel, Castaneda-Vazquez, & Castaneda-Vazquez, 2015; Ng, Saavedra-Avila, Kennedy, Carreno, & Porcelli, 2015; Parsons, Drewe, Gey van Pittius, Warren, & van Helden, 2013). Genetic analysis has indicated that members of the MTBC descended from *M. canetti* (or *M. prototuberculosis*) (Gutierrez et al., 2005). All MTBC species can cause infection

in animals; whereas *M. tuberculosis*, *M. bovis* (El-Sayed et al., 2015), *M. africanum*, *M. canetti*, and those labeled 'smooth TB bacilli' can cause human tuberculosis (Coscolla et al., 2013). *M. tuberculosis* and *M. bovis* cause the highest number of human TB cases with *M. tuberculosis* out-numbering *M. bovis* in terms of frequency and virulence (El-Sayed et al., 2015). *M. tuberculosis* and *M. bovis* share 99.95% genetic identity, with small genetic differences being localized in the regions of difference (RD) (Brosch et al., 2002). Both *M. tuberculosis* and *M. bovis* are highly pathogenic due to a large number of virulence factors (Rodriguez-Campos et al., 2014). Although not a member of the MTBC, *Mycobacterium smegmatis* also is important in that it has been used to develop live attenuated vaccines for human consumption (Ng et al., 2015). *M. smegmatis* is an extremely useful research tool because it does not cause disease in humans or cattle, is missing numerous virulence properties, and grows quickly in the laboratory (Ng et al., 2015).

M. bovis

M. bovis causes major economic impacts to farm animals, threatens the ecosystem, and is responsible for almost 3.1% of global human tuberculosis (El-Sayed et al., 2015). Because of its robustness, *M. bovis* is capable of surviving in very harsh environmental conditions (A. E. Fine, Bolin, Gardiner, & Kaneene, 2011). *M. bovis* can survive in animal carcasses for up to 6 weeks, 4 weeks in fecal matter, up to 58 days in fresh water, 2 years in covered manure, and can withstand dispersed sunlight for approximately 5 months under various environmental conditions (El-Sayed et al., 2015). Cattle are vulnerable to infection when they ingest grass from contaminated pasturelands or areas that harbor *M. bovis* bacilli (El-Sayed et al., 2015). The quantity that is required to indirectly transmit *M bovis* is higher than that of aerosolized

transmission (A. E. Fine et al., 2011). In the past, human *M. bovis* infections have been primarily due to the consumption of unpasteurized milk and dairy products. However, unpasteurized dairy products continue to be one of the most important routes of transmitting *M. bovis* in developing countries (Wilkins et al., 2008). Countries with high prevalence rates of *M. bovis* and which export unpasteurized dairy products to the U.S. are a major contributor of U.S. *M. bovis* cases (Wilkins et al., 2008). Other U.S. *M. bovis* infections are due to humans coming into contact with deer populations (El-Sayed et al., 2015), particularly those who participate in activities such as hunting, trapping, taxidermy, venison processing, and venison consumption (Wilkins et al., 2008). In animals, *M. bovis* is spread from one animal to another through aerosolization during feeding and during water drinking (El-Sayed et al., 2015). Despite the above noted burden of *M. bovis* infections in humans, this paper will focus on *M. tuberculosis* infections in humans.

M. tuberculosis

M. tuberculosis is a slow-growing organism that takes approximately 12 to 24 hours to divide and requires up to 21 days before colonies appear on solid agar medium (Sakamoto, 2012). Despite much speculation, there is no known reason why *M. tuberculosis* grows so slowly. One possible mechanism that could explain the slow growth of *M. tuberculosis* is that its waxy cell wall does not allow many nutrients to enter the cytosol and its method of synthesizing RNA molecules is very slow (Sakamoto, 2012). When the bacterium has been observed in the infectious state (*i.e.,* in the host), the metabolism of *M. tuberculosis* fluctuates from aerophilic respiration (metabolizing nutrients using oxygen) to micro-aerobic respiration and the metabolism of lipids (Sakamoto, 2012). *M. tuberculosis* replicates and survives inside phagocyte cells, including macrophages and monocytes (Sakamoto, 2012).

The only vaccine that is currently available against TB is BCG, a live attenuated version of *M. bovis* (da Costa, Walker, & Bonavia, 2015). There are 16 genetic deletions or regions of difference, designated RD1 to RD16, that exist between BCG and *M. tuberculosis* (Sakamoto, 2012). As noted above, when the BCG vaccine originally was created through continuous passage in the laboratory, the RD1 region was deleted from *M. bovis* (Sakamoto, 2012). The RD1 region contained a series of nine genes, including the early secretory antigenic target-6 (ESAT-6) (Sakamoto, 2012) and culture filtrate protein-10 (CFP-10) (Kaku, Kawamura, Uchiyama, Kurenuma, & Mitsuyama, 2007). Since BCG is the only vaccine available to prevent TB, it is surprising that BCG only provides 80% efficacy in children younger than ten years old (Mangtani et al., 2014) and is approximately 50% effective in adolescents and adults (Principi & Esposito, 2015). As noted above, these efficacy numbers may be overestimates, as other studies have noted that BCG is not effective at all (P. E. Fine, 1988).

One of the major virulence mechanisms of *M. tuberculosis* is its ability to survive inside phagosomes and arrest the normal phagosome maturation process (O'Garra et al., 2013). The normal phagosome maturation process includes addition of phosphatidylinositol (PI)3 kinase hVPS34 and the endosomal tethering molecule EEA1 to the phagosome (Vergne, Chua, & Deretic, 2003). By an unknown mechanism, *M. tuberculosis* impedes EEA1 mobilization to phagosomes when *M. tuberculosis* is inside macrophages, thereby inhibiting phagosome-lysosome fusion (Fratti, Backer, Gruenberg, Corvera, & Deretic, 2001). By blocking EEA1, *M. tuberculosis* prevents the V0-ATPase (normally responsible for acidifying the phososome) and lysosomal hydrolases (normally responsible for degrading molecules in the phagosome) from fusing with the phagosome (Fratti, Chua, Vergne, & Deretic, 2003). Without phagosome-lysosome fusion, *M. tuberculosis* surives and replicates in host cells (Vergne et al., 2003).

Another molecule that affects phagosome maturation is calcium, but the effect of calcium on phagolysosome genesis remains to be described (Malik, Denning, & Kusner, 2000; Vergne et al., 2003). Interestingly, one study has shown that the prevention of elevated cytosolic calcium hinders phagosome procurement of late endosomal and lysosomal markers (Malik et al., 2000). When *M. tuberculosis* infects macrophages, it averts the influx of calcium and obstructs calcium/calmodulin kinase II (calcium/calmodulin effectors) from being activated (Malik et al., 2000).

