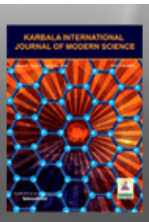

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Comprehensive Review on the Application of Bio-immunoinformatics in the Development of Highly Ef-fective New Candidate Vaccines against Tuberculosis

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Abstract

Tuberculosis (TB) remains a significant public health challenge worldwide. Currently, Bacillus Calmette-Guerin (BCG) is the only vaccine available for TB prophylaxis. However, the efficacy of the BCG vaccine against adult pulmonary TB is considered inconsistent. This condition encourages researchers to look for more effective options, such as subunit vaccines. This condition requires the development of a more effective subunit vaccine to protect active TB in productive and adult ages. There is an urgent need for more effective vaccines, as the Bacillus Calmette-Guérin (BCG) vaccine currently available has inconsistent efficacy and is only partially effective in adults. Bio-immunoinformatics, an interdisciplinary field integrating bioinformatics with immunology, offers promising strategies for vaccine development. By utilizing genomic and proteomic data from *Mycobacterium tuberculosis* (*Mtb*) genome, bio-immunoinformatics tools can precisely predict B-cell and T-cell epitopes, facilitating the design of vaccines that induce robust and long-lasting immune responses. Furthermore, integrating immunoinformatics with systems biology and machine learning enables the identification of immune escape mechanisms and variability in host responses, improving candidate selection. This review examines bio-immunoinformatics' application in identifying potential antigens, mapping epitopes, and designing highly effective TB vaccine candidates. This article highlights recent computational approaches and methodologies advancements, underscoring their pivotal role in accelerating TB vaccine research and development. Finally, we discuss the transformative potential of bio-immunoinformatics in revolutionizing TB vaccine design, ultimately contributing to more effective and widely applicable TB prevention strategies.

Keywords

Antigen Identification; Bio-Immunoinformatics; Computational Tools; Epitope Mapping; Tuberculosis; Vaccine De-velopment.

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REVIEW ARTICLE

Comprehensive Review on the Application of Bio-immunoinformatics in the Development of Highly Effective New Candidate Vaccines Against Tuberculosis

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Abstract

Tuberculosis (TB) remains a significant public health challenge worldwide. Currently, Bacillus Calmette-Guerin (BCG) is the only vaccine available for TB prophylaxis. However, the efficacy of the BCG vaccine against adult pulmonary TB is considered inconsistent. This condition encourages researchers to look for more effective options, such as subunit vaccines. This condition requires the development of a more effective subunit vaccine to protect active TB in productive and adult ages. There is an urgent need for more effective vaccines, as the Bacillus Calmette-Guérin (BCG) vaccine currently available has inconsistent efficacy and is only partially effective in adults. Bio-immunoinformatics, an interdisciplinary field integrating bioinformatics with immunology, offers promising strategies for vaccine development. By utilizing genomic and proteomic data from *Mycobacterium tuberculosis* (*Mtb*) genome, bio-immunoinformatics tools can precisely predict B-cell and T-cell epitopes, facilitating the design of vaccines that induce robust and long-lasting immune responses. Furthermore, integrating immunoinformatics with systems biology and machine learning enables the identification of immune escape mechanisms and variability in host responses, improving candidate selection. This review examines bio-immunoinformatics' application in identifying potential antigens, mapping epitopes, and designing highly effective TB vaccine candidates. This article highlights recent computational approaches and methodologies advancements, underscoring their pivotal role in accelerating TB vaccine research and development. Finally, we discuss the transformative potential of bio-immunoinformatics in revolutionizing TB vaccine design, ultimately contributing to more effective and widely applicable TB prevention strategies.

Keywords: Antigen identification, Bio-immunoinformatics, Computational tools, Epitope mapping, Tuberculosis, Vaccine development

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1. Introduction

The revolutionary advancements in computational speed and memory storage have ushered in a new era for analyzing biological data. Millions of genomes from various organisms, including prokaryotes, eukaryotes, and humans, have been sequenced and securely stored in multiple databases [1]. This vast amount of data serves as a valuable resource for numerous applications aimed to enhance human life and conserve natural resources for a better future [2]. Bioinformatics has brought significant innovations in molecular biology. It enables the organization of vast datasets, such as gene banks, protein banks, protein–protein interaction results, and pathways of protein activity [3]. Centralized data can be accessed anytime, allowing researchers and the public to retrieve information easily; bioinformatics is a rapidly growing and versatile field. Several important methods in bioinformatics, such as multiple sequence alignment, genome assembly, protein structure prediction, protein structural alignment, gene expression analysis, protein–protein interaction mapping, gene finding, and phylogenetic tree construction, have become indispensable tools, especially in life science and applied science [4].

Bio-immunoinformatics, or computational immunology, combines computer science and experimental immunology. It involves using computational methods and resources to analyze immunological data [5]. A critical aspect of bio-immunoinformatics is the development of new immunity theories [6]. Novel vaccines have been designed and evaluated utilizing in silico methodologies by applying reverse vaccinology concepts in bio-immunoinformatics. This approach holds great promise as an immunomodulation method due to the abundance of available data. The effectiveness of *in-silico* vaccine design paves the way for significant advancements in immunology [7].

With millions of new cases reported every year, TB remains one of the top ten leading causes of death globally [8]. BCG has been the sole TB vaccine approved for use for more than a century. Several studies have shown that BCG can prevent miliary and severe TB in children. Results show that BCG is administered to newborns and toddlers [9] for optimal protection. It offers a more than 70 % protection rate against severe TB forms, including TB meningitis in children and adults. However, BCG does not prevent latent pulmonary infections; its protection lasts only 10–20 years. This would clarify its variable range of efficacy from 0 % to 80 % in protecting adults from pulmonary tuberculosis [10,11].

TB treatment is increasingly complex, with the emergence of MTB strains that are resistant to TB drugs (MDR-TB). Currently, BCG is the only vaccine available for TB prophylaxis. The advantages of this vaccine are that it is relatively safe and has efficacy in preventing severe TB in children [12,13]. In several clinical trials, the efficacy of BCG to prevent active TB in adults turned out to be very low, and the lowest protection occurred in countries with high TB incidence, such as India.

Furthermore, the disadvantage of BCG is that it can cause infection if given to individuals with immunological system disorders [14,15]. In addition, the efficacy of the BCG vaccine against adult pulmonary TB is considered inconsistent. Several types of antigens from MTB have been widely studied as TB vaccine candidates. Resuscitation promotion factor D (RpfD) encoded by the Rv2389c gene is an antigen widely used in several previous studies as a TB vaccine candidate. Based on bioinformatics analysis (*In silico*), the Rpf-D gene has the highest immunogenic response compared to other epitopes [16]. The Rpf-D protein causes a humoral immune response in mice immunized orally and subcutaneously [17]. In its development, the Rpf-D and MPT83 plus ESAT6 antigens is an essential protein used in diagnosis to distinguish between TB and non-TB patients (who have been immunized by BCG), so Rpf-D is no longer used in the development of the TB vaccine [10,18–20].

