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SUPPLEMENT ARTICLE

What's Old and New in Tuberculosis Vaccines for Children

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Tuberculosis (TB) is a leading cause of global child mortality. Until the turn of the 21st century, *Mycobacterium bovis* bacille Calmette-Guerin (BCG) was the only vaccine to prevent TB. The pediatric TB vaccine pipeline has advanced in the past decade to include the evaluation of novel whole cell vaccines to replace infant BCG and investigation of subunit and whole cell vaccines to boost TB immunity during adolescence. We describe the history of BCG, current TB vaccine candidates in clinical trials, and the challenges and opportunities for future TB vaccine research in children. Children are a critical target population for TB vaccines, and expansion of the pediatric TB vaccine pipeline is urgently needed to end the TB pandemic.

Key words. child; tuberculosis; vaccine.

The year 2021 commemorated the 100th-year anniversary of *Mycobacterium bovis* bacille Calmette-Guérin (BCG), the first and only licensed vaccine against tuberculosis (TB) [1]. The first human to receive BCG was a neonate born to a mother with TB; now, over 200 million doses of BCG are given annually throughout the world [2]. BCG vaccination in newborns provides ~90% protection against disseminated TB disease during the first year of life and up to 2-fold reduction in all-cause child mortality [1, 3–5]. Despite these successes, variable BCG vaccine efficacy against pulmonary TB and waning protection beyond infancy result in over 10 million new TB cases and 1.5 million TB-related deaths (230 000 among children) each year [6]. Innovative TB vaccine candidates, trial design, and implementation strategies are needed to end the TB pandemic. This review describes the lessons learned from the first era of pediatric TB vaccines, recent advances in the pediatric TB vaccine pipeline, and challenges and opportunities for future TB vaccine research in children.

THE FIRST ERA OF PEDIATRIC TB VACCINATION: INFANT PRIMARY BCG AND SUBUNIT BOOSTERS

Infants experience higher TB morbidity and mortality than adults and accordingly have been a priority target population for TB vaccines [7]. Most countries currently administer BCG at birth or upon a child's first contact with health services [1, 8]. Despite the relative immaturity of the neonatal immune system, BCG vaccination elicits robust CD4 T cell responses producing Th1 cytokines, including interferon-gamma (IFN-γ), tumor necrosis factor (TNF), and interleukin 2 (IL-2) [9]. Vaccine-induced protection is highest and most consistent against hematogenous TB including meningitis and miliary TB among young children, but BCG efficacy to prevent pulmonary TB is variable (ranging from 0% to 80%) and differs by sex, geographic distance from the equator, and preexisting exposure to *Mycobacterium tuberculosis* and other mycobacteria [1]. BCG is a live attenuated vaccine, which poses safety risks for children with acquired or primary cell-mediated immunodeficiency who can develop disseminated or aggressive regional BCG disease after vaccination [10]. Prior to the antiretroviral therapy (ART) era, children with HIV had 3- to 4-fold increased risk for disseminated BCG resulting in high mortality [11]. The lack of standard manufacturing procedures in the early stages of distribution led to heterogeneity of vaccine strains used globally, a problem that persists today and may account for variation in BCG efficacy [12]. Moreover, an inconsistent supply chain has resulted in frequent BCG vaccine shortages, particularly in low- and middle-income countries [2].

To address these challenges, the start of the 21st century marked the development of novel TB subunit vaccines to boost BCG immunogenicity. The 2 types of TB subunit vaccines include (1) viral vectors expressing *M. tuberculosis* antigens and (2) recombinant *M. tuberculosis* fusion proteins delivered with an adjuvant (Figure 1). Subunit vaccines have more limited *M. tuberculosis* antigenic targets compared with whole cell vaccines, such as BCG, but offer an improved safety profile and standardized manufacturing. The viral vector vaccine, modified vaccinia virus Ankara expressing immunodominant *M. tuberculosis* antigen Ag85A (MVA85A), was the first novel TB vaccine to be evaluated in a large efficacy trial among infants [13]. In this placebo-controlled trial, BCG-vaccinated infants

