Review Article

Tuberculosis control by novel vaccines development and Indian initiatives

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Abstract

Pulmonary tuberculosis (TB) caused by *Mycobacterium tuberculosis* (Mtb) is one of the worst microbial diseases of humankind and an awful societal problem, especially in underdeveloped countries. The enormous TB-infected individuals were in the Southeast Asian Region (46%), about 23% in the African Region, 18% in the West Pacific region, and the rest in other regions. While TB is often effectively treatable, untreated cases can lead to drug-resistant forms that are harder to manage and may kill the infected sooner after the disease flares up. In 2021, approximately 1.6 million individuals succumbed to TB worldwide. Although the Bacillus Calmette–Guérin (BCG) vaccine is currently the only approved anti-TB vaccine, its effectiveness in adolescents and adults is limited. Globally, several vaccines are in the developmental stage. This review narrates the progress made in developing 23 candidate anti-tuberculosis vaccines (including developing more immunogenic and safer recombinant Mtb strains) by different scientific groups worldwide. Additionally, the incidence of TB in India, along with the Indian initiatives and activities in controlling the disease, has been briefly discussed.

Keywords: BCG, Indian TB programs, *Mycobacterium tuberculosis*, new TB vaccines, phagosomes, TB in India, TB vaccines, tuberculosis

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INTRODUCTION

Tuberculosis (TB) is an infectious bacterial disease caused by *Mycobacterium tuberculosis* (Mtb). Mtb usually infects the lungs but can infect any part of the body; among these are Mtb infections of the kidney, spine, brain, and heart. Tuberculosis can also infect the skin. Mtb infecting the lungs is termed pulmonary tuberculosis. TB-infected individuals can carry the microorganism but may not have active disease symptoms. Those having pulmonary tuberculosis spread the disease to others through aerosol droplets, transmitted through cough. Those infected with Mtb but not having clinical symptoms of the disease are classified as having latent tuberculosis (LTB). In individuals having LTB, the immune system fights the infection and can suppress the disease to a great extent. Persons suffering from LTB cannot transmit the disease to others. LTB is a complex immunologic issue. In LTB in pulmonary tuberculosis, the Mtb initially gets entry through the nostrils during breathing, inhaling microscopic germ droplets generated from the cough of an Mtb-infected individual who may be physically close to the non-infected. The size range of the infectious cough droplets can be from 0.65 to over 7.00 μm.[1]

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The size range of Mtb in length and width allows the cough droplets to accommodate one or more microbes within their space, thereby causing the generation of infectious aerosols, stabilized by the action of surface active amphiphilic biological substances secreted by the lung tissues at the infection site. Once the Mtb entrapped into microscopic droplets reach the lower respiratory tract of the lungs of the healthy individual with the inhaled air and touch the lung surface, the contents in the aerosol open up and diffuse inside, reaching the lungs tissues where the resident macrophages or the white blood cells which are the professional phagocytes meant for defending the invaders, engulf the Mtb and internalize it inside a vesicular body formed by itself. Pathogen-associated molecular designs on *M. tuberculosis* are recognized via diverse receptors on the macrophages that mediate opsonic and non-opsonic bacterial uptake into these immune cells. The internalized vesicular body within the cytoplasm of the macrophages is called a phagosome. It is formed as a vacuole. Phagosome formation enhances reactive oxygen species (ROS) production by activating the NADPH oxidase (NOX) enzyme complex. Entrapped Mtb develops capacities to withstand the aggressive and destructive environment of the phagosomes by mechanisms that are not yet fully understood. After creating phagosomes, they draw the resident lysosomes within the macrophage toward them. These lysosomes adhere to the outer surface of the phagosomes, poised for fusion when the appropriate conditions are met. This fusion forms a digestive structure called a phagolysosome, where the lysosomal contents are delivered into the phagosome.

During the maturation process of phagosomes, among other biochemical changes, the lowering of the phagosomal pH is obligatory. Phagosome-lysosome fusion occurs after the phagosome's membrane-surface pH changes to acidic pH. Lysosomes contain degradative enzymes that hydrolyze DNA, RNA, proteins, polysaccharides, and lipids. Once the contents of the lysosome are delivered into the phagosome, the released contents destroy the Mtb entrapped therein. In summary, in hosts having strong immune systems, the phagosome maturation involves biochemical activities of turning the phagosome and its outer membrane into acidic pH, followed by fusion with the lysosomes forming a phagolysosome and destruction of entrapped invading agents by the released contents from the lysosomes are thought to be the mechanism for clearing the infection by the healthy hosts.[2]

In relatively weaker immune hosts, a few of the engulfed Mtb residents inside the phagosomes can survive within the adverse conditions and are in a position to direct the reprogramming of the membranes of the phagosomes, preventing these from getting acidic, thereby preventing fusion with the adhered lysosomes. In such situations, the captured Mtb remains alive with minimum nutrient uptake within the phagosomes. In such immunity hosts, TB remains latent for years. During the initial phase of infection, the infected macrophages and other infected cells transmit chemical signals that attract neutrophils, lymphocytes, and monocytes towards the infected cells. However, the bacteria remain hiding within the infected macrophage and remain unapproachable to the surrounding neutrophils, lymphocytes, and monocytes, subverting the immune response of the host by the mechanism, which is not fully understood, thereby facilitating the formation of granulomatous focal lesions (which are inflammatory cell infiltrates) that provide a survival niche, and enable limiting growth and spread of Mtb. The bacilli, in a few numbers, survive inside granulomas. Of all the Mtb-infected individuals in the LTB stage, only about 10% of the infected eventually get the clinically identifiable TB disease, which implies that phagosomes also have a protective role,[3] in preventing and delaying the advancement of the disease. The onset of the disease is characterized by an initial low rise in body temperature, gradual but advancing weakness, weight loss, persistent cough accompanied by sputum production, and night sweats. A well-observed phenomenon in Mtb infection is that apoptosis of infected host macrophages is inhibited, much of the mechanism of which has been unveiled, although a great deal of it is yet unclear; virulent Mtb induces as well as inhibits infected host macrophage cell death.[4-9]

Granulomas are considered highly dynamic and are held in place in a dynamic equilibrium until the immune system of the infected is relatively strong. Individuals can remain disease symptoms-free if the immune system can stop the rapid multiplication of the Mtb, and may even win the situation by therapeutic interventions at this stage and may get free from the disease if treated with appropriate anti-tubercular chemotherapy. It is estimated that nearly 30% of the global population has LTB. Mtb cannot be detected in such infected individuals, but their blood test for interferon-γ release assays or the tuberculin skin test can provide evidence of LTB infection.[10]

The LTB-infected individuals, if untreated, and if the immune system further deteriorates for various reasons, including inadequate intake of nutritious foods and the aging process, then at the opportune time, the Mtb shall dissolve the granulomatous focal lesions and multiply by using the dissolved host-cellular materials as food for multiplication, and shall invade the non-infected lungs

tissues as also other body parts, and would eventually kill the host.[11-14]

In all types of pathogenic microbial attacks on humans, the first line of defense is the innate immune response where the macrophages, the neutrophils, and the NK cells mount an attack together, trying to destroy the invader by engulfing it after recognizing it as an invader through the recognition of the protruding patterns of the protein substances on the outer surface of the invader. After engulfing the intruder in a phagosome, its destruction follows phagosome maturation, fusion with lysosomes, and disintegration with lysosomal enzymes. Concomitantly, biochemical signals are sent through the immune cells, such as the dendritic cells and neutrophils, to the other macrophages for getting activated for sending molecular signals to the inactive $CD4^+$ T cells through MHC class II pathways to activate the adaptive immunity tracts through Th-1 and Th-2 modes of cellular pathways of immunity responses, to produce activated $CD8^+$ T cells and plasma B cells respectively. Multiple cytokines such as IL-8, IL-12, IL-17, TNF-alpha, and IFN beta get secreted by the immune cells as Mtb enters the human body. However, the Mtb cannot be fully cleared as it has developed the mechanism to hide inside the phagosome complex of the infected macrophages of relatively weaker immunity-possessing hosts, as already outlined.

In countries with a high incidence of active TB, many individuals with LTB who are otherwise healthy remain and exist. The innate immune system (IIMS) of such individuals capacitates a large percentage of such individuals to get their own IIMS activated, learning to sense more precisely the presence of multiple foreign disease-producing antigens and to fight more numbers of microbial invaders, including multiple viruses with increased efficiently. It was observed by the author[15,16] that in countries where the incidence of tuberculosis was higher, there was statistically less number of deaths from COVID-19 flu when compared with deaths in countries with less incidence of tuberculosis. The author hypothesized that in such countries with a high incidence of tuberculosis, there would also be a high percentage of Mtb-sensitized healthy individuals exposed to tuberculosis, and such individuals would have more competent innate immune capabilities to resist COVID-19 flu. A recent study on a small number of Danish healthcare workers concluded[17] that Bacille Calmette–Guérin (BCG) vaccination did not affect the incidence of COVID-19 and other infectious disease episodes. The Danish population has a very low incidence of tuberculosis,[18] so the results obtained on the effects of BCG vaccination on the incidence of other infectious diseases are not surprising.

