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Review

Tuberculosis Vaccines: An Update of Recent and Ongoing Clinical Trials

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Abstract: TB remains a global health challenge and, until now, only one licensed vaccine (the BCG vaccine) is available. The main goal of this work is to assess the progress in the development of new TB vaccines and highlight the research in nanovaccines. A review was conducted using a methodology with the appropriate keywords and inclusion and exclusion criteria. The search revealed 37 clinical trials that were further reviewed. The results available have reported good immunogenicity and safety profiles for the vaccines under investigation. Over the last five years, the vaccines, VPM1002 and Vaccae, have moved ahead to phase III clinical trials, with the remaining candidate vaccines progressing in phase I and II clinical trials. RUTI and ID93+GLA-SE involve the use of nanoparticles. This strategy seems promising to improve the delivery, efficacy, cost, and storage conditions of the existing TB vaccines. In conclusion, the use of nanovaccines may be an option for both prevention and treatment. However, further studies are necessary for the development of novel TB vaccines.

Keywords: clinical trials; infectious diseases; nanomedicine; pulmonary tuberculosis; tuberculosis prevention

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1. Introduction

Tuberculosis (TB) is an infectious disease caused by the bacillus *Mycobacterium tuberculosis* (*M. tuberculosis*). *M. tuberculosis* in inhaled droplets enters the alveolar passages of exposed humans, contacting resident cells, and is ingested by the alveolar macrophages [1]. Within host macrophages, *M. tuberculosis* resides in a phagosome (Figure 1) where the bacteria is exposed to a hostile environment consisting of an acidic pH, lysosomal enzymes, toxic peptides, and reactive oxygen intermediates [2]. However, *M. tuberculosis* has evolved mechanisms to avoid this hostile vacuolar microenvironment and evade the host defense mechanisms [2,3]. With the progression of the disease, the infected macrophages produce chemokines that attract neutrophils, lymphocytes, and inactivated monocytes, leading to the formation of granulomatous focal lesions to contain the spread of the bacteria [4]. Depending on the host immune response, the infection may arrest here, referred to as latent TB, and persist in an asymptomatic nontransmissible state [1,3]. In those with effective cell-mediated immunity, the infection may arrest permanently, with the granulomas healing and resulting in fibrous-calcified lesions [2,3]. When the initial infection in the lung cannot be controlled, or when the host with latent infection has a suppressed immune system, the granuloma center can become liquefied, serving as a medium for the newly revived bacteria to replicate in an uncontrolled manner and progress to later stages of the disease, including dissemination to other parts of the body [1,2,4].

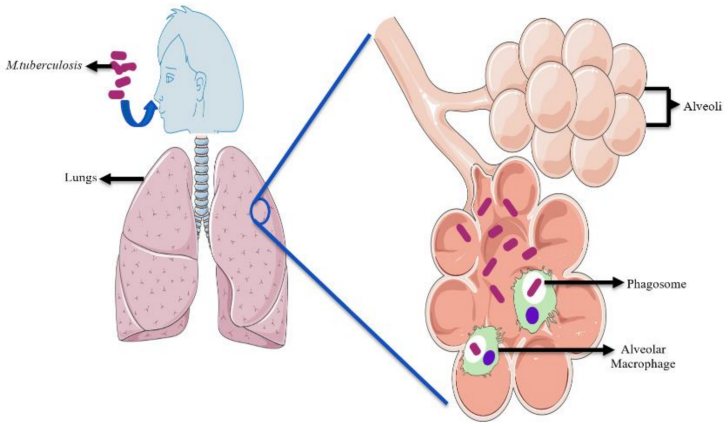

Figure 1. *M. tuberculosis* entry and presentation in alveoli.

TB is a disease that humanity has faced for millennia. In ancient Greece, Hippocrates accurately defined its symptoms and characteristic lung lesions, while Isocrates was the first to suppose its infectious nature [5]. By the eighteenth-century, TB had become epidemic in Western Europe, but was only recognized as a disease that not only affects the lungs in 1810 [5]. Further progress and insight into the disease was garnered throughout the nineteenth century, highlighted by the first successful remedy against TB in 1854, and in 1882, the successful attempt, by Robert Koch, at isolating the tubercle bacillus and reproducing the disease in inoculated animals [5]. This laid the foundation for the subsequent discoveries of tuberculin skin tests (1890), and for the development of the bacillus Calmette-Guérin (BCG) vaccine (1921) [5]. Nowadays, BCG is widely used and prevents TB in children, but has an immuno-protective effect only lasting 10 to 15 years, and is not effective in preventing TB in adults [5–8].

According to the 2020 World Health Organization (WHO) global TB report, TB remains one of the top 10 causes of death worldwide, with an estimated ten million people newly diagnosed in 2019 [9]. Globally, within the same period, over 1.2 million deaths were reported, reaffirming that, since 2007, TB has been the principal cause of death from a single infectious agent [9]. While effective drug treatments exist, the only licensed vaccine available for TB prevention remains the BCG vaccine, developed 100 years ago [5–7,9]. To fulfil the need for an effective vaccine for the adult population, provide longer lasting immunity in children, and ensure we meet the End TB Strategy target of 2035 (95% reduction in the absolute number of TB deaths per year) as set out by the WHO, it is of paramount importance to continue the research and development of new TB vaccines [4,9]. In the last decade, novel vaccines using nanoparticles (NPs) have been widely developed. These nanovaccines utilize particles smaller than 1000 nm, which can be biological or synthetic, in combination with pathogen-specific antigens [8,10]. The use of nanovaccines as a preventative option is being investigated for a host of infectious diseases, including acquired immunodeficiency syndrome (AIDS), malaria, influenza, and TB. The ability of nanovaccines to control antigen presentation, while inducing both cell-mediated and antibody-mediated immunity, make them an attractive potential tool in the fight against TB, bearing in mind its pathogenesis [10]. While not perfect, particularly with regard to the potential for nanomedicine toxicity, as well as a lack of regulatory guidelines, nanoparticles, when incorporated into vaccines, offer several advantages over traditional vaccines, as will be discussed later [8,10]. Over the last several years, numerous clinical trials have been conducted with novel vaccines against TB, but none have entered the market. The main objective of this review was to highlight the progress over the last five years in the development of new TB vaccines (Table 1), and to distinguish the potential use of nanovaccines as a novel approach.

Table 1. Vaccine candidate trials started/completed in 2015–2020.

