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Review Article

Current trends in tuberculosis vaccine

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ABSTRACT

Despite the global efforts made to control tuberculosis (TB) and the large number of available new anti-TB drugs, TB still affects one-third of the world population. The conventional vaccine bacille Calmette–Guérin (BCG) shows varying efficacy in different populations, and there are safety issues in immunocompromised patients. Hence, there is an urgent requirement for a new and better TB vaccine candidate than BCG. There are several alternate vaccines available for TB such as DNA, subunit, adjuvant, and live-attenuated vaccines. Use of auxotrophic vaccine is an emerging technology. Newer vaccine technologies include vaccine delivery methods such as adenovirus- and cytomegalovirus (CMV)-based vector delivery, chimeric monoclonal antibody, single-chain fragment variable, RNA-lipoplexes, and nanoparticle-based technology. Based on its application, TB vaccines are classified as conventional, prophylactic, booster, therapeutic, and reinfection preventive vaccines. Currently, there are 12 vaccine candidates in clinical trials. In this review, we have briefly discussed about each of these vaccines in different phases of clinical trials. These vaccines should be analyzed further for developing a safe and more efficacious vaccine for TB.

Introduction

Tuberculosis (TB) is a deadly disease infecting about one-third of the world population. In 2017, around 10 million developed TB, out of which 1.3 million deaths occurred in HIV-negative patients. About 90% of those who developed TB were adults (aged ≥15 years). Globally, close to one-fourth of the population is estimated to have a latent tubercular infection, putting them at risk of developing active infection during their lifetime according to the World Health Organization (WHO) Global TB Report 2018.[1] With the increasing drug resistance among mycobacterial strains, it is an immediate necessity for vaccine development, keeping in mind that "prevention is always better than cure." Vaccine is a priority and is likely to affect both the incidence and prevalence of disease. The End TB Strategy, one of the pillars of the WHO, aims at preventive treatment of people at high risk of TB by 2030, which involves effective vaccination strategies to lower the risk of infection.

First tested in the year 1921, an attenuated strain *Mycobacterium bovis* bacille Calmette–Guérin (BCG) is believed to be the best vaccine candidate for preventing the disease in children so

far. Fatal disseminated BCG disease was seen in less than two cases per million vaccine doses (most of whom were immunodeficient).[2] BCG has certain challenges which are of concern in today's world. BCG has shown to have varying efficacy in adult population in different parts of the world. A meta-analysis from 2010 to 2017 showed that around 51% of the immunocompromised patients developed BCG- associated complications after vaccination.[3] Genomic characterization of BCG strain and *Mycobacterium tuberculosis* has identified more than 100 distinct coding sequences that are missing from BCG, which also includes 6 kDa early secretory antigenic target protein (ESAT-6), which has potent T-cell antigenicity.[4] Thus, the generation of an improved vaccine to replace BCG is an urgent requirement. The researchers would need to keep in mind some of the attributes of an ideal TB vaccine candidate (Fig. 1).

TB vaccine technologies

Vaccine technologies include DNA, subunit , adjuvant, and live-attenuated vaccines. Each of these aforementioned vaccine types has their own advantages and challenges. Previous studies showed that DNA and subunit vaccines may not give protection against the disease for a long duration as they do not offer a complete set of antigens, and moreover, they do not replicate in the host to a level required for long-lasting immunity.[5] The attenuated organism in case of live vaccine multiplies within the target host tissue, thereby providing sustained memory T-cell responses. These attenuated vaccines are constructed by deleting the virulent genes in *M. tuberculosis* or by generating selected auxotrophs by insertional mutagenesis, so that they offer long-term immunity against the pathogen. Attenuated vaccines produced through deletion of virulence genes may lead to reversion of their virulence which may cause chronic diseases, and moreover, they cannot be administered to immunocompromised individuals.[6] Use of auxotrophic strains is increasing because of their safety in the host body. Auxotrophs are strains which are unable to synthesize certain amino acids required for their growth unless supplemented externally such as laboratory medium. They are supposed to be attenuated *in vivo* due to

deprivation of particular nutrients. Lysine auxotroph of Mtb (*ΔlysA ΔsecA2 Mtb*) was proved to be a promising vaccine platform to construct a safe combination HIV-TB vaccine for use in neonatal mice.[7] Auxotrophic recombinant attenuated *M. tuberculosis* strain mc^{2}6435 (*ΔleuCD ΔpanCD ΔsecA2* pSIV Gag) vaccine was found to be safe in non–Simian immunodeficiency virus (SIV)-infected and SIV-infected infant macaques and thus would be safe in HIV-TB patients.[8]

Apart from focusing on the construction of a successful vaccine candidate, there are researchers who focused on improving the antigen exposure *in vivo* through various vaccine delivery systems. Vaccine delivery systems shall either allow controlled release of antigen, abrogating the need for booster dose, or improve the temporal presentation of antigen to immune-competent cells.