Further, the immune response from Mtb exposure is anticipated to be much more intense as the antigens expressed by Mtb are much more diverse than those expressed by BCG. More studies are necessary to ascertain how the immune system in both innate and adaptive pathways gets modulated on exposure to Mtb and what roles such immune activation plays in postponing TB disease syndromes' development. In this context, the role of the phagosomes in immune activation, protecting the hosts in cases of LTB individuals, may also need to be understood more precisely, which may have a bearing on elevating the IIMS.

Although tuberculosis infection caused by Mtb is often curable and preventable, about 1.6 million people died in 2021, of which some 187,000 had HIV. Tuberculosis disease is presently the 13th leading cause of death and the second leading cause of kill by microbial infection after COVID-19. Worldwide, some 10.6 million people fell ill with tuberculosis in 2021. The most significant number of TB-infected individuals were in the Southeast Asian Region (46%), about 23% in the African Region, and about 18% in the West Pacific region.[19] The regions' classification is based on the World Health Organization (WHO) classification. India, Pakistan, Bangladesh, China, Indonesia, the Philippines, the Democratic Republic of the Congo, and Nigeria are the regions where people are more infected with the disease.

For the treatment of tuberculosis infection, the first line of treatment of Mtb is with rifampin, isoniazid, pyrazinamide, and ethambutol; the second line of treatment is with combinations of kanamycin (discontinued use in some countries like the USA), streptomycin, capreomycin, amikacin, levofloxacin, moxifloxacin and gatifloxacin; while treatment of multidrug-resistant tuberculosis is with one or more of the therapeutic substances such as bedaquiline, delamanid, linezolid and pretomanid.[20]

The Mtb strains causing tuberculosis are systematically classified based on their susceptibility to the disease-treating drugs. The disease can be had from Mtb strains which are resistant to one of the first line of treatment drugs, namely rifampicin, isoniazid, pyrazinamide, and ethambutol, and the disease caused by such Mtb strain is then called Mono-resistance tuberculosis (mono-resistant TB). When the Mtb strain is resistant to more than one first-line anti-TB drug, other than both isoniazid and rifampicin, the disease caused by the strain is classified as poly-resistance tuberculosis (poly-resistant TB). Multidrug resistance tuberculosis (MDR-TB) is caused by Mtb strains resistant to at least both isoniazid and rifampicin. TB can be had from Mtb strains that are multidrug-resistant

(MDR-TB) and also resistant to any fluoroquinolone (used in tuberculosis treatment) and at least one of three second-line injectable drugs (capreomycin, kanamycin, and amikacin). Tuberculosis from such Mtb strains is called extensive drug resistance tuberculosis (XDR-TB).[21]

REASONS FOR WRITING THE PAPER

The evolved research and developmental strategies on tuberculosis management through vaccination have shifted gradually towards improving the BCG strains by recombinant DNA technology and developing safe and more effective live recombinant Mtb strains for use as candidate anti-tuberculosis vaccines. Efforts are also directed towards boosting the BCG vaccine-based immunity with recombinant subunit vaccines such as viral-vectored- or protein-based vaccines. Inactivated Mycobacterium strains of different types are also on the menu. Further, the research strategy is to boost the efficacies of the protein and peptide-based subunit anti-tuberculosis antigens formulated with novel adjuvants. Knowledge of genomic sequencing of *M. tuberculosis* strains, advances in molecular genetics, and expertise in bioinformatics enables the identification of more potent protein and peptide-based Mtb antigens. Several recombinant DNA-based viral-vectored constructs are also being used to develop stronger adaptive immunity.[22-35] *M. tuberculosis* bacteria undergo mutations in their genome to resist the therapeutic drugs discovered over time, although understanding resistance-conferring mutations is incomplete.[36] Nevertheless, since resistance development to therapeutic substances is a feature of tuberculosis, one of the ways to keep the infection away in disease-prone countries is to have effective vaccines for developing an immunologically strong self-defense system in innate and acquired immunity pathways in every individual. This paper is yet another update in the form of a gist of the research work being carried out globally towards the development of one or more effective anti-tuberculosis vaccines, along with an analysis of strategies and recommendations that are thought to be beneficial towards containing the disease in various countries, and especially in India. The Indian government has plans to contain the disease on a time-bound scale. This effort is to find more scientific clues for the abatement of tuberculosis through the invention and/or use of potent anti-tuberculosis vaccines. The author has long-term experience in handling novel adjuvants based on *Mycobacterium w* (Mw) and novel p38 MAPK inhibitors involving Mw.[37,38]

TUBERCULOSIS INCIDENCE IN INDIA

The Indian Ministry of Health and Family Welfare (MOH&FW) notified that in 2021, India had a load of 2.14 million TB cases, about 18% higher than in 2020. In 2021, over 220 million people across the country were screened for TB for early detection and treatment of the disease. The pooled prevalence of bacteriologically positive pulmonary tuberculosis in India was reported[39] to be 295.9 (at 95% confidence interval (CI): 201.1–390.6) per 100,000 population, and the disease was higher among males than among females. The National TB Prevalence Survey India 2019-2021 Report has more details.[40]

The Indian government continues to support, free of cost, the treatment of over 1.045 million TB patients nationwide under the new initiative named Pradhan Mantri TB Mukt Bharat Abhiyan (PMTBMBA).[41] PMTBMBA is an initiative of MOH&FW, primed by the Prime Minister, and the program aims to eliminate tuberculosis from India by 2025. PMTBMBA was launched[42] virtually by the Hon'ble President Smt. Droupadi Murmu on September 9, 2022. Elimination of TB would mean reaching[43] a target of no more than 44 new TB cases or 65 total cases per one hundred thousand of the Indian population by 2025.

The present estimate[44] is that about 40% of the Indian population is infected with Mtb bacteria and that the vast majority of such population have latent TB rather than clinically manifested TB disease. The incidence of TB cases (including HIV + TB) was estimated at 2590,000 in 2022, about 188 per one hundred thousand population. To reach a figure of 65 total cases per one hundred thousand of the Indian population by 2025 is considered to be an enormous task to accomplish.

Combatting drug-resistant tuberculosis in India is a considerable challenge,[45] although India has improved in the situation over the years. It was estimated that in 2008, India had the second-highest number of MDR-TB cases after China, accounting for 24% of the 5.7 million TB cases (new and relapse) notified globally in 2010. There existed multiple obstacles to combat the disease, such as "too few laboratories, slow diagnostic tools, inadequate management of treatment, insufficient supplies of second-line drugs, and shortages of trained personnel," which situation has only marginally improved up to the present time. There were 88060 deaths[46] in India from tuberculosis in 2021, compared to 1.6 million deaths globally.[47] The deaths from tuberculosis in India in 2021 were about 5.5% of global deaths.

An estimated 3.5 million TB deaths occurred in 2000 globally,[48] of which approximately 0.5 million were also associated with HIV infection. In India during that time,[49] some 0.5 million died annually from the disease. When compared with these figures of 2000, while globally there

had been an improvement in the control of deaths from the disease from the year 2000 to 2021, India had made an overall improvement in the control of the disease both in terms of actual numbers of reduction in total deaths (88060 deaths in 2021 compared to 0.5 million in 2000) as well as, in terms of percentage of total death globally (5.5% in 2021 compared to 14.3% in 2000).

Indian strategy, in brief, is to vaccinate the newborn with BCG and to treat effectively the active tuberculosis cases using therapeutic medication; to adopt and use all the treatment lines of drugs, namely the first line of TB treatment for diagnosed Mtb infection; to treat MDR-TB patients; to treat Rifampicin-resistant TB cases; and to treat all the other kinds of tuberculosis-infected individuals by providing medicines free of cost through the government programs. BCG vaccines are available freely to the newborn through the Indian Expanded Immunization Program (EPI). BCG is abundantly available from the supplies of locally manufactured, WHO-prequalified industrial establishments for BCG vaccine production, namely Serum Institute of India, Pune; B C G Vaccine Lab, Guindy; and Green Signal Biopharma, Chennai.

Except for BCG vaccines, there is no other approved vaccine against tuberculosis. It is already well-known that the efficacy of the BCG vaccine to prevent pulmonary tuberculosis in adolescents and adults is very limited. It costs the government heavily to provide therapeutic substances to treat the affected free of cost. The financial burden on individuals due to out-of-pocket healthcare expenses is notably high, and frequently, the impoverished individuals who are afflicted cannot afford to cover these costs when they are expected to do so from their funds. More resistant tuberculosis strains develop in such situations, making the diseased more troubled. Therefore, there is an urgent need to develop a more effective vaccine against tuberculosis. If appropriate vaccines are available, often the government would provide such vaccines free of cost to the residents. Using an effective TB vaccine shall also reduce the expenditure burden on the national tuberculosis eradication programs. The availability of an effective anti-tuberculosis vaccine is, therefore, highly desirable in the country.