Mechanisms of Antibiotic Resistance

Bacteria typically use one or more of the following four antibiotic resistance mechanisms to prevent damage by antimicrobial drugs (Hawkey, 1998). First, bacteria can alter the site that a potential drug binds, either on the surface of the bacteria or inside the cytoplasm of the bacteria (Hawkey, 1998). Alterations of the bacterial target sites are accomplished by mutating DNA or by acquiring DNA from other bacteria that gives the bacteria the ability to alter its binding site. Second, bacteria can modify their cell wall or surface structures to decrease penetration by a drug or increase efflux of the drug away from the bacteria or out of its cytoplasm (Hawkey, 1998). Third, bacteria can inactivate or modify the antibiotic through beta lactamase enzymes (Hawkey, 1998). Beta lactamases cleave the beta lactam ring, thereby inactivating the antibiotic (Hawkey, 1998). Currently, researchers have described over 200 different types of beta lactamase enzymes (Hawkey, 1998). The majority of the beta lactamase enzymes target antibiotics that are in the penicillin and cephalosporin families, while other enzymes have specific targets (e.g. *Enterobacter* spp, encode an AmpC enzyme that targets specific cephalosporins) (Hawkey, 1998). Beta lactamase enzymes are found in both Gram-negative and Gram-positive bacteria,

and display varying degrees of antibiotic resistance. Fourth, bacteria can prevent the drug from inhibiting a specific pathway by creating an alternative pathway (Hawkey, 1998). For example, PBP2a is an alternative penicillin binding protein synthesized by methicillin-resistant *Staphylococcus aureus* strains. In addition to the normal *S. aureus* penicillin binding proteins that are inhibited by beta lactam antibiotics, PBP2a is not inhibited by beta lactam antibiotics (Hawkey, 1998).

Assessments of antibiotic resistance are based on the desired clinical outcome, the health risk of the pathogen, the area of the body that was infected, antibiotic dispersal and concentration in body tissues, and patient immune response (Hawkey, 1998). The use of antibiotics in tuberculosis is different from other infectious diseases (Gillespie, 2002) because of the slow replication and metabolic rates for *M. tuberculosis*, rendering the bacterium difficult to treat ((McKinney et al., 2000), (Pablos-Mendez, 2000), (Wayne, 1994)). After infection by *M. tuberculosis*, the bacteria may be embedded in a pulmonary cavity, empyema pus, or solid caseous material that restricts antibiotic penetration, or bacteria may be present in an area that has a low pH that inhibits antimicrobial activity ((Elliott, Berning, Iseman, & Peloquin, 1995), (Iseman & Madsen, 1991)). Microorganisms that are found in lung cavities typically multiply by aerobic mechanisms (Gillespie, 2002). Microorganisms that are found in a granuloma or necrotic caseous foci are in an environment that prevent the actions of agents like aminoglycosides due to the low pH at the site.

The Reaction of the Host

The role of cell-mediated immunity, especially secretion of interferon γ [IFNγ] by Th1 type $CD4^+$ T cells, has been considered the most principal component of host immunity against TB (Principi & Esposito, 2015). Conversely, the role of antibodies in host defense against TB has been considered to be minor since there is no association between antibody titers against TB antigens and disease outcome (Principi & Esposito, 2015). IFN-γ has been shown to recruit macrophages to the site of infection and activate those macrophages to kill *M. tuberculosis* by inducing various antimicrobial pathways. Data from both laboratories and clinical studies have reinforced the idea that IFN-γ is crucial for host defense against TB (Principi & Esposito, 2015). For example, IFN-γ deficient mice are extremely vulnerable to TB infections and patients with mutations in their interleukin-12/IFN-γ-signal transduction pathway have increased risks of having severe TB infections (Principi & Esposito, 2015). In addition, patients with T-cell deficiencies, including those that are HIV positive or are undergoing cancer treatment, often are more susceptible to TB infections (Principi & Esposito, 2015). Therefore, there is a clear need to create new TB vaccines that can induce strong cellular Th1 immune reactions, including IFN-γ, TNF-α, and/or IL-2 secretions.

Currently, new vaccine development efforts have been focused on stimulating host immunity against TB infection (Yuk & Jo, 2014). *M. tuberculosis* is capable of suppressing the normal role that alveolar macrophages play in alerting nearby cells to respond to an invasive pathogen. Instead, *M. tuberculosis* blocks macrophage antigen processing and inhibits priming of effector T cells (Shaler, Horvath, Lai, & Xing, 2012). *M. tuberculosis* also blocks phagolysosome fusion and inhibits host cell production of reactive oxygen and nitrogen radicals (Shaler et al., 2011). In theory, a new TB vaccine would initiate the correct innate immune

response that would activate macrophages and stimulate T cells to protect against TB infection (Ottenhoff, 2012).

The hydrophobic, 'waxy' surface of *M. tuberculosis,* which gives the bacteria their acid-fast property, is composed mycolic acids, phosphatidyl-myo-inositol mannosides (PIMs), arabinogalactan, and peptidoglycan (Yuk & Jo, 2014). The *M. tuberculosis* cell wall also includes mannose-containing biomolecules such as mannose-capped lipoarabinomannan (LAM), lipomannan (LM), and mannoglycoproteins (Yuk & Jo, 2014). Of these cell wall building blocks, LM and LAM play principal roles modulating the host immune response (Yuk & Jo, 2014). The destruction of normal tissue architecture surrounding the bacteria and the pathological process that occurs during TB infection is partly initiated by *M. tuberculosis* but also is the result of an immune-pathological inflammatory response of the host (Yuk & Jo, 2014). *M. tuberculosis* invades host cells by binding to receptors located on the surface of the host cell, including the toll-like receptors (TLRs) or c-type lectin family receptors (Yuk & Jo, 2014). TLRs are important for innate immunity because they recognize common antigens or molecules found on many pathogenic microorganisms (Yuk & Jo, 2014). Despite the fact that TB infections produce many bacterial molecules that should stimulate TLRs, innate immune responses do not always clear *M. tuberculosis* bacteria (Yuk & Jo, 2014). More recently, experts have proposed that B cells play an important role in determining host immunity against *M. tuberculosis*, by reducing the bacterial load in tissues and decreasing the overall inflammatory response (Yuk & Jo, 2014).

BCG Vaccine

The global TB outbreak that was noted above has occurred in spite of a licensed tuberculosis vaccine – BCG (Principi & Esposito, 2015). The vaccine is administered via the intradermal route and is a live attenuated strain of *M. bovis*. BCG was created nearly one hundred years ago, was first was used in humans in 1921, and has been utilized worldwide to limit the spread of TB infections (Principi & Esposito, 2015) (P. E. Fine, 1995). Since 1948, many case-controlled studies and clinical trials have been performed using BCG. Many countries have included BCG into the immunization schedule for children. Beginning in 1974, the World Health Organization (WHO) made it part of their expanded program on immunization to immunize infants living in high-risk areas with BCG (Principi & Esposito, 2015). However, the BCG vaccine has several shortcomings, as evidenced by several epidemiological studies and controlled clinical trials (Mangtani et al., 2014). Whereas BCG prevents miliary and meningeal TB, it has not been able to eradicate TB or prevent adult infections (Principi & Esposito, 2015). The effectiveness of BCG is negatively affected by various factors, including vaccinee age, the anatomical location of TB infection, the geographical region that BCG was given, pre-existing immune responses to the bacteria (or related *Mycobacteria*), and immune status of the vaccinee (Principi & Esposito, 2015). BCG-induced protection is very high when given to infants or young children, as well as when given to patients in the early stages of infection (before developing military or meningeal TB infection).

