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Vaccines – Recent advances and clinical trials

Marisol Ocampo C.

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1. Introduction

Short description of the chapter: The chapter gives an overall view of attempts at preventing tuberculosis; this disease has a high impact on public health worldwide. Aspects concerning the development of anti-tuberculosis vaccines being tested by different research groups around the world are analysed in this chapter. The current vaccine is described, as is the search for new antigens, the use of adjuvants, the approaches adopted to date for designing new vaccines, and current trials in their different phases of research. An analysis is made of the results obtained to date and comments are made about the future regarding the development of an effective anti-tuberculosis vaccine.

Tuberculosis is considered the second worldwide cause of mortality after AIDS; it is caused by an infectious agent (*Mycobacterium tuberculosis*) and 8.6 million people were reported as having acquired tuberculosis in 2012 and 1.3 million people died from tuberculosis in the same year [1].

Even though tuberculosis is curable and can be prevented, it has been calculated that a third of the world's population has latent tuberculosis (i.e., they are infected by *Mycobacterium tuberculosis* (*Mtb*) but have not yet become ill nor can they transmit the infection). *Mtb* usually affects the lungs (pulmonary tuberculosis) but can affect other sites (extra-pulmonary tuberculosis). People infected with the tubercle bacilli have around a 10% risk of developing the disease throughout their lifetimes, and this risk becomes much greater for smokers or people who have a deficient immune system, as happens with cases of infection caused by the human immunodeficiency virus (HIV), malnutrition or diabetes.

The best way to avoid the disease is to find a totally efficient vaccine which can prevent mycobacteria entering the target cell for infection, vaccines which can protect individuals from initial infection, those which can prevent progression to active tuberculosis in recently infected

individuals, or else those which can reduce the ability of those having active disease to transmit it to others.

Tuberculosis is currently controlled by using antibiotics; however, the socioeconomic panorama of endemic areas, the extensive period of time represented by treating it, together with intense secondary effects produced by anti-tuberculosis drugs, as well as the appearance of cases of multi-resistant tuberculosis, make this a limited solution regarding the enormous problem which this disease represents.

The publication of the H37Rv strain genome in 1998 [2], more than 100 years after Robert Koch discovered the existence of *Mtb* (in 1882), provided information with great potential for identifying its gene repertory, some of which is key when ascertaining mycobacterial virulence, pathogenesis, survival and latency. Such an advance has led to thinking about designing new drugs against this disease, overcoming resistance to drugs and, above all, obtaining an effective vaccine. However, despite around 4,000 of the genes forming this microorganism already being known and the pertinent data being already available in enormous databases, functional information regarding the proteins encoding them is still very limited.

The *Mycobacterium bovis* Bacillus Calmette–Guerin (BCG) vaccine has been used for almost 100 years for preventing tuberculosis. This vaccine may offer protection against serious forms of tuberculosis in children (tuberculous meningitis and miliary tuberculosis) [3], but it continues to be controversial regarding the variability of protection results (0% to 80%), being especially effective in preventing infant tuberculous meningitis but not pulmonary tuberculosis in adults [4]. BCG use is not recommended in HIV patients due to the risk of infection being disseminated by the *Mycobacterium bovis* strain used in this vaccine. Even though around 12 anti-tuberculosis vaccine candidates are currently in clinical trials, the following should be born in mind as obstacles preventing the development of efficient anti-tuberculosis vaccines. There is a poor correlation among protection in animal models which could be applied to studies in humans, there is the impossibility of validating an animal model since an efficient vaccine for preventing pulmonary tuberculosis has still not been found, and there is the cost and sample size regarding clinical trials due to a low regional incidence despite the disease's high prevalence. Many aspects concerning a natural immune response in humans following infection remain unknown and it is not clear which model would be suitable in choosing the best antigens for an anti-tuberculosis vaccine [5]. However, it is becoming clearer that there are substantial differences in humans' immunological responses to tuberculosis (as well as concerning other inflammatory diseases) which cannot be found in or predicted by studies in animals [6].

The complicated natural history of tuberculosis suggests that at least three vaccination strategies are possible. One would prevent primary infection and disease followed by exposure to the mycobacteria, another would prevent reactivation in cases of already infected individuals, while a third would advocate immunotherapeutic treatment complemented by normal procedures against tuberculosis directed at patients who are already ill from this disease [7, 8].