Some of the techniques include use of adenovirus-based or CMV-based vectors carrying TB-specific antigens, chimeric monoclonal antibody (CMA) usage, RNA lipoplex-mediated delivery, nanoparticle-mediated delivery, and single-chain fragment variable (ScFv) targeting specific cells.

Adenoviruses have the ability to recombine and express the heterologous genes. This property encourages their use in gene/vaccine delivery methods. Adenovirus-based vaccine delivering the antigen linked to Major histocompatibility complex (MHC) class II–associated invariant chain (Ii) has been shown to induce CD8 T-cell responses in lymphocytic choriomeningitis virus infections.[9] Some of the disadvantages include possible adenoviral infections and presence of pre-existing antivector antibodies, which limit boosting of specific T cells.

CMV vector–based vaccines are advantageous as it elicits both CD4+ and CD8+ memory T-cell responses. Its ability to cause persistent infection results in facilitation of robust, life-long immune response. A study on 68-1 RhCMV/TB-6-Ag vector expressing a single six-antigen Mtb polyprotein, by Hansen et al,[10] showed that it was able to elicit and maintain immune effector response to control Mtb at the earliest stages of infection.

CMA-mediated delivery that involves fusion of CMA to the antigen of interest are directed to dendritic cell (DC) surface receptors for improved T-cell responses. Previous studies showed that αDEC-205 CMA (mAb) containing the sequence of eight HIV-derived T-cell epitopes (αDECHIVBr8) induced both protective CD4+ and CD8+ responses in the presence of poly (I:C).[11] However, high cost and time-consuming, labor-intensive technology are major drawbacks of monoclonal antibodies.

For potential DC targeting, cell surface receptors should be identified. The cell surface molecules DEC-205 and CD11c has been widely studied for antigen targeting of DC. ScFv generation is an emerging technique for targeting DC, wherein the variable regions of heavy and light chains are linked by a flexible peptide linker. Similar approaches can be adopted for TB vaccine delivery to see if there is a chance for generating long-lasting immunity. Demangel *et al*[12] showed that antigen targeting to DCs via a DEC-205 binding ScFv leads to enhanced immunogenicity in TB DNA vaccine.

A newer technology involving RNA-lipoplexes (RNA-LPX) might provide an improved vaccine delivery to DCs. The lipoplexes play an important role in the protection of RNA from ribonucleases. Lipoplexes also mediates efficient uptake of RNA and expression of the encoded antigen by DC populations

ATTRIBUTES OF AN IDEAL TB VACCINE
- ✦ **Safety and efficacy in at-risk infants, children and adults**
- ✦ **Effectiveness against all forms of TB including pulmonary and MDR-TB**
- ✦ **Logistically practical**
- ✦ **Formulation that can be feasibly manufactured on a mass scale**
- ✦ **Stored and administered under low-technology conditions**

Fig. 1 – Attributes of an ideal TB vaccine. Adapted from the study by Orme. Clin Vaccine Immunol. 2015;22(3):249-257. TB, tuberculosis.

and macrophages in various lymphoid compartments. RNA-LPX with enhanced green fluorescent protein triggers interferon-α (IFNα) release by plasmacytoid DCs and macrophages[13]

The other emerging technology is nanoparticle (NP)-mediated delivery of which poly (lactic-co-glycolic acid)–based NP are promising tools for the modulation of immune cell activity.

Based on the applications, TB vaccines are classified into several types, which are currently in clinical trials. We can categorize them as conventional vaccines, prophylactic vaccines, booster vaccines, therapeutic/postexposure vaccines, and vaccines to prevent reinfection (Fig. 2). The currently available conventional TB vaccine is BCG, but BCG improvisation by recombinant BCG (rBCG) technology can also be included in this category. Prophylactic vaccines are those which prevent the disease when given in early age. Both conventional and prophylactic vaccines aim at possible early innate mechanisms. Booster vaccines are those which generate bigger, better, and long-lasting memory immunity when administered along with the conventional ones. Therapeutic vaccines are administered during TB treatment, and they help to improve drug therapy for both latent and pulmonary TB (PTB) by reducing the duration of the treatment. They are directed to reduce tissue destruction. Vaccines used to prevent reinfection are administered during or after the treatment for TB. Revaccination of BCG is one such technique for preventing TB reinfection.[14]