Therefore, there is an urgent need for new TB vaccines. Bio-immunoinformatics leverages computational tools to analyze biological data, facilitating the identification of novel antigens and epitopes for TB vaccine development. Identifying immune response targets against *Mtb* is challenging due to the complicated pathology of human *Mtb* infection. Aerosolized *Mtb* primarily infects the lungs, and macrophages engulf the pathogens and recruit other immune cells [21].

Granulomas are formed when immune cells such as NK cells, CD4+ T cells, γδ T cells, and neutrophils are stimulated to control the infection [22]. A small number of bacilli remained dormant within the granuloma, potentially reactivating the disease later [23]. In most cases, CD4+ T cells control the infection by managing the bacteria and preventing further spread. Reactivation of the infection may result in acute, chronic, or extrapulmonary disease. *Mtb* is adept at evading the body's immune system, allowing symptoms to remain latent for extended periods [22]. However, the factors contributing to

most asymptomatic *Mtb* exposures remain elusive [24].

Several ways to create novel tuberculosis vaccines are suggested based on what is known about host immunity to *Mtb* combined with other relevant factors. Vaccines can be administered in three different ways: before exposure (to protect uninfected people or newborns), after exposure (to protect adults and adolescents from latent tuberculosis), and therapeutically (to treat already-infected people) [17,25]. Pre-exposure vaccines aim to protect against *Mtb* by eliciting an immune response that is either more robust or rapid than the BCG vaccine [26]. Post-exposure vaccines aim to prevent the disease's reactivation or eliminate latent tuberculosis. This approach seeks to prevent the reactivation of latent tuberculosis into active disease [27]. Preventative pre-exposure is the guiding principle of the BCG vaccine. Biochemical forms are another way to classify vaccines; these include live attenuated, inactivated, adjuvanted, and recombinant proteins. Some vaccines aim to increase BCG efficacy levels [28], while others use different administration methods, like aerosol inhalers, to enhance adult immune responses [29].

Recent advancements in TB vaccinations have emphasized various biochemical forms, including recombinant, subunit, live attenuated, and inactivated vaccines. Extensive research has focused on evaluating diverse combinations of antigens and adjuvants, particularly for protein subunit vaccines. This review article examines recent advancements in the application of bio-immunoinformatics to develop new candidate vaccines against TB. We highlight computational approaches and methodologies in this field, emphasizing their potential to expedite TB vaccine research and development. Leveraging bio-immunoinformatics allows for identifying immunogenic antigens, mapping epitopes, and designing effective vaccine candidates, ultimately enhancing the efficacy and scope of future TB vaccination strategies.

2. Methods and data sources

This review article is based on the latest tuberculosis-related articles, almost all published in the last five years. A systematic review and a meticulous approach were adopted in selecting data sources according to previous researchers' methodology in bio-immunoinformatics approaches for identifying candidate TB vaccines to ensure comprehensive coverage of this review. The articles used in this review were sourced from journals published by Science Direct, MDPI, Google Scholar, Springer Link, PubMed, Scopus, Web of Science, and others. A systematic literature review is a constructive approach that can uncover, evaluate, and synthesize thorough, significant research data on a particular topic in TB vaccines [30]. This allows researchers to gain a comprehensive, profound understanding of the studies and their conclusions. In addition, this method reduces the possibility of bias due to human-induced errors. Search terms were carefully curated, combining TB vaccines, vaccine development, bio-immunoinformatics, antigen identification, epitope mapping, and computational tools to retrieve relevant publications comprehensively. Articles were then filtered based on their statistical significance, prioritizing those presenting empirical data and robust bio-immunoinformatics analysis on *Mtb*-specific antigen-based recombinant vaccine candidates with high immunogenicity. This rigorous methodology facilitated the incorporation of high-quality and credible sources, thus enhancing the integrity and reliability of the review.

3. Results and discussion

3.1. Bio-immunoinformatics approaches for identification of candidate TB vaccines

By harnessing the power of bioinformatics, researchers can now computationally predict and design peptide-based vaccines. Peptide-based vaccinations have some benefits over traditional ones, including lower cost, fewer side effects, faster production, and safer transportation and storage. Despite these advantages, developing peptide-based vaccines has proven to be a complex process that involves multiple steps: identification of potential antigens, T and B cell epitopes prediction, immunogenicity assessment, allergenicity, and toxicity evaluation, adjuvant and linker peptides selection, generating and optimizing the final vaccine design, and evaluating its features [10]. Computational tools in bioinformatics can expedite the design of optimal subunit vaccine candidates by identifying the immunogenic epitopes or peptides. By employing the organism's genetic information, these techniques effectively reduce the time and cost required to develop the vaccines [31]. It is common practice to use an adjuvant with immunoreactive biomolecules comprising subunit vaccines, usually polypeptides and glycolipids. These vaccines can be produced cheaply, offering high specificity and efficiency with minimal side effects [32]. A plethora of potential subunit TB vaccine candidates are undergoing clinical trials at this very moment. Fig. 1 illustrates the steps in identifying

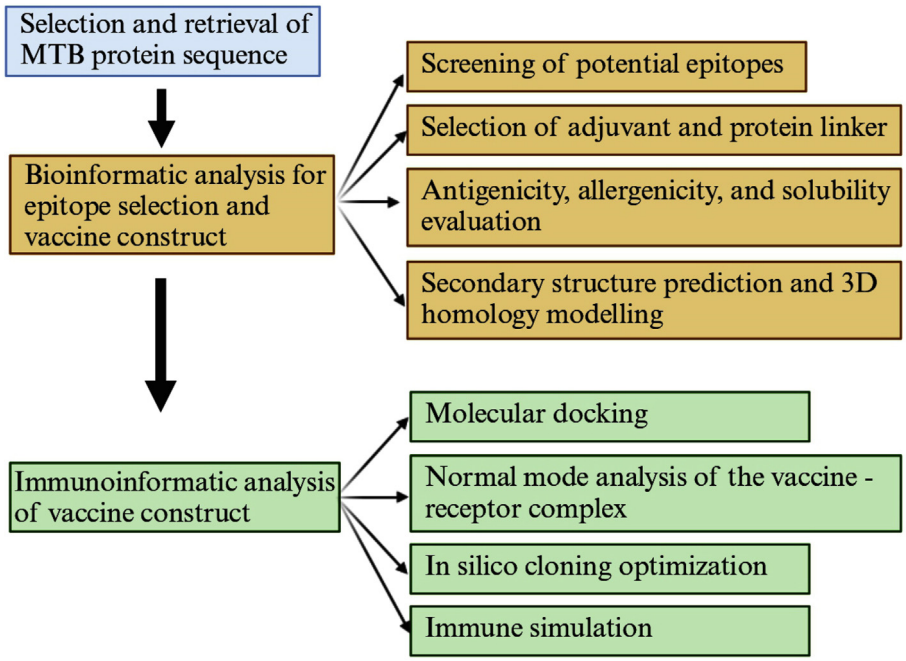

Fig. 1. Stepping to identify immunogenic epitopes or peptides for the candidate TB vaccine. Created with BioRender.com (Accessed August 12, 2024).

immunogenic epitopes or peptides for the candidate TB vaccine using bio-immunoinformatics.