Research into prophylactic methods against tuberculosis has been based on antigens which are recognized by tuberculosis patients and which produce a cellular immune response;

however, more recent results have led to the conclusion that *Mtb* is a very complex pathogen, and it seems that the T-cell response is not strong enough for controlling the disease in humans. As part of the search for antigens with prophylactic or diagnostic potential, *in vitro* culturing of *Mtb* has led to a set of culture filtrate proteins (CFPs) being recognized whose main characteristic is their immunodominance. Some antigens have been implicated in a protective immune response or in T-cell activation in infected humans and animal models, which is why they have been considered good vaccine candidates. Such antigens have been identified by biochemical approaches and have been characterized by being abundant proteins, secreted by a culture medium [9, 10]. Despite efforts having been made in the field of research into antigens with immunoprophylactic potential, only a few have passed experimentation Phase II; it has been reported recently that one of the vaccines being developed, which has been in the most advanced clinical studies (MVA85A), has not provided greater protection than that currently offered by BCG [11]. Some antigens with immunogenic potential have been identified by using synthetic peptides; their versatility has been shown in developing alternatives against tuberculosis [9, 12-14].

Another has involved bioinformatics as a recent discipline integrating the large amount of biological information which has been obtained regarding different microorganisms and which can be found in large-scale databases, along with their biological significance. This approach has been used for rationally selecting sequences from the best immunomodulator candidates from among the thousands of genes which microorganisms have. Bioinformatics can thus contribute towards limiting the amount of candidates to be tested [15, 16].

Tuberculosis (TB), a disease caused by *Mycobacterium tuberculosis* (*Mtb*) infection, remains a major public health problem. The *Mycobacterium bovis* strain-derived Bacillus Calmette–Guerin (BCG) vaccine is the only one currently available against this disease; it effectively protects against TB disseminated in younger children, but it has not managed to reduce infection prevalence and it has only shown variable protection against pulmonary TB, which constitutes the global disease burden [3]. Several facts highlight the urgent need for an efficient vaccine against this disease:

- A lack of knowledge regarding the mechanisms by which the BCG vaccine has not provided sufficient protection against *Mtb*;
- *Mtb* is responsible for almost two million deaths annually and remains in a latent stage in a third of the world's population;
- TB is one of the diseases with the greatest potential for evolutionary adaptation; and
- The slow decline in TB incidence globally and the emergence of multidrug-resistant forms of TB is devastating for patients (especially HIV-positive individuals) and healthcare providers.

WHO data reports that efforts at developing new diagnostic tests, new vaccines and drugs against TB have increased considerably during the last decade [1]. Twelve anti-TB preventative vaccine candidates were reported as being in trial Phases I, II or IIb, and two more immunotherapeutic vaccines in Phases II or III by 2013.

The Phase IIb results for vaccine candidate MVA85A published in February 2013, which was tested in children and administered as a BCG backup, were seen to provide no additional protection when comparing the results with those obtained when just applying the BCG vaccine [11]. However, this study showed that the vaccine had an acceptable safety profile in the study population, and that a high quality trial could be carried out in a high TB load setting after solid results were found.

Even though the nature of protective immunity which must be produced against TB has not been completely understood to date, and despite that biomarkers of protection have not been clearly established, it has been suggested that *Mtb* antigens included in a possible vaccine must induce a strong Th1 cell immune response. It has usually been held that antigens which are immunogenic are recognized by TB patients or else are those which are expressed during *Mtb* infection; however, more recent results have led to concluding that *Mtb* is a very complex pathogen, and it seems that the T-cell response is not strong enough for controlling the disease in humans. As part of the search for antigens with prophylactic or diagnostic potential, the *in vitro* culturing of *Mtb* has led to a set of culture filtrate proteins (CFPs) being recognized whose main characteristic is their immunodominance. Some antigens have been implicated in a protective immune response or in T-cell activation in infected humans and animal models, which is why they have been considered good vaccine candidates. Such antigens have been identified by biochemical approaches and have been characterized by being abundant proteins, secreted by a culture medium [9, 10]. Despite efforts having been made in the field of research into antigens with immunoprophylactic potential, only a few have passed experimentation Phase II; it has been reported recently that one of the vaccines being developed, which has been in the most advanced clinical studies (MVA85A), has not provided greater protection than that currently offered by BCG [11]. Some antigens with immunogenic potential have been identified by using synthetic peptides; their versatility has been shown for developing alternatives against TB [9, 12-14].