Type of Vaccine	Name of Vaccine	Vaccine Composition	Start Date	Actual Completion Date	Phase	Status	ClinicalTrials.gov Identifier
TB subunit vaccine	M72/AS01E	Fusion protein Mtb32A & Mtb39A, AS01E adjuvant	January 2011 August 2014 November 2020	July 2015 November 2018 Est.* July 2022	II II II	Completed Completed Recruiting	NCT01262976 NCT01755598 NCT04556981
	GamTBvac	Ag85A & ESAT6-CFP10 fusion with dextran-binding domain immobilized on dextran mixed with adjuvant DEAE-dextran core, with CpG oligodeoxynucleotides	January 2017 December 2018 Est.* November 2021	December 2017 May 2020 Est.* November 2025	I II III	Completed Completed Not yet recruiting	NCT03255278 NCT03878004 NCT04975737
	H56:IC31	Fusion protein of Ag85B, ESAT-6, latent Rv2660c with IC31 adjuvant	August 2013 November 2014 May 2015 November 2015 June 2018 January 2019	November 2015 October 2016 December 2016 Est.* September 2019 Est.* June 2021 Est.* December 2024	I/II I I I II II	Completed Completed Completed Unknown Withdrawn Recruiting	NCT01865487 NCT02375698 NCT02378207 NCT02503839 NCT03265977 NCT03512249
	H4:IC31	H4 antigen, IC31 adjuvant	July 2013 February 2014 May 2015	December 2017 August 2017 October 2016	I/II II I	Completed Completed Completed	NCT01861730 NCT02075203 NCT02378207
	ID93 + GLA-SE	Fusion Rv1813, Rv2608, Rv3619, Rv3620 with GLA-SE adjuvant	September 2013 June 2015 October 2015 May 2018 October 2018 April 2019	July 2015 January 2017 August 2017 Est.* June 2020 June 2020 Est.* December 2020	I II I II I I	Completed Completed Completed Active Completed Active	NCT01927159 NCT02465216 NCT02508376 NCT03806686 NCT03722472 NCT03806699
	AEC/BC02	Ag85b antigen & ESAT-6/ CFP-10, BC02 adjuvant	April 2018 May 2020	October 2019 Est.* August 2021	I Ib	Completed Active	NCT03026972 NCT04239313
Recombinant live vaccine	TB/FLU-01L	Replication-deficient recombinant influenza virus A expressing ESAT-6 antigen	October 2013	February 2015	I	Completed	NCT03017378
	TB/FLU-04L	Attenuated replication- deficient influenza virus vector expressing antigens Ag85A & ESAT-6	October 2013	February 2015	I	Completed	NCT02501421
	Ad5Ag85A	Adenovirus serotype 5 expressing Ag85A	September 2017	Est.* September 2021	I	Recruiting	NCT02337270
	ChAdOx1-85A	Chimpanzee adenoviral expressing Ag85A	July 2013 January 2019 July 2019	April 2016 August 2020 Est.* January 2022	I I I	Completed Completed Recruiting	NCT01829490 NCT04121494 NCT03681860
	MVA85A	Recombinant replication-deficient modified Vaccinia virus Ankara expressing Ag85A	October 2012 October 2013 September 2015 July 2019	May 2015 January 2016 October 2018 Est.* January 2022	II I I IIa	Completed Completed Terminated Recruiting	NCT01650389 NCT01954563 NCT02532036 NCT03681860
	VPM1002	Recombinant BCG vaccine with listeriolysin O encoding gene.	June 2015 December 2017 November 2020	November 2017 Est.* May 2022 Est.* June 2025	II II/III III	Completed Recruiting Recruiting	NCT02391415 NCT03152903 NCT04351685
Attenuated live vaccine	MTBVAC	Attenuated *M. tuberculosis* clinical isolate with ESAT6 & CFP10 & independent stable genetic deletions of *phoP* & *fadD26* genes	November 2015 January 2019 February 2019 Est.* July 2022	March 2018 Est.* March 2021 Est.* December 2020 Est.* September 2029	I/II I/II II III	Completed Unknown Unknown Not yet recruiting	NCT02729571 NCT02933281 NCT03536117 NCT0497517
Inactivated TB vaccine	DAR-901	Agar-grown SRL172 by scalable, broth-grown manufacturing technique	February 2014 March 2016	June 2016 February 2020	I II	Completed Completed	NCT02063555 NCT02712424
	RUTI	Polyantigenic liposomal vaccine of detoxified, fragmented *M. tuberculosis*	January 2019 Est.* September 2021	Est.* July 2020 Est.* September 2023	II II	Unknown Not yet recruiting	NCT02711735 NCT04919239
	Vaccae	Heat-killed *M. vaccae*	October 2013	November 2017	III	Completed	NCT01979900
DNA vaccine	GX-70	Antigen plasmids *M. tuberculosis* & Flt3 ligand	March 2018	August 2018	I	Withdrawn	NCT03159975

Est.*—Estimated.

2. Materials and Methods

A search was carried out using the ClinicalTrials.gov database (www.clinicaltrials.gov, accessed on 12 September 2021). The selected keywords were "tuberculosis" in the condition/disease category, and "vaccines", "immunization", or "vaccination" in the other term categories. Accordingly, 172 potential clinical trials were identified (Figure 2). Study titles, study start and completion dates, study conditions, and interventions were read, and

clinical trials were included based on the following inclusion criteria: with a study start date or completion date within the last five years, concerning TB, and investigating a novel vaccine. Since this review is focused on novel TB vaccines, the exclusion criterion included any clinical trial investigating the current BCG vaccine or improvements to the current vaccination regime. On the basis of these criteria, 128 clinical trials were excluded. From those: 58 trials were excluded as they did not fulfil the "period of the last five years" criterion; 21 trials were focused on the current BCG vaccine; 18 trials investigated TB drug treatment modalities; 13 trials dealt with diagnosing or screening for TB; 4 trials focused on evaluating the effect of comorbidities on TB and vaccination; 3 trials explored vaccination programs; 4 trials involved the investigation of the immune response to TB; 2 trials investigated the effect of vitamin supplementation; 3 trials were focused on epidemiology; and 2 trials were excluded for assessing the effect of HIV and/or active TB on the immune response to the influenza vaccine. In total, 44 clinical trials were selected for review.

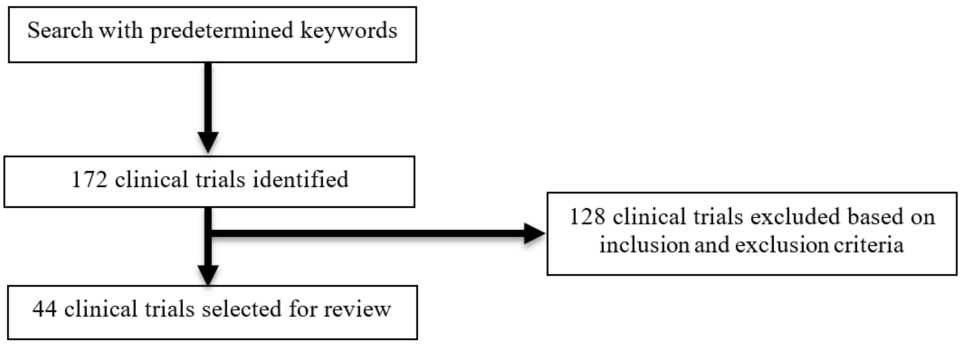

Figure 2. Literature review methodology.

These 44 clinical trials have been subdivided according to the vaccine type. The primary findings of clinical trials already completed, and with results available, have been summarized. The clinical trials that were recruiting, active, or that have been withdrawn or terminated were mentioned, and their results are discussed in the following section.