The computational steps to identify immunogenic epitopes or peptides for the candidate TB vaccine are as follows:

3.1.1. Antigen identification

Genomic and proteomic data of *Mtb* are analyzed to identify proteins that could serve as potential vaccine targets. Comparative genomics, proteomics, and transcriptomics pinpoint antigens specific to TB. The correlation between *Mtb* strain genotype and phenotype has been the subject of multiple genomic investigations in the last several years. Proteins involved in lipid and fatty acid metabolism and those encoding cell surface proteins are among the many mycobacterial virulence genes discovered [33]. Bioinformatics analysis also revealed that *Mtb* proteins involved in peptidoglycan and lysine biosynthesis, such as the pathogen-specific pathogenicity of Rv2611c and Rv1459c, make them promising vaccine antigens [34]. A mutation of Ser219 to leucine in the PhoP virulence factor may result in reduced virulence in H37Ra, according to comparative genomic investigations between the virulent H37Rv strain and the non-virulent H37Ra strain [35,36]. Similarly, *Mycobacterium bovis* BCG has attenuated virulence due to the genome's deleted region of difference 1 (RD1) [37,38].

While most comparative genomic studies have focused on virulent and non-virulent *Mtb* strains, there is a notable absence of comprehensive research examining epitope variation and its relation to the host. However, reliable and comprehensive genome sequences can only be obtained through meticulous comparisons and studies of the virtually identical *Mtb* genome sequences (with >99 % identity). Consequently, this gap persists [39]. *Mtb* genomes are notoriously difficult to sequence using Illumina's HiSeq platform and other second-generation sequencing technologies because of their high GC content and repetitive PE/PPE multi-gene family sequences [40].

3.1.2. Epitope mapping

Once the potential antigens are identified, bioinformatics tools will be used to predict B-cell and T-cell epitopes. This process involves finding regions of antigens that the immune system will likely recognize. Several tools are available online for B epitope mapping; some of them require the input of simply the sequence of the potential antigenic protein to predict continuous epitopes, for example, ABCpred [41], BepiPred-2.0 [42], and LBtope [43]. Ellipro [44] and DiscoTope [45], on the other hand, predict conformational epitopes from the submitted three-dimensional (3D) structure of the protein.

T-cell epitopes and the corresponding MHC-I and MHC-II alleles, as well as the binding IC_{50}, can be predicted using tools available on the IEDB website (http://tools.iedb.org/mhci/ and http://tools.iedb.org/mhcii/, respectively). Several prediction

methods are available on the websites; however, the default setting uses the IEDB recommended, which is updated periodically based on the availability of predictors and observed predicted performance for a given allele. During the time when this review is written, NetMHCpan EL 4.1 and NetMHCIIpan 4.1 [46] are the recommended methods to be used across all MHC-I and MHC-II alleles, respectively. Other available methods for MHC-I alleles prediction include Artificial neural network (ANN) [47], Stabilized matrix method (SMM) [48], SMM with a Peptide: MHC Binding Energy Covariance matrix (SMMPMBEC) [49], Scoring Matrices derived from Combinatorial Peptide Libraries [50], Consensus [51], NetMHCcons [52], PickPocket [53], and NetMHCstabpan [54], whereas methods for MHC-II alleles prediction include Consensus method [55], Combinatorial library, NN-align-2.3 (netMHCII-2.3) [56], NN-align-2.2 (netMHCII-2.2) [57], SMM-align (netMHCII-1.1) [58], Sturniolo [59], NetMHCIIpan-3.1 [60], and NetMHCIIpan-3.2 [56]. The website also allows analysis of only alleles occurring in at least 1 % of the human population or allele frequency of 1 % or higher. Alternatively, it is also possible to specify the analysis on alleles occurring in 97 % (in the case of MHC-I) [61] or 99 % (in the case of MHC-II) [62] of the population. In MHC-II epitope mining tool, it is also possible to select a reference panel of 7 alleles, as described in Ref. [63]. Alternatively, the tools can also analyze specific peptides with self-defined lengths of epitopes. In addition, when the locus selected in MHC-II epitope mining is either HLA-DP or HLA-DQ, α & β chains could be selected separately, making it possible to predict all different chain combinations.

The selection of epitopes for vaccine construction involves various criteria and standards to ensure immunogenicity, safety, and efficacy. A few key considerations and cut-offs commonly used in epitope selection are:

1) Immunogenicity: This response can involve both the innate and adaptive immune systems and may result in the production of antibodies, activation of T-cells, or other immune mechanisms. From this step, it is recommended that MHC binding affinity and predicted B-cell epitopes be considered [64].
2) Pathogenicity: Avoiding virulence factors by excluding epitopes from toxic or virulence-enhancing regions [65].
3) Epitope size: The size of an epitope plays a crucial role in its selection for vaccine construction, as it directly impacts the binding, recognition, and effectiveness of the immune response. Different types of epitopes (T-cell or B-cell) have specific size requirements that must be met to ensure immunogenicity and compatibility with the immune system. Linear epitopes typically range from 6 to 20 amino acids in length, facilitating recognition by B-cell receptors or antibodies. Meanwhile, conformational epitopes, formed by amino acids from different parts of the antigen, may involve more significant regions, but their functional size is critical for accurate antibody binding [44].

Epitope-based vaccine analysis for *Mtb* vaccine design involves screening and constructing potential epitopes from the *Mtb*'s outer membrane protein A (Rv0899) [27] Rv0125 (Mtb32A), Rv3804c (Ag85A), Rv2684, and Rv2608 are the four highly conserved *Mtb* antigens that were utilized in other studies which aim to develop a new multi-epitope unit vaccine [66,67].

Epitopes are selected by observing the pathogenesis of infectious diseases, immune cell involvement, and epitope selection. Many studies only selected epitopes for MHC-I and MHC-II molecules due to their distinct roles in antigen presentation and activation of specific immune responses. Understanding the differences in how these molecules interact with the immune system is critical for rational vaccine design and immunotherapy.