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It should be born in mind, here, that vaccines must perform well in a series of clinical phases, thereby establishing vaccine candidate efficacy and safety, encompassing the following phases [19]:

- *First-in-human clinical trials*: First-in-human studies are small studies in healthy adults (usually 20-80 subjects) to evaluate a vaccine candidate's safety and immunogenicity;

- *Phase I clinical trials*: Phase I clinical trials are small safety studies in different target populations. These may include a preliminary assessment of dose-range and/or age de-escalation;
- *Phase IIa clinical trials*: Phase IIa clinical trials are larger studies (usually 100-300 subjects) exploring dose-range in target populations aimed at identifying the optimum dose, the dosing schedule and/or administration route based on safety, immunogenicity and/or biological end-points. These are focused studies designed to provide evidence of biological activity in the target population;
- *Phase IIb clinical trials*: Phase IIb clinical trials are larger, well-controlled studies evaluating safety and proof-of-concept/efficacy. These trials usually include more than 1,000 subjects and are designed to demonstrate evidence of disease prevention or treatment in the target population; and
- *Phase III clinical trials*: Phase III clinical trials are pivotal registration studies to support licensure. They are designed to demonstrate statistically significant evidence of disease prevention or treatment and long-term safety in target populations as required by national regulatory authorities. These studies are conducted with the final manufactured product.

2. The existing BCG vaccine

The established vaccine referred to as 'Bacille de Calmette et Guérin' (BCG), a live-attenuated bacterial vaccine developed in 1921, efficiently protects children against disseminated disease, and this is probably the most convincing argument for its use; however, it induces highly variable protection against TB. Even though the mechanisms have not been identified to date which allow this vaccine to protect against severe forms of TB in children (TB meningitis and miliary TB), while its efficacy in preventing pulmonary TB in adults remains highly variable, it continues to be the gold standard regarding prevention against TB caused by *Mtb*, being given to more than 80% of neonates and nursing infants in countries where it forms part of their national infant immunization programme.

Continued emphasis has thus been placed on understanding the mechanisms behind the failure of BCG to provide sufficient protection against *Mtb* in the lungs, and to designing new vaccines to be used in conjunction with BCG as booster strategies for installing protective immunity at the site of infection. It seems that protective immunity with the current vaccine requires mycobacterial replication in the host, meaning that a pre-existing immune response could lead to a cross-reaction with BCG.

Trials have indicated that BCG has 60-80% protective efficacy against severe forms of TB in children, particularly meningitis [20, 21], and controversial attempts have been made to relate its efficacy against pulmonary diseases with the vaccinated population's geographical location and exposure to environmental mycobacteria [22, 23]. It has also been proposed that different BCG strains induce different levels of protection.

Greater BCG vaccine efficiency regarding protection against pulmonary, miliary and meningeal TB has been associated with the absence of prior *Mtb* infection or sensitization to envi-

ronmental mycobacteria. This fact is relevant when designing new vaccines against TB since prior infection could mask or block their effects [23]. Other authors have shown the BCG vaccine's efficacy against leprosy and against TB itself [24].

Recent systematic studies have concluded that the BCG vaccine protects against *Mtb* infection as well as its progression towards active disease, determined by interferon-gamma release assays (IGRAs) in children [25]. These IGRAs were based on INF- γ release by T-cells from individuals who had been infected by *Mtb*, and led to the clear differentiation of individuals who had been previously vaccinated with BCG or who had been infected by other mycobacteria [26].

It could be concluded that the BCG vaccine has shown great variety regarding its efficiency concerning protection against TB, thereby creating great controversy and encouraging numerous efforts aimed at producing better vaccines against this disease. However, it cannot be ignored that BCG is the most used vaccine around the world – more than three thousand million doses having been administered to date – and that this fact must be taken into account when designing new vaccines which must necessarily be compared to classic BCG efficacy.