3. TB Novel Vaccines

The novel TB vaccines under clinical trials were categorized according to their vaccine type as: subunit vaccines; recombinant live vaccines; attenuated live vaccines; inactivated vaccines; and DNA vaccines (Figure 3).

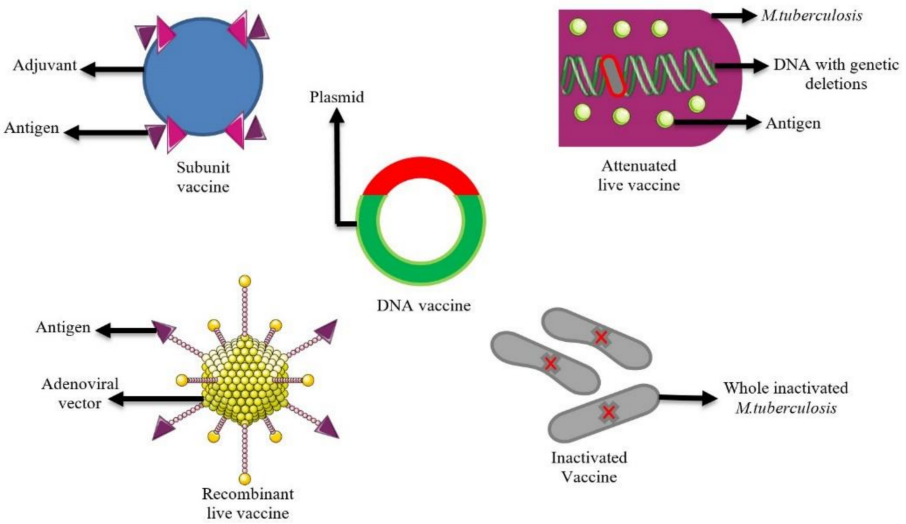

Figure 3. Vaccine types.

3.1. TB Subunit Vaccines

These vaccines have immunoactive ingredients, including proteins, isolated and purified from *M. tuberculosis* [6]. Their properties are enhanced with the addition of an adjuvant [6].

3.1.1. M72/AS01E

This vaccine is a recombinant fusion protein derivative of the *M. tuberculosis* antigens, Mtb32A and Mtb39A, in combination with the AS01E adjuvant system [11,12]. In 2010, a phase II clinical trial in infants assessed the safety and immunogenicity of M72/AS0113. An acceptable safety profile was demonstrated, with no safety concerns identified, and adverse events (AEs) and serious adverse events (SAEs) being equal to those in similar vaccine studies [13]. Further noted was the M72-specific CD4+ T cell responses that were significantly higher after two M72/AS01 doses, even up to one-year post vaccination [13]. Similar assessments were made in a phase II clinical trial, completed in 2015, assessing the safety and immunogenicity of M72/AS01E in HIV-positive (HIV+) adults, aged 18–59 years, living in a TB-endemic region (ClinicalTrials.gov: NCT01262976). In this double-blind trial, 240 participants were enrolled in three cohorts each, with a placebo comparator: HIV+ adults on highly active antiretroviral therapy (HAART); HIV+ adults not on HAART; and HIV-negative (HIV-) adults. Depending on the study group, two doses of M72/AS01E, or placebo (saline), were administered at day 0 and day 30. Across doses in the total study population, a headache preventing normal activity (3.75%) was the most reported solicited AE, followed by malaise (1.25%), myalgia (0.83%), and gastrointestinal symptoms (0.4%). SAEs (medical occurrences resulting in death, considered life-threatening, requiring hospitalization or prolongation of hospitalization, or resulting in disability/incapacity) were reported in 1.25% of the total study population. The antimycobacterium tuberculosis fusion protein (M72)-specific antibody concentrations were assessed in all groups at days 0, 30, 60, 210, and at years 1, 2, and 3, as measured by the enzyme-linked immunosorbent assay (ELISA), and given in ELISA units per millilitre (EU/mL). At day 0, all groups were found with antibody concentrations between 1.4 and 1.5 EU/mL. However, all intervention groups were found with increased antibody concentrations on the subsequent days, peaking at day 60 (range 249.4–754.5 EU/mL), and declining until year 3, where antibody concentrations within the intervention groups were found to range from 4.9–24.3 EU/mL. Within these intervention groups, the percentages of seroconverted subjects for M72-specific antibodies ranged from 66.7–97.3%. These findings were substantiated with the phase IIb clinical trial, where adults (18–50 years old) with *M. tuberculosis* infection (defined by positive results on an IFN-γ release assay), without evidence of active TB disease, were randomly assigned in a 1:1 ratio to receive two doses of either M72/AS01E or placebo, administered one month apart (ClinicalTrials.gov: NCT0175598). The results of the three-year final analysis of the efficacy, safety, and immunogenicity demonstrated that the efficacy at month 36 was 49.7% (90% confidence interval (CI), 12.1 to 71.2; 95% CI, 2.1 to 74.2) in the M72/AS01E group, whereas the concentrations of M72-specific antibodies, and the frequencies of M72-specific CD4+ T cells, increased after the first dose, and were sustained throughout the follow-up period [11]. Pyrexia, as an SAE, was recorded in the intervention group, with no deaths determined to be related to the trial regimen [11]. Potential immune-mediated diseases were reported in the intervention group [11]. Currently recruiting is a phase II clinical trial to assess the safety and immunogenicity of the M72/AS01E vaccine in virally supressed antiretroviral-treated participants with HIV (ClinicalTrials.gov: NCT04556981).

3.1.2. GamTBvac

The recombinant subunit vaccine, GamTBvac, is also under clinical trial. It consists of two mycobacterial antigen fusions (Ag85A and ESAT6-CFP10) fused with dextran-binding domain (from *Leuconostoc mesenteroides*) immobilized on dextran, and mixed with an adjuvant consisting of a DEAE-dextran core, and with CpG oligodeoxynucleotides (TLR9 agonists) [14]. The vaccine was assessed for immunogenicity and protective efficacy

in GamTBvac-prime/boost and BCG-prime/GamTBvac-boost murine and guinea pig TB models [14]. The results showed that GamTBvac had strong immunogenicity and significant protective effects against the *M. tuberculosis* strain, H37Rv, under inhalatory and intravenous routes, with a strong protective effect as a BCG booster vaccine [14]. Such results led to the completion of a phase I clinical trial evaluating the safety and immunogenicity of this vaccine against TB among 60 healthy volunteers, aged 18–49 years (ClinicalTrials.gov: NCT03255278). The study was designed as a comparative placebo-controlled study, with a two-fold increase of an applied dose among the participants. First, the safety of the vaccine was evaluated, followed by the optimal dose and vaccination administration scheme. The trial was completed in December 2017, but no results have been posted yet (ClinicalTrials.gov: NCT03255278). Recently completed was a phase II double-blind randomized (in 3:1 ratio vaccine: placebo) clinical trial to assess the safety, reactogenicity, and immunogenicity of GamTBvac in 180 healthy BCG-vaccinated adults (ClinicalTrials.gov: NCT03878004). Results are, however, yet to be posted. Although not yet recruiting, a multicentre double-blind randomized placebo-controlled phase III clinical trial is underway that aims to assess the safety and efficacy of GamTBvac against the development of primary respiratory TB not associated with HIV infection in healthy volunteers between the ages of 18 and 45 years (ClinicalTrials.gov: NCT04975737).