MHC-I molecules present peptides derived from intracellular proteins (e.g., viral or tumor antigens) to CD8+ cytotoxic T lymphocytes (CTLs). This process enables the immune system to identify and eliminate infected or abnormal cells. MHC-I molecules typically bind peptides 8–11 amino acids long. This length ensures a snug fit into the binding groove of MHC-I molecules [68]. Moreover, selected epitopes must have a high binding affinity for specific MHC-I alleles to ensure stable presentation.

MHC-II molecules present peptides derived from extracellular proteins to CD4+ helper T cells. This activates and regulates B-cell antibody production, macrophage activation, and other immune processes. MHC-II molecules bind longer peptides, typically 13–25 amino acids. Moreover, MHC-II alleles are highly polymorphic, so epitope selection must account for allele-specific binding across diverse populations [69].

The selection process for MHC epitopes differs between bacteria and viruses due to the distinct biological and immunological characteristics of these pathogens. These differences influence how antigens are processed and presented and the types of immune responses required for effective immunity. Antigen processing and immune responses are

differentiated between viruses and bacteria. The cytosolic pathway processes viral proteins. Proteasomes degrade viral proteins, and peptides are transported into the endoplasmic reticulum by the TAP (Transporter Associated with Antigen Processing) system for loading onto MHC-I molecules. Viral particles or extracellular viral components can also be processed in endosomal compartments for presentation on MHC-II molecules. A study by Sette and Rappuoli in 2010 showed that epitopes from influenza nucleoprotein and hemagglutinin are commonly targeted for MHC-I and MHC-II presentation [70].

In extracellular bacteria, antigens are processed in the endosomal pathway and presented on MHC-II molecules to activate CD4+ T cells, which coordinate antibody production and macrophage activation. For intracellular bacteria, bacterial proteins processed through the cytosolic pathway can also be presented on MHC-I molecules to activate CD8+ T cells. A study by Delogu & Brennan 2001, showed that Epitopes from *Mycobacterium tuberculosis* proteins ESAT-6 and Ag85B are commonly selected for both MHC-I and MHC-II presentation [71]. Differences in Epitope Selection are summarized in Table 1.

3.1.3. Vaccine design

Computer models that predict the stability and immunogenicity of vaccine candidates support the development of peptide-based, subunit, and multi-epitope vaccines. *In silico* models help optimize the vaccine constructs for a more effective immune response [74,75].

When selecting an amino acid sequence for inclusion in vaccine construction, several critical characteristics ensure its ability to trigger an immune response effectively. These characteristics are influenced by the type of vaccine (e.g., peptide-based, protein subunit, DNA, or mRNA) and the immune mechanisms that need to be activated (e.g., humoral, cellular immunity). Below are key factors that contribute to the suitability of an amino acid sequence for vaccine design.

1) Immunogenicity
   Immunogenicity refers to the ability of an antigen to provoke an immune response. For a sequence to be included in a vaccine, it must be recognized by the immune system and trigger an adaptive immune response [70].
2) Binding Affinity to MHC Molecules (MHC-I and MHC-II)
   For a sequence to be recognized by the immune system, it must bind effectively to MHC (Major Histocompatibility Complex) molecules. This is particularly crucial for the presentation of the antigen to T cells [72].
3) Epitope identification
   The selected amino acid sequence should contain T-cell epitopes (both CD4+ and CD8+ T-cell epitopes) or B-cell epitopes (for stimulating antibody responses) [68].
4) Adjuvanticity
   Some sequences possess intrinsic adjuvant properties, which enhance the immune response. Intrinsic immunostimulatory motifs, such as CpG motifs in bacterial DNA or specific motifs in viral proteins, can enhance the effectiveness of a vaccine by promoting the activation of innate immune responses [76].
5) Structural Stability and Flexibility
   The sequence should maintain its structural integrity so antigen-presenting cells can correctly process it. It should also allow for flexibility in binding to MHC molecules. Sequences that are rigid or have large hydrophobic regions might not be suitable because they may hinder the optimal presentation of MHC molecules [77].
6) Size of the Amino Acid Sequence
   The size of the selected amino acid sequence plays a role in its ability to bind to MHC molecules and stimulate an immune response [78].

Table 1. Differences in epitope selection [72,73].

Feature	Viruses	Bacteria
Primary Pathway	Cytosolic (MHC-I) and Endosomal (MHC-II)	Endosomal (MHC-II); Cytosolic (MHC-I for intracellular bacteria)
Immune Response Focus	CD8+ T cells (killing infected cells), CD4+ T cells (antibody assistance)	CD4+ T cells (antibody and macrophage activation), CD8+ T cells for intracellular bacteria
Epitope Source	Structural and non-structural viral proteins	Surface proteins, secreted toxins, conserved intracellular proteins
Epitope Length	8–11 amino acids (MHC-I); 13–25 (MHC-II)	13–25 amino acids (MHC-II); 8–11 (MHC-I for intracellular)
Conservation	Highly conserved due to smaller genome size	Variable: conserved regions of key virulence factors are prioritized
Cross-Presentation	Common due to viral infections	Limited; occurs in intracellular bacteria like *Listeria monocytogenes*

For an amino acid sequence to be suitable for inclusion in vaccine construction, it must exhibit characteristics such as strong immunogenicity, effective MHC binding, presence of T-cell and B-cell epitopes, ability to enhance the immune response, and avoidance of self-reactivity. Combined with computational predictions and empirical validation, these factors guide the selection of the best candidates for vaccine development.

3.1.4. Machine learning

Machine learning algorithms are increasingly being used to predict epitopes and antigenicity, enhancing the accuracy of vaccine candidate identification. Teahan et al. employed a machine learning-based reverse vaccinology strategy to determine whether each protein in the proteome of the *Mtb* laboratory reference strain H37Rv would serve as a protective antigen (PAg) [79].

3.1.5. Structural bioinformatics

Structural analysis of antigen-antibody interactions helps understand the molecular basis of immune recognition, guiding the design of more effective vaccines.

3.1.6. Systems biology

Integrating systems biology approaches with bio-immunoinformatics allows for a holistic understanding of host-pathogen interactions, identifying key pathways and targets for vaccine development.