In countries with endemic TB infections, infants are vaccinated with BCG soon after birth because it provides approximately 80% protection against pulmonary infection (Mangtani et al., 2014). Conversely, BCG only protects about 50% of adults against TB infections (Principi & Esposito, 2015), and some studies have noted no real protection for adults (P. E. Fine, 1988).

Although the effectiveness of BCG has been inconsistent, some reports have indicated protection as high as 80% against TB infection (Moliva, Turner, & Torrelles, 2015). BCG vaccination generally appears to provide approximately 10 to 15 years of immunity, but studies in Native Alaskan Indian communities demonstrated that BCG provided 50 years of immunity (Aronson et al., 2004). Conversely, BCG has been noted to confer virtually no immunity to some communities in India, particularly the Chengalpattu region (Moliva et al., 2015). In addition, the effectiveness of BCG has been noted to vary depending on geographical location and population age (Moliva et al., 2015). Together, these data show the need for new studies that closely examine the human genetic pool and the environment when evaluating BCG protection (Moliva et al., 2015). Currently, new vaccines are being developed, but these new vaccines face hurdles as they are tested globally (Moliva et al., 2015).

Active TB Treatment

A Randomized Controlled Trial (RCT) was performed between the 1950s to 1960s by health authorities in the U. S. and the British Medical Research Council (BMRC) in Great Britain to assess the blending of streptomycin (SM), isoniazid (INH), and para-aminosalicylic acid (PAS) for treating tuberculosis (Chang, Yew, & Sotgiu, 2015). Other drugs that were developed in 1952 and 1959 are pyrazinamide (PZA) and rifampicin (RMP) respectively, and these two drugs were evaluated through an RCT studies in Europe, East Africa, India, Singapore, and Hong Kong (Chang et al., 2015). The RCT conclusion revealed the scientific evidence in favor of authorizing shot-course drug therapy of 6 months (Chang et al., 2015). The length of treatment for TB is significantly compressed to 6 months due to the sterilizing effect of both PZA and RM (Blumberg et al., 2003). The studies indicated that PZA can only be used for 2

months of the 6 months therapy (Blumberg et al., 2003). The 6 months therapy is divided into 2 months and then a 4 months therapy. The 2 months therapy includes RMP, INH, PZA, and SM; while the 4 months therapy includes RMP and INH (Blumberg et al., 2003). In other to prevent parenteral inoculation and decrease the chances of transmitting Human Immunodeficiency Virus (HIV) Ethambutol (EMB) has substituted SM (Chang et al., 2015). The above emphasizes the treatment of active TB.

Preclinical and Clinical Testing of Vaccines

The following sections (below) describe TB vaccines that are either in preclinical or Phase I-IV clinical testing. Table 1 provides a brief summary of each vaccine described below.

Table 1. TB Vaccines in different clinical trials

Vaccine	Strategy	Type	Sponsorship (s)	Status
BCGΔ*zmp1*	Prime	Protein/adjuvant	U. S. Department of Health and Human Services-National Institutes of Health, and National Institute of General Medical Sciences	Preclinical Phase
MtbΔ*secA2*	Prime	Protein/adjuvant	U. S. Department of Health and Human Services-National Institutes of Health, and National Institute of General Medical Sciences	Preclinical Phase

rBCG30	Prime	Recombinant vaccine with plasmid pMBT30	UCLA, NIH, NIAID, Aeras	Phase I
MTBVAC	Prime	Live genetically attenuated MTB	University of Zaragoza, Biofabri, TBVI	Phase I
AERAS-422	Prime-boost	Protein/adjuvant		Phase I
AdAg85A	Prime-boost	Viral vector	McMaster University, CanSinoo	Phase I
Dar 901	Prime-boost	Whole-cell M. obuense	Dartmouth University, Aeras	Phase I
ID93+GLA-SE	Prime-boost	Protein/adjuvant	Infectious Disease Research Institute, Aeras	Phase I
Crucell Ad35/MVA85A	Prime-boost	Viral Vector	Crucell, Oxford University, Aeras	Phase I
TB/FLU-04L	Prime	Viral vector	Research Institute for Biological Safety Problems and the Research Institute on Influenza	Phase I
VPM1002	Prime	Live recombinant Bacille Calmette-Guérin (rBCG)	Serum Institute of India, Vakzine Projekt management, TBVI, Max Planck Institute for Infection Biology	Phase II
RUTI	Immunotherapeutic	Fragmented Mtb	Archivel Farma	Phase II
MVA85A	Prime	Live genetically attenuated *M. tuberculosis* (Mtb)	University of Zaragoza, Biofabri, TuBerculosis Vaccine Initiative (TBVI)	Phase II
H1:IC31	Prime-boost	Protein/adjuvant	SSI, Valneva	Phase II
H56:IC31	Prime-boost	Protein/adjuvant	SSI, Valneva, Aeras	Phase II
H4:IC31	Prime-boost	Protein/adjuvant	Statens Serum Institut (SSI), Sanofi Pasteur, Valneva, Aeras	Phase II
Crucell Ad35/AERAS-402	Prime-boost	Viral vector	Crucell, Aeras	Phase II
$M72+AS01_E$	Prime-boost	Protein/adjuvant	GlaxoSmithKline, Aeras	Phase II

M. Vaccae	Immunotherapeutic	Whole-cell *M. vaccae*	AnHui Longcom	Phase III
M. indicus pranii	Immunotherapeutic	Whole-cell *M. indicus pranii*	Ministry of Science and Technology (Government of India), Cadilla Pharmaceuticals	Phase III
BCG	Prime	Live attenuated *M. bovis*	Statens Serum Institute	Phase IV

Preclinical Vaccine Studies

Preclinical testing of new TB vaccines will be discussed in a limited manner in this review, due to the large number of preclinical vaccines being tested and excellent reviews published by others (Ng et al., 2015). Researchers have identified mutations in BCG and *M. tuberculosis* that restore the normal phagosome maturation process, possibly enhancing processing of the microbe and enhancing antigen presentation (Ng et al., 2015). One of those mutations has resulted in a preclinical vaccine, BCGΔ*zmp1*, which carries a deleted *Rv0198c* gene that results in the loss of the zinc metalloprotease 1 (Zmp1) (Ng et al., 2015). Zmp1 is important in blocking phagosome maturation because it was shown to be responsible for the destruction of host cellubrevin (VAMP3), an element of v-SNARE that participates in fusion of endosomal vesicles (Fratti, Chua, & Deretic, 2002). BCGΔ*zmp1* stimulates high immunogenicity and elicits very strong memory immune responses in mice and bovine models, compared to BCG (Ng et al., 2015). Importantly, BCGΔ*zmp1* also was found to be attenuated in SCID mice, indicating that it is likely to be safe in human studies. BCGΔ*zmp1* also was found to provide better protection against TB than BCG vaccine in guinea pigs exposed to *M. tuberculosis*.

