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REVIEW

Current status of new tuberculosis vaccine in children

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ABSTRACT

Pediatric tuberculosis contributes significantly to the burden of TB disease worldwide. In order to achieve the goal of eliminating TB by 2050, an effective TB vaccine is urgently needed to prevent TB transmission in children. BCG vaccination can protect children from the severe types of TB such as TB meningitis and miliary TB, while its efficacy against pediatric pulmonary TB ranged from no protection to very high protection. In recent decades, multiple new vaccine candidates have been developed, and shown encouraging safety and immunogenicity in the preclinical experiments. However, the limited data on protective efficacy in infants evaluated by clinical trials has been disappointing, an example being MVA85A. To date, no vaccine has been shown to be clinically safer and more effective than the presently licensed BCG vaccine. Hence, before a new vaccine is developed with more promising efficacy, we must reconsider how to better use the current BCG vaccine to maximize its effectiveness in children.

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Introduction

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* complex (MTBC), is one of the top 10 causes of death among children worldwide.[1] It is estimated that one million cases of TB occur among children at ages of less than 15 y globally each year, 75% of which emerge in the 22 high TB-burden countries.[2] Although pediatric tuberculosis significantly contributes to the burden of disease, it has been neglected, when compared to other focuses of National TB Control Programmes (NTP) in most settings.[3,4] The lower priority afforded to pediatric tuberculosis is mainly due to its lower infectivity. This is commonplace in most NTPs, despite the fact that TB is a major cause of childhood morbidity and mortality, especially in the developing countries with poor public-health infrastructure.[2] Recently, pediatric tuberculosis has received greater attention, and in 2013 the World Health Organization (WHO) developed a road map aiming to achieve zero deaths due to childhood TB by 2025.[5] In order to accomplish this goal, an effective TB vaccine is urgently needed to prevent TB transmission in children.[6] At present, *Mycobacterium bovis* bacillus Calmette-Guerin (BCG) is the only licensed tuberculosis vaccine, which has been recommended by WHO for neonatal inoculation in the countries with a high TB prevalence.[7] BCG vaccination can protect the children from severe types of TB such as TB meningitis and miliary TB, while its efficacy against pediatric pulmonary TB has ranged from no protection to very high protection (0–80%).[8-10] Many new vaccines show promising results against *M. tuberculosis* infection in preclinical trials.[8,11,12]

In this review, the references were retrieved by searches of Pubmed, and website associated with TB vaccines including WHO, Aeras and ClinicalTrial.gov with key words: "tuberculosis vaccine," or "tuberculosis vaccination" or "tuberculosis prevention," or "BCG" and "children." The search was limited to reports published from January, 2000 to May, 2015. More than 200 articles were found, while only studies reporting data on current preventive TB vaccine candidates for the pediatric population will be reviewed.

BCG

BCG is now the most widely used vaccine worldwide.[13] As of 1974, the WHO Expanded Programme on Immunization recommends BCG should be given as soon as possible after birth in high TB-prevalence countries, with coverage in infants exceeding 80%.[7] Although the efficacy of BCG in preventing the development of adult pulmonary TB is controversial, BCG vaccination clearly protects infants and children from tuberculosis meningitis and severe forms of disseminated TB.[7] In a prospective community-based study from Turkey, child household contacts of smear-positive adult pulmonary TB cases with a BCG scar had a much lower risk of latent tuberculosis infection than those with no BCG scar.[14] Similar findings were observed from an outbreak in a nursery in the UK, which showed a significant protective effect of BCG vaccination against *M. tuberculosis* infection among infants.[15] A meta-analysis by Trunz et al demonstrated that the BCG vaccine had

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prevented an estimated 73% of TB meningitis and 77% of miliary disease in children from birth to 5 y of age.[16] Considering the low cost at US$2–3 per dose, BCG vaccination is a highly cost-effective intervention against childhood tuberculosis.[16]

Several published papers from various sources, and from various global locations, have shown that the effectiveness of BCG vaccine among children varies notably.[17-20] The variation of protection by BCG may be attributed to different types of BCG, to genetic differences between populations, and to cold-chain maintenance of BCG.[18] In addition, another important issue affecting this variability is the exposure to environmental mycobacteria.[20] A systematic review of 21 randomized controlled trials evaluating the protection by BCG revealed that BCG imparted greater protection in northern latitudes, which may be due to less exposure to non-tuberculous mycobacteria (NTM) faced by vaccine recipients in these areas.[20] In another study from China, the researchers also found that the immune response to BCG vaccination varied according to the NTM exposure among neonates.[21] Although the exact reason for these observations is currently unclear, it is hypothesized that prior exposure to NTM may produces antigens which may block the replication of BCG.[22] Further studies are needed to illuminate the effects of NTM exposure on BCG efficacy, which may be helpful in the future, in the development of the candidate vaccines which can be free from the compromise of NTM exposure.