3.1.3. H56:IC31

Clinical trials are also ongoing in post-exposure vaccines, including H56:IC31, which is specifically targeted at the *M. tuberculosis*-infected population. H56:IC31 contains a fusion protein of three mycobacterial antigens (early secreted Ag85B and ESAT-6, and the latent Rv2660c) formulated in the Th1-stimulating IC31 adjuvant [15]. Within healthy HIV-adults, previous trials showed no SAEs, with transient AEs when using the vaccine [15]. In addition, within these same trials, antigen-specific IgG responses and Th1 cytokine-expressing CD4+ T cells were observed [15]. A completed phase I trial evaluated the safety and immunogenicity of H4:IC31, H56:IC31, and BCG vaccination in previously BCG-vaccinated healthy adolescents (ClinicalTrials.gov: NCT02378207). Within the H56:IC31 group, 20/24 participants presented AEs. After administration of the vaccine (5 mcg H56/500 nmol IC31 administered as 0.5 mL) at days 0 and 56, 45% of the group presented a response rate to Ag85B on day 70, with this decreasing to 9.5% on day 168. Similarly, response rates to TB10.4 were higher at day 70 (5.6%) compared to day 168 (0%). A similar safety profile was found in another phase I trial testing the safety and immunogenicity of the vaccine in adults who had recently been successfully treated for drug-susceptible pulmonary TB (ClinicalTrials.gov: NCT02375698). Within the H56:IC31 group, 87.5% of the participants reported an AE, however no SAEs were found. The immunogenicity was assessed via a mean percent change from the baseline of participant responses to the TB antigens, Ag85A and ESAT-6, which were found to be 0.014% and 0.026%, respectively, at day 224. A further phase I clinical trial is currently active and is further evaluating the safety and immunogenicity of H56:IC31, in conjunction with a COX-2 inhibitor, to test the hypothesis that this may strengthen the vaccine response (ClinicalTrials.gov: NCT02503839). The optimal dose of the vaccine was also investigated in a phase I/II trial, which found that two or three vaccinations at a dose of 5 mg:500 nmol (H56:IC31) induced durable antigen-specific CD4 T cell responses with acceptable safety and tolerability profiles in TB-infected and uninfected adults (ClinicalTrials.gov: NCT01865487). A phase II clinical trial to evaluate H56:IC31 in preventing TB reinfection is currently recruiting (ClinicalTrials.gov: NCT03512249), while another phase II trial was withdrawn prior to study procedures being done (ClinicalTrials.gov: NCT03265977).

3.1.4. H4:IC31

H4:IC31, another candidate vaccine, is composed of two active components: H4 antigen (fusion protein of *M. tuberculosis* antigen 85B and TB10.4), and an immunological adjuvant called IC31®, which is a combination of the antimicrobial peptide KLK and

oligodeoxynucleotide (ODN) 1a (ODN1a) [12,16]. Previous phase I clinical trials have proven an acceptable safety profile with immunogenicity, capable of triggering multifunctional CD4+ T cell responses in previously BCG-vaccinated healthy individuals [16]. These same trials provided the optimal antigen-adjuvant dose combinations [16]. The previously mentioned clinical trial (ClinicalTrials.gov: NCT02378207) also investigated the safety and immunogenicity of H4:IC31 in healthy adolescents. In the H4:IC31 vaccine group, 24 participants were administered 15 mcg H4/500 nmol and IC31 IM as 0.5 mL at days 0 and 56. 91.6% of these participants reported AEs (compared with 83.3% in the H56/IC31 group), with the most common being pain at the injection site and a headache. No SAEs were reported in this group. However, the response rate at day 168 for both Ag85B and TB10.4 was the highest, registering 21.7% and 8.7%, respectively, compared to 9.5% Ag85B and 0% TB10.4 in the H56:IC31 group. A phase II clinical trial assessing the safety, immunogenicity, and prevention of TB with the vaccine in a similar population group, in comparison to BCG revaccination, was also carried out (ClinicalTrials.gov: NCT02075203). The 989 healthy adolescents were enrolled, of which 330 made up the H4:IC31 cohort. This group received two doses of 15 mcg H4/500 nmol IC31 each on days 0 and 56. The results indicate that 35.76% of participants reported AEs in this cohort, compared with 99.7% of participants reporting AEs in the BCG revaccination group (n = 330). The H4:IC31 cohort also showed a higher percentage of participants with an immune response compared to the BCG revaccination group. At day 70, 81.8% of participants showed an immune response measured by a 13-colour intracellular cytokine staining assay performed on peripheral blood mononuclear cells to assess CD4+ T cells expressing IFN-γ, TNF, IL-2, IL-17, IL-22, CD107a, and/or CD154, alone or in combination, in response to stimulation with peptide pools representing the entire amino acid sequence of the TB mycobacterial antigens, Ag85B and TB10.4, and BCG antigens, compared with 16% in the BCG revaccination group. Although not available yet, results have been submitted for another phase I/II clinical trial to assess the safety and immunogenicity of H4: IC31 in BCG-primed infants (ClinicalTrials.gov: NCT01861730).

3.1.5. ID93 + GLA-SE

Over the last half decade, much research has also been conducted on ID93+GLA-SE. This is a subunit vaccine comprising four antigens representing different families of *M. tuberculosis* proteins. These proteins include two predicted outer membrane proteins, Rv1813 and Rv2608, and two secreted proteins, Rv3619 and Rv3620, belonging to the ESAT-6 family [17]. In combination with these is the Th1-inducing synthetic TLR4-agonist adjuvant, Glucopyranosyl Lipid A (GLA), formulated in a stable oil-in-water nanoemulsion (SE) [12,17]. In animal models, this vaccine demonstrated prophylactic and therapeutic immunization potential and, ultimately, human trials followed thereafter [17].