MtbΔ*secA2* mutant is another example of a preclinical vaccine that is attenuated for virulence, yet is highly immunogenic and has altered effects on host and has altered effects on host cells thereby increasing the likelihood of cell death (Ng et al., 2015). This phenotype was

associated with decreased secretion of bacterial SodA and KarG enzymes that lead to enhanced production of superoxide radicals and hydrogen peroxide by host cells (Braunstein, Espinosa, Chan, Belisle, & Jacobs, 2003). A *M. tuberculosis* mutant with decreased SodA expression was found to be impaired for intracellular growth and was highly vulnerable to destruction by hydrogen peroxide, similar to the MtbΔ*secA2* mutation (Braunstein et al., 2003). Vaccination studies with the MtbΔ*secA2* mutant in guinea pig and murine models demonstrated that that this strain is more potent in priming antigen-specific $CD8^+$ T cells and better triggers immune responses against *M. tuberculosis* challenge than BCG (Hinchey et al., 2007).

Whereas hundreds of other studies have reported on preclinical testing of various *M. tuberculosis* attenuated mutants or vaccines that limit *M. tuberculosis* infection, the above noted two studies offer a small snapshot into what may be tested in Phase I-IV clinical trials in the future. Although many of these new vaccines have demonstrated efficacy in animal models, their effectiveness and/or safety in human subjects are poor, have not been studied, or currently are under evaluation.

Phase I Clinical Trials

rBCG30 (Ng et al., 2015)

rBCG30 is a live mycobacterial vaccine that was constructed to enhance the immunogenicity of the existing BCG vaccine. rBCG30 was created by transforming BCG with an expression plasmid encoding antigen 85B (Ag85B; an immunogdominant antigen) (Hoft et al., 2008). The *M. tuberculosis* antigen 85 (Ag85) complex is composed of three secreted enzymes, (Ag85A, Ag85B, and Ag85C) (Ohara et al., 1995; Wiker & Harboe, 1992). All Ag85 complex proteins have more than one biological activity, such as attaching to fibronectin (Kuo,

Bell, Hsieh, Ptak, & Chang, 2012; Naito, Ohara, Matsumoto, & Yamada, 1998), attaching to elastin (Kuo, Ptak, Hsieh, Akey, & Chang, 2013), and playing a role in mycolic acid synthesis (Belisle et al., 1997); and all three are immunodominant antigens in humans and mice (Huygen, 2014). In particular, Ag85B has been reported to stimulate autophagy in phagocytes (Jagannath et al., 2009) and has been shown to induce strong $CD4^{+}$ T cell responses associated with interferon-γ secretion. The latter point provided the rationale for using Ag85B as a vaccine candidate (Ng et al., 2015).

The effectiveness and immunogenicity of rBCG30 have been analyzed in investigational studies in various animal species (Principi & Esposito, 2015) (Ng et al., 2015).. rBCG30's ability to generate immunity in guinea pigs was found to be much higher than that of BCG vaccination (Ng et al., 2015). rBCG30 also has been shown to be well-tolerated by guinea pigs, similar to BCG, and stimulates anti-TB responses (Ng et al., 2015; Principi & Esposito, 2015). The efficacy of rBCG30 is believed to be due to generation of high levels of Ag85b-specific T cell lymphoproliferation and gamma interferon (IFN-γ) responses (Principi & Esposito, 2015). rBCG30 also has been found to increase the number of Ag85b-specific CD4+ and CD8+ memory T cells and the magnitude of antigen-specific T cell responses to inhibit intracellular mycobacteria (Principi & Esposito, 2015).

Safety and efficacy of rBCG30 was established in a phase I clinical trial in healthy adult humans, where it was found that rBCG30 induced immune response to Ag85B (Hoft et al., 2008). Although the rBCG30 vaccine appears to be superior to the standard BCG vaccine in humans, it has not progressed to phase II clinical trials due to concerns over the level of attenuation of rBCG30 and interest in generating a broad immunogenic vaccine (Kaufmann & Gengenbacher, 2012). More recently, the phase I clinical trial of rBCG30has been halted for

unknown reasons and the vaccine is being re-examined in a preclinical studies. New preclinical efforts include the creation of rBCG30 strains that will include supplementary attenuating mutations, such as Δ*mbtB* (deletion of the mycobactin synthesis pathway leading to detects in iron acquisition) (Ng et al., 2015).

MTBVAC (Ng et al., 2015)

The MTBVAC vaccine strain was derived from the MT103 strain (a virulent Mtb strain that was isolated from an immunocompetent TB patient) (Arbues et al., 2013). MTBVAC is a weakened strain of *M. tuberculosis* that has been proposed to be more safe and efficacious than BCG (Principi & Esposito, 2015). The MTBVAC vaccine strain has two stable unmarked deleted genes (*phoP* and *fadD26*) and thus meets the WHO guidelines for new TB vaccines [minimum of two independent mutations; new live vaccine strains must be severely attenuated] (Arbues et al., 2013). The first deleted gene, *phoP*, is a transcriptional regulator that regulates 2% of the Mtb genome and responds to various stress stimuli, including hypoxia, and regulates lipid metabolism, respiration, and virulence (Gonzalo-Asensio et al., 2008). The second deleted gene, *fadD26*, encodes acyl-CoA synthase (needed for lipid metabolism) and phthiocerol dimycocerosates biosynthesis (needed for the mycobacterial cell wall integrity) (Camacho, Ensergueix, Perez, Gicquel, & Guilhot, 1999; Infante, Aguilar, Gicquel, & Pando, 2005). As a result of these two mutations, the *M. tuberculosis* virulence factor ESAT-6 cannot be secreted, which leads to diminished production of phtiocerol dimycocerosate, a component of the *M. tuberculosis* cell envelope that is used to defend the bacterium against the host immune system (Principi & Esposito, 2015). MTBVAC also is a good vaccine candidate because it does not assemble complicated cell wall lipids that are controlled by *phoP*, which inhibit the host immune

response (Principi & Esposito, 2015). When MTBVAC was tested in animal models, it was noted to be as safe as BCG, but was more protective than BCG to virulent Mtb challenge (Arbues et al., 2013). The phase I clinical trial of MTBVAC vaccine began at the end of 2013 and is still ongoing (Ng et al., 2015).