3. Different approaches

Bearing in mind the results obtained in animal models regarding protective immunity concerning TB, most vaccine candidates have been based on vectors, adjuvants and antigens, inducing classical cytokines for a Th1 profile, such as INF- γ and TNF- α , produced by CD4+ and CD8+ T-cells. The main immunization strategies used for developing vaccines against TB would include:

- Prime vaccines are those seeking to be more efficient than the current BCG and which might replace it. These would include those which have improved on the *M. bovis* BCG strain or new vaccines based on live attenuated *M. tuberculosis*. Attempts have also been made to improve the safety of the actual BCG so that it can be used with children exposed to HIV, since using the actual vaccine is not recommended. They prevent infection and are considered pre-exposure, are prophylactic and their intention is to obtain a longer-lasting and more effective immune response;
- Vaccines administered after BCG has been applied to the new born are known as boost or prime-boost vaccines for improving efficiency and extending the protection time produced with the first immunization with classical BCG. They form part of a prophylactic booster strategy for classical BCG vaccination for prolonging the immunity induced by it;
- Post-exposure vaccines include those which prevent the disease's primary progression or latent TB reactivation. They are directed towards individuals with latent tuberculosis infection (LTBI), meaning that it should be considered that any *Mtb* infection could become a clinically active disease or a latent infection (as it is believed that mycobacteria could persist in metabolic stages of slow replication until practically remaining inactive), depending upon the host's immune state. The antigens require careful selection; if the antecedents provoked

by immunization with tuberculin in the past are born in mind [27], proteins are selected in this strategy which are expressed during the infection's latent state and which are recognized by individuals with dormant *Mtb* infection. It is thought that vaccination is a more effective means than antibiotics for eliminating mycobacteria in a latent state. A consideration to be born in mind when selecting antigens is the fact that it has been described how antigens recognized by the immune systems of individuals with latent infection differ from those recognized by patients with active disease [28]; and

- Immunotherapeutic vaccines include whole-cell and fragmented mycobacteria and might become synergized with chemotherapy to shorten treatment for active TB or LTBI.

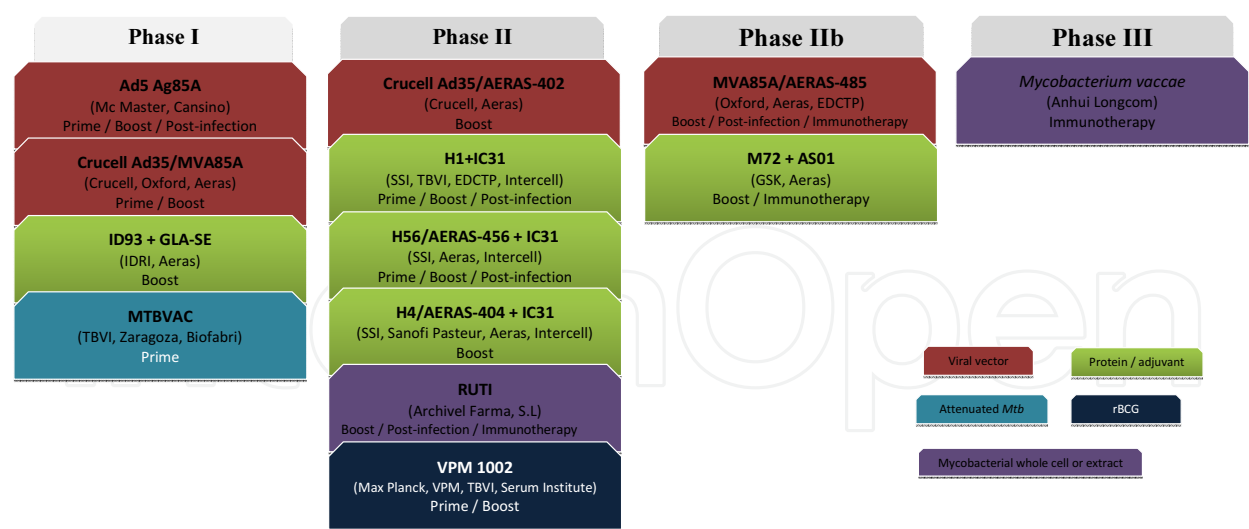
Subunit vaccines offer great advantages in all cases as they allow for the selective design of antigens which could induce an effective protective immune response. Using these vaccines is justified as only a few antigens are needed to achieve the same protection obtained with complete bacteria, and their use leads to obtaining safe, reproducible vaccines, and no problems have been foreseen regarding their application to immunocompromised individuals.