3.2. M. tuberculosis-specific antigen-based recombinant vaccine candidates with high immunogenicity

Several potential TB vaccine candidates have been identified using bio-immunoinformatics methods. For instance, computational techniques enhanced their efficacy while developing the M72/AS01E and GamTBvac vaccines. These vaccines undergo phase 3 clinical trials, completing phases 1 and 2 [80,81]. Additional *Mtb* proteins/antigens with strong immunogenicity have been identified, including Rv1031, Rv1198, Rv2016 [82], Rv3619c [83], Rv3620c [84], PE35 [85], PPE39 [86], PPE68 [87], Mtb39A (Rv1196), Mtb32A (Rv0125), Mtb9.8 [88], MPT63 (Rv1926c) [89], MPT83 (Rv2873) [90], LppX [91], MPT64 (Rv1980c) [92], and the A60 complex. According to recent studies, Rv0569 enhances the secretion of Th1 cytokines IFN-γ, TNF-α, and IL-12p40 [93].

Mtb antigens exhibit differential patterns throughout infection, which presents a significant challenge in developing a vaccine that would elicit the correct immune response [25]. Furthermore, while Ag85B expression is primarily observed early in the infection process, ESAT-6 exhibits a more consistent expression pattern [11]. The Ag85B, ESAT-6, and Rv660c protein fusion known as H56 promotes immunological responses to antigens produced throughout the *Mtb* infection lifecycle. An animal study using mice demonstrated innate and adaptive immune activation induction after receiving a vaccination that contained H56/CAF01 [94]. Several TB vaccine candidates are based on *Mtb* cross-reactive antigens and are now being tested in clinical studies. False positive results in tuberculin skin tests utilizing *Mtb*'s pure protein derivative (PPD), comparable to the BCG vaccine, can occur when immunization with these antigens is administered. Furthermore, problems like blocking or masking effects might arise from these potential vaccines' cross-reactivity with mycobacteria in the environment. Hence, pre-vaccinated individuals may not benefit from these vaccines regarding booster shots [95]. The masking hypothesis states that while early sensitization with environmental mycobacteria offers some protection against TB, cross-reactive antigens in vaccinations administered later in life reduce their effectiveness. According to the blocking theory, a new vaccine cannot work because the immune system has already cleared its system of cross-reactive antigens with those it has encountered in its exposure to mycobacteria in the environment [96]. It is possible to circumvent the effects of blocking or masking by using *Mtb*-specific antigens in new TB vaccines [97]. Therefore, scientists are looking into developing novel subunit and/or recombinant vaccines based on *Mtb*-specific antigens to provide better TB vaccines than BCG [98]. Based on research [16], it was identified that through a bio-immunoinformatics approach, 30 possible *Mtb* OMP (Outer Membrane Protein) candidates have been found with immunogenic potential epitopes (Table 2).

The immunodominant epitopes developed for Rv2264c, Rv0172, Rv1006, and Rv0295c are projected to be antigenic, non-allergenic, and able to bind maximally with B cells and a wide range of MHC alleles. Overexpressed versions of these epitopes are soluble, non-transmembrane, solvent-exposed, and have little to no sequence resemblance to the human proteome. Additionally, their study improved molecular docking and molecular dynamics studies of the Rv0295c and Rv1006 epitope-HLADRB1*04:01 complexes. Therefore, suggesting that these in silico-derived epitopes may be valuable in developing modular VLP-based vaccines displaying immunogenic peptides or epitopes against

Table 2. List of potential immunogenic epitopes from OMP (Outer Membrane Proteins) of Mtb by bio-immunoinformatics approach (Modified from Ref. [16]).

No	Putative OMPs of *Mtb*	Amino acid position		B cell epitopes	T cell prediction			
		Start	End	Amino Acid Sequence	Class I	Class II	VaxiJen Score	AlgPred Score
1	Rv0172[a]	382	432	ASTASTLPKEIAYSEPRLQPPN GYKDTTVPGIWVPDTPLSHRN TQPGWVVA	27	27	0.5031	−0.7961
2	Rv0257	21	52	GLRGSLPGDSGGTAPDSHRLP ASSSPDGKNIG	19	0	0.778	−0.4938
3	Rv0259c	75	90	AASAHPHVT	18	0	0.945	−0.4234
		174	188	RRVAVASFL	7	3	1.057	−0.4159
4	Rv0295c[a]	228	264	AIGQDPKLAPAPMLERQANQR SDEWVDRYRAEAPRLG	26	27	0.8884	−1.2448
5	Rv0506	125	137	VKDERSETSPRAL	6	2	1.3782	−0.7865
6	Rv1006a	24	59	LNGCSSSASHRGPLNAMGSP AIPSTAQEIPNPLRGQ	26	27	0.4214	−0.7621
7	Rv1351	1	34	MTPRSLPRYGNSSRRKSFPMH RPSNVATATRKKS	24	23	0.6134	−0.7983
8	Rv1477	238	269	SSEGGQGAPPFRMWDPGSGP AGGRAWDGLWDP	22	12	1.189	−0.5473
9	Rv1478	211	230	MLEASGSAGKVTVSPVRKAG	18	10	0.9246	−0.7297
10	Rv1488	312	343	GKPGEDGVFRFEPSPVEDQPK HAADGDDAEVA	21	27	1.1083	−0.4204
11	Rv1906c	110	135	CQPWQNTGSEGAAPAGVPGP EAGAQL	18	17	0.8377	−0.4423
12	Rv1910c	28	60	YGGNGDSRKAAPLAPKAAAL GRSMPETPTGDVL	22	22	0.726	−1.3588
13	Rv2075c	392	403	SWAPDEPRAGAG	5	1	1.2283	−0.6243
14	Rv2112c	184	204	VTGSGRVGIGPSGDEPGFQLS	9	3	1.5556	−0.4154
15	Rv2232	1	40	MSSPRERRPASQAPRLSRRPP AHQTSRSSPDTTAPTGSGL	24	5	0.7629	−0.5237
		49	79	GIVTDTTASGTNCPPPPRAAA RRASSPGESP	17	10	0.6672	−0.7378
16	Rv2264c[a]	383	425	AWSEADEDSHIGPAPGYTAAR PSLSFDHDAHAEPEPKSPPIPW	27	27	0.7743	−1.0082
17	Rv2307c	115	135	GYGGNPGRPSEQGLAADARAA	12	2	0.9422	−0.7492
18	Rv2525c[a]	102	153	YGKGSTADWLGGASAGVQHA RRGSELHAAAGGPTSAPIYASID DNPSYEQYK	27	27	0.9026	−0.4884
19	Rv2672	33	72	AFGADPRFATYSGAGPQGAAT TTPPPAGPPPLAAPKNDLS	21	27	0.7615	−0.6531
20	Rv2891	33	52	PAHADDSRLGWPLRPPPAVV	21	4	1.2467	−0.6453
21	Rv2956	199	247	AGALAGAGHRKSPKQGVFRG AAQGGDIVARQPPGRWVCPSS AGGPIGWH	22	25	0.4568	−0.9657
22	Rv2980	33	55	NRQPPERPVVIPAVPAPQATGPG GEYRRAPVAEPTTAGATAWRTG	16	27	0.5001	−0.4498
		68	93	PNST	23	21	0.7059	−0.7536

