1. Introduction

Moving ahead of the World Health Organization Millennium Development goal targets for the tuberculosis (TB) set up till year 2015, the Sustainable Development Goals (SDGs) adopted by the United nations (UN) in 2015 has set one more goal i.e. point 3.3, as to eradicate the TB epidemic by the year 2030 [1]. Similarly, the World Health Assembly approved the WHO strategy to eradicate the TB in 2014. The strategy aims for 95% reduction in TB deaths and 90% reduction in TB incidence rate by 2035 [2].

As per the WHO reports, an estimated 10.4 million cases occurred in 2015 including the 1.2 million people living with HIV/AIDS (PLHA). There were 1.8 million deaths due to TB of which 0.4 million were PLHAs. An estimated 480,000 developed multi drug resistant TB (MDR-TB) and an additional 100,000 were rifampicin resistant TB amenable for the second line treatment in 2015 [2].

Above data and the very fact that an open case of TB will infect 10-15 contacts leadsto an inference that despite the growth in diagnostic and therapeutic modalities, we may not be able to achieve the elimination of TB by 2050 [3]. This necessitates the availability of effective vaccines for TB control.

Till date Bacilli Calmette-Guerin (BCG) is the only available vaccine for TB. Since 1921 it has been administered to over 4 billion individuals, and almost 120 million children receive BCG annually [4]. BCG has limitations in terms of its efficacy in pulmonary TB while there are also safety concerns in the era of HIV. Therefore, there has been a need for a more effective and safer TB vaccine across the world. The fourth Global Forum for TB Vaccines in
the year 2015 witnessed at least 15 TB vaccine candidates in the clinical trials as compared to its first forum in 2001, when there was not a single candidate in worldwide clinical portfolio[5,6].

Multiple vaccine development strategies are the need of hour and can be simply grouped in one of the following: First option is to administer prior to exposure to prevent the initial infection, next option is to give the vaccine after exposure, to individuals who are infected but may be asymptomatic and at a risk to develop the disease in future. This will protect them against disease manifestation and therefore transmission. Finally it can be given after the treatment of active disease to prevent reactivation and subsequent transmission [7,8].

Accordingly, currently available BCG and the vaccines in the clinical trials can be grouped into one of the three categories:

a. Priming vaccines: This type of vaccine is intended to prevent TB infection and the disease in infants who have not been infected with the TB bacilli. BCG comes under this category.

b. Booster Vaccines: This type of vaccine is are to be delivered during adolescence to prevent infection or to prevent latent infection to be developed into active disease as the BCG protection wanes.

c. Immunotherapeutic vaccines: This type of vaccine is are given to individual with active TB along with the TB chemotherapy intended to shorten the duration of therapy and/or prevention of recurrence after treatment [8].

2. Bacilli Calmette-Guerin (BCG)

It was obtained by Albert Calmette and Camille Guerin sustained efforts of 230 in vitro passages of strain of Mycobacterium bovis over a period of 13 years. It is a live attenuated vaccine and was first administered orally to an infant in 1921. Currently it is recommended as a single intra-dermal dose at the site of insertion of deltoid, in the healthy babies as close to the time of birth as possible in countries where TB is common. This is in accordance to the World Health Organization (WHO) recommendation for the children born in countries with TB as endemic disease. This measures leads to the protection against miliary TB and tubercular meningitis during childhood. Evidences do not support additional booster dosing. Adults who do not have tuberculosis and are not previously immunized and are frequently exposed to tuberculosis may get the immunization [9,10] Babies diagnosed of having HIV/AIDS should not be vaccinated [11].

Many countries have different immunization policies. India and Pakistan were the first countries outside the Europe to adopt universal BCG immunization policies since 1948, while the United States and Netherland have never adopted it as a routine policy [12]. United King-
dom has scrapped its use as a routine immunization in 2005 because of decreasing cost effectiveness secondary to decreasing annual incidences of TB [13].

Protective efficacy of BCG varies widely. A 1994 systemic review concludes that the BCG offers a protection of over 50% against getting TB [14]. But this efficacy falls, as one approaches the areas near equator [14,15]. According to one review in 2014, reduction in infection rate was between 19-27% while reduction in progression to active TB was by 71% [16]. Importantly the vaccine has positive effects against the severe forms of childhood TB, especially the meningeal and miliary TB. As per one meta-analysis, the BCG vaccination in the year 2002 reduced one case of tubercular meningitis for every 3435 vaccinations and one case of miliary Tb for every 9314 vaccinations, and it was deemed to be highly cost effective in endemic region [17]. There occurs a great deal of variations in terms of protection against pulmonary TB which is the most contagious form of TB. Clinical data have shown it to be anywhere between nil to 80% [14,1]. The differences in effectiveness of the vaccine has been attributed to multiple reasons including proximity to equator, genetic differences in the host populations, exposure to other bacterial infections, laboratory conditions including the strain being cultured and the growth media used [15,19].