A randomized double-blind placebo-controlled phase I trial to assess the safety and immunogenicity of ID93+GLA-SE in healthy BCG-vaccinated adults was completed in 2015 (ClinicalTrials.gov: NCT01927159). This trial enrolled 66 healthy HIV-adults, with an average age of 25 years, who were randomly assigned to five groups: one placebo and four cohorts, with varying doses of the vaccine [18]. Following randomization, the study population was divided as follows: the placebo group (saline, n = 12); Cohort 1 (ID93 (10 µg) + GLA-SE (2 µg, n = 9); Cohort 2 (2 µg ID93 + 2 µg GLA-SE, n = 15); Cohort 3 (10 µg ID93 + 2 µg GLA-SE, n = 15); and Cohort 4 (10 µg ID93 + 5 µg GLA-SE, n = 15) [18]. On days 0, 28, and 112, participants were injected intramuscularly as per their enrolled cohort [18]. All participants were followed over a six-month period, and injection site reaction and AEs were assessed at days 1, 3, 7, 14, and 28 after each injection [18]. Antibodies and T cell responses were also measured [18]. The AEs reported were mild or moderate in nature, with no SAEs in the vaccine groups [18]. In assessing vaccine-induced immunogenicity, ID93+GLA-SE vaccination induced marked rapid increases in the frequencies of total cytokine-expressing CD4+ T cells specific to Rv1813, Rv2608, Rv3619, and Rv3620 in all four cohorts [18]. ID93-specific IgG responses also increased significantly after the three ID93+GLA-SE vaccinations in participants from all four cohorts, with levels maintained

above those of prevaccination, and of those in the placebo recipients at the end of the study [18]. Humoral responses were also detected against all four antigens specifically dominated by IgG1 and IgG3 [18]. A further phase I randomized double-blind clinical trial was completed in which the safety, tolerability, and immunogenicity of ID93+GLA-SE was evaluated (ClinicalTrials.gov: NCT02508376). While no results have been released yet, this study differed in that ID93 was administered alone and in combination with GLA-SE, as well as in combination with the AP10-602 adjuvant. Recently, a phase I clinical trial evaluating the safety, immunogenicity, and tolerability of ID93+GLA-SE in adults (ClinicalTrials.gov: NCT03722472) was completed, with the results yet to be posted. A similar clinical trial evaluating the same parameters, but in adolescents and in the phase II stage (ClinicalTrials.gov: NCT03806686), is underway. Further clinical trials have also progressed to phase II. A triple-blind phase IIa clinical trial in TB patients following completion of treatment has been conducted (ClinicalTrials.gov: NCT02465216). After enrolment and randomized allocation, 60 participants were divided into four groups with differing doses of antigen and adjuvant: Group 1: low-dose antigen and adjuvant; Group 2: high-dose antigen, low-dose adjuvant; Group 3: low-dose antigen, high-dose adjuvant; Group 4: low-dose antigen, high-dose adjuvant; and one placebo group (saline). Group 4 differed in that it received the vaccine three times, each one at days 0, 28, and 56, whereas all the other groups received the vaccine twice, once on day 0, and once on day 56. Within the vaccine groups, no SAEs were reported throughout the study duration. However, AEs were reported by all participants in Groups 1 and 2, and by 92.8%, 85.7%, and by 75% of the participants in Groups 3, 4 and placebo, respectively. At day 70, the IgG antibody responder rate was 100% throughout all vaccine groups; however, the CD4+ T cell responder rate varied between 11.1% and 90.9%. Presently, a phase IIa-clinical trial to assess the safety, immunogenicity, and efficacy of ID93+GLA-SE in BCG-vaccinated healthcare workers, is underway (ClinicalTrials: NCT03806686).

3.1.6. AEC/BC02

AEC/BC02 represents another promising vaccine candidate. It adds a new adjuvant system, BC02, based on BCG-derived CpG and aluminium salt, to the Ag85b antigen and the ESAT-6/CFP-10 (EC) fusion protein [19]. Previous studies have demonstrated the safety of BC02, and have observed an increase in both antigen-specific IL-12 secretion by peritoneal macrophages, and the number of antigen-specific T cells that release IFN-γ [19]. An investigation in animal models re-emphasized the induction of a strong cellular immune response by the vaccine, but found it did not protect against *M. tuberculosis* when used as a pre-exposure vaccine [19]. In the latent infection animal model, however, the vaccine was found to successfully control the reactivation of *M. tuberculosis* [19]. A recently completed phase I clinical trial assessed the degree of immunity provided by the vaccine and also its safety profile (ClinicalTrials.gov: NCT03026972), while a further phase Ib clinical trial is currently active (ClinicalTrials.gov: NCT04239313).

3.2. Recombinant Live Vaccines

In these vaccines, a live vector is used to deliver heterologous antigens. An immune response is elicited towards the heterologous antigen being presented, as well as because of the viral vector's capacity for infection and its immunological properties [20]. Thus, viral vectors act as delivery units for TB antigens.

3.2.1. TB/FLU-01L

A phase I clinical trial, completed in 2015, investigated TB/FLU-01L, a vaccine comprising a replication-deficient recombinant influenza virus A expressing ESAT-6 antigen (ClinicalTrial.gov: NCT03017378). In this 42-day trial, consisting of 36 healthy male or female volunteers, aged 18–50 years old, the safety and systemic immune response elicited by the vaccine were assessed after intranasal (n = 18), or sublingual (n = 18), administration at day 1 and day 21 (two doses total) [21]. In both groups, seven days following the first

dose, only two participants reported AEs, namely, nasal congestion and sneezing [21]. In the sublingually administered group, a further two participants reported pharynx hyperaemia [21]. Similarly, only one participant in each intervention group reported systemic AEs, being fever (sublingual vaccine group), and sore throat (nasal vaccine group) [21]. After the second dose (seven days), only one participant reported pharynx hyperaemia in the sublingual vaccine group [21]. In no case was shedding of the vaccine virus detected [21]. After analyzing the immunogenicity, we found that 72.2% of the subjects from the sublingual group, and 77.8% subjects from the immunized intranasal group, demonstrated detectable responses (defined as any cytokine response at any time point) [21].

3.2.2. TB/FLU-04L

Another mucosal-vectored vaccine in the clinical trials is TB/FLU-04L. It is based on an attenuated replication-deficient influenza virus vector expressing the antigens Ag85A and ESAT-6 [9]. It was designed as a prophylactic boost vaccine for infants, adolescents, and adults [9]. While no results were made available, a phase I double-blind randomized placebo-controlled trial exploring the safety and immunogenicity of two doses (day 1 and day 21) of TB/FLU-04L versus the matched placebo in BCG-vaccinated healthy adults, aged 18–50 years, was conducted and completed in 2015 (ClinicalTrials: NCT02501421). Currently, a phase II trial concerning latent TB infection is in implementation [9].

3.2.3. Ad5Ag85A

Adenovirus, as a vector, is also being used to develop vaccines for TB. Ad5Ag85A is an adenovirus serotype 5 vector expressing Ag85A [22]. Initially, animal models with Ad5Ag85A demonstrated better protection over the BCG vaccine alone, with immunization via the respiratory passages, providing better immunity [22]. In phase I human trials, intramuscular vaccination was observed to be safe and immunogenic, stimulating polyfunctional T cell responses [22]. Currently recruiting is a phase I trial involving healthy volunteers previously immunized with BCG (ClinicalTrials.gov: NCT02337270). The trial intends to evaluate the safety and immune responses in the blood and the lungs after administration by aerosol of Ad5Ag85A, with completion estimated in 2021.