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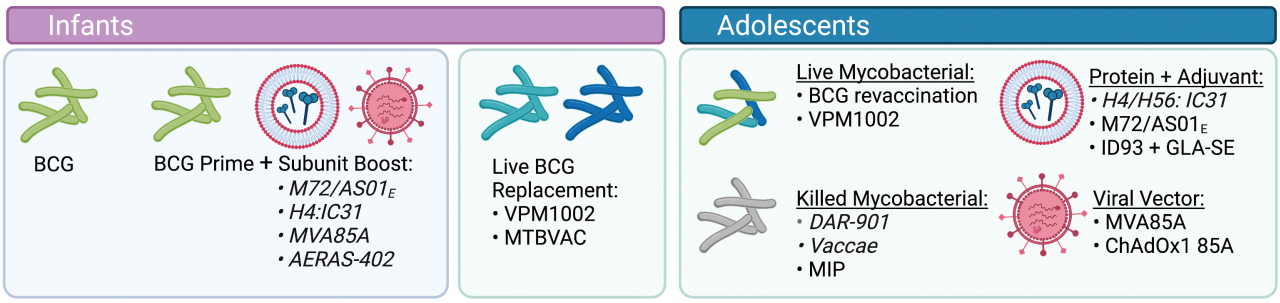

Figure 1. Pediatric TB vaccine approaches. Summary of previous and current approaches to TB vaccines in children under 18 years of age. Vaccine candidates that have completed testing in phase 1 or higher trials are indicated in italics. Created with BioRender.com.

who received a MVA85A booster at 4-6 months of age demonstrated modest induction of Ag85A-specific Th1 and Th17 CD4 cellular immunity, but no additional protection against clinically asymptomatic *M. tuberculosis* infection or TB disease compared with BCG alone. Other subunit vaccines, including M72/AS01$_E$, AERAS-402, and H4:IC31, have been evaluated as BCG boosters among infants (Table 1). While these subunit candidates were safe, infant immunogenicity was modest in comparison to parallel studies in adults [14, 15, 16]. A reverse prime-boost strategy has also been evaluated among HIV-exposed uninfected infants. In this study, primary vaccination with MVA85A at birth only elicited modest immunity and did not significantly enhance immunogenicity of an infant BCG booster [17].

RECENT STRATEGIC ADVANCEMENTS IN PEDIATRIC TB VACCINATION TRIALS

In the past decade, 11 new candidates have been evaluated in pediatric TB vaccine trials (Figure 1). Strategic advancements in trials in children have included: (1) evaluation of novel whole cell candidates to replace infant BCG vaccination, (2) prioritization of adolescents as a target population, and (3) use of innovative trial endpoints beyond prevention of TB disease. While infants are particularly vulnerable to TB disease and need a vaccine with better safety and efficacy than BCG, they are not the main drivers of *M. tuberculosis* transmission. Adolescents have emerged as a target age group for new TB vaccine trials due to the sharp increase in TB incidence between 15 and 25 years of age [28]. Adolescents develop adult-type cavitary TB disease that is more readily transmissible compared with infants [29]. Modeling studies suggest that a TB vaccine with modest efficacy would have a larger impact on the TB epidemic when administered to adolescents and adults instead of infants, preventing 17 million TB cases vs 0.89 million cases over a 25-year time period, respectively [30].

Prevention of microbiologically confirmed TB disease remains the gold standard efficacy endpoint for TB vaccine trials [31, 32]. Prevention of TB disease can be measured more easily among adolescents using culture or polymerase chain reaction (PCR)-based microbiological assays from respiratory specimens. However, this endpoint is particularly difficult to ascertain in younger children due to the paucibacillary nature of TB and challenges of respiratory sample collection in this age group [29, 31].

Prevention of clinically asymptomatic *M. tuberculosis* infection is an endpoint used more recently in TB vaccine trials, which provides opportunities to lower cost and more rapidly inform the advancement of vaccine candidates to larger trials measuring prevention of TB disease. *Mycobacterium tuberculosis* infection is measured indirectly by blood-based immunodiagnostic tests and interferon-gamma release assays (IGRA; eg, QuantiFERON-TB Gold Plus or T-Spot TB) [33]. Measuring incident *M. tuberculosis* infection (ie, IGRA conversion) as a trial endpoint is biologically relevant, since individuals with recent *M. tuberculosis* infection have a higher risk of developing TB disease than those with remote infection [34]. *Mycobacterium tuberculosis* infection also occurs at much higher rates than TB disease; therefore, prevention of *M. tuberculosis* infection trials can provide proof of vaccine-mediated effects using smaller sample sizes than trials powered to detect TB disease. Limitations of trials using *M. tuberculosis* infection as the primary endpoint include imprecision of IGRA assays to measure *M. tuberculosis* infection, resulting in possible misclassification of *M. tuberculosis* infection status at enrollment or post-vaccination, and interpretation of longitudinal changes in IGRA results, which can revert from positive to negative [33]. The potential epidemiological impact of preventing *M. tuberculosis* infection and its correlation with preventing TB disease must still be determined [35].