India has no unique lead in developing a novel anti-tuberculosis vaccine. Nature has provided multiple viruses. Viruses are essentially proteins-expressing nucleic acid assemblies, wrapped with protective protein coats. Viruses that can infect human cells are potential opportunities for scientists to choose and pick up from, modify, and use the modified nucleic acid stretches for expressing chosen antigens for developing novel anti-tuberculosis vaccines. Such modified viruses would lose the capacity to infect human cells. Several novel recombinant DNA-based viral-vectored constructs exist globally for anti-tuberculosis vaccine development, none of which have been invented in India. India has acquired competence in expressing multiple recombinant DNA-based antigen proteins in transgenic hosts. Indian research can, therefore, be directed towards inventing novel and competent viral vectors and packaging such vectors for expressing Mtb antigens to develop novel anti-tuberculosis candidate vaccines.

Further, as high levels of expression of antigens admixed with suitable adjuvants can make novel protein and peptide-based anti-tuberculosis vaccines, India can also venture to develop such adjuvanted novel vaccines. In doing so, India needs to develop novel adjuvants where Indian expertise is presently feeble. However, the country is highly competent in expressing multiple kinds of recombinant DNA-based antigens in different hosts.

VACCINATION: A COST-EFFECTIVE WAY OF KEEPING THE POPULATION HEALTHY

There is a lot of rich literature to show that the economic benefits of effective vaccination are much more when compared with the costs of treatment of a disease. The impact of effective vaccines is broad and far-reaching. Vaccination reduces morbidity and mortality from infections. Effective vaccination is the cost-effective method for societies preparing to prevent infectious disease outbreaks at all levels, but it is most useful for the socially underprivileged poor. The cost-effectiveness and other economic benefits of vaccination against infectious diseases are extensively reviewed.[50-53]

The efficacy of the only approved anti-tuberculosis vaccine namely bacillus Calmette–Guérin (BCG), with limited fruitfulness in preventing pulmonary tuberculosis in adults, urges the need for the availability of one or more new, novel, effective, broad-spectrum anti-tuberculosis vaccines for controlling the disease.

Tuberculosis caused by Mtb infection is one of the most threatening infectious diseases in the world. The diseases cause high mortality in high-burden regions, where large sections of people residing there are weakened by poverty, such as Asian countries including India, China, Pakistan, Bangladesh, and other neighboring countries; African countries; and a large stretch of South American countries. The disease may be curable if diagnosed early, the recommended anti-tuberculosis therapeutic substances

are used for the recommended period, and the patients are adequately fed with nutritious foods. Research carried out during the decades of 2000 and 2010 has enabled us to understand more about the immune responses against the disease. With increased knowledge, several vaccines yet in the developmental stage have been devised. A couple of vaccines to prevent the disease are anticipated to be soon available.

ANIMAL MODELS IN TUBERCULOSIS RESEARCH

Each research strategy for developing a vaccine includes the determination of immunogenicity of the chosen antigens, their adequate safety on usage, and their adorable efficacy, tested and determined in preclinical animal models, followed by moving through phases I, IIa, IIb, and III clinical trials. Preclinical immunogenicity, safety, and efficacy have been determined in various kinds of animals by different investigators. Although Mtb can cause infection in several species of animals besides humans, no animal model of the disease can completely simulate the occurrence and development of human tuberculosis. Animals like mice, guinea pigs, rabbits, rats, pigs, goats, cattle, and nonhuman primates have been used under different experimental settings to generate information, which provides insights into understanding the immune responses, pathogenesis, and pathological changes.[54] The use of mice requires incurring less expenditure, a short experimental cycle, mature immunological evaluation indicators, and the availability of inbred strains. BALB/c and C57BL/6 mice strains have most frequently been used.

Guinea pigs are also commonly used in many studies. Guinea pigs are more susceptible to tuberculosis caused by Mtb. Granulomas similar to humans are formed in guinea pigs.

Several investigators have used pigs to study the response of antigen-specific T cells to mycobacterium lipids and lipopeptide-rich antigen preparations. Due to their high similarity to humans in terms of anatomy, genetics, and immune response, these animals have been used in numerous studies where the mechanism of the spread of tuberculosis is being studied to understand several safety aspects of vaccines, particularly in infants.

Immunological responses to nonhuman primates are thought to be closer to human responses, and therefore, assessments of safety and efficacy have been carried out in these animals too.

Other animals, such as zebrafish, drosophila, certain humanized animal models, and ameba have also been used in some studies.

None of the above animals are ideal for predicting the precise host-immune correlates, except that studies in specific animal models provide considerable immunological insights, based on which studies can be elaborated further during the phase trials. Humanized animals must be developed in this context to obtain more precise insights into host-immune correlates. Nevertheless, the present methods of advancements are based on various animal studies of the chosen new antigens along with comparative evaluations using the long-approved BCG vaccines, based on which intelligent conclusions are drawn to end animal studies and move into phases of clinical trials on human subjects.

TB VACCINES DEVELOPMENT PATHWAYS AND MAJOR SUCCESS IN R&D

Certain nonclinical studies must be carried out for a vaccine to be competent for clinical phase studies. Based on the results of such studies, investigators must seek the regulators' permission to conduct preclinical and clinical phase studies and proceed step by step. While research on the development of effective vaccines has initially been empirical in the context of understanding the basis of protection immunologically, the comprehension of the molecular basis of protection through vaccination has been gradual, and the scientific knowledge of protection through vaccination continues to grow.

Vaccination against an infection aims to generate efficient and long-lived immune memory to shorten the time gap between the infection and the onset of adaptive immune response at the site to control the infection and its dissemination to the secondary areas. Vaccination aims to prevent Mtb infection and ward off the microbes to advance up to the stage of clinical disease manifestation from the LTB stage. The target vaccines can be prophylactic vaccines administered to healthy individuals to prevent Mtb infection, which may be used singularly or with booster doses as determined experimentally.

Another type of vaccine development includes therapeutic vaccines where the recipient individuals are administered with processed disease-causing inactivated pathogens (antigens) admixed with suitable adjuvants. Such composite formulations primarily target host macrophage response modification and activation, resulting in successful phagosome maturation.[2] The macrophages and the innate immune cells are the first lines of host defense against any infection, and the Mtb infection is no exception. Incidentally, the macrophages are also the hosts of Mtb, providing them as the residence of the infecting pathogens. The Mtb infection passes through several complex stages,

maintaining an asymptotic status in the infected after being phagocytosed and remaining in the patient macrophage-phagosome assembly as LTB.

One can assume that if a very low dose of the recombinant Mtb cells (or its killed form or even the inactivated wild strain, inactivated at low temperature) or its identified antigens[55,56] are used to sensitize the macrophage-phagosome assembly with an added immunomodulator known to induce strong phagocytosis, then there might be priming of the macrophages, both infected and non-infected, activating these cells to proceed further to switch on themselves to act as antigen-presenting cells (APCs) for vaccination.

The phagosomes are formed at the lungs-resident macrophages. The phagosomes tending to change to late phagosomes with lowering of their pH undergo maturation after being fused with lysosomes present in the cell cytosol and finally form phagolysosomes, leading to digestion and clearance of the infection, thereby making the host free of tuberculosis. This process is rendered inactive by Mtb infection. It is thought that the blood-borne non-infected macrophages on being primed as above may assist in phagosome maturation. To achieve the goal in the vaccinated individuals with the new vaccines by the aforesaid priming process, the fate of granuloma formation must be assessed with careful monitoring of the new vaccine recipients through rigorous monitoring of diagnostic signatures and clinical validation. If such a concept can be established then a number of therapeutic vaccines might also emerge.

Live-attenuated vaccines are created using safe live microbes, which are similar to the natural infective microbes. Therefore, it is thought that the immune response by using such microbes shall be strong and long-lasting. Attenuated strains activate the recipients' immune system through well-defined and unmistakable subsets of T helper cells in the Th-1, Th-2, and Th-17 pathways to create clonal antibodies, cytotoxic CD 8+ T cells, and memory immune cells. BCG is the only live-attenuated anti-tuberculosis vaccine approved for vaccination against tuberculosis. However, many live-attenuated Mtb strains are in the R&D stage.

