Vaccine research and development: tuberculosis as a global health threat

MOHAMMED MAIKUDI USMAN[1,2], SALMAH ISMAIL[1], TEOW CHONG TEOH[1]

[1]Institute of Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur, Malaysia
[2]Department of Biotechnology, School of Pure and Applied Sciences, Modibbo Adama University of Technology, Yola, Nigeria

Abstract

One of the aims of the World Health Organisation (WHO) Millennium Development Goals (MDG) is to reduce the number of cases of tuberculosis (TB) infection by the year 2015. However, 9 million new cases were reported in 2013, with an estimated 480,000 new cases of multi-drug resistant tuberculosis (MDR-TB) globally. Bacille Calmette-Guérin (BCG) is the most available and currently used candidate vaccine against tuberculosis; it prevents childhood TB, but its effectiveness against pulmonary TB in adults and adolescents is disputed. To achieve the goal of the WHO MDG, the need for a new improved vaccine is of primary importance. This review highlights several articles that have reported vaccine development. There are about 16 TB vaccines in different phases of clinical trials at the time of writing, which include recombinant peptide/protein, live-attenuated and recombinant live-attenuated, protein/ adjuvant, viral-vectored, and immunotherapeutic vaccine. Further studies in reverse vaccinology and massive campaigns on vaccination are needed in order to achieve the target for TB eradication by 2050.

Key words: *tuberculosis, Mycobacterium tuberculosis, vaccine, clinical trials, BCG vaccine.*

Introduction

Tuberculosis is a disease caused by an infectious agent called *Mycobacterium tuberculosis* (*Mtb*). Despite reports of declining TB cases in recent years, it also remains a leading deadly infectious disease globally, second only to HIV [1]. In 2012, an estimated 8.7 million new infections in the year 2011, out of which 1.4 million people died of tuberculosis, was published by the WHO. The report also showed highest burden in Asia and Africa, while China and India accounted for almost 40% of the total TB cases globally [2]. Although there are great achievements globally regarding the threat of TB, in Sub-Saharan Africa tuberculosis remains a major cause of morbidity and mortality [3]. Some factors are responsible for the growth of TB in Africa, the HIV epidemic being the most important one [4, 5]. An increase in the incidence of MDR-TB is another factor that threatens the efforts towards TB control throughout the world [6]. At latency stage of TB infection the containment of infection is achieved by the host, indicated by survival of mycobacteria in relatively stable numbers [7]. Patients with latent tuberculosis typically do not feel ill and are not infectious [8].

World Health Organisation (WHO) Millennium Development Goals (MDG) are a set of targets aimed at expressing key points of human development [9]. The goals of MDG were generated at the United Nations Millennium Summit in September 2000 [10]. In addition to a target associated with MDG and endorsed by the Stop TB partnership for reducing its prevalence and death as a result by 50%, the target was also aimed at eradication of TB as a worldwide health threat by the year 2050 [11].

Bacille Calmette-Guérin is a vaccine against tuberculosis, developed in 1921 by Albert Calmette and Camille Guérin, caused by attenuation of the *Mycobacterium bovis* strain [12]. The novel tuberculosis vaccines need to be better in efficacy and safety, or both, than BCG. Therefore, the urgent need for alternative anti-tuberculosis vaccines is of paramount importance. Bacille Calmette-Guérin immunisation is still in used because of the protection it gives against the infant form of tuberculosis [13, 14]. Administration of diverse booster vaccines later to BCG prime strengthens the protection induced by BCG. Also, other vaccines serve as replacements to Bacille Calmette-Guérin for the generation of a superior immune response up-front. In both cases, the aim of vaccination is the generation of long lasting protection against the most prevalent form of pulmonary tuberculosis in all age groups [13, 14].

Complete-genome sequencing, and comparative and system biology lead to new knowledge into the origin and evolution of *Mtb* and the molecular principle of its pathogenicity. These have vital implications about our perspective of the new vaccine development [15]. Progress in the fields of molecular biology, genomics, proteomics, and

transcriptomics contributed tremendously in the search for a new and enhanced tuberculosis vaccines [16]. Some TB vaccines have entered different stages of clinical trials in recent years. An attempt to review recent research and development of TB vaccines is given below.