CURRENT NOVEL PEDIATRIC TB VACCINE CANDIDATES

Subunit Vaccines

Subunit TB vaccines currently being evaluated in adolescent phase 1 and 2 trials include M72/AS01$_E$, ID93 + GLA-SE, and ChAdOx1 85A prime + MVA85A boost (Figure 2; Table 1).

M72/AS01$_E$ is a fusion of 2 proteins (Mtb39a and Mtb32a) delivered in an adjuvant containing an organic saponin and lipid.

Table 1. TB Vaccine Trials in Pediatric Populations[a]

Vaccine	Phase	Purpose	Age Range	Start Date	NCT Number [Reference]
Infant BCG Prime + Boost					
MVA85A	2	Safety & Immunogenicity	24 wks to 11 y	2008	NCT00679159 [18]
MVA85A	2	Safety, Immunogenicity, & Efficacy (prevention of disease)	18-26 wks	2009	NCT00953927[13]
AERAS-402	1, 2	Safety & Immunogenicity	16-26 wks	2010	NCT01198366 [19]
M72/AS01$_E$	2	Safety & Immunogenicity	8-28 wks	2010	NCT01098474 [20]
MVA85A[b,c]	2	Safety & Immunogenicity	0-4 days	2012	NCT01650389 [17]
H4:IC31	1, 2	Safety & Immunogenicity	24-28 wks	2013	NCT01861730
Infant BCG Replacement					
VPM1002	2	Safety & Immunogenicity	0-8 days	2011	NCT01479972 [21]
VPM1002[c]	2	Safety & Immunogenicity	0-12 days	2015	NCT02391415 [22]
MTBVAC	1, 2	Dose-Escalation Safety & Immunogenicity	0-4 days	2015	NCT02729571 [23]
MTBVAC	2	Dose-Defining Safety & Immunogenicity	0-4 days	2019	NCT03536117
VPM1002[c]	3	Safety & Efficacy (prevention of infection)	0-14 days	2020	NCT04351685
MTBVAC[c]	3	Safety, Immunogenicity, & Efficacy (prevention of disease)	0-7 days	2022	NCT04975178
Child & Adolescent Vaccination					
M72/AS01$_E$	2	Safety & Immunogenicity	13-17 y	2009	NCT00950612 [14]
M. vaccae (Vaccae)	3	Safety & Efficacy (prevention of disease)	15-65 y	2013	NCT01979900
MVA85A	2	Immunogenicity	12-17 y	2014	NCT02178748 [24]
BCG H4:IC31	2	Safety, Immunogenicity, & Efficacy (prevention of infection)	12-17 y	2014	NCT02075203 [25]
BCG H4:IC31 H56:IC31	1	Safety & Immunogenicity	12-17 y	2015	NCT02378207 [26]
DAR-901	2	Safety & Efficacy (prevention of infection)	13-15 y	2016	NCT02712424 [27]
ID93 + GLA-SE	1	Safety & Immunogenicity	14-18 y	2019	NCT03806699
BCG ChAdOx1 85A MVA85A	1, 2	Safety & Immunogenicity	12-49 y	2019	NCT03681860
BCG	2	Safety, Immunogenicity, & Efficacy (prevention of infection)	10-18 y	2019	NCT04152161
VPM1002 *M. indicus pranii*	3	Safety & Efficacy (prevention of disease)	6-99 y	2019	CTRI/2019/01/017026
M72/AS01$_E$[d]	2	Safety & Immunogenicity	16-35 y	2020	NCT04556981

[a]Excluding trials of primary BCG vaccination and trials evaluating a new manufacturing source of BCG.

[b]Reverse prime boost (MVA85A prime + BCG boost).

[c]Trial included infants born to mothers living with HIV.

[d]All participants in the trial are living with HIV.

Two doses of M72/AS01$_E$ administered 1 month apart showed 49.7% (95% CI 2.1%-74.2%) efficacy to prevent TB disease among IGRA-positive adults and were safe and immunogenic among adolescents [14, 36]. Although this candidate has not been further tested in infants, a phase 2 trial among adolescents ≥16 years and adults with HIV is ongoing (NCT04556981), and a large phase 3 trial is being planned.