While DNA-based vaccines offer the induction of robust MHC class I-restricted cytotoxic T-lymphocyte (CTL) cells, due to their strong potential for inducing memory responses, DNA vaccines are particularly suited for priming immune responses. The co-administration of BCG with plasmid DNA encoding immunodominant, subdominant and phase-specific antigens – poorly expressed by BCG – may lead to the development of improved TB vaccines [29].

4. Vaccines currently being developed

Regarding vaccine candidates currently in experimental clinical phases (Figure 1), there are a great variety of strategies for inducing the desired protective response; these would include complete microorganisms, cell extracts and non-pathogenic mycobacteria (*M. vaccae*, RUTI, *M. smegmatis*) [30, 31], candidates included with viral vectors (MVA85A, AERAS-402 and AdAg85A) [11, 32], fusion proteins with Th1 response-inducing adjuvants (M72/AS01, Hybrid 1/CAF01, Hybrid 1/IC31 and HyVac 4) [33, 34], and BCG-based live recombinant vaccines (VPM 1002, Aeras 422, rBCG30) [35, 36] and those based on *Mtb* (MTBVAC) [37].

A broad range of vaccine candidates are already being evaluated pre-clinically, which contributes towards broadening clinical portfolio diversity and filling current scientific gaps. Consensus has been achieved among investigators regarding new TB vaccines to rationalize and streamline advancing TB vaccine candidates, with increased emphasis being placed on global coordination among key stakeholders to advance a common research agenda. A re-prioritized focus on early-stage research is also underway to supplement existing efforts. In this way, resources and research efforts must be devoted to the search for new alternatives for vaccine design, the deepening of immunological mechanisms in humans, and the development of reliable biomarkers that would predict vaccine efficacy and disease progression; using these strategies together we can find new vaccine alternatives and progress towards successful future clinical trials [5].



Based on Global tuberculosis Report.WHO. 2013 [1]

Figure 1. The development pipeline for new TB vaccines, 2013

4.1. Phase I clinical trials:

Ad5 Ag85A is a human adenovirus serotype 5 vector expressing Ag85A (*fbpA*, Rv3804c, secreted antigen 85-A, mycolyl transferase 85A), an immunodominant antigen produced by all mycobacterial species. It has been developed by McMaster University with support from Tianjin CanSino Biotechnology Inc. The vaccine was evaluated in a Phase I trial in 12 BCG-naïve and 12 previously BCG-immunized, healthy, Canadian adults, which demonstrated no vaccine-related serious adverse events. It showed that Ad5Ag85A was immunogenic in both groups and that it stimulated polyfunctional T-cell responses, but it more potently boosted both CD4+ and CD8+ T-cell immunity in previously BCG-vaccinated volunteers compared to BCG-naïve volunteers, supporting its further clinical development as a booster vaccine after BCG priming [38, 39].

ID93/GLA-SE is being developed by the Infectious Disease Research Institute (IDRI) in collaboration with Aeras. It consists of a recombinant fusion protein expressing the antigens Rv2608, Rv3619 and Rv3620 associated with virulence and the Rv1813 *Mtb* latency antigen; all of them are combined with a stable oil-in-water emulsion incorporating glucopyranosyl lipid adjuvant, a synthetic TLR-4 agonist (GLA-SE), together with the recombinant protein. ID93ID93/GLA-SE induced multifunctional CD4+ Th1 cell responses (IFN- γ , TNF- α , IL-2) in mice and protected both mice and guinea pigs against *Mtb* [40]. It is beginning a Phase Ib trial in adults to assess safety and immunogenicity in such a population. The purpose of this Phase I trial is to determine ID93/GLA-SE safety, tolerability and immunogenicity in BCG-vaccinated healthy adult subjects in South Africa for preventing pulmonary TB; results have yet to be published for this clinical trial.

MTBVAC is being developed by the University of Zaragoza, Institut Pasteur, BIOFABRI and the Tuberculosis Vaccine Initiative (TBVI). It is a live *Mtb* strain which has been attenuated via

deletion of the *phoP* gene encoding a transcription factor, which is key to virulence regulation, and *fadD26* which is essential for the synthesis of the lipid complex involving phthiocerol dimycocerosate (DIM), one of the major mycobacterial virulence factors [37]. MTBVAC has been shown to be safe in all preclinical studies and has conferred better protection than BCG in mice. It has been the first live attenuated *Mtb* vaccine to enter a Phase I clinical trial in Lausanne; 36 healthy volunteers participated to ensure the vaccine candidate MTBVAC's safety and immunogenicity.