Inactivated or killed microbes-based vaccines are created using different types of killed Mycobacterium species as recombinant Mtb strains. The advancements in the understanding of the fundamentals of the role of the innate immune system in recognizing the antigenic patterns from the evolutionary knowledge and communicating the immune system in the adaptive pathways for creating preparedness through the Th-1 and Th-2 routes on a structured mode are the basis of immunization while using inactivated microbes-based vaccines. Innate immune cells cannot precisely recognize a specific invader but possess knowledge of pattern recognition through evolutionary development. Specific adjuvants assist in intensifying the propagative and promotional roles of the innate immune system to be more effective. The immune evasion strategies of *M. tuberculosis* have not been fully understood yet. Identifying key protective epitopes against Mtb strains is a complex issue that has not yet been satisfactorily resolved, although much knowledge has been gained. Investigators have focused on precisely identifying potent antigens that stimulate the $CD8^+$ T cells through the $CD4^+$ T helper cells in the Th-1 pathways for destroying the invading *M. tuberculosis* strains and also for identifying other potent antigens to effectively neutralize the disease-causing *M. tuberculosis* strains, again for enabling the activated macrophages to clear such microbes from the body of the infected.

It is well-established that the structure of an effective antigen would determine the specificity, affinity, and accessibility of the binding sites to major histocompatibility complex (MHC) proteins involved in antigen presentation to T cells or the sensitized antibodies and, thereby, the specific immune response can be facilitated. Using the present knowledge in immunology and the advancements in protein structures, primarily through crystallography, structural biology, bioinformatics, and genomic analysis of multiple *M. tuberculosis* strains, several of the more potent protein antigens have been identified, many of which have been produced either singularly or a number of these have been biologically recombined and joined, and after that produced by recombinant DNA technology, purified and formulated with more potent adjuvants. For an antigen to efficiently work, the antigen admixed with an efficient adjuvant work complements one another. The combined effect should elicit immune responses in innate and adaptive immunity pathways to identify and eliminate pathogens efficiently. Protein and peptide antigens often require selecting more effective adjuvants for the cohort to be more effective, highlighting the importance of selecting adjuvants. Several such novel combination-based formulations have been tested as potent anti-tuberculosis vaccine formulations.

In viral vector-based vaccines, a genetically modified harmless piece of a virus construct is created to smuggle the pre-packed nucleic acid instructions that code for making antigens from the disease-causing microbes into human cells so that on being translated into proteins (antigens) within the cytoplasm of the recipient, these are secreted out of the cytoplasm, and are available to the immune cells, thereby triggering protective immunity against it. Viral vectors can be replicated, in which case multiple copies of the vectors are produced upon entry into cells. Consequently, because

of replication, more cells can be infected and therefore more antigens shall be produced. In certain situations such properties are disadvantageous, causing concerns of safety for the recipients. The viral constructs can be non-replicating types of viral vectors also. Vaccine formulations can be designed using non-replicating types of vectors also. In such situations, the antigen production is limited, but is enough for eliciting immune responses within the recipients. The anti-tuberculosis viral vector-based vaccines are developed in non-replicating types of viral vectors. These vectors cannot integrate into the genome of the recipient cells.

In summary, it can be said that the vaccine development strategies by adopting the use of live-attenuated microbes as well as the inactivated microbes admixed with adjuvants are considered to be rather blind shots for protection, though experimentation-based because enough precise basis of understanding of protection at the molecular level of immunological pathways are not fully understood nor the clonal developments of protective T and B cells can be controlled precisely. The development of proteins and peptides-based adjuvanted vaccines as well as the viral vector-based developmental strategies are more knowledge-based in the context of more immunological insights at the molecular level. Numerous Mtb membrane and cell wall glycolipoproteins, crucial for novel vaccine discovery, are heavily glycosylated (at N and O sites). Selecting judiciously from these diverse menus and combining a few through recombinant DNA technology is a daunting task. New TB vaccine candidates are believed to be identified from such studies. Viral cassettes expressing such optimized combinations of TB antigens are thought to be coming out as novel future anti-TB vaccines. The present stage of knowledge is yet not adequate however, for conquering the invasion of Mtb.

The gist of present global developments in search of effective anti-tuberculosis vaccines is provided below:

LIVE-ATTENUATED TB VACCINES

The following are, in gist, the global developments in live-attenuated anti-tuberculosis vaccines:

Bacillus Calmette–Guérin (BCG), the only approved live-attenuated vaccine

The bacillus Calmette–Guérin (BCG) is the only vaccine to humankind to prevent tuberculosis by vaccination. While live BCG was used for the first time in July 1921 by the oral route to immunize people against the disease, the microbial strains of BCG that are used presently have undergone change over the years in the hands of scientists. The present-day route of administration of BCG vaccination in the Expanded Program of Immunization (EPI) for the countries using BCG is intradermal, injected into the left upper arm in the deltoid region. The vaccine is reconstituted with the appropriate diluent before administration. The vaccine is given within a few days of birth and can be given up to six months of age and even later. In countries where TB is more common, one dose is recommended in healthy babies soon after birth. There is, however, no universal uniform BCG global vaccination policy. In some countries with rampant tuberculosis, BCG vaccination is included in their EPI. In many countries, BCG vaccination is only optional.

The attenuated BCG strains are derived from *Mycobacterium bovis*. The BCG vaccine's initial live-attenuated microbial strains are reportedly derived from *M. bovis* through 230 successive passages of the wild strain (*M. bovis*) grown on media-impregnated beef bile.[57,58] Presently, several attenuated BCG strains are used for vaccination in different settings, the major ones among which are the BCG-Denmark strain, BCG-Russia strain (genetically identical to the BCG-Bulgarian strain), and BCG-Japan strain. In India, at least three manufacturers[59] are WHO-prequalified for the supply of the vaccine, namely Serum Institute of India, Pune (use Russian 368 strain); B C G Vaccine Lab, Guindy, Chennai (Danish 1331 strain); and Green Signal Biopharma, Chennai (Danish 1331 strain).

All the approved BCG vaccines do not perform as similar in enhancing immunity; some are more efficacious than others. In a relatively recent study,[60] for the comparative efficacy of different BCG vaccines, using three widely used BCG vaccines, namely the BCG-Denmark strain, BCG-Japan strain, and BCG-Russia strain on a pool of 209 infants immunized with these BCG vaccines, the data from 164 infants were included in the final analysis, consisting of BCG-Denmark, $n = 54$; BCG-Japan, $n = 54$; and BCG-Russia, $n = 57$. The results indicated that the BCG-Denmark or BCG-Japan vaccine induced higher frequencies of mycobacterium-specific polyfunctional and cytotoxic T cells and higher concentrations of Th-1 cytokines. The results were indicative of the fact that there were differences in protection levels of different BCG vaccines against tuberculosis, although no differentiation is made by the WHO or by any agency between and among the WHO-authorized BCG vaccines and their immunization effects on the children; all the children immunized by the deployment of a WHO-authorized BCG vaccine is assumed to have been equally protected from tuberculosis.

The BCG vaccine is one of the most widely used of all current vaccines and has existed for over 100 years, immunizing a very high percentage of neonates and infants

in countries where the incidence of tuberculosis is very high, such as in Southeast East Asian Countries, African countries, and certain South American countries through their national childhood immunization program. However, BCG vaccination does not adequately prevent primary tuberculosis or latent pulmonary infection reactivation.[61] It is now established that the current BCG vaccines cannot induce sufficient $CD8^+$ T cell responses, especially in the lung, implying that the immune response in the Th-1 pathway is weak. Therefore, the impact of BCG vaccination on the prevention or transmission of tuberculosis is limited, especially among adolescents and adults. There is a need to invent more effective anti-tuberculosis vaccines. However, BCG vaccination just after birth effectively protects[62] from tuberculous meningitis.

LIFE-ATTENUATED CANDIDATE TB VACCINES IN R&D

The significant number of candidate live-attenuated vaccines in the R&D stage is given below:

VPM1002

VPM1002 is a live-attenuated, recombinant BCG vaccine (rBCG). At Max Planck Institute for Infection Biology, Berlin, the BCG was genetically modified by replacing the urease C encoding gene with the listeriolysin encoding gene obtained from *Listeria monocytogenes*. The evolved rBCG disrupted the phagosomal membrane at acidic pH. The depletion of the phagosomal membrane allows for rapid phagosome acidification. It promotes phagolysosome fusion, resulting in the release of mycobacterial antigens into the cytosol, thereby enabling the killing of the hiding Mtb microbes therein.