ID93 + GLA-SE is a fusion protein of 4 *M. tuberculosis* antigens associated with virulence and latency (Rv2608, Rv3619, and Rv3620 and Rv1813) with a synthetic toll-like receptor 4 adjuvant in oil-in-water emulsion [37]. Three doses of ID93 + GLA-SE elicited cellular and humoral immune responses in adults [38]. A phase 1 safety and immunogenicity trial is ongoing in adolescents (NCT03806699). Testing of the efficacy of ID93 + GLA-SE to prevent *M. tuberculosis* infection (NCT03806686) or recurrent TB disease is being planned in adults [39].

A heterologous "prime-boost" vaccination strategy using adenoviral vector-based vaccines demonstrated promising

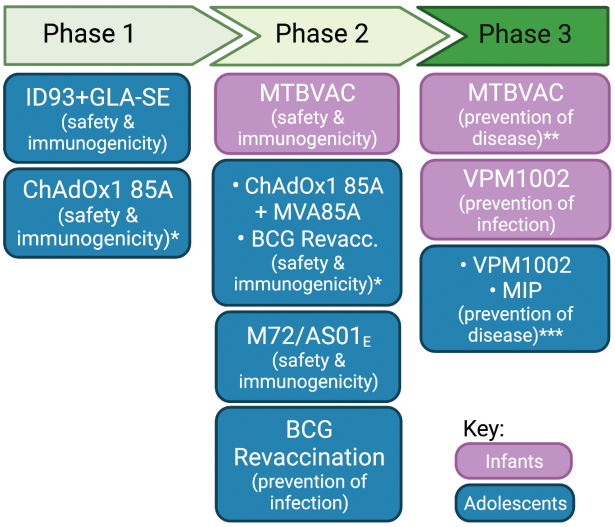

Figure 2. Currently active pediatric TB vaccine trials. Trials are indicated according to trial phase, age group, and primary endpoint. Bullet points indicate 2 separate arms within a given trial. *Combined phase 1/2 trial; **Not yet recruiting; ***Includes children ≥6 years of age. Created with BioRender.com.

immunogenicity in a malaria vaccine trial of young African children, and this approach is now being applied to the TB vaccine field [40]. While MVA85A alone was only modestly immunogenic as a booster to BCG among infants, administration of chimpanzee adenovirus expressing *M. tuberculosis* antigen 85A (ChAdOx1 85A) followed by an MVA85A boost generated *M. tuberculosis*-specific CD4 and CD8 immune responses in adults and is being evaluated in a phase 1/2 trial among adolescents (NCT03681860) [41].

Whole Cell Vaccines

Four whole cell vaccines are being assessed in phase 2 and 3 trials in children, including live attenuated *M. tuberculosis* (MTBVAC), live recombinant BCG (VPM1002), BCG revaccination, and heat-killed non-tuberculous mycobacterium *Mycobacterium indicus pranii* (Figure 2).

Among infants, trials to replace primary BCG vaccination with either MTBVAC or VPM1002 are ongoing (Figure 1). MTBVAC is an attenuated clinical strain of *M. tuberculosis*, modified by removing 2 virulence genes *phoP* and *fadD26* [42]. MTBVAC contains a higher number of *M. tuberculosis* antigenic targets than BCG, as it retains approximately 25% of *M. tuberculosis* human T cell epitopes that are deleted in BCG [43]. MTBVAC was safe in an infant phase 1 trial and achieved a higher frequency of CD4 cells expressing Th1 cytokines than BCG at 1-year follow-up [43]. A dose-finding phase 2 trial has been completed (NCT03536117), and a phase 3 trial to prevent TB disease is due to begin in 2022 (NCT04975178). A consideration for the MTBVAC vaccine is that it contains antigens that cross-react with currently available IGRAs, which may interfere with the clinical utility of IGRAs to identify *M. tuberculosis* infection after vaccination and could limit the use of *M. tuberculosis* infection as an endpoint until new immunodiagnostic tests are available.

VPM1002 is a genetically modified strain of BCG subtype Prague (BCGΔureC::hly), expressing the listeriolysin gene (hly) from *Listeria monocytogenes* in the absence of urease C. These modifications enhance the translocation of mycobacterial antigens into the cytosol, which improves antigen presentation to CD8 T cells and elicits robust Th1 and Th17 responses [44]. A phase 1 trial among infants demonstrated no severe adverse events and lower rates of injection site abscesses than BCG [21]. A multi-center phase 2b safety trial was completed in 2018 [22], and a phase 3 trial evaluating the efficacy of VPM1002 to prevent *M. tuberculosis* infection in HIV-exposed and unexposed infants is ongoing (NCT04351685).