Epidemics of HIV/AIDS have increased the global prevalence of TB.[23] Due to the low CD4+ T cells, HIV-infected infants are more prone to develop disseminated BCG disease following neonatal inoculation.[24,25] Based on these findings, the WHO recommended that BCG vaccine, a live attenuated *Mycobacterium bovis*, should not be given to children diagnosed as HIV positive.[26] Although this strategy is essential to reduce the emergency of BCG-related diseases, it may be difficult to implement, and is rarely employed. There is an urgent need to develop a non-live alternative bacterial vaccine suitable for preventing TB transmission among children living in HIV-epidemic regions.

New TB vaccine candidates

In recent studies, findings from basic research have focused on antigens which are immunodominate, essential for virulence, containing recognized T cell epitopes and to which T cell responses are protective in animal models.[27,28] Based on these findings, many new TB vaccine candidates have been developed, many showing moderately increased efficacy or/and safety over BCG in preclinical trials (Fig. 1, Table 1).[29] In terms of strategies of the candidates, vaccines can be divided into 3 groups, including live or killed recombinant mycobacteria, viral-vector and protein-adjuvanted vaccines.

Live Recombinant Mycobacteria for primary immunization

rBCG30

In general, the rationale of live recombinant mycobacteria is to add certain genes to BCG, or to remove specific genes from the natural mycobacterial genome, which will create a new vaccine to directly replace BCG.[28] The first recombinant BCG vaccine was rBCG30, developed at the University of California, Los Angeles.[30] By overexpressing the *M. tuberculosis* protein Ag85B, the recombinant BCG stimulated a strong immune response to *M. tuberculosis* in guinea pig models.[31] The animals immunized with rBCG30 survived significantly longer after challenge from a highly virulent strain of *M. tuberculosis* than those immunized with BCG.[31] The Phase I clinical trial completed in 2011 demonstrated that rBCG30 was safe and immunogenic. Unfortunately, this vaccine is not being further developed while awaiting the development of the next

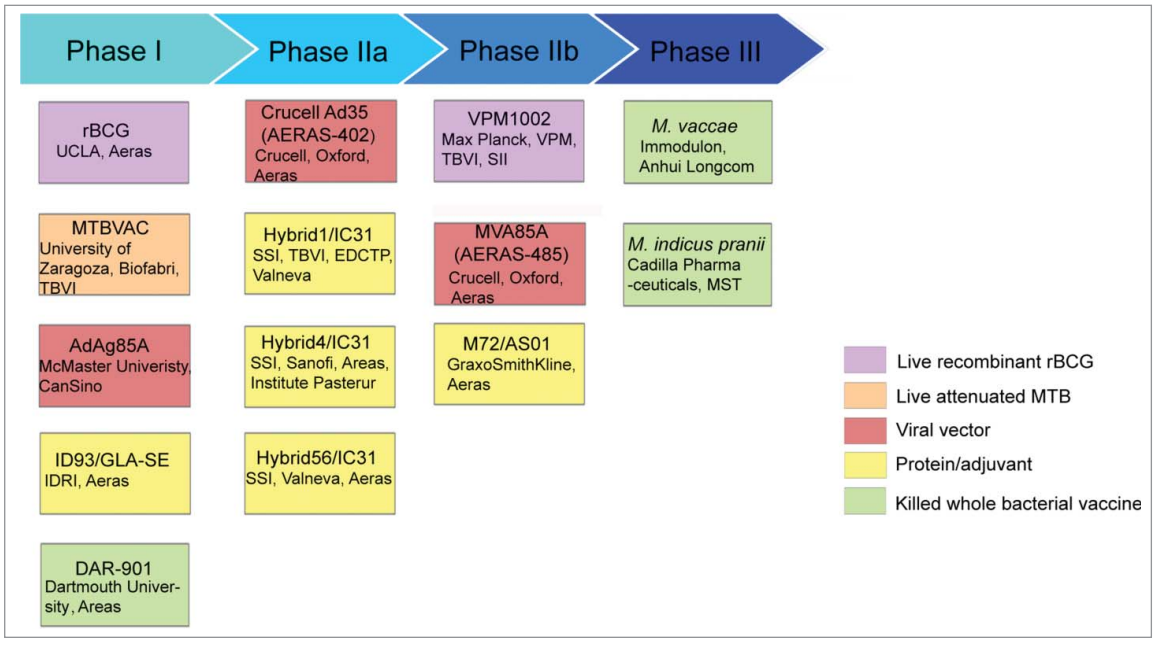

Figure 1. TB vaccine candidates in clinical trials in 2015. Based on the Tuberculosis Vaccines Pipeline and AREAS website.[29]

generation of auxotrophic recombinant BCG strains which avoid the inclusion of antibiotic resistance genes.[11]