Further developments into a vaccine formulation were done by the Hannover-based Vakzine Projekt Management GmbH (VPMG). The rBCG, VPM1002, was used as a candidate anti-tuberculosis vaccine. In preclinical studies, VPM1002 was far more efficacious and safer than BCG. VPM1002 was subjected to phases I, IIa, and IIb evaluation in Germany and South Africa, demonstrating its safety and immunogenicity.[63] The intricacies of TB's fundamental immunology and pathology, with a focus on the involvement of T cells in triggering both Th-1 and Th-2 pathways, have been expounded upon. These complexities play a significant role in shaping acquired immunity against tuberculosis and have implications for infection prevention, disease prevention, and recurrence prevention. These discussions are presented in the context of VPM1002 and other potential vaccine candidates. VPM1002, a vaccine candidate, was created by Kaufmann and his research team at the Max Planck Institute. The candidate vaccine VPM1002 had completed phases I and IIa clinical trials establishing the safety and immunogenicity of the candidate vaccine in adults and neonates. Max Planck Society licensed the VPM1002 vaccine to the company Vakzine Projekt Management (VPM), and the latter with VPMG had teamed up with Serum Institute of India Pvt. Ltd, Pune, and together conducted a phase II trial in South Africa, evaluating the candidate vaccine as a prime vaccine in healthy HIV-exposed and HIV-unexposed infants. A phase III trial is undertaken in India.[64-66] The Department of Biotechnology of the Government of India supported the Indian phase III clinical trial project. A phase III clinical trial is currently underway in another location to evaluate the vaccine's efficacy in safeguarding the elderly against various infectious respiratory illnesses.[67]

MTBVAC

MTBVAC, developed by the University of Zaragoza, Spain; Institut Pasteur, France; and Biofabri,[68] Spain, is a live-attenuated Mtb strain currently in clinical development, and its discovery, construction, and characterization have followed the principles of Pasteur.[69-72] Biofabri and IAVI, an international nonprofit research organization, signed an agreement[73] in May 2023 for end-to-end development of the candidate vaccine MTBVAC.

rBCGΔais1/zmp1

The anti-tuberculosis vaccine by the name rBCGΔais1/zmp1 is a recombinant live BCG developed by the University of Zürich. The proof of concept of the candidate vaccine on immunogenicity, safety, and protective efficacy was successfully performed in mice, guinea pigs, and cattle.[74] The vaccine aims to replace BCG in newborns who are HIV-exposed. The protective efficacy of BCG zmp1 deletion mutants in a guinea pig model of tuberculosis infection was studied. It was found that the zmp1 deletion mutants of BCG could provide enhanced protection by reducing the bacterial load of tubercle bacilli in the lungs of infected guinea pigs. The results were independent of the BCG sub-strains (BCG Pasteur and BCG Denmark).[75] Another study also found that the *zmp1*-deleted BCG mutants were more immunogenic in the mouse model; however, the *zmp1* deletion did not affect the survival of mice with severe combined immunodeficiency.[76] The candidate vaccine was in the preclinical stage[77] of development till 2016 and is included in the active anti-tuberculosis programs of TAVI along with other candidate anti-TB vaccines of TAVI. TBVI[78] is a nonprofit making foundation in the Netherlands. TAVI is involved in discovering and facilitating new, safe, and effective anti-tuberculosis vaccines that are affordable.

Several anti-tuberculosis vaccines are in various stages of development[79] with TAVI initiatives, which include recombinant BCG vaccine where the gene *zmp1* is deleted from the strain; a combination of H64 (a fusion protein of six antigens) and CAF01, an adjuvant which induces strong CMI responses; a therapeutic anti-tuberculosis candidate vaccines platform based on Modified Vaccinia Ankara (MVA); a recombinant replication-deficient chimpanzee adenovirus constructed in Oxford, UK, expressing the mycobacterial protein PPE15, and named as ChAdOxPPE15, and which is to be used towards a booster BCG vaccination regimen; and a combination of a TB antigen CysVac2, which is a fusion protein of the *M. tuberculosis* antigens Ag85B and CysD along with an adjuvant, delta inulin (a naturally-derived carbohydrate), where the combination had shown good safety profile and induction of significant T and B cell responses in animal models. The latest stage of development of the candidate vaccine rBCGΔais1/zmp1 is not known.

Mtb *sig E* Mutant

An attenuated mutant strain of Mtb H37Rv lacking the sigma factor E was evaluated as a potential live vaccine strain against TB.[80] Mtb strains are thought to code 13 sigma proteins encoded in the *M. tuberculosis* genome, where several of these are known to be important for the virulence of the bacterium.[81,82] In a mouse model, the Mtb mutant strains lacking the sigma factor E (*sigE* mutant) were found to secrete more gamma interferon (IFN-γ), tumor necrosis factor-alpha (TNF-α), inducible nitric oxide synthase (iNOS), and β-defensins than in animals infected with the parental or complemented mutant strains. The conclusions were that the *sigE* mutant strain could be used as a potential candidate for developing an anti-tuberculosis vaccine. In the guinea pig model of tuberculosis, the strain was shown to induce a strong Th-1 immune response, reduce colony-forming units, and prolong survival[83] on the challenge. The strain has not yet been used in clinical trials.

Sig H (Mtb deleted *sigH*)

It was shown that aerosol immunization of macaques with *sig H*-deleted mutant Mtb strain, designated as Sig H (Mtb deleted *sigH*) mutant, resulted in significant recruitment of inducible bronchus-associated lymphoid tissues and elevated expression of $CD4^+$ T cells and $CD8^+$ T cells indicating that immunization of macaques led to elevated pulmonary protection of these animals. Vaccinated animals could resist significant lethal TB challenges. It was thought that anti-tuberculosis vaccine candidates could be developed[84] using *sig H-deleted* mutant Mtb strains. However, the research results for developing a candidate vaccine have not been adequately pursued.

Deleted lysA deleted pan CD mutants

The safety and immunogenicity of a double lysine and pantothenate gene mutant of Mtb strain were studied in immunocompromised SCID and gamma interferon knockout mice. It was found that single-dose subcutaneous vaccination of the auxotrope in the experimental mice induced short-term and long-term protection.[85] The Mtb strain is an unlinked double deletion mutant of Mtb H37Rv, where both the primary attenuating mutation of BCG (DeltaRD1) and two genes required for synthesizing pantothenate (Delta pan CD) are deleted. The genes *panC* and *panD* are necessary for the biosynthesis of pantothenic acid (vitamin B5), which is essential for enabling the metabolism of fatty acids through synthesizing enzymes such as coenzyme A and acyl carrier protein. Therefore, their deletions should result in a strain of less virulence. Safety and efficacy of two live-attenuated *M. tuberculosis* double deletion vaccine strains designated as $mc^2$6020 (*ΔlysA ΔpanCD*) and $mc^2$6030 (*ΔRD1 ΔpanCD*) were evaluated in cynomolgus macaques. It was observed that both the mutants were safe and well tolerated in macaques. After vaccination, following a high-dose intrabronchial challenge with virulent *M. tuberculosis*, on macaques vaccinated with $mc^2$6020 (*ΔlysA ΔpanCD*), the results showed a level of protection that was intermediate between those elicited by BCG vaccination and no vaccination.[86] Further developments on the mc2 6020 (*ΔlysA ΔpanCD*) (deleted lysA and deleted pan CD) mutant are unknown.

INACTIVATED OR KILLED CANDIDATE TB VACCINES

The following summarizes the more prominent ones, either in the R&D stage or the inconclusive experimental results:

DAR-901

SRL172, an inactivated, whole-cell mycobacterial vaccine, was safe and immunogenic and reduced the incidence of culture-confirmed tuberculosis in a phase III trial in HIV-infected and BCG-immunized adults in Tanzania.[87] Using the same seed, a vaccine formulation by the name DAR-901 was developed. The process for large-scale vaccine formulation manufacture would be accessible by this process.

DAR-901 formulation was tested among the BCG-immunized adolescents in Tanzania, a randomized controlled, double-blind phase IIb trial. The vaccine is also termed the Dartmouth TB Vaccine. The formulation was evaluated as a three-dose product to find if the formulation in 3 three-dose regimen was well tolerated, safe, and immunogenic. The results[88] were appealing; therefore,

DAR-901 shall move to the next phase for evaluation in Phase III to determine its efficacy.

Primary phase IIb trial funding came from the Global Health Innovative Technology Fund, Japan; the Jack and Dorothy Byrne Foundation, USA; and Oxford Immunotec, England.[89]

Mycobacterium indicus pranii (MIP) [Formerly known as Mw]

Mycobacterium indicus pranii (MIP), also known as Mw, shares T cell and B cell epitopes with Mtb and *Mycobacterium leprosy*. Mw formulations were approved for use as an immunomodulator for leprosy treatment, and the technology was procured[90] by Cadila Pharmaceuticals, Ahmedabad, in the early 1990s. In a prospective, randomized, double-blind, placebo-controlled, multicentric clinical trial using a heat-killed formulation based on MIP as an adjunct to anti-tubercular treatment in Cat II pulmonary TB (PTB) patients, the sputum smear conversion did not show a statistically significant difference.[91] MIP or Mw formulation, or IMMUVAC, is presently in phase III clinical trials[92] (CTRI/2019/01/017026) for treating tuberculosis; it targets adolescents and adults. Depending on the results of phase III trials, the utility of the formulation in tuberculosis management in India shall be assessed.