4.2. Phase II trials:

AERAS-402/Crucell Ad35 is an adenovirus-vectored vaccine formed by replication-deficient adenovirus serotype 35 (Ad35), which functions as a viral vector expressing a fusion protein with three *Mtb* antigens: Ag85A, Ag85B and TB10.4. The antigen 85 complex is highly conserved among different species of mycobacteria and consists of multiple secreted components, Ag85A and Ag85B being highly homologous and the most immunogenic. The TB10.4 antigen (low molecular weight protein antigen 7 ESXH, *cfp7* or Rv0288) has been shown to be an immunodominant antigen. AERAS-402/Crucell Ad35 is designed as a booster vaccine for infants, adolescents and adults. It has been shown to induce polyfunctional CD4T cells and strong CD8T-cell responses when given to adults after priming with BCG, which were fiftyfold higher than those detectable pre-boost [41]. A single AERAS-402 dose has induced CD4T cells predominantly expressing single IFN- γ , whereas two doses induced CD4T cells predominantly expressing IFN- γ , TNF- α and IL-2 together. Although begun as a Phase IIb proof-of-concept trial, preliminary data has led to it now being revised as a smaller Phase II trial, having safety and immunogenicity in healthy infants previously vaccinated with BCG at birth as its primary end-points [42]. AERAS-402/Crucell Ad35 and MVA85A are also being tested in combination to try to drive a balanced CD4+/CD8+ immune response. One or two doses of AERAS-402/Crucell Ad35 followed by a dose of MVA85A are being evaluated for safety and immunogenicity in a combined Phase I/Phase II trial in adults in the United Kingdom. Three vaccines are protein subunit adjuvanted vaccines which were initially developed by the Statens Serum Institute in Copenhagen, Denmark.

Hybrid 1 + IC31 is based on a fusion protein containing Ag85B and ESAT-6 in adjuvant IC31. IC31 (Intercell) is a new adjuvant containing two components (an immunopotentiating cationic peptide (KLKL₅KLK) and a single-stranded oligodeoxynucleotide ODN₁) and has been shown to induce potent, sustained, antigen-specific cellular immunity via the Toll-like receptor-9/MyD88 signalling pathway [43], as well as humoral responses to a variety of peptides and antigens. ESAT-6 (in contrast to antigen 85 complex members) is a virulence factor which is restricted to mycobacteria from the *Mtb* complex. The two proteins fused in Hybrid 1 contain epitopes recognized by TB patients' T-cells with a broad range of HLA from different types; this has been determined by the high number of INF- γ -secreting T-cells specific for these antigens. The results from a clinical Phase I study have shown that the vaccine is well-tolerated, highly immunogenic in naïve individuals, and that it has induced strong Th1 responses persisting for more than two-and-a-half years after vaccination [34].

Hybrid 56 + IC31 contains a fusion protein which includes the antigens 85B and ESAT6 (as Hybrid 1) as well as antigen Rv2660c, which has been described as a hypothetical protein, originally identified on the basis of enhanced transcription in a starvation model of *Mtb* growth arrest [44]. The strong induction of Rv2660c has been associated with hypoxia-induced, non-replicating persistence and an enduring hypoxic response. However, it has been proposed, recently, that there could have been a fortuitous crossed reaction between the response of T-cells induced by recombinant protein Rv2660c and a yet-to-be-defined *Mtb* antigen, and it is expected that results will be obtained for Hybrid 56 + IC31 providing relevant information about latent infection physiology [45]. This vaccine combines antigens characteristic of early infection and latency, and seems to have protected mice against TB – as would be ideal – before and after exposure to infection [46].

Hybrid 4 + IC31 uses adjuvant IC31. However, this Sanofi Pasteur candidate includes a fusion protein candidate which expresses Ag85B and TB10.4; the latter antigen is from the same gene family as ESAT-6 and has been included in vaccine candidate AERAS-402.