VPM1002

A promising substitute candidate with substantially greater protection than BCG in animal models is VPM1002, which is currently in a Phase II trial in newborn infants.[11,32] A total of 3 organizations participated in the development ofVPM1002, including Vakzine Projekt Management GmbH, the Max Planck Institute for Infection Biology, and the Tuberculosis Vaccine Initiative (TBVI). The vaccine is now owned and being aggressively developed by the Serum Institute of India. The vaccine contains a new BCG strain expressing the listeriolysin (hly) from the bacterium *Liusteria monocytogenes*.[33,34] The exogenous listeriolysin facilitates the perforation of the phagosome membrane, allowing the release of recombinant BCG antigens into the cytosol of host cells.[35] Hence, the vaccine is able to stimulate the CD8 T cells via major histocompatibility complex (MHC) I presentation, and further activate both T helper (Th)1 and Th17 cytokine responses.[35,36] In addition, an endogenous gene *ureC*, encoding urease C, has been deleted in VPM1002.[35] Urease C is crucial for the hydrolysis of urea, resulting in the ammonia production and a basic environment in the milieu.[32] Because hly has a stringent optimum pH of 5.5, inactivating urease C is necessary to producing the acidic pH environment for hly activity.[32] In early animal testing, VPM1002 showed encouraging immunogenicity, safety and tolerability in comparison with BCG vaccine.[36] Further clinical trials demonstrated that VPM1002 could induce multifunctional CD4 and CD8 T cell subsets.[36] Recently, a Phase II trial has been completed in South Africa to evaluate the immunogenicity and safety of VPM1002 compared with the BCG vaccine in newborn infants, while the clinical trial data is not published till now (http://ClinicalTrials.gov identifier NCT01479972, Table 2). Of note, this vaccine is produced by standard fermentation methodology, which overcomes the very poor production yield and lot to lot variability associated with the standard pellicle method for the growth of BCG.

MTBVAC

MTBVAC, developed at the University of Zaragoza, is the first live attenuated *M. tuberculosis* strain in Phase I clinical trial.[37] In order to generate a both safer and more effective vaccine, *phoP*, which encodes the transcriptional regulator associated with the regulation of *M. tuberculosis* virulence, and *fadD26*, which is crucial to major mycobacterial surface virulence factors (PDIMs) of *M. tuberculosis*, were knocked out.[37,38] In preclinical studies, the recombinant MTBVAC vaccine exhibited similar safety and biodistribution profiles, and superior protection in animal model as compared with *Mycobacterium bovis* BCG.[39,40] Recently, a highly attenuated MTBVAC-based live vaccine was developed by Solans et al. through an additional gene inactivation generated in *erp* of MTBVAC.[40] Although the virulence of the MTBVAC *erp*(-) strain was hyper-attenuated, the results from immunocompetent mice revealed that it did not compromise its protective efficacy profile as compared with BCG.[40] These findings indicate that it can be used as a potential vaccine candidate for high-risk children with immune suppression.[39]

Mycobacterium vaccae

Mycobacterium vaccae, a saprophytic *Mycobacterium* containing numerous antigenic epitopes common to *M. tuberculosis*, has been used as an immunotherapeutic vaccine in combination with drug therapy.[41,42] There are 3 available preparations of *M. vaccae* currently, including a heat killed product from Immodulon of U.K, a related heat killed strain developed by Dartmouth and recently identified to be *M. obuense* (a close relative of *M. vaccae.)* and a lysate vaccine from AnHui Zhifei Longcom of China.[43] Interestingly, a recent clinical trail from Tanzania has demonstrated that the protective effectiveness of *M. vaccae* against TB was observed among HIV-infected and BCG-vaccinated adults with CD4 counts of not less than 200 cells/μl, suggesting that *M. vaccae* can be used as a preventive vaccine for TB.[44] Further clinical studies on the usefulness of *M. vaccae* for preventing infant population from TB infection are warranted.