Disease burden of tuberculosis

Tuberculosis is likely to remain in a position of a major public health problem in the coming decades because of its large global load [17]. According to a 2014 (WHO) report on TB, an estimated figure of 1.5 million deaths were recorded in 2013 (0.4 million were HIV-infected people and 1.1 million were HIV-negative) [18]. *Mtb* and HIV infections act in a collaborative manner [19]. The role of CD4 T cells is of paramount importance in *Mtb* infection control; in HIV and TB co-infection there is continuous loss of CD4 T cells, leading to an advancement to active form of TB [20]. Most cases of TB infection and death prevail among men, but the TB burden is also high among women. In 2012, the South-East region and Western Pacific region accounted for about 58% of the world's TB cases. Also, the African region had nearly one quarter of the world's cases; India and China recorded the highest number of cases with 29% and 12% of the global total respectively [21]. In Sub-Saharan Africa, TB has increased markedly over the past two decades and there was a reported doubling of annual incidence from 173.6 to 351.7 per 100,000 population between 1990 and 2007 [22]. MDR-TB can be defined as TB found to be resistant to both rifampicin and isoniazid drugs with or without resistance to other anti-TB drugs [23]. MDR-TB is on the increase; extensively drug-resistant tuberculosis (XDR-TB) and totally drug-resistant tuberculosis have already been reported. These obstacles impose rising threats to tuberculosis control [2, 24, 25].

Bacteriology

In 1882, Robert Koch discovered *Mtb*, the intracellular pathogen [26]. Mycobacteria are non-motile, aerobic bacteria that have characteristics of acid fastness (Zeihl-Neelsen staining) because of mycolic acid enriched cell wall [27]. *Mtb* are rod-shaped bacilli [28]. The presence of a wide array of complex lipids and lipoglycans on the cell surface of *Mtb* make it unique among the bacterial pathogens [29]. The hydrophobic nature and the complexity of the cell wall, which is composed of the following: arabinogalactan, peptidoglycan, fatty acid (mycolic acids), and glycolipids layered on top of the plasma membrane critically cause *Mtb* to deceive the immune system of the host [30].

Immune response to tuberculosis infection

Tuberculosis infection is airborne, its cycle starts when a host inhales infectious airborne particles, usually of less than 5 µm diameter, containing infectious pathogen [31]. Alveolar macrophages engulf mycobacteria when they enter the lung, where *Mtb* reproduces, and inhibit macrophage killing mechanisms. Despite the inhibitory influence of *Mtb*, infected macrophages secrete chemokines and cytokines, leading to the recruitment and activation of many immune cell populations to the lung [32]. The innate immunity activation depends on recognition of *Mtb* components of the cell wall as mycolic acid, mannan, and peptidoglycans through toll-like receptors [33]. Mycobacterial antigen recognition, macrophages, and dendritic cell (DC) activation as well as other cells involved in innate immunity need toll-like receptors [34]. The bacilli is engulfed by macrophage through phagocytic receptors, of which the complement and mannose receptors play an important role [35]. The immune cells enclose the pathogenic bacteria in the first stage of tuberculosis infection, intracellular multiplication occurs, and the bacteria-overloaded cells may traverse the alveolar barrier, affecting other tissues and organs [36]. Survival of *Mtb* in macrophages is achieved by inhibiting acidification of the phagosomal complement and also by inhibition of the fusion of the phagosome with lysosomes [35]. The cell-mediated immunity is effectively involved in regulating *Mtb* limitation in granulomatous lesions of the lungs, usually without eliminating the bacteria that prevail in the latent stage [37]. Besides macrophages, it is understood that DCs also play a role as an important intracellular niche for *Mtb* [38]. Dendritic cells are key regulators of adaptive immunity and are potent antigen presenting cells [39]. Dendritic cells have the unique ability to migrate to draining lymph nodes from the site of infection and afterwards recruit T cells of infection where they effectively activate the acquired immune response [40].