Mycobacterium vaccae (Mv) vaccine

Mycobacterium vaccae (Mv) vaccine formulation is used for treating Mtb infection of specific types. It contains heat-killed Mv microorganisms, similar to the MIP formulation, which treats patients suffering from multidrug-resistant tuberculosis (MDR-TB). The Mv formulation has also been used as an adjunct therapy and anti-TB therapeutics, with the idea of enhanced clearing of Mtb from patients suffering from TB. It was observed in a couple of studies that Mv formulations were safe and had considerable positive effects in clearing Mtb from MDR-TB patients. However, the results were inconclusive while using Mv formulations alone for treating MDR-TB.[93] In mice–model evaluation of the *M. vaccae* vaccine formulation, it was found that the formulation up-regulated some 2326 genes and downregulated some 2221 genes in vaccinated mice, which were linked to changes in the expression (up-regulation and downregulation) of some 123 signaling pathways, implying thereby that the impact of *M. vaccae* vaccine formulation was very complex.[94] The Mv formulations based on heat-killed *Mycobacterium vaccae* were being evaluated as a multi-country (Ukraine and Mongolia), placebo-controlled, randomized phase III trial in patients with drug-sensitive, multidrug-resistant tuberculosis (MDR-TB) and tuberculosis patients who were also infected with HIV (TB-HIV). This study was to be followed by trials in other countries like China, Russia, and South Africa. The results of the phase III trials have not yet been published.[95]

RUTI

Archival Farma, a company based in Spain, developed a novel anti-tuberculosis RUTI vaccine. This vaccine consists of heat-inactivated, fragmented, and purified components derived from *Mycobacterium tuberculosis* bacilli (FC Mtb) and is delivered in a liposomal formulation.

The active ingredients contain cell wall-based nano fragments of Mtb. FC Mtb was manufactured under good manufacturing practices. In a phase I study, RUTI was evaluated at each of the four tested doses, namely 5, 25, 100, and 200 μg, and placebo, a double-blind, randomized, and placebo-controlled trial conducted in healthy volunteers. The RUTI formulations were well tolerated, and the results were satisfactory[96] for further trials. Production of antigen-specific response toward the antigen is measured over time, and production of IFN-γ is traced and followed by ELISA tests. In the following phase II trials to test tolerability and immunogenicity of 4 increasing doses (5, 25, 100, and 200 μg of FC Mtb), RUTI was administrated to volunteers (randomized, placebo-controlled phase II clinical trial in healthy volunteers with latent tuberculosis infection), it was found that the vaccination was reasonably well tolerated[97] for further trials. A phase IIb study was also conducted in India.[98]

CANDIDATE PROTEIN/PEPTIDE-BASED SUBUNIT TB VACCINES ADJUVANTED WITH APPROPRIATE ADJUVANTS

The following provides several candidate protein/peptide subunit vaccines adjuvanted with appropriate accessary attendants:

H4:IC31 (AERAS-404)

H4:IC31 (AERAS-404) is a peptide–based anti-tuberculosis vaccine candidate composed of a fusion protein of *M. tuberculosis* antigens Ag85B and TB10.4, combined with an adjuvant by the name IC31. The adjuvant IC31 combines an 11-mer antibacterial peptide [KLKLLLLLKLK-NH2] and a synthetic oligodeoxynucleotide (ODN1a). The ODN1a is a Toll-like receptor nine agonists without containing cytosine phosphate guanine (CpG) motifs.[99] H4:IC31 was demonstrated to have an acceptable safety profile. It was immunogenic,[100] which was capable of triggering multifunctional $CD4^+$ T cell responses in previously BCG-vaccinated healthy individuals in doses of fusion protein combinations of 5, 15, or 50 μg of H4

antigen (purified fusion protein of *M. tuberculosis* antigens Ag85B and TB10.4) and 500 nmol of IC31 adjuvant.[101] The adjuvanted subunit vaccine was in phase 2b clinical trials.[102] The phase 2b results were satisfactory,[103] encouraging the candidate vaccine to move to phase III clinical trials.

H56:IC31

The candidate vaccine formulation H56:IC31 is an adjuvanted subunit vaccine, where three *M. tuberculosis* antigens, namely Ag85B, ESAT6, and Rv2660c, were combined as a fusion protein and formulated with the adjuvant IC31. Statens Serum Institut, Germany, had developed the vaccine.[104] The vaccine candidate was used in several phase I and II trials in Africa (South Africa and Tanzania) and was found to be safe with good immunogenicity characteristics.[105] Details about its entering into phase III trials are not known.

AEC/BC02

AEC/BC02 is a formulation consisting of two antigens: a subunit protein, Ag85B, and a fusion protein, ESAT6-CFP10, which is admixed with an adjuvant BC02. The formulation is finished as a freeze-dried material. BC02, also known as BCG CpG DNA compound adjuvants system 02, comprises BCG CpG DNA biological adjuvant with aluminum hydroxide. The vaccine formulation was developed by the National Institutes for Food and Drug Control, China, and is manufactured by Anhui Zhifei Longcom Biologic Pharmacy Co., Ltd, China.[106] The formulation triggered enduring, antigen-specific cellular immune responses in mice and guinea pigs. It proved effective in challenge tests conducted on vaccinated guinea pigs.[107] In April 2018, a phase I clinical trial[108] was initiated for this vaccine (NCT03026972). There is currently no information available regarding the most recent developments.

GamTBvac

The candidate anti-tuberculosis vaccine GamTBvac is made up of two Mtb antigen fusions Ag85A and ESAT6-CFP10 (combined with dextran-binding domain immobilized on dextran) and formulated with an adjuvant, made up of DEAE-dextran core, and with CpG oligodeoxynucleotides. The CpG oligodeoxynucleotides are TLR9 agonists. The candidate vaccine was assessed for immunogenicity and protective efficacy in murine and guinea pig TB models. In both models, the candidate had strong protective and immunogenic effects against challenges with Mtb strain H37Rv under aerosol and intravenous challenge conditions.[109] A phase II clinical study was conducted[110] in healthy adults to evaluate the safety and immunogenicity in 180 previously vaccinated with BCG vaccine in healthy volunteers (without *M. tuberculosis* infection). The results were satisfactory, encouraging the candidate vaccine for further clinical testing.

M72/AS01E candidate vaccine

The tuberculosis candidate vaccine M72/AS01E contains a recombinant fusion protein by the name M72, which is derived from two Mtb antigens, namely Mtb32A and Mtb39A, and this recombinant fusion protein is combined and formulated with the proprietary adjuvant system, AS01E of GlaxoSmithKline plc (GSK), UK.

In the composite experimental vaccine formulation of M72/AS01E, one adult dose[111] is a 0.5-mL sterile liquid containing 10 μg M72 protein and AS01E Adjuvant System. The Adjuvant System in 0.5-mL injection contains 25 μg monophosphoryl lipid A and 25 μg "QS-21 Stimulon," all in a liposomal suspension. GSK had teamed up with Agenus Inc, USA, and obtained its adjuvant QS-21, which is registered as a trademark of Agenus Inc [by the trademark notation "QS-21 Stimulon"] for use in various formulations requiring enhanced immune response.[112] Using QS-21, GSK modified the adjuvant and named it the AS01E adjuvant system, a proprietary substance.

QS-21 is chemically an acylated 3, 28-bisdesmodic triterpene glycosides (1,3) or "saponin" with a molecular formula of C92O46H148, obtained from the bark of a Chilean tree, [*Quillaja Saponaria*] as an active fraction using a reverse-phase chromatography (RP-HPLC), where QS denotes *Quillaja Saponaria,* and the number 21 stands for the identity of the RP-HPLC peak].[113] QS-21 has been widely used as a potent adjuvant in elucidating an antigen to the immune system, thereby manifesting increased immune response in both Th-1 and Th-2 pathways.

GSK developed the composite vaccine formulation candidate M72/AS01E in association with Aeras and IAVI up to the stage of proof-of-concept phase.

Aeras, USA is a nonprofit product development organization. Aeras is extensively working to develop effective biologics and vaccines to prevent TB across all age groups affordably and sustainably.[114] On October 01, 2018, Aeras announced[115] the transfer of all its TB vaccine clinical research programs and assets to the International AIDS Vaccine Initiative (IAVI). Later, IAVI and Aeras collaborated with GSK to develop the vaccine up to the proof-of-concept phase.

The M72/AS01E vaccine candidate has been under development since the early 2000s. The proof-of-concept phase

(phase IIb) is stated to have been partly funded by the Gates Foundation. A randomized, double-blind, placebo-controlled, phase IIb trial was conducted by GSK and its collaborators using the M72/AS01$_E$ tuberculosis vaccine of GSK and its collaborators in Kenya, South Africa, and Zambia on human immunodeficiency virus (HIV)-negative adults 18–50 years of age with latent *M. tuberculosis* infection (by interferon-γ release assay) where the vaccine recipients and the placebo were randomly assigned (in a 1:1 ratio) to receive two doses of either M72/AS01$_E$ or placebo intramuscularly one month apart.