DAR-901

DAR-901, developed at Dartmouth University and Areas, is a whole-cell mycobacterial vaccine consisting of inactivated *Mycobacterium obuense*.[45] Different from an earlier therapeutic TB vaccine candidate SRL-172, the primary component of

Table 1. Preventive tuberculosis vaccine candidates in clinical trials.

Name[a]	Composition	Classification	Strategy
rBCG30	BCG overexpressing Ag85B	Recombinant BCG	Prime
VPM1002	Recombinant BCG strain	Recombinant BCG	Prime
MTBVAC	Live-attenuated *Mycobacterium tuberculosis*	Attenuated *M. tuberculosis*	Prime
DAR-901	*M. obuense lysate*	Inactivated mycobacterium	Prime-boost
Mycobacterium vaccae	*M. vaccae lysate*	Inactivated mycobacterium	Prime-Boost
MVA85A(AERAS-485)	Modified vaccinia virus Ankara expressing MTB antigen Ag85A	Viral vector	Prime-Boost
Crucell Ad35(AERAS-402)	Replication-deficient adenovirus 35 expressing MTB antigens 85A, 85B and TB10.4	Viral vector	Prime-Boost
AdAg85A	Replication-deficient adenovirus 5 expressing Ag85A	Aerosol Viral vector	Prime-Boost
Hybrid 1/IC31	Ag85B-ESAT6 fusion protein + IC31 adjuvant	Protein/adjuvant	Prime-Boost
Hybrid 4/IC31	Ag85B-TB10.4 fusion protein + IC31 adjuvant	Protein/adjuvant	Prime-Boost
Hybrid 56/IC31	Ag85B-ESAT6-Rv2660c fusion protein + IC31 adjuvant	Protein/adjuvant	Prime-Boost
M72/AS01	Mtb39a-Mtb32a fusion protein + AS01 adjuvant	Protein/adjuvant	Prime-Boost
ID93/GLA-SE	Rv2608-Rv3619-Rv3620-Rv1813 fusion protein + GLA-SE adjuvant	Protein/adjuvant	Prime-Boost

[a]Based on the Tuberculosis Vaccines Pipeline and AREAS website.

Table 2. Clinical trials of current preventive tuberculosis vaccine candidates in children.

Name	Identifier[a]	Objective	Locations	Status
VPM1002	NCT01479972	To evaluate the safety and immunogenicity of VPM1002 in comparison with BCG in newborn infants	South Africa	Completed
	NCT02391415	To evaluate the safety and immunogenicity of VPM1002 in comparison with BCG in HIV-exposed/-unexposed newborn infants	South Africa	Recruiting participants
MVA85A	NCT00953927	To evaluate the safety, immunogenicity and efficacy of MVA85A in BCG vaccinated infants without tuberculosis or HIV infection	South Africa	Completed
Crucell Ad35(AERAS-402)	NCT01198366	To evaluate the safety and immunogenicity of AERAS-402 in BCG-vaccinated, HIV-uninfected infants without evidence of tuberculosis	Kenya, Mozambique, South Africa	Completed.
Hybrid 4/IC31	NCT01861730	To evaluate the safety and immunogenicityof Hybrid 4+IC31 in BCG-vaccinated infants	South Africa	Recruiting participants
	NCT02075203	To evaluate the safety, immunogenicity, and prevention of infection with *Mycobacterium tuberculosis* of Hybrid4/IC31 and BCG revaccination in healthy adolescents	South Africa	Recruiting participants
M72/AS01	NCT01098474	To evaluate the safety and immunogenicity of M72/AS01in healthy infants	Gambia	Completed

[a]Referenced from the website of ClinicalTrials.gov.

which was also inactivated *M. obuense*, DAR-901 is grown broth rather than agar, a more scalable production method.[45-47] Recently, a Phase I trial of DAR-901, is currently conducted in HIV negative and HIV positive adults previously vaccinated with BCG to assess the safety, tolerability, and immunogenicity of multiple doses of DAR-901 at different dose levels. Further clinical trials need to be performed to determine the role of DAR-901 in the prevention of TB infection among children.[45]

Viral-vector and protein-adjuvanted vaccines that boost bcg prime

There are several new subunit TB vaccine candidates in preclinical and clinical trials that are used to complement the immune response following priming with BCG in early infant.[8] These candidates are based on dominant antigens that are expressed by metabolically active *M. tuberculosis*.[11] Compared with BCG, all the adjuvanted protein vaccines which contain fusion proteins of one or more antigens showed similar or better efficacy to protect mice and guinea pigs against *M. tuberculosis* infection.[11] Two types of products have been developed, including viral-vectored vaccines and adjuvanted subunit vaccines.