Protective immunity against *Mtb* depends critically on T lymphocytes, due to its intracellular lifestyle [41]. In tuberculosis, cellular responses are the mediators of both pathogenesis and protection, which involves primarily interactions of phagocytes of macrophage lineage and lymphocytes [42]. Production of cytokines like interferon γ (INF-γ) and tumour necrosis factor (TNF) establish protective immune responses against *Mycobacterium tuberculosis* infection; both cytokines activate macrophage toward *Mtb* control [43]. The cytokines play an essential role in controlling mycobacterium growth by expression of reactive nitrogen and oxygen [44]. In the presence of oxygen, an enzyme associated with macrophage functions in tuberculosis, called nitric oxide synthese-2, catalyses the metabolism of L-arginine into L-citrulline and nitric oxide, which takes part in killing the intracellular pathogen [45]. The *Mtb* immune response consists of great number of different cell kinds, including T cells, neutrophils, B-cells, and natural killer cells, and the roles played by CD4 T helper type 1 cells are the best understood [46]. Other interleukin (IL) producing T lymphocytes such as CD8 T lymphocyte and CD4 cells are likely take part in protective immunity

[47]. After infection, *Mtb* stimulates both CD4 and CD8 T cells and other immune cells; secretion of INF-γ dominates a strong type 1 immune response [48]. During adaptive immune response to *Mtb* infection, CD4 cells are the primary source of INF-γ, which are required for the survival of the host during both phases of acute and chronic infection [49]. The need for INF-γ in immune protection of tuberculosis is well established both in animal models and in humans [50]. Interferon γ is the key cytokine in humans and also in mice, the role of which is to activate the bactericidal actions in the host cell, macrophage [51]. It has been reported that individuals with genetic deficiency in the INF-γ receptor are more likely to be infected with mycobacterial [52]. Several studies have revealed an increased susceptibility to mycobacterial diseases in INF-γ-deficient mice and also in humans having INF-γ receptor abnormalities [38]. INF-γ expresses protein peptides cathelicidin and defensin-β 2, which are delivered to *Mtb* phagosomes via vitamin D-dependent pathway [53], defensins, and a single cathelicidin, LL-37, which are major groups of host defensive peptides in humans. Susceptibility to infectious diseases including tuberculosis has been reported due to alteration in the synthesis of these molecules [53-55].

Vaccines

Currently, most tuberculosis vaccines under different stages of clinical trials are focused on either replacement of BCG or as a booster following vaccination with prime BCG [56]. Subsequently to the failure of MVA85A in the last two years, there has been no new, prominent TB vaccine entering clinical testing. There are 16 TB candidate vaccines in clinical testing, classified into priming vaccines, prime boosters, and immunotherapeutic vaccines [57]. Most of these candidates are subunit vaccines; selected antigens of *Mtb* are expressed using recombinant viral vectors or are administered in combination as protein/adjuvant [58]. The developmental pipeline of new TB vaccines is shown in Table 1.

Bacillus Calmette-Guérin

BCG vaccine was first developed from a virulent strain [68]. Attenuation of the original BCG strain of *M. bovis* led to the establishment of a BCG vaccine resulting from subcultures in a media aimed at preserving its immunogenicity [69]. BCG play a protective role against *Mtb* because it induces CD4 (T helper type 1) and CD8