The data about the duration of protection is highly inconsistent. British MRC study showed that the protection waning occurred to 59% subjects after 15 years and to zero subject after 20 years; while another study analyzing the evidence of protection in native Americans immunized in 1930s found vaccine efficacy to be 52% without statistically significant waning over 50-60 years of time [20,21].

BCG has happened to be a safe vaccine in healthy infants. Local site reactions including pain and scarring may occur. Regional suppurative or non-suppurative adenitis may happen and are self-limiting. Suppurative case may sometimes require needle aspiration or surgical excision [22,23]. Sometimes breast or gluteal abscess or regional osteomyelitis or osteitis may happen which may be potentially life threatening needing anti-tuberculous treatment [24]. Disseminated BCG infection (BCG-osis) though rare i.e. less than one per million immunization, may happen if the vaccine is given accidentally to an immuno-compromised patient e.g. in SCID patients [25]. The same may happen with HIV-infected children, even if the child is asymptomatic at the time vaccination [26,27]. Therefore, WHO has stopped recommending the BCG in the children with HIV [11].

Apart from the TB, BCG may find its use in leprosy, buruli ulcers and bladder and colorectal malignancies. In leprosy protective efficacy has been 26-41% in controlled trials to as high as 60% in case control and cohort studies though it is not specifically used to control leprosy [28,29,30,31]. Also, an added benefit of BCG vaccination in developing countries has been the provision of non-specific protection, what is called as heterologous effect or off-target
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effect, against sepsis and respiratory infections in neonates.

3. Other Priming Vaccines in Pipeline

3.1. VPM1002

It is a recombinant BCG strain expressing membrane perforating listeriolysin which is encoded by the gene \textit{hly} derived from Listeria monocytogenes. This strain lacks the urease \textit{C} gene and has a hygromycin resistance marker \cite{32}. The candidate vaccine showed a better protective effect in animal model as compared to the parental BCG. In its phase 1 trial it was noted safe to be administered in healthy adults and had similar adverse effect profile in comparison to BCG and had no human to human transmission. It induced good CD4+ and CD8+ T cell responses \cite{32,33}. Phase 2 trial to study its effects in newborn is on the way \cite{34}.

3.2. MTBVAC

This vaccine has the credit of being the first live attenuated Mtb vaccine to be entered in Phase I study. MTBVAC is a new vaccine strain having mutations in \textit{phoP} and \textit{fadD26} genes. \textit{fadD26} translational product is required to synthesize phthiocerol dimycocerosates, a cell wall content that protect the bacilli from host defenses. Following the promising Phase 1 results, it is now in the Phase 2a trial \cite{35,36}.

4. Therapeutic Vaccines

4.1. RUTI

This is an immunotherapeutic vaccine composed of detoxified liposomal fragments of Mycobacterium tuberculosis. It is supposed to improve the treatment of latent Tb after a short course of anti-tubercular treatment. In its animal model it showed a good safety profile and elicited TH1, TH2, TH3, CD8+ T cells and antibodies responses. In Phase 1 trial it was well tolerated after being given subcutaneously in BCG naïve healthy adults. There were local reactions reported, without any serious adverse events. It also induced specific cellular and antibody based responses \cite{37,38}. Now it is in Phase 2a trial.

4.2. Mycobacterium vaccae

This is a whole cell inactivated Mycobacterium vaccae (MV) meant to be administered intra-dermally. A Meta-analysis showed that MV when added to the anti-tubercular chemotherapy in TB patients who have received no treatment earlier showed improvement in sputum conversion and X-ray picture. Another meta-analysis also revealed that it was able to prevent the TB in high risk categories and is safe and immunogenic in people living with HIV/AIDS. This can have a role as prophylactic vaccine in HIV infected patients. Phase 3 trial is going on and has shown to induce a variable IFN-γ response \cite{39,40}.
5. Virus Based Booster Vaccines

Viral vector based vaccine technology has an ease of cloning large or multiple immunodominant antigens and can be exploited for a high titer scale up production. Vaccinia, a wild type pox virus qualifies the scenario but has safety issue in HIV subjects due to its high replicating capacity. A Modified vaccinia virus Ankara (MVA) has been made replication deficient retaining its ability to express protein. Adenovirus also has type1 immuno-adjuvant property and has been rendered replication deficient by deletion of gene E1. This has an additional advantage of natural respiratory tropism but is limited by neutralizing antibodies in human plasma [41,42].