Recent modifications of MTBVAC include the hyper-attenuated MTBVAC Δ*erp* mutant (Solans et al., 2014). The *erp* gene encodes for an exported repetitive protein (ERP), which is a secreted antigen that is often localized to the bacterial surface (Ng et al., 2015). In addition, the antigenic effects of MTBVAC have been enhanced by the suppression of *mcr7*, which is an antisense RNA that controls the secretion of a twin arginine translocation (TAT) protein of *M. tuberculosis* (Principi & Esposito, 2015). Suppressing *mcr7* leads to enhanced production of the Ag85 family (described above), and over-expression of these antigenic molecules enhances host immunity against TB (Principi & Esposito, 2015). The clinical trial for MTBVAC is still ongoing with 3 different identification numbers (ClinicalTrials.gov Identifier: NCT02729571; NCT02013245; and NCT02933281).

AERAS-422 (Ng et al., 2015)

AERAS-422 is a recombinant BCG strain (Danish strain 1331) that overexpresses two Ag85 antigens (Ag85A [*fbpA*] and Ag85B [*fbpB*]) and the latent TB antigen Rv3407 (Ng et al., 2015). AERAS-422 also encodes the *Clostridium perfringens* perforin perfringolysin O to disrupt the phagosomal membrane (Ng et al., 2015) and expose the vaccine strain to the host cell cytoplasm. Perfringolysin O was inserted into the AERAS-422 *ureC* gene to eliminate urease activity but express porin activities at neutral pH (Kaufmann & Gengenbacher, 2012). Preclinical studies in mouse models revealed that the AERAS-422 vaccine is highly attenuated, possess

improved immunogenicity, and stimulates better protection than BCG (Sun et al., 2009). A phase I clinical study was halted because healthy participants contracted shingles (reactivation of varicella-zoster virus) ((Kaufmann & Gengenbacher, 2012), (Kupferschmidt, 2011)). There was no clear reason as to why the reactivation occurred, but researchers believe that AERAS-422 may have suppressed the participant's immune systems (Ng et al., 2015). Before continuing the phase I clinical trial, further investigations are needed in animal models to uncover the reason for the varicella-zoster virus reactivation (Ng et al., 2015). The phase I double-blind/dose-escalated randomized controlled trial of AERAS-422 vaccine was completed, which was comprised of 24 healthy HIV-negative adult subjects who had not been exposed to *M. tuberculosis* before being selected. In this study, the safety and immunogenicity of intradermal administration of AERAS-422 was evaluated (ClinicalTrials.gov Identifier: NCT01340820).

AdAg85A (Ahsan, 2015)

AdAg85A is derived from a recombinant replication-deficient adenovirus serotype 5 vaccine vector (Ahsan, 2015). The phase I clinical trial for AdAg85A vaccine was performed in animal models (mouse, guinea pigs, and cattle) and results indicated that it was safe and has higher degree of protection against Mtb infection compared to the standard BCG vaccine (ClinicalTrials.gov identifier: NCT00800670). Infant goats showed less TB infection in lungs and lymph nodes when vaccinated with a combination of BCG and AdAg85A, compared to BCG alone (Ahsan, 2015). Vaccine studies with AdAg85A also revealed that antigen-specific IFN-γ and antibodies were predictive biomarkers of immunity (Perez de Val et al., 2012). Intranasal administration of BCG with AdAg85A markedly enhanced the long-term viability of BCG-primed guinea pigs after *M. tuberculosis* lung infection (Xing et al., 2009). In summary,

AdAg85A appears to provide better protection than BCG alone and AdAg85A can enhance the immunogenicity of BCG. Low dose clinical studies with AdAg85A have been terminated but the reason for study termination is unclear (ClinicalTrials.gov Identifier: NCT00800670).

Dar-901 (Ahsan, 2015)

Dar-901 is a heat-inactivated *Mycobacterium obuense* strain that was generated by researchers at Darmouth University (studies supported by the nonprofit biotechnology organization Aeras) (Ahsan, 2015). A Phase I clinical study was comprised of 77 adult subjects, of which 56 were HIV-negative and 21 were HIV-positive. The study evaluated the safety, tolerability, and immunogenicity of the number of Dar-901 immunizations and doses (from 0.1 to 1 mg) (ClinicalTrials.gov Identifier: NCT02063555). Although the phase I clinical trial is now complete, a phase II clinical trial is now underway (Ahsan, 2015) (ClinicalTrials.gov Identifier: NCT02712424).

ID93+GLA-SE (Ahsan, 2015)

Four antigenic M. tuberculosis recombinant proteins, Rv2608, Rv3619, Rv3620, and Rv1813, are included in the ID93+GLA-SE vaccine. The vaccine was created by the Infectious Disease Research Institute (IDRI, Seattle, Washington) in conjunction with Aeras (Ahsan, 2015). A Phase I clinical trial was conducted to evaluate the safety and immunogenicity of ID93+GLA-SE (ClinicalTrials.gov Identifier: NCT01599897) (Orr et al., 2013). GLA-SE is a TLR4 agonist that stimulates polyfunctional T helper type 1 (T_H1) cells to produce IFN-γ, TNF and IL-2 when co-administered with ID93 (Orr et al., 2013). The clinical trial for ID93+GLA-SE is complete with two different identification numbers (ClinicalTrials.gov Identifier: NCT01927159; and

NCT01599897). Although phase II clinical trials are pending, subjects currently are not being recruited (ClinicalTrials.gov Identifier: NCT02465216; and NCT02508376).

Crucell Ad35/MVA85A (Ahsan, 2015)

Crucell Ad35 (also referred to as AERAS-402) and MVA85A are two separate tuberculosis vaccines but have been tested together in phase I clinical trials, where one vaccine was given after the other (Ahsan, 2015). Crucell Ad35/AERAS-402 is a replication-deficient adenovirus (Ad35) that expresses TB antigens Ag85A, Ag85B, and TB10.4. Crucell Ad35/AERAS-402 was created by Aeras (noted above) and Crucell, a Dutch pharmaceutical company (Ahsan, 2015). MVA85A is an altered Modified Vaccinia Ankara (MVA) [a poxvirus unable to reproduce in human tissues] viral vector expressing Ag85A of *M. tuberculosis* (Ahsan, 2015). Crucell Ad35 has been shown to stimulate $CD4^+$ and $CD8^+$ T cell responses in mice with IFN-γ playing an important role in protection (Radosevic et al., 2007). Similarly, AERAS-402 has been demonstrated to stimulated a potent $CD8^+$ T cell response with IFN-γ and TNF-α being expressed by these cells (Abel et al., 2010). Given the similarity of the two vaccines (AERAS-402 and MVA85A), the reason for conducting this trial is to investigate potential benefits of administering two vaccines sequentially (one after the other), verifying vaccine safety, and examining enhanced immunogenicity (Ahsan, 2015). The clinical trial for Crucell Ad35/MVA85A has been completed (ClinicalTrials.gov Identifier: NCT01683773).

TB/FLU-04L (Ahsan, 2015)

TB/FLU-04L is a recombinant influenza vaccine created by the Research Institute for Biological Safety Problems (Kazakhstan) and the Research Institute on Influenza (Russia). It is

comprised of influenza virus strain (A/Puetro Rico/8/34 H1N1) and *M. tuberculosis* antigens Ag85A and early secretory antigenic target 6 (ESAT6) (Ahsan, 2015). Phase I clinical trial have been completed and the vaccine will soon enter phase II clinical trials (Ahsan, 2015) (ClinicalTrials.gov Identifier: NCT02501421).