TB vaccines in clinical trials

In present, multiple vaccine candidates are pursued by researchers in clinical trials, each showing a different kind of immune response and level of protection (Fig. 3). Overexpression of immunodominant antigens through rBCG technology is one of the approaches to improve CD8+ T-cell induction and this has helped some of the rBCG vaccines to reach early clinical evaluation. Some of the vaccines in clinical trials used live-attenuated *M. tuberculosis* strains as vaccine candidates.[15]

TB vaccines in phase I

As mentioned earlier, adenoviral vector–based delivery of vaccine candidate helps in building a successful vaccine candidate. Ag85A is one of the components of antigen 85 complex, which is secreted and found in the phagosome and on the cell wall of the bacilli. All components of Ag85 function as mycolyl transferases, catalyzing the transfer of mycolic acid from one trehalose 6-monomycolate to another, forming trehalose 6,6'-dimycolate, a glycolipid located at the external layer of the cell wall.[16] This has been identified as a potential target for drug designing and also exploited as an adjuvant/antigen with a rationale of improving the BCG vaccine as BCG lacks this complex. The following vaccines in the pipeline in phase I clinical trials exploit the usage of Ag85A in different means.

Ad5Ag85A

Ad5Ag85A includes a non-replicating adenovirus serotype 5 (Ad5) expressing mycobacterial antigen 85A. It was developed by McMaster University and supported by Tianjin CanSino Biotechnology Inc. Preliminary studies of Ad5Ag85A in a murine model showed that it was a potential BCG booster vaccine candidate, when administered through intranasal route. Increased protection by intranasal Ad5Ag85A was correlated with the numbers of IFN-γ producing T cells in the lungs.[17] A major challenge to Ad5 vector–based vaccine trials is that, in TB endemic countries, neutralization by pre-existing Ad5 antibodies is frequent because of prior exposure.[14] Hence, clearance from the tissue within smaller duration after vaccination may not provide required immunity and memory cell production.

MVA85A

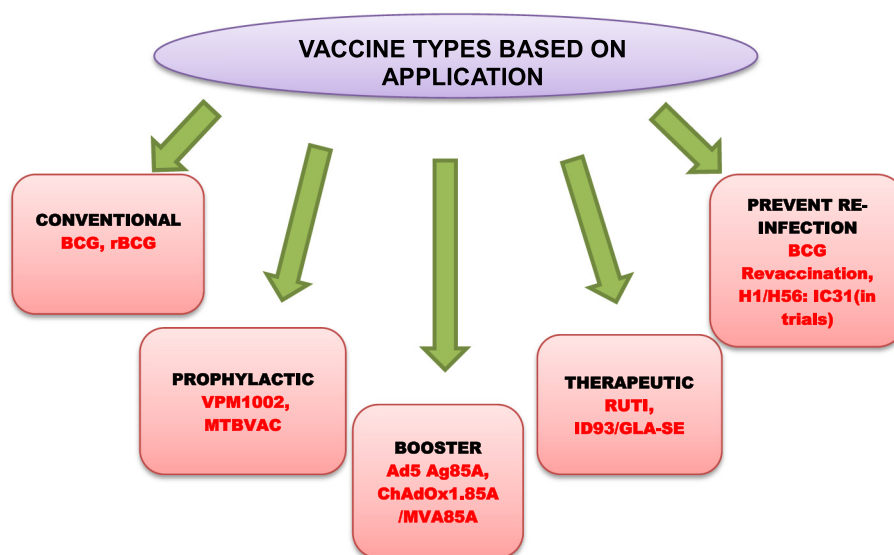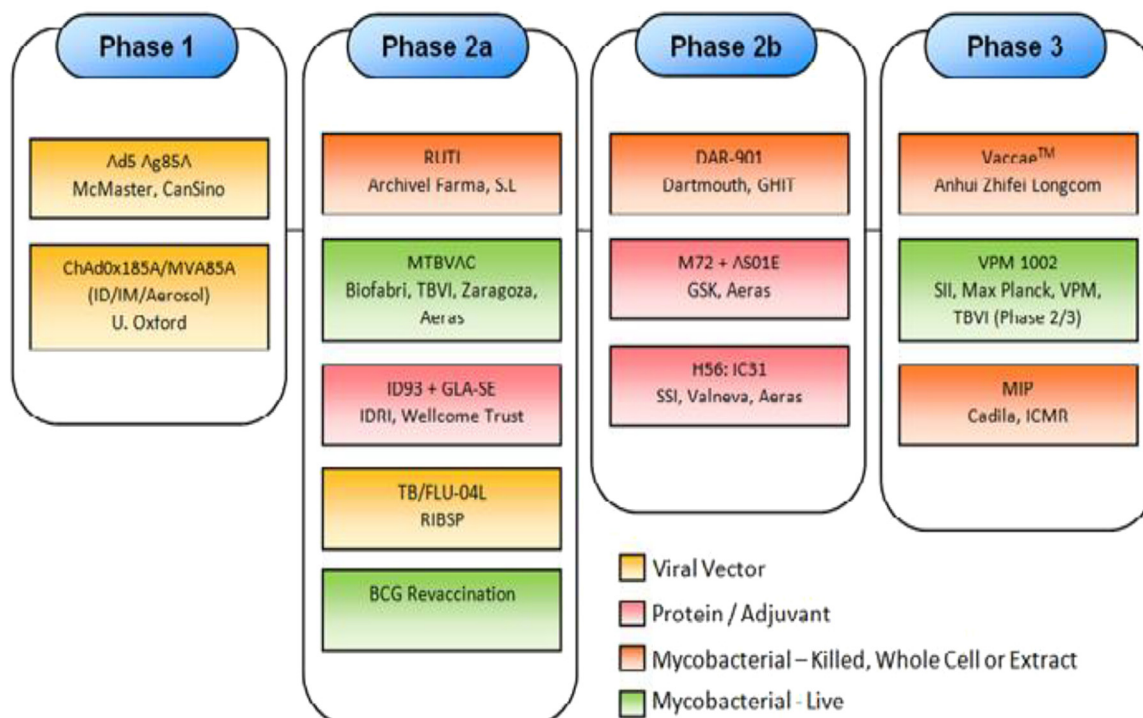The modified vaccinia Ankara (MVA) virus technology has been developed to be used as a booster for BCG and was found