RUTI is a therapeutic vaccine developed in Badalona (Catalonia, Spain) by Archivel Farma; it is formed by biotransformed *Mtb* cell fragments, delivered in liposomes. The original idea of introducing this vaccine was to boost the dominant immunological response against growing *Mtb* which already exists in a host. RUTI has already demonstrated its efficacy in controlling LTBI in experimental mice and guinea-pig models after a short period of chemotherapy; such experiments in animals have shown the induction of a mixed Th1/Th2/Th3, polyantigenic response involving no local or systemic toxicity [30].

The Phase I clinical trial showed that vaccination was reasonably well-tolerated as judged by local and systemic clinical evaluation, although dose-dependent local adverse reactions were noted; it also triggered a specific immunological response against *Mtb* in healthy subjects compared to a placebo [47]. It has also been shown that it can induce a cellular immune response measurable as IFN- γ secretion.

The results of a double-blind, randomized, placebo-controlled Phase II clinical trial for assessing the safety, tolerability and immunogenicity of three doses of RUTI vaccine administered after completion of one month of isoniazid treatment in HIV-infected and -uninfected subjects with LTBI have been reported recently [48]. The study involving 111 subjects was carried out at three South African sites (Bloemfontein, George and Port Elizabeth); the RUTI safety profile was considered acceptable. As some HIV-positive individuals were found in the group, this suggested that the RUTI vaccine did not cause any variation in the HIV viral load or CD4 count. Vaccination thus did not affect HIV infection evolution.

VPM 1002 is a recombinant $\Delta ureC$ *hly*+ BCG (rBCG) strain which expresses *Listeria monocytogenes* membrane-perforating listeriolysin (*hly*) and which is devoid of urease C. This rBCG construct induces superior protection against aerogenic challenges with MTB compared to parental BCG. Phase I clinical trials in adults in Germany and South Africa have proven safe and immunogenic for B-cell and T-cell responses [49], and a current Phase IIa trial is ongoing for assessing immunogenicity and safety in its target population (i.e., the new born in a high TB incidence setting). A second Phase II trial will assess the vaccine's safety and immunogenicity in HIV-exposed and -unexposed new-borns.

4.3. Phase IIb studies:

M72/AS01E is a protein subunit vaccine, formulated in a novel adjuvant system which induces type I to enhance cell-mediated immunogenicity. It contains a fusion protein containing *Mtb* antigens Mtb32A (*pepA*, probable serine protease *pepA*, Rv0125) and Mtb39A (Rv1196, a PPE family protein) in adjuvant system AS01E, which is a liposome formulation with monophosphoryl lipid A (MPL) and QS21 immunostimulants. The M72 antigen expressed in BCG and *Mtb* mycobacteria contains human CD4⁺ and CD8⁺ T-cell epitopes and induces proliferation and INF- γ production by T-cells from PBMC in TB-infected individuals. Although no immune-correlate regarding protection has been identified, T-cells expressing Th1 cytokines (INF- γ and TNF α) are associated with protection. Safety and immunogenicity were tested in different populations: as a booster to BCG in Gambian infants [50], in HIV-infected adults on combination antiretroviral therapy in Switzerland [51] and healthy PPD-positive adults in the Philippines [52]. The Phase IIb study will be the largest trial of a novel TB vaccine in adults, aiming to enrol 4,500 HIV-negative adults in TB-endemic countries in Africa. The primary end-point will be the protective efficacy of two doses of M72/AS01E against pulmonary TB. Secondary end-points will include safety and immunogenicity.

MVA85A is the first virally vectored TB vaccine to be tested in humans. It is a recombinant, attenuated vaccinia-vectored vaccine candidate expressing *Mtb* Ag85A. It was designed as a booster vaccine for BCG-vaccinated infants and this vaccine's first Phase IIb trial was conducted in South Africa from 2009 to 2012, results being published in early 2013 [11]. An additional MVA85A Phase IIb trial was conducted in adults infected with HIV-1, at two clinical sites, in Cape Town, South Africa and Dakar, Senegal; the trial concluded that MVA85A was well-tolerated and immunogenic in adults infected with HIV-1. However, it was detected as offering no efficacy against *Mtb* infection or disease [53].

Although the MVA85A vaccine has produced some protection in murine models of TB, the recent trial in BCG-vaccinated children in South Africa revealed that, although it could generate a high level of multifunctional CD4⁺ T cell response in the host and produce INF- γ , failed to prevent either TB infection or disease in vaccinated subjects.