MVA85A(AERAS-485)

MVA85A is a modified vaccinia virus Ankara (MVA) expressing the major secreted antigen Ag85A (MVA85A, AERAS-485) of *M. tuberculosis*.[48] With the support from Aeras, the Oxford-Emergent Tuberculosis Consortium developed this virally vectored TB vaccine. As a heterologous boost for BCG, MVA85A moderately improved BCG-induced protective efficacy against *M. tuberculosis* challenge in animal models,[49-52] which was predominantly attributable to better induction of CD4 and CD8 T cell responses, as well as antigen-specific Th1 and Th17 cells responsible for protection against *M. tuberculosis*.[53] Several clinical trials have demonstrated that MVA85A appears to be safe and well tolerated.[53,54] However, an underpowered Phase II trial of MVA85A in adults infected with HIV revealed that MVA85A showed no trend in efficacy against *M. tuberculosis* infection or disease.[55] Similar results were observed in another Phase IIb trial of MVA85A in infants conducted in South Africa. Healthy infants aged 4 to 6 months who had been previously inoculated with BCG shortly after birth received a dose of MVA85A or a placebo between 4 and 6 months of age. In the follow-up period, the incidence of TB between the experimental and placebo groups was not different. The further vaccine efficacy analysis revealed that the low protective efficacy of 17.3% with no significance to placebo indicated that a single dose of MVA85A was unable to confer significant protection against tuberculosis disease or *M. tuberculosis* infection in infants (http://ClinicalTrials.gov identifier: NCT00953927).[56] Further work is underway with the vaccine being used either by the aerosol route or given as a heterologous boost to an adenoviral vector prime.[57]

Crucell Ad35(AERAS-402)

CrucellAd35 (AERAS-402), developed by Crucell, is a replication-deficient adenovirus vector that produces 3 natural *M. tuberculosis* antigens 85A, 85B and TB10.4.[58] The one-piece fusion polyprotein containing 3 antigens could be expressed upon immunization because the vector seroprevalence, and levels of neutralizing antibody titers, to Ad35 are relatively low in people living in developing countries.[59] In mouse models and monkey, the Crucell Ad35 (Aeras-402) has been shown to elicit robust CD4 and CD8 T cell responses, producing multiple cytokines and other immune effector molecules.[58] Studies in adults revealed that Crucell Ad35 (Aeras-402) was safe and immunogenic in healthy adults previously vaccinated with BCG and with no previous *Mycobacterium tuberculosis* infection.[60] Multiparameter flow cytometric assays demonstrated that the vaccine could induce a robust CD8 T cell response as well as a polyfunctional CD4 T cell response after BCG priming.[60] Another

clinical Phase IIb trial with the planned recruitment of over 400 infants revealed that AERAS-402 has an acceptable safety profile in infants; however the polyfunctional T cell responses were lower than those previously measured with this vaccine in adults (http://ClinicalTrials.gov identifier: NCT01198366).[61] Therefore the trial was stopped after the first 400 subjects were enrolled and did not move on to the efficacy phase.

AdAg85A

Similar to MVA85A, AdAg85A consists of a replication-deficient serotype 5 adenoviral vector containing the natural *M. tuberculosis* antigen 85A.[62] It has been developed by McMaster University. Primary data showed thatAdAg85A provided promising protection against TB infection in mice when priming as booster vaccine for BCG when administered intranasally.[62,63] Compared with intramuscular injection, intranasal administration induced stronger CD4 and CD8 T cell responses.[63,64] More recently, a literature from Mu et al. reported that a new intranasally bivalent adenovirus-vectored vaccine expressing both Ag85A and TB10.4 antigen conferred a significantly improved level of protection against *M. tuberculosis* challenge comparable to Ag85A alone or BCG immunization.[65] In a Phase I clinical trial evaluating safety and immunogenicity of AdAg85A administered intramuscularly, the vaccine was found to be safe and well tolerated.[66] Although the recombinant Ad5 vaccine has shown good safety profile, the prevalence of neutralizing antibody titers against Ad5 was up to 90% in sub-Saharan Africa, which may limit the usefulness of this vaccine.[66] Concern also remains on the increased rate of HIV acquisition seen in the HIV STEP trial, and the use of intramuscular adenoviruses in areas with high HIV rates is unlikely to be acceptable.