Phase II Clinical Trials

VPM1002 (Ng et al., 2015)

VPM1002 is a live, attenuated vaccine strain constructed in the Danish BCG strain, subtype Prague (Hawkridge & Mahomed, 2011). VPM1002 is a recombinant vaccine because it was manipulated to encode the *Listeria monocytogenes* pore-forming protein listeriolysin O (LLO), encoded by the *hly* gene (Ng et al., 2015). In VPM1002, LLO was inserted into the urease gene. The strain was created based on the hypothesis that LLO expression would form pores in the phagosome and allow the mycobacteria to access the host cell cytosol (Ng et al., 2015). Phagosomes normally are responsible for engulfing foreign objects (e.g. bacteria) and digesting them (Hawkridge & Mahomed, 2011). In theory, cytosolic exposure of the vaccine strain could lead to both MHC class I and class II antigen presentation, which then lead to global immune responses by inducing cells like CD4 and CD8 (Hawkridge & Mahomed, 2011). The expression of LLO appeared to promote apoptosis in infected macrophages and lead to increased CD8+ T cell responses via cross-priming by means of alternate mechanism (Ng et al., 2015). Eradication of the urease gene allowed the intra-phagosomal pH to be more favorable to LLO function (Hawkridge & Mahomed, 2011). However, genetic insertion of LLO into the urease gene resulted in introduction of an antibiotic (hygromycin) resistance gene in VPM1002 that the researchers planned to remove during experimentation (Hawkridge & Mahomed, 2011). The

vaccine was created by Vakzine Projekt Management GmbH, based in Germany (Hawkridge & Mahomed, 2011).

Enlistment of 80 healthy subjects into a phase I clinical trial in Bloemfontein, South Africa was concluded in June 2009, where the study focused on dose-escalations and comparison of VPM1002 to BCG in healthy male subjects (Hawkridge & Mahomed, 2011). All subjects tolerated the VPM1002 vaccine and were able to mount immunity against *M. tuberculosis* (Hawkridge & Mahomed, 2011). Safety and efficacy findings from this phase I clinical trial prompted an ongoing phase IIa clinical trial in HIV-unexposed neonates (80 subject in Germany and 24 subject in Bloemfontein, South Africa), revealing that VPM1002 has the same safety profile as BCG and triggers comparable immune response as that reported in phase I studies (Ng et al., 2015) (ClinicalTrials.gov Identifier: NCT01479972). A phase IIb trial also is being considered for VPM1002 in HIV-infected neonates (Ng et al., 2015). Additional clinical trial information also is available for VPM1002 (ClinicalTrials.gov Identifier: NCT01113281; NCT01479972; NCT00749034; NCT02391415; and NCT02371447).

RUTI (Ahsan, 2015)

RUTI is an inactivated vaccine composed of fragmented, detoxified *M. tuberculosis* bacteria that is cultivated under stress conditions to stimulate latency antigens, which are usually concealed from immune surveilance (Principi & Esposito, 2015). Furthermore, RUTI is inactivated to decrease the risk of adverse effects and has been fragmented to promote the antigen processing and presentation to antigen presenting cells (Principi & Esposito, 2015). RUTI contains lower levels of lipoarabinomannan (LAM), a *M. tuberculosis* molecule that has been compared to endotoxin and has been correlated with cell death in granulomas (Principi &

Esposito, 2015). RUTI stimulates a powerful IFN-γ response from $CD4^+$ cells and $CD8^+$ cells against derivatives of tuberculin such as ESAT6 and Ag85B (Ahsan, 2015). Preclinical studies in mice and guinea pigs found that it was more effective to use RUTI after antibiotic treatment without any observed toxic effects (Ahsan, 2015). In a phase I clinical study, healthy subjects displayed local reactions in a dose-dependent manner and increased T-cell responses correlated with local adverse reactions (Ahsan, 2015). RUTI was intended to decrease the length of therapy needed to treat latent TB infection with isoniazid (INH) (Ahsan, 2015). Based on these findings, RUTI has the potential of enhancing existing antibiotic therapies such as INH and help clear TB infections (Ahsan, 2015). A phase II clinical trial of RUTI has been completed, where latent TB infections were first treated for 1 month with standard chemotherapy, then RUTI was administered (instead of the standard 6-9 months of TB chemotherapy) (ClinicalTrials.gov Identifier: NCT01136161). Additional clinical trial information is available for RUTI (ClinicalTrials.gov Identifier: NCT00546273; NCT02711735; and NCT01136161).

MVA85A (Ahsan, 2015)

As noted above (Phase I Clinical Trials), MVA85A is an altered Modified Vaccinia Ankara (MVA) [a poxvirus unable to reproduce in human tissues] viral vector expressing Ag85A of *M. tuberculosis* (Ahsan, 2015). MVA85A was created as a booster for the BCG vaccine in 2002. When the BCG vaccine was boosted with MVA85A, increases in BCG-induced responses against TB infection were observed various animal experiments (Hawkridge & Mahomed, 2011). Oxford Emergent Tuberculosis Consortium (OETC) and Aeras are the two entities that are developing MVA85A as a vaccine (Hawkridge & Mahomed, 2011). The vaccine has been shown to stimulate robust T-cell responses and offer protection against Mtb infection (Hawkridge &

Mahomed, 2011). Ag85A of *M. tuberculosis*, also referred to as mycolyl transferase, is an important target antigen that is well known to stimulate strong immune responses in small animals (Hawkridge & Mahomed, 2011). The benefit of having a TB antigen expressed from a viral vaccine is that it likely will prevent cross-reactivity or false-positives in future diagnostic tests (e.g. IFN-γ release assays) for TB infections (Hawkridge & Mahomed, 2011). When the MVA85A vaccine was tested in phase I clinical trials, the vaccine was well-tolerated by HIV positive subjects in Senegal and these patients developed immunity with little systemic or local side effects to aerosol administration (Ahsan, 2015). Given these encouraging findings, researchers have proposed assessing other MVA85A administration routes (Ahsan, 2015). In one study with 2797 infants, MVA85A was well-tolerated by the subjects and diagnostic testing indicated that the subjects developed immunity to TB. However, follow-up analysis has indicated that MVA85A offered poor protection to the infants against TB infections (Ahsan, 2015). Other phase I clinical trials have been completed in United Kingdom, West Africa, and South Africa (Hawkridge & Mahomed, 2011) (clinicalTrials.gov Identifier: NCT00395720). More recently, a phase II clinical trial to evaluate the safety and immunogenicity of MVA85A has been completed in 24 healthy children and 36 healthy infants in South Africa. This study revealed that MVA85A is safe and immunogenic in an area that has a high incidence of TB infections (ClinicalTrials.gov Identifier: NCT00679159). Also, there are other clinical trials of MVA85A that assesses different parameters such as route of administration and stages of TB infection/resistance (ClinicalTrials.gov Identifier: NCT02532036; NCT01683773; NCT01497769; and NCT01151189).