23	Rv3096	134	168	DPLPRPGRQRAPRAGVHNSGW VQSPGAERLDDRRY	24	11	0.4054	−0.4096	
24	Rv3212	1	13	MVKPERRTKTDIA	7	0	0.5383	−0.4733	
		188	199	DARVKPSNRGLQ	4	2	1.3432	−0.5699	
25	Rv3484	358	380	AFGSAPPTSQTAAAAKPNPSTVV	17	13	0.6267	−0.4196	
26	Rv3492c	354	381	KTAQNDPSTVRGARNYPCQE FPGKRAPT	26	6	0.7796	−0.4882	
		529	552	GAFADPAGGTGIFAPGMTGA SSAE	21	15	0.5891	−0.4566	
27	Rv3587c	43	116	SSAGAKPVSADKPASAQSHPG SPAPQAPQPAGQTEGNAAAAPP QGQNPETPTPTAAVQPPPVLKEGD DCPDSTL	22	27	0.7745	−0.7618	
28	Rv3693	242	258	RVGVDPTAADPAGWPRL	17	0	0.5311	−0.6480	
29	Rv3796	139	182	LAPPGRAPVLVYGPGPAGGLPP SEVGNPNPATVNPANPTPGLAA	23	27	0.5826	−0.4584	
30	Rv3909	374	388	STRGATVLPDGPLTG	12	2	0.5078	−0.4393	

TB is plausible. Table 2 demonstrates that the immunodominant epitopes (IDE) can bind B-cells and many MHC alleles; each IDE can bind to more than 25 Class I and II alleles. The selection of B-cell epitopes was based on factors such as antigenicity score, allergenicity, epitope length, and amino acid sequences within a range of 9–51 residues. The B-cells chosen for vaccine development demonstrated higher antigenicity scores (Table 1). We also tested the predicted T cell numbers, solvent accessibility, transmembrane architecture, and overexpression solubility of these IDEs.

Healthy tuberculin reactor's peripheral blood mononuclear cells produce a considerable amount of interleukin (IL)-12p40 and the newly discovered 11-kDa protein (Rv3204, MTSP11) [99]. This peptide stimulates splenocytes collected from vaccinated mice to release much higher levels of IFN-γ [10]. Antigen (Possible DNA Methyltransferase) MTSP11 warrants additional evaluation as a subunit vaccine component due to the substantially high interleukin (IL)-12p40 production. Since non-reactor tuberculin-derived Peripheral Blood Mononuclear Cells (PBMCs) do not produce enough IFN-γ, the T cell memory system's response to mycobacterial antigens is the main source of IFN-γ testing. The cellular immune response is critical for protection against *Mtb* infection. Studies in mice have demonstrated the essential roles of CD4+ and CD8+ T cells in preventing TB disease through cellular depletion and transfer experiments. Furthermore, protection against TB relies heavily on IFN-γ. At the same time, the more susceptible trait to TB infection is caused by mutations in the IFN-γ receptor in humans and disruption of IFN-γ in mice. The proteins actively released by *Mtb* during growth are believed to cause particular expansions, stimulating cell-mediated immune responses and protective immunity. T cells that produce IFN-γ can identify macrophages that contain intracellular mycobacteria and launch antimicrobial attacks against them [100]. Based on the methods depicted in Fig. 2, antibody-mediated protection against *Mtb* might rely on CD4+ T cell-dependent mechanisms [101]. Fig. 2a shows that after being exposed to *Mtb* bacteria, CD4+ T cells may assist B cells in initiating antibody response development. As illustrated in Fig. 2b, CD4+ T cells can mediate antibody formation, activating natural killer (NK) cells and enhancing antibody-dependent cellular cytotoxicity, eliminating *Mtb* bacteria from host cells. Fig. 2c shows that one possible outcome of *Mtb* antigen-antibody interactions is an increase in the presentation of *Mtb* antigens to $CD4^+$ T epitope by professional phagocytes such as dendritic cells or