Hybrid 1/IC31

With backing from Statens Serum Institut (SSI), TBVI, and the European & Developing Countries Clinical Trials Partnership (EDCTP), a recombinant subunit vaccine named Hybrid 1/IC31(H1/IC31) was developed. It contains the hybrid protein of Antigen 85B (Ag85B) and Early Secretory Antigenic Target 6 (ESAT6), and is adjuvanted with IC31, an adjuvant system combining an antibacterial peptide (KLK) and a synthetic Toll-like receptor 9 agonist (ODN1a).[67,68] Numerous studies have demonstrated that the fusion subunit vaccine was safe in HIV-infected adults with a CD4 Lymphocyte count greater than 350 cells/mm^3, and no serious adverse reactions associated with the vaccine were observed.[69] In addition, H1/IC31 resulted in a robust CD4 T cell response, as well as the secretion of IFN-γ.[68,70,71] These strong responses persisted over 2.5 y of follow-up in BCG-naïve volunteers.[70] However, because ESAT6 was also the most important antigen used in the diagnosis of latent TB, the inclusion of ESAT6 in the vaccine may increase the risk of interference with the ESAT-6-based diagnostic assay. A recent study found that 17% of the participants administered with a high dose of H1/IC31 showed positive test results with Quantiferon Gold.[70]

Hybrid 4/IC31

Hybrid 4/IC31 (H4/IC31), originally developed by SSI and now under development by Sanofi Pasteur, is a subunit vaccine that consists of a recombinant fusion protein of Ag85B and TB10.4, and the adjuvant IC31.[72] Similar to H1/IC31, it provided promising safety and tolerability, while H4/IC31 could avoid the interference with the result of IFN-γ release assay (IGRA).[72,73] When administered as priming or booster vaccine, H4/IC31 showed moderate protective efficacy against pulmonary TB in mice and guinea pigs.[72,73] The inoculation of H4/IC31 as a booster for BCG in the mouse model could elicit the multifunctional CD4 T cells, which was associated with higher expression of IFN-γ, TNF-α and IL-2.[73] A Phase II trial sponsored by Sanofi Pasteur, Aeras and the HIV Vaccine Trials Network to evaluate its safety and immunogenicity in BCG vaccinated health infants is currently in progress (http://ClinicalTrials.gov identifier: NCT01861730).

Hybrid 56/IC31

Hybrid 56/IC31 (H56/IC31), designed by the Statens Serum Institut (SSI) in Denmark, is an immunogenic fusion protein containing Ag85B, ESAT6 and the latency-associated protein Rv2660c, as well as the adjuvant IC31.[74] In BCG-vaccinated non-human primate models, H56/IC31 has been shown to be well-tolerated and immunogenic.[74] Moreover the vaccine showed excellent protective effectiveness against TB reactivation after animals were given with BCG vaccine.[74] This booster vaccine is currently undergoing Phase I/IIa clinical trials to evaluate its safety and immunogenicity in HIV-negative, BCG vaccinated volunteers with/without latent TB. Unfortunately, no evaluation results have been reported on the efficacy of this vaccine to protect children against TB infection.

M72/AS01

Developed by GlaxoSmithKline, the M72/AS01 vaccine is a recombinant vaccine comprising Mtb39a and Mtb32a antigens, which are only expressed in *M. tuberculosis* and BCG rather than in other mycobacteria.[75] AS01 is an adjuvant consisting of immunostimulants MPL and Quillaja saponaria fraction 1 (QS21) combined with liposomes, which induced humoral andTh1 cellular responses.[76] A clinical trial in 110 volunteers completed in Belgium found that M72/AS01 was clinically well tolerated and induced high magnitude and persistent cell-mediated and humoral immune responses.[76] In addition, there was no report of serious adverse events related to the vaccination.[76,77] A Phase IIa trial from South Africa was completed in 45 *M. tuberculosis* infected or uninfected adults, which demonstrated that M72/AS01 elicited a novel T cell responses different from Th1 and Th17 responses.[12] Although the exact function of these novel T cell populations was unknown, these cells may mediate the inflammation induced by Th1 and Th17.[12] Another Phase II trial performed in South Africa found that M72/AS01 showed clinically acceptable safety and immunogenicity profile in the adolescents aged 1317 y.[78] In a Phase II study, the

assessment for the safety and immunogenicity of M72/AS01 has been completed in Gambia, which showed that M72/AS01 was acceptably tolerated with no vaccine-related serious adverse events reported in infants (http://ClinicalTrials.gov identifier: NCT01098474).[79] A Phase 2b proof of concept efficacy study of the vaccine is underway in 3500 latently infected young adults in 3 countries in Africa, with results likely available in 2018.