Fig. 2 – Vaccine types based on application and timing. BCG, bacille Calmette–Guérin; rBCG, recombinant BCG.

Fig. 3 — The TB vaccine pipeline 2018. Adapted from AERAS Global clinical portfolio of TB vaccine candidates. www.aeras.org/pages/global-portfolio as available on 21.12.2018. BCG, bacille Calmette—Guérin; MIP, *Mycobacterium indicus pranii*; TB, TB, tuberculosis.

to induce Th1 and Th17 antigen specific response for the MVA85A vaccine candidate. MVA85A was the first novel TB vaccine to enter safety and immunogenicity studies in patients with latent TB infection (LTBI). It has been T-cell response.[18] Both intradermal and aerosolized routes of administrations is practiced. However, the aerosol mode of administration was found to induce a better Ag85-specific CD4 cell cytokine response than intradermal route.[19] MVA85A showed variable immune responses in different populations of the world and also with respect to different age groups as it showed low levels of immunogenicity in South African infants compared with UK adults when given as a booster for BCG.[20] Similar studies using chimpanzee adenovirus carriers, which contain deletions of E1 and E3 genes, revealed that they are subjected to low neutralization rate of antibodies in human population, hence making it a promising vaccine.

TB vaccines in phase IIa

MTBVAC
MTBVAC is a derivative of MT103 which is a live-attenuated *M. tuberculosis* isolate, constructed by generating two independent insertional deletions of virulence-causing genes *phoP* and *fadD26* without antibiotic resistance markers. Both

of these are involved in cell wall lipids synthesis.[21] MTBVAC was the first live-attenuated MTB vaccine to get approval for human trials. MTBVAC was found to show similar immunogenicity as that of BCG,[22] and it provided a longer lasting immunity when administered as a booster vaccine to BCG compared with BCG alone in guinea pig model.[23] The major challenge to this vaccine is that its efficacy and safety in immunocompromised populations (such as HIV-infected populations) is uncertain.

TB/FLU-04l
TB/FLU-04L is a recombinant vaccine containing the influenza virus strain A/Puerto Rico/8/34 H1N1 and Ag85A/ESAT-6 antigens of *M. tuberculosis*.[24] After the success of phase I trial, it is currently undergoing phase IIa clinical trials.