Table 1. The developmental pipeline trend for new vaccines against tuberculosis

TB vaccine	Vaccine Type/Strategy	Phase	Sponsors	Reference
MTBVAC	Live-attenuated vaccine/ priming vaccine	Phase I	University of Zaragoza, Biofabri, The Tuberculosis Vaccine Initiative (TBVI)	[59]
VPM1002	Recombinant live/prime	Phase I	Max Planck, Vakzine Projekt management GmbH, The Tuberculosis Vaccine Initiative (TBVI)	[60]
Ad5 Ag85A	Viral-vectored vaccine Prime booster	Phase I	McMaster University, Supported by Tianjin Cansino Biotech. Inc	[61]
M72 + AS0	Protein and adjuvant/prime booster	Phase IIb	GlaxoSmithKline, Aeras	[62]
MVA85A	Attenuated Mycobacterium tuberculosis strain	Phase I	The Tuberculosis Vaccine Initiative (TBVI), Zaragoza, Biofabri	[63]
Crucell Ad35+ MVA85A	Viral vector/Prime booster	Phase I	Crucell, Oxford University, Aeras	[62]
Hybrid 1 + IC31	Recombinant protein/ Prime-boost	Phase I	Statens Serum Institut/Tuberculosis Vaccine Initiative/Intercell	[64]
Hybrid 4 + IC31	Recombinant and adjuvant	Phase I	Statens Serum Institut (SSI), Tuberculosis Vaccine Initiative (TBVI)	[65]
Hybrid 56 + IC31	Adjuvanted subunit/Prime-Boost	Phase II	Statens Serum Institut	[66]
ChAdOx1 85A + MVA85A	Viral vector/Prime-boost	Phase I	Oxford University	[62]
ID93 + GLA-SE	Adjuvanted subunit/Prime-Boost	Phase I	Infectious Diseases Research Institute	[66]
DAR-901	Mycobacterial-whole cell or Extract	Phase I	Darmouth, Aeras	[63]
TB/FLU-04l	Viral vector/prime booster	Phase I	Research Institute for Biological Safety Problems	[63]
Mycobacterium vaccae	Therapeutic/Boost, Post infection	Phase III	NIH, Aeras, Immodulon	[60]
RUTI	Immunotherapeutic/ Fragmented MTB	Phase II	Archivel Farma	[67]

(T cell) responses [70]. BCG gives efficient protection against tuberculosis in new-borns, but does not provide prevention of latent infection or reactivation of TB in adults [71]. BCG is still used for children in many countries as part of the WHO expanded program on immunisation, but it is surrounded with controversy regarding its efficacy in protecting against adult and adolescent forms of TB [72]. BCG vaccine evaluation began formally in the 1930s, but scientists became aware of the degree of dissimilarity between the various results in the 1950s. In the UK, major trials carried out by the Medical Research Council indicated that more than 75% protection, whereas trials carried out by the US Public Health Services recorded less than 30% protection. Following trials and other studies resulted in a persistent broad range of estimates [73]. BCG has shown protective efficacy in adult pulmonary tuberculosis with reported variability in efficiency ranging from 0 to 80 per cent [74]. There is need of both Th1 and Th17 responses for an ideal host protection against tuberculosis; BCG happens to induce Th1 response and fails to induce Th17 response in the lung. The ability of BCG to induce Th1 but not Th17 mostly leads to inferior efficiency of BCG vaccine [75]. Interferon γ is produced as a result of Th1 response I sand required for protection. Marchant *et al*. demonstrated that Th1 memory response is induced at birth upon BCG immunisation in a similar way when administered later in life [76]. An improved understanding of the reasons behind variability of BCG efficacy to such a great extent is important to assess new vaccines against tuberculosis, which are undergoing clinical trial [77]. The different levels of protection could be due to variations in BCG strain from different locations [77]. Immune response in individuals might be influenced by exposure to other environmental mycobacteria (EM), which, as a result, interfere with the effectiveness of BCG. Efficacy of BCG > 70% was reported in populations from nations situated far away from the equator having no or less prevalence of EM [78]. The effectiveness of BCG was found to be significantly lower in individuals of countries located near to the equator [79]. It has been reported that BCG has a protection time ranging from 10 to 20 years in the majority of cases [80]. Recently, Nguipdop-Djoma Patrick *et al*. demonstrated that BCG vaccination exhibited long-lasting protective ability against TB in individuals aged 12-50 years with negative tuberculin skin test and vaccination carried out between the years 1962 and 1975. Their results revealed that the rates of TB in unvaccinated and vaccinated participants are 3.3 and 1.3 per 1000 person-years, respectively [81]. BCG has been used in neonates since 1974, and it provides protection against tuberculosis and tuberculosis meningitis with a 50% reduced risk of disease development in young children [82]. Despite being a safe vaccine, BCG vaccines are considered among the most reactogenic vaccines, with reactogenicity depending on variation with different strains and the number of viable bacilli

[83]. Immunocompromised children infected with HIV or immunosuppressed individuals are especially vulnerable to complications of BCG vaccine [84]. A study was carried out on 349 BCG-immunised patients having severe combined immunodeficiency in 17 countries; the results indicated a high rate of complications of BCG vaccine [79].