5.1. MVA85A

Here mycobacterial antigen 85A is delivered by MVA delivery system. It was found to improve the BCG induced protection in animal model. In Phase I trial in UK in healthy adults including BCG vaccinated adults and subjects with LTBI, the results regarding safety and immunogenicity were promising and further trials are on the way [43,44,45]. It has additional advantage of demonstrating safety in HIV infected individuals or HIV-TB co-infection and infants [46]. Also the frequency of antigen specific cells remain significantly higher from the baseline at least for a year [47]. In its Phase 2b clinical trial, done on 2797 HIV-negative healthy infants of four to six months and previously vaccinated with BCG, the vaccine met the safety objectives but the TB rates in vaccinated children was not statistically significant as compared to the placebo. Thus the results described were disappointing in terms of preventing TB in BCG vaccinated infants [48].

5.2. AERAS402/CrucellAd35

AERAS is a replication deficient adenovirus 35 expressing the mycobacterial antigens 85A, 85B and TB10.4. Ad35 offers an advantage of low level of immunity, and hence the pre-existing neutralizing antibodies. Clinical trials after their promise in Phase1 trials, regarding the safety and efficacy are on the way in BCG vaccinated subjects in both HIV infected as well as HIV non-infected population [49,50,51].

5.3. Ad5Ag85A

This utilizes the Ad5 virus to express mycobacterial antigens 85A. The vaccine showed good results in mice and guinea pigs when administered intranasal but not intramuscularly. Currently in the clinical trials, the vaccine has the problem of facing pre-existing neutralizing antibodies as well as concern of acquiring HIV infection while vaccinating the Ad5 seropositive individual in the STEP trial [52,53,54].

Others MVA85A based viral vector in phase 1 evaluation are the simian virus based vac-
cine ChAdOx1.85A+ MVA85A and MVA85A-IMX313 [55].

Another initiative currently in phase 1 is the live influenza vector expressing the mycobacterial antigen Ag85A and ESAT6 [55].

6. Protein Adjuvant Vaccines

Protein has the advantage of production in bulk but requires some agent called adjuvants to induce immune responses of desirable potency and durability. These agents can be antigen delivery systems like aluminium based adjuvants or may be immunopotentiating agents like toll-like receptor ligands, cytokines or the bacterial toxins. Recent development of immune-stimulating adjuvants have made the TB protein subunit vaccines a feasible approach and many are in the clinical trials too [56].

6.1. M72AS01

M72 is related to Mtb72F protein which is a fusion protein comprising up of Mtb39a and Mtb32a antigens. A point mutation carried out in the Mtb32a improved the long term stability of vaccine. AS01E adjuvant system contains the immunostimulant MPL and QS2 combined with liposome to induce humoral and TH1 cellular responses [57,58].

The vaccine was well tolerated and immunogenic in adults with or without previous exposure to BCG or Mtb. Concerns regarding the effectiveness are there owing to strain related variations in antigen sequences. Unlike MVA85A, CD8+ T cell response was not seen with this preparation [57,59]. Local adverse events usually resolved within a week and may have been due to the adjuvant. Phase 2 trial is on the way including the HIV and TB individuals.

6.2. Hybrid 1 + IC31

This is a recombinant subunit vaccine where antigens ESAT6 and Ag85B are adjuvanted with IC31 system (polyaminoacid KLK and oligodeoxynucleotide ODN1a). It has been shown to provide durable TH1 responses in mycobacterially naïve subjects, BCG vaccinated individuals or previously TB infected adults. Though a week immune response is seen against ESAT6, but had a more potent protective effect than the Ag85B alone vaccine. The vaccine has been safe. Few local and systemic events were milder and resolved in less than 48 hours. No serious adverse events have been reported. Concern regarding the interference of vaccine with Interferon Gamma Release Assay (IGRA) test due to ESAT6 involvement in few subjects needs further studies to validate [60,61,62].

6.3. Hybrid 4 + IC31

This vaccine was developed on the same line as H1/IC31, but the antigen ESAT6 was replaced by TB10.4 to avoid IGRA related interferences. The vaccine is in the Phase 2 trial
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evaluation and is a good candidate in pipeline [63,64].

6.4. Hybrid 56 + IC31

H56 is a fusion protein of Ag85B, ESAT6, and the latency associated protein Rv2660c. The vaccine uses this H56 combined with the adjuvant IC31. It was safe and showed excellent control on latent infection in non-primate model. Moreover, in vaccinated monkeys anti-TNF antibodies did not induce reactivation of latent Tb [65]. In its first human Phase 1 trial the vaccine induced polyfunctional (IFN-γ+, TNF α+, IL2+) CD4+ T cells) response, that too after the low doses. CD45RA-CCR7+ central memory phenotype cells were also expressed. No serious adverse events were reported though few subjects had transient cardiovascular events [66].