ID93/GLA-SE

ID93, developed by the Infectious Disease Research Institute (IDRI) in Seattle, is a fusion of 4 *M. tuberculosis* proteins, including Rv2608, Rv3619, Rv1813 and Rv3620.Rv2608, Rv3619 and Rv1813 confer the virulence of *M. tuberculosis*, while Rv3620 is associated with the latent growth of *M. tuberculosis*.[80] Combined with the TLR adjuvant glucopyranosyl lipid adjuvant-stable emulsion (GLA-SE), ID93/GLA-SE induced polyfunctional CD4 Th1cell responses characterized by secretion of antigen-specific IFN-γ, TNF and IL-2in a mouse model.[80,81] In addition, boosting BCG-vaccinated guinea pigs with ID93/GLA-SE leaded to reduced pathology and fewer bacilli within the lungs, and prevented the death of animals challenged with virulent *M. tuberculosis*.[81] This vaccine activated CD4 and CD8 T cell responses in BCG-vaccinated or TB-exposed human peripheral blood mononuclear cells.[80] In a recent preclinical publication, it was shown that the use of ID93/GLA-SE vaccine may result in cross-protection against *M. leprae* infection.[82] A Phase I clinical trial to evaluate the safety, tolerability and immunogenicity of the vaccine in healthy adults is currently in progress (http://ClinicalTrials.gov identifier: NCT02508376).

Potential strategy with bcg vaccination against tuberculosis in children

The development of novel vaccines against TB has shown tremendous growth in the past decade.[11] Many of the vaccine candidates mentioned above have already entered, or will enter into clinical trials among infants.[8] Although the preclinical experiments of these vaccine candidates such as MVA85A are always encouraging in safety and immunogenicity, the protective efficacy evaluated by clinical trials in humans may be disappointing.[56] The conflicting results between preclinical and clinical trials indicate that our knowledge on the interplay between human host and *M. tuberculosis* pathogen is still limited. Previous development of TB vaccines has focused on achieving cell-mediated immunity by inducing Th1 cytokines (including IFN-γ, IL-2, and TNF-α) expression by CD4 or CD8 T cells.[83] However, a clinical study in South Africa infants found that there was no correlation between the magnitude of expression of Th1 cytokines and protection against TB disease.[83] These results highlight that the field should look beyond Th1 cytokines as primary indicators of immunogenicity and correlates of vaccine-induced protection. Thus, in conjunction with the progression of further TB vaccine trials, we should reconsider how to make better use of the current BCG to yield its full effectiveness as an alternative in children.[84]

An appropriate time for BCG vaccination

Due to the high risk of disseminated BCG disease after vaccination in HIV-infected infants, HIV infection is a relative contraindication to BCG vaccination in infants.[85,86] Since infant HIV status is usually unknown at birth, it is relatively dangerous to use BCG to immunize neonates of HIV positive mothers living in regions of high HIV endemicity. Recently, Tchakoute et al. performed a study to determine whether administering the delayed BCG vaccination altered BCG-specific T-cell responses.[87] Their findings revealed that the levels of polyfunctional T cells and IFN-γ produced by CD4 T cells were higher in infants giving vaccination at 14 weeks of life compared with those giving vaccination at birth.[87] Hence, delayed BCG vaccination could be used as a safer alternative to vaccination at birth for HIV-infected infants or infants in the HIV-prevalent region.[88] Concerns about this approach have been raised, however, as in some studies BCG has lowered all-cause mortality.[89]

In addition to HIV-infected infants, a recent study by Kagina et al. demonstrated that delaying BCG vaccination from birth to 10 weeks of age in HIV-unexposed infants resulted in higher frequencies of BCG-specific, polyfunctional CD4 T cells at 1 y of age.[90] In contrast, Burl and his colleagues found that delaying BCG vaccination from birth to 18 weeks of age led to decreased IFN-γ and IL-17 production in the delayed vaccinated group.[91] They hypothesized that the decrease might be attributed to the exposure to NTM prior to BCG vaccination, conferring the induction of Tregs, which would reduce the immune response to BCG vaccination.[91] The findings from several other publications supported this hypothesis, that the increased efficacy of BCG vaccination was observed in locations farther from equator, where the infants suffer from lower exposure to NTMs.[22] This conflicting data provides us several competing factors to keep in mind during development and testing. First, all studies to date were all based on the measurement of immunologic responses. Although the production of BCG-specific T cell responses may be used as a crucial mediator of protection in TB, it is unknown whether there is any resulting difference in clinical outcomes. Second, considering the different prevalence of NTM worldwide, the vaccination time after birth in different regions may be different. Another issue needed to be considered is the actual adherence of the parents to the vaccine schedule and guidelines. Numerous reports have shown that adherence to vaccination can be poor among rural or migrating populations. Hence, it is necessary to balance the dilemma that exists between delayed time and worsened vaccine uptake.