RUTI
RUTI, one of the therapeutic vaccines, is made of detoxified, fragmented *M. tuberculosis* cells. RUTI is delivered in liposomes. When tested in mice and guinea pigs with latent form of TB, it was found to induce a combined Th1/Th2/Th3 polyantigenic response after a short period of chemotherapy.[25] In a long-term experiment, RUTI proved to show similar efficacy as that of BCG.[26]

ID93 + gla-se

ID93/GLA-SE is a protein/adjuvant vaccine currently in phase IIa human clinical trials containing four *M. tuberculosis* proteins including Rv2608 (PPE protein, outer membrane associated), Rv3619 (EsX protein family of secreted virulence factors), Rv1813 (upregulated under hypoxic conditions), and Rv3620 (EsX protein family of secreted virulence factors) that is combined with GLA-SE, which is a synthetic toll like receptor (TLR)-4 agonist adjuvant formulated in a squalene oil-in-water nanoemulsion.[27] In several independent studies, ID93/GLA-SE was found to provide long-lasting immunity through induction of polyfunctional Th1 cell responses.[28]

TB vaccines in phase IIb

H1/H56: IC31

H1/IC31 is an adjuvant-based TB subunit vaccine composed of H1 antigen, a hybrid protein consisting of Ag85B and ESAT-6, along with an adjuvant, IC31, which can induce potent cellular and humoral responses.[29] Dose toxicity studies of H1/IC31 by Agger *et al* and Kamath *et al* in animals proved that the vaccine has caused no safety concerns. Preclinical vaccine studies in mice and guinea pigs indicated that it was highly immunogenic.[30,31] It is now poised for phase IIb proof-of-concept testing for the prevention of recurrence in treated TB patients, prevention of infection/disease, and also safety testing in TB patients during treatment.

DAR-901

DAR-901 vaccine is an inactivated whole cell vaccine manufactured using *Mycobacterium obuense* isolate, which is a rough variant of an environmental non-tuberculous mycobacterium (NTM) through high-yield fermentation process. Among animals primed with BCG, boosting with DAR-901 at 1 mg provided greater protection against aerosol challenge than a homologous BCG boost.[32] Currently, DAR-901 is in phase IIb trials, aiming for the prevention of infection adolescents in Tanzania (DAR-PIAT).[33]

M72 + AS01$_E$

A randomized, double-blind, placebo-controlled phase IIb trial using the M72/AS01$_E$ vaccine was carried out in Kenya, South Africa, and Zambia for the prevention of TB in adults. The vaccine efficacy was found to be 54%. This vaccine contains the M72 recombinant fusion protein, resulting from two immunogenic *M. tuberculosis* antigens (Mtb32A and Mtb39A) combined with the AS01 adjuvant system.[34]

TB vaccines in phase III

Mycobacterium vaccae

Mycobacterium vaccae is a non-pathogenic species developed initially as an immunotherapeutic vaccine by inactivation of the whole cell strain of *M. vaccae*. *M. vaccae* showed variable humoral responses in phase III trial in terms of HIV viral load and CD4 T-cell count.[35] *M. vaccae* when provided in multiple doses to HIV-positive patients, immunized with BCG in childhood, showed protection against HIV-associated TB.[36] The major challenge for *M. vaccae* is inconsistency in providing protection against the disease in some geographical regions but not in other places.[37]

Mycobacterium indicus pranii

Mycobacterium indicus pranii (MIP) is a non-pathogenic bacteria developed by the National Institute of Immunology, India, which has received approval as a leprosy vaccine from the Indian drug regulator, Central Drugs Standard Control Organisation, and the US Food and Drug Administration. In a recent study, immunomodulator MIP was given as an adjunct therapy along with standard ATT. MIP was found to be safe in category II PTB patients undergoing retreatment for TB, when they are administered once in 2 weeks for 2 months with six intradermal injections (2 + 4) of heat-killed MIP at a dose of 5×108 bacilli.[38]

Conclusion

Despite newer vaccine technologies and drug therapies, TB remains one of the deadliest diseases in the world. An effective TB control strategy should include proper diagnosis, treatment, and prevention of TB. Designing much faster and cost-effective diagnostic tools for identification of different types of TB (LTBI, PTB, multi drug-resistant tuberculosis [MDR-TB], and extensively drug-resistant tuberculosis [XDR-TB]) along with NTM diseases is crucial for better treatment strategies. With increase in drug-resistant TB strains and diseases associated with them, effective therapeutic vaccine candidates may be required to aid in the treatment of the TB. Similarly, new drug targets have to be identified for designing newer drugs with a safety profile, as existing new TB drugs were found to be having detrimental side effects. Currently, research on TB vaccine is focusing on recombinant vaccine production using both BCG and *M. tuberculosis* strains, keeping in mind the challenges and benefits of the vaccines undergoing clinical trials. Newer emerging technologies need to be used in combination with targeted delivery systems to improve the existing TB vaccine candidates. On the whole, there is an urgent requirement for the development of an effective, low-cost TB vaccine with a safer profile, to prevent the world's most deadliest disease.

Conflicts of interest

The authors have none to declare.

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