3.2.4. ChadOx1-85A

A novel chimpanzee adenoviral-vectored vaccine expressing Ag85A is currently being explored in the ChAdOx1-85A vaccine. Researchers believe that simian adenoviral vectors are advantageous because of the low prevalence of antivector antibodies in humans, a consideration that has limited adenovirus use to date [23]. Murine studies show that ChAdOx1-85A is protective when it is part of a BCG-ChAdOx1 85A-MVA85A immunization plan [23]. The first human phase I trial to evaluate the safety and immunogenicity of ChAdOx1-85A alone, and as a prime-boost regime with MVA85A, in healthy BCG-vaccinated adults has been conducted (ClinicTrial.gov: NTC01829490). A total of 42 healthy BCG-vaccinated adults were divided into four groups: six adults receiving ChAdOx1-85A alone (group 1); 12 receiving ChAdOx1-85A at a higher dose (group 2); 12 receiving a ChAdOx1-85A prime–MVA85A boost (group 3); and 12 receiving a ChAdOx1 85A–ChAdOx1 85A prime–MVA85A boost combination (group 4) [23]. Groups 1 and 2 (n = 18) were followed for 168 days, while Groups 3 and 4 were followed for 224 days and 287 days, respectively [23]. Most AEs reported were mild to moderate, with no SAEs [23]. By assessing immunogenicity, it was found that Ag85A-specific ELISpot and intracellular cytokine CD4+ and CD8+ T cell responses were induced, while polyfunctional CD4+ T cells, and IFN-γ, TNF-α, and CD8+ T cells were induced by ChAdOx1-85A and boosted by MVA85A23. ChAdOx1-85A also induced serum Ag85A IgG responses, which were boosted by MVA85A [23]. A further phase I trial was recently completed comparing aerosol and intramuscular administration (ClinicalTrials.gov: NCT04121494), while another evaluating the safety and immunogenicity of ChAdOx1-85A in the adult and adolescent population is currently recruiting (ClinicalTrials.gov: NCT03681860). A phase IIa randomized clinical trial is set to follow.

3.2.5. MVA85A

The vaccinia virus was also identified as a promising vector to be used in the development of a TB vaccine. MVA85A has been, and continues to be, in clinical trial. This is a vaccine comprising a recombinant replication-deficient modified vaccinia virus, Ankara (MVA), expressing the *M. tuberculosis* Antigen 85A (Ag85A) [24]. Previous clinical trials found that MVA85A is well-tolerated and highly immunogenic when administered as a boost to BCG-primed individuals, as well as capable of intramuscular, intradermal, and aerosol administration [24,25]. With this information, a clinical trial was carried out to ascertain whether altering these administration and intradermal vaccination routes, and if specifically altering the aerosol route, would boost cellular immunity to the Ag85A (ClinicalTrials.gov: NCT01954563). In this phase I blinded trial, 36 BCG-vaccinated adults, aged 21–42 years, were randomized equally between three groups to receive two MVA85A vaccinations, one month apart, using the following regime: Group 1, aerosol–intradermal immunisation; Group 2, intradermal–aerosol immunisation; and Group 3, intradermal–intradermal immunisation [26]. Peripheral blood was collected, and AEs recorded over a six-month period [26]. Most AEs reported were mild injection site reactions after intradermal vaccination [26]. Short duration systemic AEs after vaccination by both routes were mild, as were respiratory AEs following primary aerosol MVA85A (Group 1) [26]. The most significant AEs were seen when boosting an intradermal MVA85A prime with an aerosolized MVA85A boost one month later (Group 2), which led to transient moderate, or severe respiratory, and systemic AEs [26]. No SAEs were reported [26]. A modest significant boosting of the cell-mediated immune response to Ag85A was verified, while all three groups were found to have systemic cellular immune responses to the MVA vector [26]. Serum antibodies to Ag85A and MVA were only found after intradermal vaccination [26]. Aerosolized MVA85A induced significantly higher levels of Ag85A lung mucosal CD4+ and CD8+ T cell cytokines compared to intradermal vaccination, while boosting with aerosol-inhaled MVA85A enhanced intradermal primed responses in Group 2 [26]. With the BCG vaccine contraindicated in HIV-infected infants, the MVA85A vaccine was studied as a candidate vaccine for HIV exposed infants [27]. A double-blind randomized controlled trial, in which 248 HIV-exposed infants were enrolled, compared MVA85A prime vaccination against a Candin® control, followed by selective deferred BCG vaccination at the age of eight weeks for HIV-uninfected infants, and a twelve-month follow-up for safety and immunogenicity (ClinicalTrials.gov: NCT01650389). Throughout the study, mild–moderate reactogenicity events were seen after newborn MVA85A vaccination, but no significant difference was observed in the rate of SAEs, HIV acquisition, or incident TB disease compared to the control group [27]. Vaccination with MVA85A resulted in significantly higher Ag85A-specific IFNγ and CD4+ T cells compared to the control, at weeks 4 and 8 ($p < 0.0001$) [27]. BCG did not further boost this response in those vaccinated with MVA85A, whereas the BCG-induced Ag85A-specific IFNγ+ CD4+ T cell response at weeks 16 and 52 was similar between the control group and the intervention groups [27]. A clinical trial evaluating the immunogenicity of the vaccination after aerosol and intramuscular administration in adults with latent TB was terminated (ClinicalTrials.gov: NCT02532036), while another phase IIa randomized clinical trial in adolescents and adults to assess the vaccines immunogenicity is currently recruiting (ClinicalTrials.gov: NCT03681860).

3.2.6. VPM1002

VPM1002 presents another opportunity with recombinant live vaccines. VPM1002 is a recombinant BCG vaccine in which the *urease C* gene (responsible for the inhibition of phagolysosomal maturation) has been replaced by the *listeriolysin O*-encoding gene from *Listeria monocytogenes* [28]. It has shown increased immunogenicity, efficacy, and safety in preclinical studies, as well as in phase I and II clinical trials in both infants and adults [28,29]. A further phase II clinical trial was completed in 2017 investigating the safety and immunogenicity of VPM1002 in comparison with BCG in HIV-exposed and unexposed newborn infants (ClinicalTrials.gov: NCT02391415). In this double-blind randomized

controlled trial, 416 participants were enrolled. Although results have not yet been made available, further progress is being made, as evidenced by the currently recruiting phase II/III clinical trial in which two groups of adults successfully cured of category 1 pulmonary TB will receive either VPM1002 or placebo (ClinicalTrials.gov: NCT03152903). The single dose of VPM1002/placebo will be administered, and the efficacy of the vaccine calculated against TB recurrence. In addition, a phase III double-blind randomized clinical trial has begun recruitment in which the efficacy, safety, and immunogenicity of VPM1002, in comparison to BCG, in preventing TB infection in newborn infants will be evaluated (ClinicalTrials.gov: NCT04351685).

3.3. Attenuated Live Vaccines

These vaccines contain a version of the living pathogenic organism, which has been weakened so as not to cause serious disease when administered [30].