Aside from the aforementioned considerations, the interaction between BCG and other vaccines is another potential concern affecting the immunogenicity and protective efficacy. Children suffer a high frequency and severity of microbial infection leading to millions of deaths worldwide. Many children have more than 9 infections in their first year of life; thereby the need for combined vaccines has been endorsed as a feasible solution to improve the compliance of vaccination for this high-risk population. BCG is usually co-inoculated with other vaccines such as those for hepatitis B in the regions with high TB prevalence at the

neonatal stage. Because of the potential interactions between live vaccines or immunological interference, there may be loss of protective efficacy andthe induction of adverse reactions by BCG vaccination. Unfortunately, the data on the interaction between BCG and other vaccines is limited, and further experiments on BCG-vaccine interactions if the timing of BCG vaccination is moved will be essential for future clinical BCG studies.

Revaccination of BCG

BCG revaccination used to be an integral part of many national tuberculosis programmes to maintain the protective efficacy of primary BCG vaccination in the tuberculin-negative school children.[92] To date, little data on the efficacy of BCG revaccination is available.[92-94] The first study to evaluate its efficacy was in Karonga District of Malawi.[93] The researchers found that both primary vaccination and revaccination protected children and adults against leprosy, while neither the first vaccination nor revaccination showed protection against TB.[93] Similarly, a cluster-randomized trial from Brazil revealed that the efficacy of BCG revaccination was 9%, suggesting that the revaccination given to children aged 7–14 y in this study did not provide substantial additional protection.[94] Based on this and other data, WHO recommend not revaccination BCG in children.[95] An important consideration herein is that both studies have enrolled children with one BCG scar. The detailed infection background of these participants, including infection by NTM or latent TB, was unknown. Several clinical trials have proved that both *M. tuberculosis* and NTM infection have diverse effects on BCG efficacy against *M. tuberculosis*.[22,96] Thus, future studies aiming to evaluate the protective effectiveness of revaccination may wish o enroll children clearly documented to have received primary BCG vaccination, and who also have a negative PPD result to prevent the potential interference of *M. tuberculosis* and NTM infection.

Another important consideration raised by these 2 studies is the relative short follow-up period to observe the protective efficacy of revaccination. With the extended 4 y of follow-up and the additional cases accrued, the previous study in Brazil revealed that the overall vaccine efficacy was 12% as compared with9% for the 5-year follow up,[97] indicating that the revaccination with BCG provided the additional protective efficacy against TB, and this efficacy varied with distance from equator, ranging from 1% of Manaus (with short distance from equator) to 19% of Salvador (with long distance from equator).[97] This difference further strengthened the earlier hypothesis that BCG vaccination offers higher efficacy in low NTM prevalence. Taken together, revaccination with BCG may have some available protective effectiveness in some certain settings. A large cohort study would be required to assess the efficacy of BCG revaccination given to adolescent children, and to explore the factors influencing the protection against TB of BCG revaccination. An ongoing randomized trial in South Africa is examining BCG revaccination in IGRA-negative school age adolescents to study whether BCG revaccination has potential effect on TB infection rather than TB disease (http://ClinicalTrials.gov identifier: NCT02075203).

Conclusion

Pediatric tuberculosis contributes significantly to the burden of TB disease worldwide.[1] Thanks to a deeper understanding of the host–pathogen relationship, impressive strides have been made in TB vaccine development in the past decades. Several TB vaccine candidates have already entered, or will enter into clinical trials among infants. Unfortunately, although the preclinical experiments of these vaccine candidates such as MVA85A are encouraging in safety and immunogenicity, the protective efficacy evaluated by clinical trials in the infants may be disappointing. The conflicting results between preclinical and clinical trials indicate that the complexity of the protective immune response induced by *M. tuberculosis* is currently beyond our knowledge, and the vaccine containing antigens that induce simple Th1 cell-mediated immune responses may have unsatisfactory protective efficacy against TB. Hence, future vaccine strategies may need to be focus on more variable parts of the *M. tuberculosis* genome and structure, rather than the conserved T-cell epitopes. To date, no vaccine has been shown to be safer and more effective than BCG vaccine. Hence, before the appearance of a new vaccine with more promising efficacy, we should reconsider how to make better use of the current BCG to yield its full effectiveness in children. Delaying BCG vaccination may be a safer alternative to vaccination at birth for HIV-infected infants or in HIV-prevalent region. A large cohort group study would be required to help us to generate the appropriate strategies for use of BCG vaccine in children.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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