After a mean follow-up of 2.3 years, the results showed that among the recipients of M72/AS01$_E$, the vaccine efficacy[116] was 54.0% (90% CI 13.9 to 75.4; 95% CI 2.9–78.2; $P = 0.04$). The final data analysis showed[117] that the vaccine had protected against progression to active pulmonary tuberculosis among the recipients for three years. *Tuberculosis*-infected but HIV-negative adults had shown a vaccine efficacy at month 36 as 49.7% (90% CI, 12.1 to 71.2; 95% CI, 2.1–74.2).

Following the results of the publication of phase IIb investigation on the candidate tuberculosis vaccine M72/AS01E TB of GSK and its collaborators, the WHO became more interested[118] in getting itself involved in further development of the formulation and to explore its use to contain tuberculosis using this candidate vaccine. The interest of WHO emanates from the developments of the high level meeting of the General Assembly of the United Nations on the all-country fight against tuberculosis, held on 26 September 2018, where a Resolution[119] A/RES/73/3 was adopted by the United Nations General Assembly on October 10, 2018, which was an essential resolution of the global community together to fight the menace of tuberculosis jointly in every possible way in different parts of the world. The Resolution identified multiple strategies and factors, including making effective therapeutic substances and vaccines available to combat the disease. The World Health Organization (WHO) started taking further actions to implement the Resolution step by step after that. WHO convened[120] its meetings in Geneva on July 30–31, 2019 to generate consensus among its stakeholders on the clinical development pathway for the M72/AS01E TB vaccine candidate developed by GSK and its collaborators. The participants included stakeholders involved in tuberculosis and other diseases research on vaccines, financing organizations, regulators and the policy-makers of different countries, manufacturing establishments, and many other relevant stakeholders. It was agreed that WHO would take an active part, to the extent feasible, to promote the development of an effective anti-tuberculosis vaccine to benefit the global community.

In the meantime, two organizations interestingly came forward for further developing the candidate tuberculosis vaccine M72/AS01E TB after the phase IIb efficacy information was published to take the project further forward, one of which was the Wellcome Trust and the other was the Bill & Melinda Gates Foundation. The Wellcome Trust (Trust) was founded in 1936. Henry Wellcome made a will to improve people's health by supporting scientific research and study in medicine, based on which the Trust was founded.[121] The Bill & Melinda Gates Foundation (Foundation) is an American private foundation established by Bill Gates and Melinda French Gates.[122] The Foundation is based in Seattle, Washington; It is one of the most extensive charitable foundations in the world.

On June 28, 2023, Wellcome and the Foundation together announced[123] that the Trust and Foundation together shall fund a phase III clinical trial testing of the M72/ASO1E vaccine in several countries where there are high rates of tuberculosis, such as in Africa and South East Asia. People with and without latent tuberculosis, as well as those living with HIV, will be included in the trial. Wellcome further announced that the phase III clinical trial may cost an estimated US$550 million, of which up to US$150 million shall be funded by the Trust, and the Foundation will fund the remainder, of about US$400 million. The study intends to ascertain if the experimental anti-tuberculosis vaccine M72/ASO1E shall be effective for deployment to treat tuberculosis worldwide.

VIRAL VECTOR-BASED CANDIDATE TB VACCINES

The following is the gist of the significant viral vector-based anti-tuberculosis vaccines in development:

AdAg85A

The viral vector-based candidate vaccine AdAg85A was developed by McMaster University, Hamilton, Ontario, Canada. In preclinical trials, the safety and protective efficacy of the vaccine delivered via the respiratory mucosal route in rhesus macaques was established.[124] The safety and immunogenicity of the candidate vaccine were evaluated in both BCG-naïve and previously BCG-immunized healthy adults using the vaccine through the intramuscular immunization route in phase I only. The results were satisfactory,[125] necessitating further evaluation. In another clinical trial (phase Ib), the safety and immunogenicity of the vaccine, delivered to humans via inhaled aerosol or intramuscular injection route, were found to be a safe and better way of eliciting respiratory mucosal immunity.[126]

The investigators believed that the prevailing notion that preexisting anti-AdHu5 immunity might significantly dampen the potency of the AdHu5Ag85A vaccine was inexact.

AERAS-402

The vaccine formulation AERAS-402 based on the AdHu35 construct is being developed jointly by Crucell, Netherlands, and Aeras organization, USA. Crucell is a biotechnology company which specializes in vaccines and biopharmaceutical technologies. Aeras is a nonprofit biotechnology organization involved in developing new anti-tuberculosis vaccines. The recombinant fusion protein-based construct of *M. tuberculosis* antigens Ag85A, Ag85B, and TB10.4, designated as AdHu35, is formulated as AERAS-402 was shown to confer protection[127] against *M. tuberculosis* in a mouse model when the animals were sensitized through vaccination in either an intranasal or an intramuscular route. The protection also correlated with the manifestation of multiple immunological parameters. The safety and immunogenicity of the vaccine delivered to the lungs in nonhuman primates were evaluated, and the results were also satisfactory[128] with the manifestation of immunological protective parameters. Following these results, a phase I clinical trial under multiple conditions was undertaken at different places, and the candidate vaccine was safe.[129-134] After these assessments, phase II clinical trials were successfully conducted on diverse populations, as indicated by the clinical trial registration numbers NCT01017536, NCT02414828, and NCT01198366. The candidate vaccine was found to be safe. The vaccine candidate is expected to soon progress into phase III trials.

ChAdOx1 85A prime-MVA85A

ChAdOx1.85A is a replication-deficient chimpanzee adenovirus expressing the antigen Ag85A. MVA85A refers to a genetically altered form of Vaccinia Ankara that additionally expresses Ag85A. ChAdOx1.85A alone, as well as in combination with MVA85A, had been tested for safety, immunogenicity, and protective efficacy against an *M. tuberculosis* challenge in mice, where the animals were BCG-primed and vaccinated or boosted with ChAdOx1.85A and MVA85A. Later, the evaluation of safety, immunogenicity, and protective efficacy were carried out in chimpanzee. The results were satisfactory.[135,136] The safety and immunogenicity of the candidate vaccine ChAdOx1 85A prime – MVA85A was carried out in healthy UK adults in phase I, and the results demonstrated[137] that the vaccine was well tolerated, was immunogenic, and safe for further studies.

Further study in phase I was extended to a wider range of participants, where participants included adults aged 70 years and older. This study's results[138] were also similar, if not better, indicating that the vaccine was better tolerated in older adults than younger ones. Further clinical evaluation is anticipated to be taken up soon.

CMV-vectored MCMVA 85

Oxford University, UK, developed the candidate vaccine MCMV85A expressing Ag85A. When tested in mice, this vaccine showed that the animals vaccinated with the candidate could reduce the mycobacterial load[139] after challenge with *M. tuberculosis*. MCMV85A is a cytomegalovirus (CMV)-vectored viral cassette of non-proliferating type. CMV-cassettes can induce high levels of responses of host $CD8^+$ T lymphocytes against pathogens and are therefore considered an added advantage for developing candidate anti-tuberculosis vaccines as *M. tuberculosis* infection creates a chronic disease syndrome in the infected where more and prolonged activated $CD8^+$ T lymphocyte-responses are needed. The candidate vaccine has not yet entered into clinical studies.

CMV-vectored RhCMV/TB and HCMV cassette-based vaccines

The recombinant CMV vector vaccine by the name RhCMV/TB encoding nine *M. tuberculosis* antigens, namely Ag85A, Ag85B, ESAT6 (acute phage antigens), Rv1733, Rv2626, Rv3407 (latency antigens), and RpfA, RpfC, RpfD (antigens coded by resuscitation promoting factor [rpf] genes) combined and expressed through recombinant DNA technology, on vaccination on rhesus macaques showed high levels of T cells mediated immune responses. They performed well on the *M. tuberculosis* challenge.[140] RhCMV/TB is a vaccine for rhesus monkeys. Rhesus cytomegalovirus (RhCMV) and human cytomegalovirus (HCMV) have closely conserved homologous gene sequences.[141] Therefore, recombinant RhCMV viral cassettes are anticipated to work efficiently and closely related to developing human anti-tuberculosis vaccines. CMV cassettes can harbor significantly larger segments of foreign DNA at many different genomic sites, and therefore, multiple antigen-coding genes can be inserted. More on the advantages of using CMV cassettes can be seen in recent reviews.[142,143] It is anticipated that the candidate vaccine RhCMV/TB and/or HCMV cassette-based candidates, when developed, are likely to be explored to ascertain if these could be used for developing candidate human anti-tuberculosis vaccines.

MVA85A

The genetically MVA virus-vectored candidate anti-tuberculosis vaccine called MVA85A expresses *M. tuberculosis* immunodominant antigen Ag85A. The vaccine was

developed by the University of Oxford, UK. Intradermal or intramuscular immunization with this vaccine in animals, starting with mice, and followed by other animal models such as guinea pigs, cattle, and rhesus monkeys, resulted in induction of a high level of antigen-specific immune response in all the animal models, and responded efficiently to *M. tuberculosis* challenges.[144-149] Significantly higher levels of IFN-gamma secretion and the boosting of CD4(+) and CD8(+) T cell responses in these experiments were positive indications of the utility of the candidate vaccine. additionally, the beneficial indications in the data generated in macaque, which has a close phylogenetic relationship with humans, reinforce hopes for further candidate development.