MTBVAC

MTBVAC is a live attenuated mycobacterial vaccine. It is based on a rationally attenuated *M. tuberculosis* clinical isolate belonging to modern lineage 4 (one of the most widespread lineages among humans), that conserves most of the T cell epitopes described for TB, including the antigens ESAT6 and CFP10 of the RD1, while incorporating two independent stable genetic deletions of the *phoP* and *fadD26* genes [31]. It is hoped that this vaccine may replace the BCG vaccine in newborns and also be utilised as a preventative vaccine in adolescents and adults. The preclinical studies highlighted that MTBVAC induced immunity to ESAT6 and CFP10 and demonstrates improved efficacy in comparison to BCG [31]. As a result, a randomized controlled double-blinded dose escalation trial in adults and neonates was conducted (ClinicalTrials.gov: NCT02729571). In this trial, eighteen adults were enrolled and randomly assigned, nine each, to the BCG and MTBVAC groups [32]. In addition, thirty-six infants were enrolled and randomly assigned: eight to the BCG group; nine to the 2.5×10^3 CFU MTBVAC group; nine to the 2.5×10^4 CFU group; and ten to the 2.5×10^5 CFU group [32]. Safety and immunogenicity analyses were completed in those receiving a dose of vaccine. Mild injection-site reactions occurred only in infants in the BCG and the 2.5×10^5 CFU MTBVAC groups [32]. Systemic AESs were evenly distributed across BCG and MTBVAC dose groups and were mostly mild in severity [32]. Moreover, eight SAEs were reported in seven vaccine recipients (one adult MTBVAC recipient, one infant BCG recipient, one infant in the 2.5×10^3 CFU MTBVAC group, two in the 2.5×10^4 CFU MTBVAC group, and two in the 2.5×10^5 CFU MTBVAC group), and one infant died as a result of possible viral pneumonia [32]. Vaccination with all MTBVAC doses induced durable antigen-specific Th1 cytokine-expressing CD4 cell responses in infants, peaking 70 days postvaccination, and detectable 360 days after vaccination [32]. For the highest MTBVAC dose, the response exceeded responses induced by an equivalent dose of the BCG vaccine up to 360 days postvaccination [32]. Dose-related IGRA conversion was noted in 38% of infants in the 2.5×10^3 CFU MTBVAC group, in 75% of infants in the 2.5×10^4 CFU MTBVAC group, and in 78% of infants in the 2.5×10^5 CFU MTBVAC group, at day 180, compared with 0% in the BCG group [32]. By day 360, IGRA reversion had occurred in all infants in the 2.5×10^3 CFU MTBVAC group, in 67% of infants in the 2.5×10^4 CFU MTBVAC group, and in 43% of infants in the 2.5×10^5 CFU MTBVAC group [32]. In the adult groups, only secondary outcomes, including local injection-site, systemic reactions, and haematology and biochemistry, at days 7 and 28 were measured [32]. A phase II clinical trial to assess the dose-defining safety and immunogenicity of MTBVAC in neonates (ClinicalTrials.gov: NCT03536117), and a further phase II clinical trial assessing the same parameters in adults with and without latent TB, had been recruiting, however, the current status is unknown (ClinicalTrials.gov: NCT02933281). Not yet recruiting is a phase III clinical trial in which the safety, immunogenicity, and efficacy of the MTBVAC vaccine will be evaluated in HIV-uninfected infants

born to HIV-infected, and HIV-uninfected, mothers, as compared to the standard BCG vaccination regime (Clinicaltrials.gov: NCT04975178).

3.4. Inactivated Tuberculosis Vaccines

Inactivated TB vaccines, long since established for TB prevention and treatment, continue to be investigated. These are vaccines with inactivated whole bacteria or cleavage fragments thereof, prepared physically or chemically [6].

3.4.1. DAR-901

DAR-901 is a vaccine prepared from the Master Cell Bank of agar-grown SRL172 by a new scalable broth-grown manufacturing technique. Murine trials comparing a BCG booster to the DAR-901 booster indicated that DAR-901 conferred superior protection from a TB challenge [33]. A phase I clinical trial measuring the CD4+ T cell cytokine response to the DAR-901 booster vaccine in BCG- primed adults was completed (ClinicalTrials.gov: NCT02063555). In this clinical trial, 28 adults with negative IFNγ release assays were subdivided into three groups: ten participants received three intradermal doses of DAR-901, nine participants received three intradermal doses of saline placebo, and the other nine received two doses of saline followed by a single intradermal dose of BCG [33]. All intradermal doses were given at 0, 2 and 4 months. The results found that DAR-901 recipients exhibited increased DAR-901 antigen-specific polyfunctional or bifunctional T cell responses compared to baseline [33]. Vaccine-specific CD4+ IFNγ, IL2, TNFα, and any cytokine responses, peaked at seven days after Dose 3 [33]. Th1 responses predominated, with most responder cells exhibiting a polyfunctional effector memory phenotype [33]. However, the BCG vaccine induced greater CD4+ T cell responses than the placebo, while the DAR-901 responses did not differ from the placebo [33]. Neither the DAR-901 vaccine, nor the BCG vaccine, induced substantial or sustained Th17 /Th22 cytokine responses [33]. A phase II clinical trial has been completed to assess DAR-901 as a booster vaccine to prevent TB in BCG-primed adolescents (ClinicalTrials.gov: NCT02712424). The results have been submitted but have not yet been made available.

3.4.2. RUTI

Phase II clinical trials have also been conducted and are ongoing in the use of another inactivated TB vaccine. RUTI is a polyantigenic liposomal vaccine made of detoxified fragmented *M. tuberculosis* cells, indicated for the prevention of active TB in subjects with latent TB infection [34]. Murine models demonstrated that RUTI could be given as an adjunctive intervention to already proven therapeutic agents [35]. The TB therapy with rifampicin and isoniazid was markedly more effective when treatment was administered concurrently with a regime of RUTI administration in weeks 17, 19, and 21 post infections [35]. RUTI triggered a Th1/Th2 response, as demonstrated by the production of IgG1, IgG2a, and IgG3 antibodies against a wide range of peptides [35]. Previous clinical trials revealed that RUTI was reasonably well-tolerated, while triggering specific immunological responses against *M. tuberculosis* in healthy subjects, compared to the placebo [35]. This was further confirmed in people with latent TB infection [34]. A phase II clinical trial, to test the safety of RUTI vaccination in those with multidrug-resistant TB after successful treatment is registered, however, its status is unknown (ClinicalTrials.gov: NCT02711735), while a further phase II clinical trial to evaluate the efficacy of RUTI vaccination in drug sensitive and multidrug-resistant patients is planned, but not yet recruiting (ClinicalTrials.gov: NCT04919239).

3.4.3. Vaccae

Vaccae is a vaccine composed of heat-killed *Mycobacterium vaccae*, which has been found to enhance anti-TB mycobacterial infections in patients with cellular immune function and, combined with chemotherapy, can enhance the efficacy of chemotherapy in the adjunctive treatment of TB [36]. Studies have proven the efficacy of Vaccae as an adjunctive

therapy associated with a significant increase in the closure situation, and with being curative in conjunction with the current therapy [36]. There was also an improvement in symptoms when used as an adjunctive, with an associated increase in CD4+ counts [36]. Its role in prevention was assessed in a phase III clinical trial assessing the safety and efficacy of Vaccae in TB prevention, enrolling 10000 participants, although results have not been made available (ClinicalTrials.gov: NCT01979900).