6.5. ID93/GLA-SE

This vaccine uses ID93, a fusion protein of four Mtb antigens Rv2608, Rv3619, Rv3620 and Rv1813 adjuvanted with glucopyranosyl lipid adjuvant-stable emulsion (GLA-SE). It induced a significant multifunctional CD4+ response in BCG vaccinated or nonvaccinated mice and guinea pigs. In non-human primates it was well tolerated and induced both TH1 and TH2 type responses [67]. Moreover it also protected from MDR-TB in animal models [68]. Clinical trials regarding safety and efficacy in human is ongoing [69,70].

7. Whole Cell Inactivated Vaccine

7.1. DAR-901

It is an inactivated whole cell non-tuberculous mycobacterial vaccine from the master cell bank of previous vaccine SRL-172. In its phase 1 trial with participants who were BCG primed and included HIV positives- negatives and IGRA positives-negatives, it induced both cellular and humoral responses and had acceptable safety and tolerability profile [71]. Currently the phase 2 trial is ongoing [72].

8. Conclusion

It is indeed hopeful to have a number of vaccine candidates in the active phases of development for this dreaded disease, TB. The search will continue and may get modified with time till an ideal TB vaccine is available to make the epidemiology of the disease favorable, including the drug resistance and its nexus with the HIV. Till then, we have to rely on BCG especially for the prevention of serious manifestations of TB in pediatric population and preventing the mortality.
Table 1: BCG and other vaccine candidates.

<table>
<thead>
<tr>
<th>Name</th>
<th>Type</th>
<th>Composition</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Priming vaccines</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>BCG</td>
<td>Live attenuated</td>
<td>Live attenuated <em>Mycobacterium bovis</em></td>
<td>In use since 1921</td>
</tr>
<tr>
<td>MTBVAC</td>
<td>Live attenuated</td>
<td>Live attenuated <em>Mycobacterium tuberculosis</em></td>
<td>Phase IIa</td>
</tr>
<tr>
<td>VPM1002</td>
<td>Live attenuated</td>
<td>Live attenuated recombinant bacille Calmette-Guerin (r-BCG)</td>
<td>Phase IIa</td>
</tr>
<tr>
<td><strong>Booster Vaccines</strong></td>
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<tr>
<td>M72/AS01</td>
<td>Protein/Adjuvant</td>
<td>Mtb39a-Mtb32a fusion protein with AS01 adjuvant</td>
<td>Phase IIb</td>
</tr>
<tr>
<td>Hybrid 4 + IC31</td>
<td>Protein adjuvant</td>
<td>TB10.4-Ag85B fusion protein with IC31 adjuvant</td>
<td>Phase II</td>
</tr>
<tr>
<td>Hybrid 56 + IC31</td>
<td>Protein Adjuvant</td>
<td>Ag85B-ESAT6-Rv2660e fusion protein with IC31 adjuvant</td>
<td>Phase IIa</td>
</tr>
<tr>
<td>Hybrid 1 +IC31</td>
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<td>Phase IIa</td>
</tr>
<tr>
<td>ID 93+ GLA-SE</td>
<td>Protein Adjuvant</td>
<td>Rv2608-Rv3619-Rv3620-Rv1813 fusion protein with GLA-SE adjuvant</td>
<td>Phase I</td>
</tr>
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<td>Ad5Ag85A</td>
<td>Viral Vector</td>
<td>Recombinant adenovirus expressing Ag85A</td>
<td>Phase I</td>
</tr>
<tr>
<td>Crucell Ad35+ MVA85A</td>
<td>Viral Vector</td>
<td>Recombinant adenovirus 35 expressing Ag85A-Ag85B-TB 10.4 fusion protein</td>
<td>Phase IIa</td>
</tr>
<tr>
<td>ChAdOx1.85A+ MVA85A</td>
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<td>Simian virus ChAdOx1.85A plus MVA85A</td>
<td>Phase I</td>
</tr>
<tr>
<td>MVA85A</td>
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<td>Modified Vaccinia virus Ankara expressing Ag85A</td>
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</tr>
<tr>
<td>MVA85A-IMX313</td>
<td>Viral Vector</td>
<td>MVA85A combined with carrier protein IMX313</td>
<td>Phase I completed</td>
</tr>
<tr>
<td>TB/FLU-04L</td>
<td>Viral Vector</td>
<td>Live recombinant influenza vectored tuberculosis vaccine expressing Ag85A and ESAT 6</td>
<td>Phase I</td>
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<tr>
<td>Dar-901</td>
<td>Inactivated whole cell vaccine</td>
<td>Whole cell <em>M.obuense</em></td>
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<td>Fragmented M.tuberculosis</td>
<td>Detoxified liposomal fragments of <em>M.tuberculosis</em></td>
<td>Phase IIa</td>
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9. References