Live attenuated TB vaccines

Improvement of the vaccine depends on strengthening the immunogenicity and persistence of a genetically modified recombinant strain of BCG (rBCG). Hence, a genetically manipulated rBCG could be more efficient compared to the parental BCG due to introduction of some parts of DNA (genes) lost during *in vitro* attenuation [85]. The loss of the RD1 region is the genetic principal behind BCG attenuation – the region encoding the machinery needed to synthesise and export the major T-cell antigen/virulence factor ESAT-6/CFP-10 [86]. The first recombinant BCG was generated by Horwitz *et al*. [87] and Horwitz and Harth [88]. rBCG3 overexpressed antigen Ag85b, which induced protection against TB significantly in animals. Compared with parental BCG, rBCG30 significantly increased Ag85b-specific T cells that inhibit intracellular mycobacteria [89].

VPM1002 is the second recombinant BGC vaccine candidate [79], formed because of two variations of live *Mtb*. The gene encoding for Listeriolysin (Hly) from *Listeria monocytogenes* incorporated into the genome of BCG [59], rBCGUre:CHly*, conferred high protection against *Mycobacterium tuberculosis* challenge through aerosol. This improved protection was because of efficient perforation of the phagocyte phagosomal membrane by Listeriolysin (Hly) [90]. rBCGUre:CHly* is now in the phase of clinical trial due to its enhanced protection against tuberculosis [91]. Recombinant rBCGUre:CHly* constructs movement from endosomes to cytosol due to the activity of Listeriolysin with concomitant deletion of the urease gene. Loss of the urease gene leads to improved mycobacterial antigen processing via MHC I pathway as well as improved CD8 cytotoxic T cell activity [92].

BCG::ESAT-L28A/L29S improved BCG strain with modifications at amino acid residues. Leu[28]-Leu[29] of the ESAT molecule showed strong attenuation in mice and high protective efficiency both in mouse and guinea-pig vaccination-infection models [93]. Chun Wang *et al*., 2012 [94] constructed three recombinant BCG strains that overexpressed immunodominant antigens of *Mycobacterium tuberculosis,* Ag85B (rBCG::85B) and Ag85A (rBCG::85A). Both recombinants (rBCG::AB) provided stronger and longer-lasting protection compared to the BCG containing vector without insert pMV261(rBCG::261) using mice.

In January 2013 MTBVAC entered a phase I clinical trial, and it is the live-attenuated *Mycobacterium tuberculosis* vaccine that entered the phase I trial. It is a derivative

of attenuated strain SO2 obtained by insertion of a ka-namycin-resistance cassette in the phoP (phoP is a tran-scription regulator) gene of *Mtb* transcription. Mutation of phoP causes a lack of expression of several genes, includ-ing ESAT6, a virulence factor [59]. In preclinical studies it was found that MTBVAC showed the same safety and biodistribution profiles as BCG and indicated superior pro-tection [86]. The satisfactory safety of MTBVAC could be explained based on the following factors: lack of front-line lipids, loss of ESAT-6 expression, and down-expression of the PhoP regulon, essentially for pathogenicity and viru-lence of *Mycobacterium tuberculosis* [86]. Highly attenu-ated MTBVAC could be a potential vaccine for populations with high-risk immunosuppression, due to inactivation of an additional gene-generated repeated protein (Erp) [63].

Subunit and viral vector-based vaccines

Live-based vaccines are not products chosen by most manufactures because of safety considerations, especially in immunosuppressed individuals, and technical challenges regarding reproducibility [16]. The main reasons for devel-oping a recombinant protein-based vaccine are as follows: they develop less reactogenicity and are considered more potent, safer, and better characterised vaccines [95]. *Mtb* secretes proteins during *in vitro* growth. One of the possi-ble ways of improvement towards a tuberculosis vaccine would involve use of such secreted proteins. Some of these proteins are immunogenic; these proteins or their agreeing genes could serve as a major part of either a DNA-based vaccine or a subunit vaccine. Identification of antigens secreted in the culture fluid is important for establishing protective immune response against TB [85]. Several studies carried out have shown promising results for DNA vaccination against tuberculosis. DNA vaccines express different *Mtb* antigens; these include: Ag85A, Ag85B, ESAT-6, MTP-64, PstS-3, and 65kDa heat-shock protein. These proteins were all found to be effective in inhibiting the growth of *Mtb*-infected mice [96]. We produced a po-tential peptide/protein vaccine (Myt272-3) from a clone contracted by shotgun cloning in the University of Malaya Molecular Bacteriology and Toxicology laboratory. The protein has an approximate molecular mass of 10.58kDa, which conformed to the computed MW by EXPASY MW bioinformatics tool. Both protein blast and MALTI-TOF analysis indicated homology with phenolpthiocerol synthe-sis polyketide synthase I PpSA of *Mtb*. *In silico* analysis of the protein also indicated non-allergenicity and antigenic-ity of the query protein sequence, which serves as a good guide for the design of a vaccine against TB.