SeV85AB

SeV85AB is a recombinant *Sendai virus* (SeV)-vectored anti-tuberculosis candidate vaccine. It expresses two *M. tuberculosis* immunodominant antigens, namely Ag85A and Ag85B. SeV does not cause human disease. In a mouse model, the immunogenicity and protective efficacy of the candidate were established[150] through the measurement of T_{RM}-mediated immune response in mucosal tissues. Tissue-resident memory T cells (T_{RM}) foster the site-specific responses mediated by tissue-adapted memory T cells, enhancing tissue immunity and preventing infection.[151] Such T_{RM}-mediated responses are the first line of defense in the lung against *M. tuberculosis* infection. To ascertain if using SeV85AB singularly or combined with a prime-boost agent can be more beneficial, experiments were mounted using a recombinant DNA vaccine encoding the same antigens expressed by SeV85AB. The DNA vaccine was constructed in bacterial plasmids that encode the target antigens sequence and placed under a strong eukaryotic promoter. The two vaccine cassettes (SeV85AB and the DNA vaccine) were used one after the other in forward and reverse order, and the effects resulted in improved protective efficacy.[152] While using the SeV85AB for future developments, the above strategy could be considered.

TB/FLU-04L and PR8.p25

Two recombinant influenza virus-vectored vaccine candidates are described,[153,154] namely, TB/FLU-04L, which expresses the antigen ESAT6, and the other expresses Ag85B and is named PR8.p25. The first one, the TB/FLU-04L, experimented in a mouse model, showed that after two intranasal immunizations, the animals had manifested high levels of Th-1 $CD4^+$ T cell immune responses. The use of the vaccine, along with treatment with isoniazid, resulted in a heavy reduction in the load of *M. tuberculosis* in the lungs of infected mice, depicting strong synergistic effects of the vaccine. Intranasal immunization of mice with the second one, namely the PR8-p25 vaccine, resulted[155] in boosting BCG-induced protective immunity. Further developments in these two candidate vaccines are awaited.

THE WAY FORWARD AND CONCLUDING REMARKS

The experimental anti-tuberculosis vaccine M72/ASOE1 is set to enter a phase III trial in high incidence tuberculosis disease areas across Africa, aiming to enroll an estimated 26,000 participants. The assessment will include people never infected with tuberculosis and people with the latent form of the disease. The trial is anticipated to take three years for recruitment of the participants and 5 years for follow-up. The trial's final results are anticipated to come by 2030.[156] If the results are significantly and unstintingly positive, this anti-tuberculosis vaccine is anticipated to be available to humankind as the second vaccine after BCG. In the meantime, other measures of containing tuberculosis are anticipated to be stepped up globally, including compulsory vaccination of children at birth with a potent BCG vaccine and deployment of the available therapeutic substances to treat the disease in the infected.[157]

In the Indian context, there are a few new tuberculosis vaccine development initiatives from Indian entrepreneurs. However, the use of the $M72/AS01_E$ vaccine when approved by the regulators and available in India seems to be one important anticipation in the background for containing the disease during the future years[158] because vaccination with $M72/AS01_E$ in phase IIb trials had protected about 50% of the recipients against progression to pulmonary tuberculosis disease for at least three years during phase IIb trials, bringing considerable hopes.

In the global context, in addition to the above anti-tuberculosis candidate vaccine M72/ASOE1, it is anticipated that several other candidate vaccines may also evolve from other research and developmental establishments if government support is generously available for their turning out from the research stage to applications. These candidates include live-attenuated VPM1002 and MTBVAC; inactivated whole-cell vaccine DAR-901 and RUTI; the protein/peptide subunit adjuvanted candidate H4:IC31 (AERAS-404); and the viral-vectored AERAS-402. The primary goal of using new vaccines would be to protect the adults from contracting the disease that Mtb did not previously infect. Concomitantly, it would also be explored if any one or more of the approved vaccines could also be used as an immunomodulator along with chemotherapeutic substances to make the infected Mtb free faster.

The development of effective tuberculosis vaccines is anticipated to be reliant more on promoting T-cell-mediated Th1 responses over Th2 pathways. Macrophages and innate immune cells are crucial in early defense against infections. In Mtb infected T-cells, perhaps the immune cells have exhausted their source of energy demand through glycolysis in the cytoplasm and mitochondria, and therefore are unable to draw energy to reach phagosome maturation. The hiding Mtb has either learnt through signals about it or are able to intervene and prevent the process, of which enough is not known. The author proposes an innovative method before using a target TB vaccine: sensitize non-infected macrophages and other immune cells with a low dose of either of the below-mentioned antigens (a) recombinant Mtb cells, (b) wild Mtb strains, inactivated at 80 degrees Centigrade or (c) identified more potent Mtb antigens, each with an effective immunomodulator. At least one sensitization session of one week before start of vaccination. Anticipated outcome: activation of non-infected macrophages, neutrophils and other immune cells generating cytotoxic CD8+T cells, and multiple cytokines such as IL-8, IL-12, IL 17, TNF-alpha, IFN beta and others secreted by the immune cells, leading to either destruction of macrophage-phagosome assembly and destruction of hiding Mtb by the activated immune cells and/or activation of macrophage-phagosome assembly, phagosome maturation, pH lowering, fusion with lysosomes, and clearance of infection. Monitoring granuloma kinetics and diagnostic signatures in vaccine recipients is crucial for clinical validation and potential therapeutic vaccine development.

India has a high-class infrastructure in research, development, diagnosis, treatment, and surveys of tuberculosis in the country, which includes among others the ICMR-National Institute for Research in Tuberculosis [formerly Tuberculosis Research Centre (TRC)], Tamil Nadu; the National JALMA Institute for Leprosy & Other Mycobacterial Diseases, Agra, under ICMR; and the National Tuberculosis Institute, Bangalore under the Directorate General of Health Services, Ministry of Health and Family Welfare, New Delhi. However, the incidence of tuberculosis patients has remained high. The total number of TB patients, including new and relapse cases, notified[159] by the Government in 2021 was 1,933,381, while in 2020, it was 1,628,161. The total number of bacteriologically positive pulmonary tuberculosis continues to be much more, close to 300 per one hundred thousand population. There exist several barriers to winning the war against the disease; some among these are the existence of a sizable portion of the nutritionally under-fed poor population, inadequacy in terms of social support to the diseased, compelling the patient to work to earn to meet both ends; the chemotherapeutic treatment causes multiple toxic syndromes among the treated; a sizable number of diseased remain unidentified; treatment of resistant cases is centralized and is not easy to avail of the facilities, while healthcare of individuals is a state subject matter.

Further, the government budget for researching newer developments in diagnosis, therapy, and vaccine development is considered low. Private investment from the industry side for newer developments is also low. Under these circumstances, while several clinical trials in multiple aspects of therapy have been carried out in India, enabling the generation of more knowledge for cost-effective rational treatment of the disease, no breakthroughs, either in chemotherapy or in vaccine development, have yet been achieved. Under such circumstances, achieving breakthrough success in containing the disease would not be easy. However, much may be achieved because of the strong attention of the Prime Minister to contain the disease through the PMTBMBA program.

For India, in the intervening period from 2023 onwards, new and novel anti-tuberculosis vaccine development strategies based on novel viral vectors should be explored, and packaging such vectors for expressing one or more of the already established Mtb antigens to develop novel anti-tuberculosis candidate vaccines, originating out of Indian research. Proceeding with more advanced knowledge in immunology in choosing the effective Mtb antigens and an in-depth understanding of immunity development through the activation of the immune cells into and out of innate and adaptive immunity pathways for vaccine development would be more desired rather than exploring blind shots of vaccine development through attenuated live or inactivated non-disease-producing mycobacterium species. Instead of only relying on and using the BCG vaccine at birth, which provides inconsistent protection against pulmonary Mtb infection in adolescents and adults, a more durable, knowledge-based development or access to one or more effective anti-tuberculosis vaccines is thought to be the long-term right pathway to choose.

Long-term financing for basic and application-oriented research needs to be guaranteed for tuberculosis research in the country with sizable assurance of spending. Multilateral cooperation for knowledge development and fund-seeking should be explored. Further, as the country has expertise in high levels of expression of antigens in transgenic host cell lines and purifying such antigens in high order of purity, such purified antigens can be admixed with suitable novel adjuvants to make novel protein and peptide-based anti-

tuberculosis vaccines. In doing so, India needs to develop novel adjuvants, where Indian expertise is feeble, although the country has high competence in chemistry and chemical technologies.

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Conflicts of interest

There are no conflicts of interest.

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