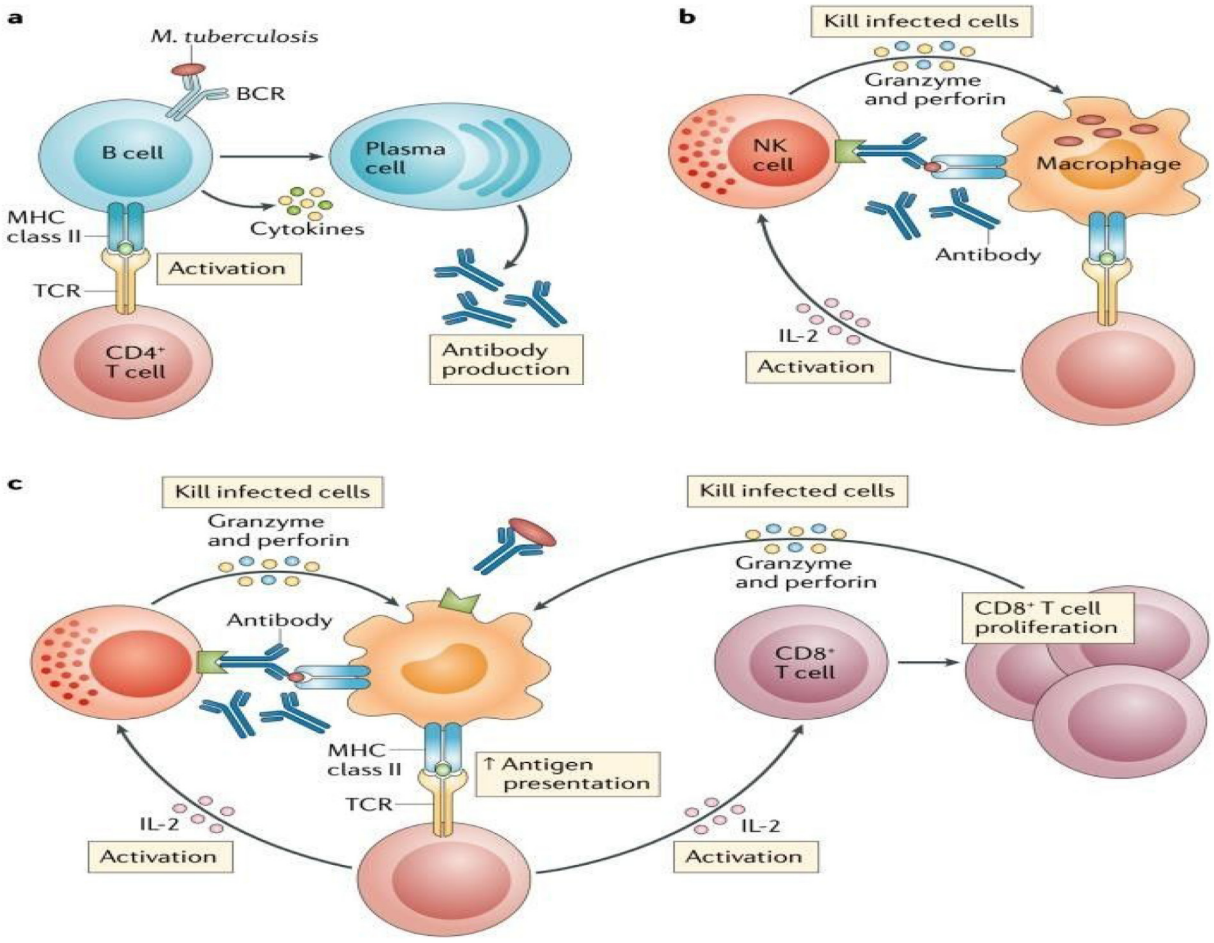

Fig. 2. Possible ways in which antibody-mediated protection in Mtb depends on $CD8^+$ T cells [101].

macrophages. Therefore, identifying T-cell epitopes is an essential step in vaccine development. T lymphocytes and B lymphocytes are the primary cellular components mediating an individual's immune response. Cytotoxic $CD8^+$ T lymphocytes (CTLs) are critical in pathogen control by recognizing and eliminating infected cells or secreting antiviral cytokine proteins.

Protein Rv1009, also known as resuscitation-promoting factor type B (Rpf-B), plays a role in reactivating dormant *Mtb* bacteria. The Rpf-B protein has a molecular weight of 38.0784 kDa (kDa) and comprises 362 amino acids, expressed by a gene of 1089 base pairs. This protein has an isoelectric point of 5.22 and is located in the cell wall, where it functions as a lipid-lysing enzyme in bacterial growth under dormant conditions. Rv1009 has an N-terminal region that binds to lipoprotein lipids [102]. Rpf-B and Rpf-E are always associated with Rpf-A and localized within the cell septa. Deletion of Rpf-A and Rpf-B in *Mtb* leads to defects in reactivation from dormancy. The presence of macrophages infected with Rpf-A and Rpf-B mutants in the bone marrow results in a two-fold increase in the production of IL-6 and TNF- α compared to wild-type *Mtb* infection. Protein Rv1009 could be a candidate vaccine for dormant *Mtb* because it has cell surface-exposed regions containing Rv1009 that can be detected as antigens [103].

Rv1926c encodes the MPT63 protein, abundantly secreted by pathogenic bacteria *M. bovis*, *Mtb*, and *M. bovis* strain BCG. MPT63 is a reasonable candidate for inclusion in the *Mtb* complex. As a target for the host's immune response, MPT63 could be significant for TB pathogenesis. Recombinant MPT63 protein from Rv1926c has been purified from *Escherichia coli* cells and can enhance the humoral immune response [104]. An abundance of T-epitopes in the immunodominant N-terminal region of MPT63 may contribute to its immunogenic characteristics. Hence, MPT63 has been suggested as a potential target for vaccine development. Rv1926c (MPT63 protein) is immunoreactive in both human and animal antibody tests and protects a

vaccine candidate that elicits a protective response. In TB patients, MPT63 triggers Th1 cell reactivity and promotes proliferation and IFN-γ production from peripheral blood mononuclear cells, but this effect is not observed in patients infected with *Mycobacterium avium* [105]. The MPT63 protein is found exclusively in the *Mtb* complex, including *Mtb* and *M. bovis*. Detection of T cells secreting IFN-γ specifically can be useful for diagnosing TB. The T cell $CD8^+$ epitopes induced by MPT63 may contribute to the immunological diagnosis of *Mtb* infection and could provide components for designing effective TB vaccines. One effective tool for TB diagnosis is a multi-antigen complex that includes MPT-64, MPT-63, ESAT-6, and CFP-21 [106].

Research on the gene Rv2770c, now referred to as PPE44, involves studying *Mtb* H37Rv expressed from the attenuated H37Ra strain. Recent studies have focused on the PPE-SVP protein PPE44, where the PPE44 antigen, combined with *M. bovis* BCG, was injected into mice. To understand the role of PPE44 in human infection, case studies on the genetic diversity of PPE44 in clinical isolates have been conducted. Genetic variation provides different potentials for isolates to evade the host immune system. No polymorphisms in the PPE44 protein were found using PCR tests; the length of the polymorphism fragments required three enzymes for this analysis. Nucleotide sequencing of the PPE44 gene isolates from *Mtb* phylogenetic lineage showed no nucleotide variations except for isolates from the Beijing genotype. PPE44 (Rv2770c) is a protein from *Mtb* and is a potential candidate for future vaccines [107].

3.3. Recent development of TB vaccines

One of the three pillars of the WHO TB strategy is research and development, which will be essential in accelerating the reductions in TB incidence and mortality needed to meet the global targets. These targets aimed to reduce new infections by 90 % and TB deaths by 95 % between 2015 and 2035. Vaccines that protect people against *Mtb*, prevent the infection from becoming active, or lessen the ability of infected individuals to spread the disease could provide a more effective level of TB control than that offered by BCG. Most TB preventive and control experts concur that even a vaccine with only 50–60 % efficacy over a 10-year period could have a profound impact. The most significant vaccine benefit would be the mass immunization of all adolescents and young adults in high-burden countries regardless of their infection status. In high-burden countries, an initial vaccination strategy could prevent up to 50 million incidents of TB in the first 35 years following its implementation [108,109]. This approach can save millions of lives and billions of dollars in treatment and control expenses. Furthermore, 23 potential vaccines have advanced to clinical trials in the past ten years. A summary of the more than a dozen candidates undergoing clinical trials is provided in Table 3. As shown in Table 3, the 23 TB vaccine candidates currently under development involve various approaches and platforms, including recombinant vaccines, inactivated vaccines, viral vector vaccines, attenuated vaccines, subunit vaccines, and whole-cell vaccines. Each candidate is at a

Table 3. The summary of the more than a dozen candidates undergoing clinical trials of candidate TB vaccines in phases I to III on the word [17,110].