Wu Li *et al*. reported a recombinant adenovirus (Ad5-CEAB) expressing *Mtb* antigens Ag85A, Ag85B, CFP10, and ESAT6 proteins combined in a mixture [97]. Ad5-CEAB resulted in a strong antigen-specific immune re-sponse as well as heightened humoral responses with a dra-matically antigen-specific serum immunoglobulin (IgG).

Viral vector

Ad5Ag85A is a viral vectored adenovirus serotype 5 vector vaccine expressing Ag85A developed by Mc-master University and supported by Tianjin CanSino Bio-technology Inc. The vaccine went through Phase I trial in 24 Canadian adults: 12 from BCG naïve and 12 from pre-viously BCG-vaccinated, healthy adults. No vaccine-related serious adverse effects were recorded. Ad5Ag85A had im-munogenicity in both groups with stimulation of polyfunc-tional T-cell responses, but found more effectively boosted CD4 and CD8 T-cell immunity in a group of previously-im-munised subjects compared to a BCG-naïve group, which is reassuring for its further clinical development serving as a booster vaccine candidate after BCG priming [61].

A phase I trial involving MVA85A combined with Crucell Ad35 (Crucell Ad35 + MVA85A) was carried out among 40 adult participants at Oxford University [62]. Research Institute for Safety Problems and the Research Institute on Influenza, in Russia, developed a recombinant influenza vaccine called TB/FLU-04L, which is composed of influenza virus strain A/Puetro Rico/8/34 H1N1 and *Mtb* antigens Ag85A and ESAT6. A phase IIa trial is be-ing planned for this vaccine candidate, and a phase I trial was completed [63]. ChAdOx1.85A is another adenovirus vaccine that expresses *Mtb* antigen Ag85A; a phase I clin-ical study is currently testing the safety of ChAdOx1.85A vaccination along with infusion with MVA85A in adults vaccinated with BCG in the United Kingdom [62].

Subunit adjuvant

Adjuvants includes compounds and molecules/macro-molecular complexes capable of boosting the potency and effective duration of specific immunological response to antigens [98]. The major hindrance in developing vaccines against bacteria has been attributed to a lack of adjuvant that adequately stimulates cell-mediated immunity [99]. It is therefore essential to administer subunit vaccines with an adjuvant to enhance immune responses to subunit vac-cines. The adjuvants approved for human use include Alu-minum salts, AS03/04 and MF59. They are primarily pro-moters of a humoral or Th2 rather than Th1 response [99].

Hybrid 1 + IC31 is a subunit adjuvant vaccine devel-oped by the Statens Serum Institute, TBVI, and Intacell. It is a hybrid of ESAT6 and Ag85B antigen with IC31, the components of the adjuvant system are oligodeoxynu-cleotide ODN1a and the cationic protein polyamino acid KLK [63]. Reither *et al*. [100] evaluated vaccine candidate H1/IC31in 48 patients infected with HIV, and the results showed durable Th1 immune responses.

Hybrid 4 + IC31 vaccine has a fusion of *Mtb* anti-gens (Ag85B and TB10.4) with adjuvant IC31, owned by Valneva. In 2014, a three-arm, phase IIa study was an-nounced by Aeras in order to determine the safety and immunogenicity of H4+IC31 and BCG revaccination in approximately 1000 BCG-immunised, non-HIV adolescent

South Africans [62]. Hybrid 4 + IC31 adjuvant vaccine effectively boosted and lengthened immunity induced by BCG, leading to enhanced protection against *Mtb* due to domination of immune response by INF-γ, TNF-α, IL-2 or TNF-α, IL-2, and CD4 cell [101].