3.5. DNA Vaccines

DNA vaccines consist of: a plasmid containing one origin of replication of *Escherichia coli* for the amplification of the plasmid; a strong promoter, generally from cytomegalovirus; multiple cloning sites in which the gene to be expressed is inserted; and an antibiotic as a selection marker [20]. With this system, the antigen can be expressed directly by the cells of the host, and can be processed as proteins synthesized in the cytoplasm, with the fragmented peptides presented to the immune system by class I MHC molecules [20]. Should the protein be exported or secreted, it can be processed by class II MHC molecules and mount a specific antibody response [20]. DNA vaccines can be delivered through a wide variety of routes, such as the mucosal, intramuscular, intradermal and transdermal routes. Because of their high efficiency and low cost, DNA vaccines have potential advantages in disease prevention. However, their instability and inability to be sufficiently immunogenic have limited their development, this being one of the greatest challenges in clinical trials [37].

GX-70

In the fight against TB, one important candidate is the GX-70 vaccine, which consists of four antigen plasmids from *M. tuberculosis*, together with recombinant Flt3 ligand. A phase I clinical trial to determine the safety and immunogenicity of the vaccine in pulmonary TB patients with high risk factors for treatment failure or relapse was planned. However, it was withdrawn (ClinicalTrials.gov: NCT03159975).

4. Discussion

TB remains a global health problem [9]. In response to this worldwide challenge, numerous novel candidate vaccines have been developed and trialed. While many have shown promising results in animal models, human clinical trials are still necessary to ensure the safety and efficacy of these new arsenals in the combat against this infectious disease [6]. A variety of new vaccines have been developed but, broadly speaking, they can all be grouped into one of five vaccine subgroups. Recombinant live vaccines and TB subunit vaccines are the subgroups with the greatest number of candidates, each consisting of six vaccines in trial. The inactivated TB vaccine subgroup consists of three vaccines in trial, followed by the attenuated live vaccine and DNA vaccine subgroups, with one vaccine each. The clinical trials conducted within the last five years, as per ClinicalTrials.gov, reflect the varying progress each candidate vaccine has made; 45% of these have been phase I studies; 9% have been phase I/II studies; 35% have been phase II studies; and 11% have been phase II/III or phase III clinical trials. The clinical trials which fall within the phase II/III or phase III categories belong to VPM1002, Vaccae, GamTBvac, and MTBVAC. On the basis of this progress, these four vaccines are perhaps our nearest candidates to join the BCG vaccine as indicated preventions for TB. Promisingly, while most clinical trials reported AEs, no SAEs were seen. Furthermore, most trials reported immunogenicity to the vaccines. Advances have been made, but progress is still needed before we can finally claim to have a vaccine capable of fighting, preventing, and treating TB. The vaccines, RUTI and ID93+GLA-SE, support the potential of using NPs as a promising delivery system. NPs in vaccination (nanovaccines) can be administered in a variety of manners: through subcutaneous and intramuscular injections, through mucosal sites (oral and intranasal), through penetrating capillaries, as well as mucosal surfaces [38]. This variety allows the possibility of pain-free delivery via the incorporation of nanovaccines in sprays, patches, and microneedle

arrays, providing psychological and physical benefits over traditional vaccines [39]. NPs, consisting of a variety of substances, from those that are lipid-based, including liposomes and lipid NPs, to carbon nanotubes, can be biodegradable and used passively or actively in vaccine delivery [38,39]. With their inherent properties, they also possess the added benefit of eliciting an immune response, and their use can be preventative or therapeutic [40]. Beyond these intrinsic properties, nanovaccines offer further advantages over traditional vaccines. Unlike traditional vaccines, the use of NPs offers greater targeting ability, being able to direct and control the release and delivery of the vaccine to a desired location, all the while being traceable [39]. NPs are also capable of being used as adjuvants and excipients [39]. The particular nature of NPs increases cross-presentation and plays an important role in the activity of antigen-presenting cells (APCs) [8]. With NPs, APCs can be modulated to promote dendritic cell activation, triggering particle-specific immune recognition and, thus, antigen processing [8]. As mentioned above, their inherent properties elicit an immune response, but it has also been found that those NPs smaller than 100 nm are capable of transferring from subcutaneous tissue to lymph nodes, where the antigen can be presented to mature immune cells and stimulate an innate immune response [40]. Research has also indicated that vaccines produced with NPs enhance antibody production when compared to the same vaccine without the use of NPs [39]. Their user-friendly nature allows the development of devices for their administration, such as a spray via the nose and, moreover, their relative low cost and the ability to alter their physicochemical composition to create tailored biological properties, make NPs in vaccination highly advantageous in comparison to traditional vaccines [39,41]. This ability to tailor NPs allows a variety of antigens to be incorporated into the NPs via encapsulation or conjugation, which may protect and also enhance the antigen's properties [41]. By being able to encapsulate the antigen, NPs not only protect it from degeneration, but also decrease the necessary dose as a single slow release of the antigen is capable and efficient [38]. More interestingly, the ability to tailor NPs opens up the possibility for personalization, where formulations can be easily altered to target a number of individual bacterial strains, or for specific population groups [40]. Nanovaccines are also advantageous in terms of storage and transport, as nanoemulsions do not require refrigeration, often being stable for several weeks [38]. With greater adherence associated with the use of NPs, a solution to creating more effective and safe TB vaccines, both in prevention and treatment, may be found.

5. Conclusions

It is evident that much work is still necessary to find a preventative and/ or therapeutic vaccine with the potential to be used instead of, or in conjunction with, the BCG vaccine. With the number of vaccines currently undergoing clinical trials with promising results, it is imperative to continue these studies towards completion and, ultimately, towards the generation of a new vaccine should the results continue in a positive trend throughout each trial. Merging NPs and vaccination is now one area of interest with great promise due to the myriad of advantageous features, including increased immunogenicity, flexibility of use, as well as cost-effectiveness. In the future, pulmonary administration as a route of TB treatment application should be explored. It may be possible to adopt an NP strategy to delivery mycobacterial antigens to the lung mucosa and protect against disease [42]. In murine models, the *M. tuberculosis* heparin-binding haemagglutinin antigen has been adsorbed in wax particles and has been shown to provide immunization [42]. Delivery of particles of this nature to the lungs may be a means of inducing an enhanced innate immune response [42]. Such an approach may prove a worthy pursuit in future human trials. When considering NPs, future prospects must also try to eliminate or limit their potential negative effects. The process for producing NPs can be technically complex, and their scaled-up production in sterile conditions may also be challenging [39]. Additionally, while their small size is beneficial in some regards, it is harmful in others, as this may lead to access to other tissues and organs not intended to be targeted, including crossing the blood-brain barrier [39]. In terms of administration, more research is needed to safely conclude

that no AEs, such as gastrointestinal symptoms, respiratory problems, and cardiovascular disorders do not follow after administration [39]. Concerns about the accumulation of NPs and the development of thrombotic events also need to be addressed [39]. Finally, it is important to note that, in generating a future vaccine, cost must also be factored in, since TB is present in both developed and developing countries. In the coming years, progress is expected to be made in finding an answer to the treatment and prevention of TB. Research and development must continue, with the aim of finding an equitable solution for all.

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