H1:IC31 (Ahsan, 2015)

H1:IC31 is a protein subunit vaccine that is composed of fusion protein (H1) of ESAT6 and Ag85B, along with IC31 (an adjuvant that contains both the cationic polyamino acid KLK and the oligodeoxynucleotide ODN1a) (van Dissel et al., 2011). Phase I clinical trials revealed no adverse reactions either locally or systemically, while inducing very strong antigen-specific T-cell responses (IFN-γ) against both ESAT6 and Ag85B elements that lasted for 2.5 years (Ottenhoff et al., 2010; van Dissel et al., 2011). Similarly, Phase II clinical trials noted that H1:IC31 was safe and immunogenic in healthy BCG-vaccinated subject and latently-infected subjects, with little local or systemic adverse reactions, and induction of very strong antigen-specific T-cell responses against ESAT6 and Ag85B throughout the 32 week study (Reither et al., 2014; van Dissel et al., 2011). More information about clinical trials for HI:IC31 can be found using ClinicalTrials.gov Identifiers: NCT01003093, NCT00929396, NCT01049282, NCT02378207, and NCT02375698).

H56:IC31(Ahsan, 2015)

The H56:IC31 vaccine contains two components: H56 is a fusion protein is comprised of Ag85B, ESAT6, and Rv2660c (nutrient stress-induced antigen); and IC31 is an adjuvant (Ahsan, 2015). H56:IC31 is a booster vaccine developed to manage late-stages of TB infections and control latent TB (Lin et al., 2012). H56:IC31 is presently undergoing assessments in phase II clinical trials in Africa (Ahsan, 2015). More information about H56:IC31 can be found using ClinicalTrials.gov Identifiers: NCT02378207, NCT02503839, NCT02375698, NCT01967134, and NCT01865487).

H4:IC31 (also known as AERAS-404) (Ahsan, 2015)

H4:IC31 (also known as AERAS-404) is protein subunit vaccine that is comprised of Ag85B and TB10.4 (Ahsan, 2015). This vaccine enhances and prolongs the immune responses induced by BCG, leading to elevated defenses against *M. tubcerulosis* infection due to the responses of IFN-γ, TNF-α, IL-2, and $CD4^{+}$ cells (Billeskov, Elvang, Andersen, & Dietrich, 2012). H4:IC31 is presently undergoing assessment in phase II clinical trials in Africa (Ahsan, 2015). AERAS-404 has both H4 and IC31 in differing doses, and the following identification numbers represent all the clinical trials for the AERAS-404 vaccine (ClinicalTrials.gov Identifier: NCT02378207; NCT02420444; NCT02075203; NCT02066428; NCT02109874; NCT02074956; and NCT01861730).

Crucell Ad35/AERAS-402 (Ahsan, 2015)

As noted above (Phase I clinical trials), Crucell Ad35A/AERAS-402 is an adenovirus-vector vaccine containing the following Mtb antigens (Ag85A, Ag85B, and TB10.4) (Ahsan, 2015). This vaccine influences strong CD4+ and CD8+ T-cell immune reactions in mice, especially when given intranasally (da Costa et al., 2015). Experimental trials that were conducted in healthy formerly BCG-vaccinated adults revealed Crucell Ad35/AERAS-402 to have a sufficient safety profile, without any reported vaccine-related major adverse reactions (da Costa et al., 2015). In other studies, mild to moderate local side effects to Crucell Ad35A/AERAS-402 were observed, but these side effects did not prevent future testing in clinical trials (Abel et al., 2010; Hoft et al., 2012). In healthy adults, Crucell Ad35/AERAS-402 stimulated potent CD4+ and CD8+ T-cell reactions, with production of IFN-γ, TNFα, and IL-2, and no major vaccine-related side effects (da Costa et al., 2015). Although tested along with

MVA85A, this vaccine has been tested by itself in both phase I and phase II clinical trials (Ahsan, 2015). The phase I clinical trial for Crucell Ad35/AERAS-402 is complete (ClinicalTrials.gov Identifier: NCT01683773). The phase II clinical trial conducted in healthy infants in Kenya, Mozambique, and South Africa has been completed, and it revealed a safe and immunogenic vaccine with minimal side effects (ClinicalTrials.gov Identifier: NCT01198366).

M72+AS01$_E$ (Ahsan, 2015)

M72+AS01$_E$ is a protein subunit vaccine comprised of the 32A and 39A antigens, which are fusion proteins from *M. tuberculosis* (Ahsan, 2015). The phase II clinical trial of M72 and the liposome-based AS01$_E$ adjuvant system demonstrated that the vaccine was safe and induced both CD4$^+$ T-cell and humoral responses to TB antigens (Montoya et al., 2013). The safety and immunogenicity of M72:AS01$_E$ also was demonstrated in a phase II clinical trial in infants who were already had been vaccinated with the BCG vaccine (Idoko et al., 2014). Overall, M72:AS01$_E$ appears to be a safe and potent vaccine to use in either infected or uninfected people (Day et al., 2013). Phase II clinical trials for this vaccine are still ongoing (ClinicalTrials.gov Identifier: NCT02097095).

Phase III Clinical Trials

M. vaccae (Ahsan, 2015)

M. vaccae is a whole organism, heat-killed, immuno-therapeutic agent that is being tested as a new TB vaccine (Ahsan, 2015). *M. vaccae* has been reported to be non-toxic, produce immunity in HIV-positive adults, and stimulate CD4+ T-cell production of IFN-γ and IL-10 in vaccinated mice (Rodriguez-Guell et al., 2008; Yang, Chen, Cui, Yu, & Li, 2010). The *M.*

vaccae vaccine appears to be well tolerated, with no reports of serious adverse events (da Costa et al., 2015). *M. vaccae* provided a 72.5% protection rate in latent TB infections (LTBI) in Chinese children, adolescent, and adults, and limited progression to active TB disease (da Costa et al., 2015). A phase III efficacy and safety trial currently is in progress, which is testing a six-dose intramuscular regimen in individuals with LTBI for the prevention of TB (ClinicalTrials.gov Identifiers: NCT01977768 and NCT01979900) (da Costa et al., 2015).