Hybrid 56 + IC31, a protein adjuvanated vaccine composed of H56, a fusion protein consisting of Ag85B, ESAT6, and Rv2660c (latency-associated protein), incorporated with the adjuvant IC31 [59]. Hybrid 56 + IC31 subunit adjuvant vaccine showed an ability to control the late-stage of TB infection, and it contains the latent stage of tuberculosis [102].

Thacher *et al.* evaluated M72/AS01 candidate vaccine [103] on 37 HIV-infected adults on cART (combination anti-retroviral therapy) in Switzerland in a ratio 3:1:1 to vaccine, adjuvant (AS01), and saline placebo. The vaccine was found to be immunogenic with induction of persistent CD4 T-cell responses specific to M72.

The Infectious Disease Research Institute came up with the ID93 vaccine, which is the most recent tuberculosis vaccine entering clinical trials, and it was designed to target both forms of active and latent tuberculosis [47]. IDR93 is a protein/adjuvant vaccine that combined four novel sets of antigens including Rv2608, Rv3619, Rv3620, and Rv1813 in addition to the adjuvant (synthetic MPL formulated in a glucopyranosyl lipid stable emulsion). Preclinical studies with mice revealed that IDR93 vaccine is protective almost at BCG levels, and in guinea pigs a combination of ID93 and BCG reduced the mortality rate [47].

Immunotherapeutic vaccines

The main targets of therapeutic vaccine design are to prevent latent infection or to reduce the need of chemotherapy [104]. RUTI is one of the therapeutic vaccines made of detoxified, fragmented *Mtb* cells delivered in liposomes. A previous study revealed that RUTI showed efficacy in controlling latent form of TB infection in mice and guinea-pigs, inducing a combined Th1/Th2/Th3 polyantigenic response after a short period of chemotherapy [105]. Nell *et al.* [106] evaluated a RUTI vaccine in a placebo-controlled clinical trial involving 95 patients infected latently with TB. The RUTI vaccine showed its immunogenicity. A phase 1 clinical trial (randomised, placebo-controlled) of RUTI vaccine was carried out in Spain using healthy white males without history of tuberculosis infection and prior BCG vaccination, to find the tolerability and immunogenicity of four RUTI doses (ranging between 5 μg and 200 μg). After completion of the trial in October 2008, the results showed that all the doses were tolerable, but moderate pain was noted with higher doses. Thirty-five days after vaccination four specific antigens were traced [58].

Mycobacterium vaccae was developed as an immunotherapeutic vaccine initially by inactivation of the whole cell strain of *M. vaccae* [107]. In a phase III trial, variable INF-γ and humoral responses were induced by Mycobacterium vaccae, according to CD4 T-cells count, HIV viral load, and previous TB treatment [59]. In Tanzania, vaccination with a multiple-dose series of *Mycobacterium vaccae* to HIV-positive adults immunised with BCG at childhood was associated with significant protection of HIV-associated TB. These results revealed that vaccination with *M. vaccae*, aimed at HIV-associated tuberculosis prevention, showed no detrimental effect on CD4 cells [108]. In 2001, *M. vaccae* was approved for sale in China for immune therapeutic purposes against TB. It appeared to provide a measurable improvement in some geographical settings but not in other places; the inconsistency led to doubts about its efficacy by some researchers [61].

Conclusions

Despite the emergence of new tuberculosis drugs and diagnoses, there is a need for new, safe, and effective vaccines to hasten the progress towards eradication of TB [109]. The trend of the tuberculosis vaccine development pipeline is in progress, with about 16 vaccine candidates at various stages of clinical trial. Despite several TB vaccines in phase, I, II and III of clinical trials, there is also a need to consider research on reverse vaccinology that involves computational analysis of proteome of the *Mycobacterium tuberculosis* in order to select new antigens that could serve as novel and more effective/efficient vaccines against tuberculosis. In addition to research development towards an improved vaccine for TB infection, mass campaigns on vaccination are needed in order to achieve the target of eradication of tuberculosis by the year 2050.

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