4.4. Phase III studies:

Vaccae consists of a heat-inactivated *Mycobacterium vaccae* preparation, developed by Anhui Zhifei Longcom Biopharmaceutical. It has been approved for TB adjuvant therapy and is the only drug recommended for TB immunotherapy by the WHO [54]. Killed *M. vaccae* is safe and does not present any adverse side-effects, except for local reaction at the injection site. Injectable *M. vaccae* (Vaccae) was approved in 2001 for sale in China as adjunct immunotherapy for TB drugs; nevertheless, *M. vaccae* appeared to produce a measurable improvement in some geographical regions but not in others; such inconsistency has led some researchers to doubt its efficacy [55]. The cell wall seems to be responsible for *M. vaccae* immunostimulating effects, which have been greater than those produced by inactivated complete mycobacteria, and this would partly explain the variability of results obtained in different studies.

The purpose of the Phase III study is to add new indications for Vaccae, mainly to prevent TB for high-risk groups of infection; it has passed this phase, including testing in MDR-TB and HIV co-infected individuals.

5. Conclusion

Even though significant achievements have been made in research into different aspects related to the prevention, diagnosis and treatment of TB, and the group of researchers dedicating their time to the topic has grown worldwide, it has been recognized that a more effective vaccine is needed for reducing the high levels of mortality caused by this disease.

Several development stages and clinical trials are involved in developing new vaccines and, while new vaccine candidates emerge, it is essential that work continues on discovering new approaches. It is considered that immunization is one of the most profitable interventions regarding health, despite this leading to significant investment in terms of time and money. Candidates selected during the design phase must pass the preclinical phase to advance to clinical Phase I, clinical Phases IIa and IIb and clinical Phase III.

Research in this field has involved different approaches regarding the selection of the best antigens, adjuvant use, antigen dose, and immunization strategy. For the development of TB vaccines, two strategies have been mainly suggested: in the first case, the widely-used BCG vaccine is substituting by an improved vaccine, either a recombinant BCG or an attenuated *Mtb* strain. The other strategy implies a boosting vaccine which improves the protection provided by the actual BCG vaccine. It must thus be born in mind that different groups of the population are exposed, so that emphasis is placed on vaccines to be administered to children, the new born and suitable vaccine candidates for adolescents and adults. A search is also being made for therapeutic vaccines which will lead to the potentiating treatment of drug-resistant TB. All vaccines must be safe for people affected by HIV and those suffering latent TB infection.

Trying to find effective vaccines against TB continues to be a significant challenge as a greater understanding of a protective immune response is still required and there remain gaps regarding the mechanisms regulating *Mtb* immunopathology in human hosts [56]. An additional complication concerns the fact that TB may be the only pathogen manipulating the host's immune response in promoting its transmission. Some aspects needing to be clarified are related to protective immunity in humans, making surrogate end-points inadequate for evaluating TB vaccine efficacy. To date, no biomarker has been validated in TB, thereby hampering selection during the early phase and the clinical evaluation of new vaccine candidates.

Even though the systematic study and selection of vaccine antigens has led to the development of promising candidate vaccines [40, 46], these vaccines' efficacy in preventing TB in the human population remains to be determined. Studies carried out so far have suggested that TB vaccine development should not be limited to the most antigenic proteins during natural infection. It is hoped that Phase I and II clinical trials data with different antigens and vaccine delivery systems will be crucial to understanding which immunological parameters are important for vaccine efficacy.

The animal models which have helped during the early phases of research into protective antigens against TB and which have provided valuable information about vaccine candidate

safety, immunogenicity and efficacy have not so far led to predictive results being obtained in developing vaccines which can be used with human beings.

Despite consensus having been achieved regarding the fact that only a vaccine is the solution which will lead to eradicating TB from the world, there remain many unanswered questions in this field which must be resolved to ensure obtaining an efficient and safe vaccine against this emergent disease, which causes a large number of deaths annually. Significant advances have been made regarding basic and applied research directed towards finding definitive solutions against this disease, showing that a vaccine against TB can be obtained in the mid-term.

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Author details

Marisol Ocampo C.^{1,2}

Address all correspondence to: marisol.ocampo@urosario.edu.co

1 Head of the Tuberculosis Department, Fundación Instituto de Inmunología de Colombia, FIDIC, Carrera, Bogotá, Colombia

2 School of Medicine and Health Sciences, Universidad del Rosario, Bogotá, Colombia

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