***M. indicus pranii* (Hawn et al., 2014)**

M. indicus pranii (MIP), previously known as *Mycobacterium w*, is a cultivable, non-pathogenic and rapidly-growing saprophyte categorized into Runyon group IV alongside other fast growers such as *M. fortuitum, M. smegmatis, M. chelonae,* and *M. vaccae* (Hawn et al., 2014). *M. indicus pranii* grows faster (6 to 8 days) than *M. tuberculosis* (>3 weeks) and is affiliated with the MAC complex (including *M. intracellulare* – growth in >2 weeks) (Hawn et al., 2014). *M. indicus pranii* shares some biochemical attributes that are typically found in fast growing strains such as *M. smegmatis* and *M. vaccae*. For example, fast growers typically lack pigments, nitrate trimming processes, aryl sulfatase, and catalase (D. C. Alexander & Turenne, 2015). *M. indicus pranii* is phylogenetically distinct , making it the immediate predecessor of opportunistic mycobacterial species represented by the *M. avium complex* (D. C. Alexander & Turenne, 2015). Live *M. indicus pranii* is being tested via aerosol delivery (Hawn et al., 2014). In mice treated with *M. indicus pranii,* along with the TB therapeutic drug INH for 4 to 6 weeks, TB bacterial loads in lung and other tissues were dramatically decreased (Faujdar et al., 2011). The clinical trial for this vaccine has been terminated for unknown reasons, but the vaccine also has been tested for efficacy against for other disease such as severe sepsis.

Phase IV Clinical Trials

BCG

The BCG vaccine is administered to infants and adults to protect against TB infection. However, the reported efficacy of BCG is 80% in children (Mangtani et al., 2014) and 50% in adults (Principi & Esposito, 2015). However, there are other studies that note than BCG provides no real protection for adults (P. E. Fine, 1988). BCG is the only vaccine that is widely and routinely used in endemic areas to limit TB infections. The vaccine was named after French scientists Albert Calmette and Camille Guérin, who discovered that repeated laboratory passage of *M. bovis* resulted in a strain that was less virulent. Since its first use in humans in 1921, the BCG vaccine has been utilized worldwide to limit the spread of TB infections (P. E. Fine, 1995). BCG vaccination generally appears to provide approximately 10 to 15 years of immunity, but studies in Native Alaskan Indian communities demonstrated that BCG provided 50 years of immunity (Aronson et al., 2004). Conversely, BCG has been noted to confer virtually no immunity to some communities in India, particularly the Chengalpattu region (Moliva et al., 2015). In addition, the effectiveness of BCG has been noted to vary depending on geographical location and population age (Moliva et al., 2015). Together, these data show the need for new studies that closely examine the human genetic pool and the environment when evaluating BCG protection (Moliva et al., 2015). Currently, new vaccines are being developed, but these new vaccines face hurdles as they through experimentation globally (Moliva et al., 2015).

Discussion

Humans are known to be vulnerable to TB and MTBC strains have been reported in every human environment. Despite the major economic and health burden of TB, there is still a clear need for a safe, effective vaccine that prevents infection and limits disease. In this scholarly project, a number of clinical trials (phase I to IV) were reviewed. Although too numerous to accurately review, a number of preclinical vaccines also have been tested, including the BCGΔ*zmp1* and MtbΔ*secA2* strains which soon may enter phase I clinical trials. Whereas the types of vaccines highlighted in this review vary widely, from viral vectors, to proteins, to whole cell live attenuated vaccines, to fragmented cells, many of these vaccines offer significant hope for future success.

Many different *M. tuberculosis* antigens are being tested as new vaccine candidates and many of these antigens share characteristics of being located on the surface of MTBC organisms, are the molecules responsible for causing TB infection, and/or induce strong immune responses after TB infection. Although the true functions for many of these molecules still are unknown, many appear to be induce protective immune responses, including Rv0125 (Mtb32A), Rv1196 (Mtb39A), Rv0288 (TB10.4), Rv1813, Rv2608, Rv3619, Rv3620, Rv1886 (Ag85A), Rv2660, Rv3804 (Ag85A), and Rv3875 (ESAT 6)] (Ahsan, 2015).

Based on immune responses induced by the BCG vaccine, new vaccines typically are expected to stimulate cell-mediated immunity (CD4 or CD8 T cells) and produce Th1 cytokines (IFN-γ, TNF-α, and IL-2) (Ahsan, 2015) that stimulate macrophages to kill *M. tuberculosis*. Although the importance of cell-mediated immunity and Th1 cytokines responses primarily resulted from studies of human TB infections, pre-clinical vaccine studies in animals also support the roles of these cells and cytokines (Ahsan, 2015). However, there is increasing

evidence that induction of strong Th1 responses alone are not adequate for new vaccines to surpass the BCG vaccine. To develop a new safe and immunogenic vaccine to replace the BCG vaccine, investigators will need more than just cytokines and T cell responses.

Conclusion

The outlook for future TB vaccines appears to be promising and robust, with hundreds of vaccines in preclinical trials, 8 vaccines in phase I clinical trials, 8 vaccines in phase II clinical trials, 2 vaccines in phase III clinical trials, and 1 vaccine in phase IV clinical trials. Currently, there are 15 vaccines in different stages of development (phase I to III), 3 vaccines that have been halted due to either adverse reactions or lack of safety and immunogenicity data, and 1 vaccine (BCG) that currently is on the market but offers less than 80% protection. Complicating vaccine development efforts are the unknown immune responses needed to prevent infection and limit TB replication and limited information about protective TB antigens. Taken together, there are substantial hurdles to overcome to develop successful vaccines against TB but the worldwide infection rate, number of global deaths, and rise of antibiotic-resistant strains make these challenges important. Finally, it is imperative for researchers to have sufficient funding to continue clinical trials on current vaccines, along with funding to develop new vaccines that are both safe and efficacious. Overall, the cost of developing new TB vaccines is far less than the cost of caring for and treating patients with TB infections.

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Abstract

Tuberculosis (TB) remains a one of the top five infectious diseases around the world, with an estimated 9.6 million people becoming ill in 2014 and approximately 1.5 million people having died from active infection (World Health Organization, 2015). TB is now the number one killer of people around the world and the countries that are most severely affected are developing countries due to a lack of healthcare accessibility and affordability. Moreover, multidrug/extensive drug resistance (MDR/XDR-TB) and HIV co-infection exacerbate the disease. The World Health Organization's goal to eliminate TB by 2050 will be difficult to accomplish without new vaccines that are safe, efficacious, and immunogenic. Presently, the live attenuated Bacille Calmette-Guérin (BCG) is the only TB vaccine licensed to be used in neonates and adults (although it is not used in the U.S.). However, it has been reported that BCG is only about 80% effective in children (Mangtani et al., 2014) and 50% effective in adults (Principi & Esposito, 2015). The focus of this review is to highlight recent progress in new TB vaccines that are in various stages of clinical trials, including 8 vaccines in phase I clinical trials, 8 vaccines in phase II clinical trials, 2 vaccines in phase III clinical trials, and 1 vaccine in phase IV clinical trials. Of these, 3 vaccines have been halted due to either adverse reactions or lack of safety and immunogenicity in subjects. While the current clinical trials are encouraging, more work needs to be done to optimize and improve vaccine efficacy.

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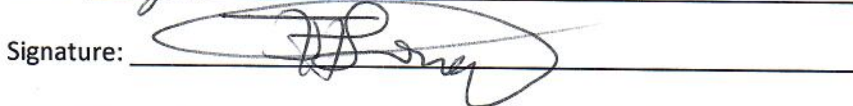
Signature: 

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