No.	Platform	Vaccine Candidate	Phase I	Phase IIa	Phase IIb	Phase III
1	Viral Vector	TB/FLU-05E	√			
		TB/FLU-04L		√		
		AdHU5Ag85A	√			
		ChAdOX1.85A + MVA85A	√			
		H107/CAG10b	√			
		H107/ CAF®01		√		
		AEC/BC02		√		
2	Protein/Adjuvant	GamTBvac				√
		M72/AS01E				√
		ID93+GLA-SE		√		
		H4/IC31		√		
		H56/IC31		√		
3	Live Attenuated Vaccine	MTBVAC				√
		VPM1002			√	
		BCG (revaccination)			√	
		BCG (traveler vaccine)			√	
		VPM1002				√
4	Inactive Vaccine	RUTI			√	
		Immuvac (MIP)		√		
		DAR-901			√	
		Vaccae				√
5	RNA	BNT164a1	√			
		BNT164b1	√			

different stage of clinical testing to evaluate its effectiveness, safety, and ability to protect against TB. The results of these clinical trials are expected to lead to improved and more effective TB vaccines for prophylaxis and TB prevention.

Members of the *Mtb* Complex (MTBC) are the causative agents of TB disease. When MTBC bacteria are actively multiplying or reproducing in the lung tissue of a patient, these bacterial cells actively secrete and release protein molecules that damage lung tissue. The secretory molecules produced by actively multiplying MTBC bacterial cells include proteins such as Antigen 85 A, B, and C, ESAT-6 protein, CFP-10, CFP-21, and other proteins [111]. Proteins Ag85 A, B, and C are secretory products of MTBC bacterial cells. These virulence factors can damage lung tissue cells as dominant secretory molecules from actively multiplying MTBC bacteria in lung tissue. These proteins have large molecular weights and complex molecular structures. These protein molecules benefit the bacteria by protecting them from the patient's body's phagocytic cells and maintaining their survival within the host's phagocytic cells. This prevents the bacteria from being destroyed by phagocytic cells, making TB disease challenging to cure through immune function.

In the vaccine development, Ag85 protein serves as an antigen based on its potential to stimulate the proliferation and differentiation of individual immune cells, including T and B lymphocytes, and to activate cells to produce increased levels of antibodies, granzyme B, and perforin, which play a role in the individual's or patient's immunity. According to recent experimental studies, using a combination of recAG85A and recAg85B proteins with the BCG vaccine can significantly enhance protection against MTBC bacterial infection by effectively stimulating the response to increased levels of IgG antibodies, granzyme proteins, and perforin proteins [112].

M72/AS01E subunit vaccine was developed by combining the mycobacterial antigens Mtb32A and Mtb39A in conjunction with the AS01E adjuvant. The vaccine's clinical safety profile and its long-lasting durability have been investigated in many studies. Infants vaccinated with BCG, adults infected with HIV, and adults infected with *Mtb* were found to display the polyfunctional CD4+ T cells that release IFN-γ, IL-2, and TNF-α. The M72/AS01E vaccine formulation demonstrated 54 % protection in healthy adults against disease activation for at least three years in Phase 2b trials [113]. Furthermore [114,115], higher vaccine-induced seroconversion rates were observed, with a sharp increase in seroconversion after administering the second dose, thereby enabling optimal protection against TB.

Although M72/AS01E seems to be well-tolerated, the vaccinated group reported more occurrences of mild to moderate local adverse events (AEs) and severe injection site pain than the placebo group. The systemic adverse event profile resembled flu-like illness in the immediate post-vaccination period. AEs usually disappear within a week or two. Vaccine participants who were infected with *Mtb* at the time of enrolment had some common symptoms more often than placebo receivers or uninfected individuals. The results on safety and immunogenicity cannot be definitively stated due to the small sample size of this early-stage study.

The following are some further details about what is known regarding the effectiveness of the M72/AS01E vaccine: Phase 1: Phase 1 clinical trials evaluate the safety and dosage of the vaccine. The Phase 1 clinical trial of M72/AS01E showed that the vaccine is safe and well-tolerated. Phase 2: Phase 2 clinical trials assess the vaccine's effectiveness in eliciting an immune response. The Phase 2 clinical trial of M72/AS01E demonstrated that the vaccine induces a strong immune response in healthy adults. Previous phase II and phase IIb clinical trials of the M72/AS01E vaccine demonstrated that its safety and immunogenicity were favorable for both HIV-positive and HIV-negative groups in adults and adolescents [81,116–118], as well as a booster for BCG in infants [119]. The final analysis on phase 2 clinical trials of M72/AS01E showed efficacy of around 54.0 %; 95 % CI: 2.9–78.2; $p = 0.04$ over a 2-year observation period and 49.7 %; 95 % CI: 2.1–74.2; $p = 0.04$ over a 3-year observation period [113]. Phase 3: Phase 3 clinical trials evaluate the vaccine's effectiveness in preventing disease. The ongoing Phase 3 trial, supported by the Gates Medical Research Institute and partners, involves up to 20,000 participants across seven countries, including South Africa, Zambia, Malawi, Mozambique, Kenya, Indonesia, and Vietnam. The trial will include individuals living with HIV to assess the vaccine's effectiveness in this vulnerable population. The diverse participant pool aims to provide comprehensive data on the vaccine's performance across different demographics and geographical regions. The trial is expected to span five years, during which participants will receive either the M72/AS01E vaccine or a placebo.

4. Conclusions

Currently, the only vaccine that can prevent or eradicate TB in humans at the moment is BCG.

However, there are many problems with BCG, and it cannot prevent TB infections in adults and people of childbearing age. Therefore, scientists are collaborating together to find a more effective vaccination strategy. Scientists are employing bio-immunoinformatics tools to investigate strategies for developing novel TB vaccines, including recombinant protein and subunit techniques. Bio-immunoinformatics has revolutionized vaccine development by providing powerful tools to identify and design new candidate vaccines against TB. Continued advancements in computational methods and interdisciplinary collaboration will be crucial in overcoming challenges and achieving the goal of eradicating TB. The discovery and cloning of several *Mtb* bacterial cell proteins or antigens has broadened the efforts to create novel subunit vaccine candidates. Vaccine administration routes have also been the subject of extensive research and published extensively. It is encouraging that researchers are making progress in producing more effective TB vaccinations; with any luck, in the not-too-distant future, we will be able to eradicate TB completely off the face of the earth. Despite the advances, there are challenges, such as validating the *in-silico* bio-immunoinformatic predictions through experimental studies, the complexity of host-pathogen interactions, and the genetic diversity of *Mtb* strains. Future advancements require integrating multi-omics data, improving computational models, and accelerating translational research to bring candidate vaccines from lab to clinic. Interdisciplinary collaboration and technological progress in AI (artificial intelligence) and machine learning will further enhance antigen discovery, immune response modelling, and epitope prediction, offering a promising outlook. With sustained efforts and innovative approaches, achieving TB eradication remains an attainable goal.

Ethics information

This review study did not involve any *in vivo* or animal experimentation, ethical permission not necessary.

Conflicts of interest

All authors declared that there is no conflict of interest.

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