The current TB vaccine pipeline for adolescents includes BCG revaccination, VPM1002, and *M. indicus pranii* (Table 1). While BCG revaccination did not offer protection from clinical TB disease in historical trials of adolescents and adults, a recent trial measuring the prevention of *M. tuberculosis* infection among IGRA-negative adolescents demonstrated that BCG revaccination had ~45% efficacy to prevent 2 endpoints: (1) sustained IGRA conversion (>0.35 IU/mL through 6 months post-conversion) and (2) high magnitude of IFN-γ (>4.0 IU/mL) among IGRA converters [25, 45, 46]. High magnitude of IFN-γ (>4.0 IU/mL) has been associated with a 30- to 40-fold increased risk for TB disease among *M. tuberculosis*-infected children and adults [47, 48], suggesting that BCG revaccination may have clinical benefits. A confirmatory phase 2b trial of BCG revaccination among children aged 10-18 years is underway (NCT04152161), and a phase 1/2 trial comparing BCG revaccination and VPM1002 is being planned among IGRA-negative and IGRA-positive children aged 8-14 years with and without HIV [49].

Heat-killed *M. indicus pranii* (previously named *Mycobacterium w*) shares antigens from *M. leprae* and *M. tuberculosis*. *Mycobacterium indicus pranii* prevented leprosy among household contacts and shortened treatment duration in leprosy patients [50]. Secondary analysis of a large leprosy household contact trial demonstrated lower rates of TB disease over 13 years of follow-up of study participants in the *M. indicus pranii* arm [51]. VPM1002 and *M. indicus pranii* are currently being evaluated in a placebo-controlled prevention of TB disease trial among child and adolescent TB household contacts (CTRI/2019/01/017026).

CURRENT CHALLENGES AND PRIORITY AREAS FOR FUTURE PEDIATRIC RESEARCH

Immune Correlates of Protection and Animal Models

Human immune correlates of protection against *M. tuberculosis* infection and TB disease have not been clearly defined, which continues to hinder the rational design and evaluation of novel TB vaccine approaches. Given the dynamic nature of immune system

development during early childhood, consideration of age at the time of vaccination is needed to better define immune correlates of vaccine-induced protective immunity, which may differ across the lifespan [52]. There is an abundance of immune cell populations with suppressive activity in neonates, including regulatory T cells, regulatory B cells, regulatory neutrophils, and myeloid-derived suppressor cells [53], which may differentially shape vaccine immunogenicity in neonates compared with adolescents and adults. Animal models afford an opportunity to identify candidate immune correlates of protection yet have not been extensively developed in the context of pediatric TB. Recently, *M. tuberculosis*-specific immunoglobulin M (IgM) was identified as a potential immune correlate of protection in rhesus macaques vaccinated intravenously with BCG [54]. This finding points to the great potential to further develop infant non-human primate models to more rapidly advance the preclinical evaluation of novel TB vaccine candidates. *Mycobacterium tuberculosis* challenge studies in young animals could facilitate the identification of immune correlates of protection that may be unique to children.

High-Risk Populations: HIV

HIV is the leading risk factor for TB disease, especially in children. In the pre-ART era, the incidence of TB was incredibly high among children with HIV; for example, in South Africa, a high TB burden setting, TB incidence was reported at 23 TB cases per 100 person-years among children with HIV [55]. While early ART initiation halved the incidence of TB in a randomized ART trial of young South African children with HIV, older children with HIV maintain a 4-fold increased risk for TB disease despite prolonged ART and immune reconstitution (median CD4 >700 cells/μL) [56, 57]. Although HIV-exposed uninfected infants, who are at higher risk for *M. tuberculosis* infection [58], are included in several current clinical trials, no TB vaccines have been adequately evaluated in children with HIV (Table 1). Observational studies showed that HIV infection and, to a lesser extent, vertical exposure are associated with impaired BCG immunogenicity in children, which may be associated with the lower vaccine-mediated protection against TB reported in retrospective epidemiological studies [59–62]. In addition to universal early initiation of ART, TB prevention in children with HIV has been limited to index case identification and TB preventive therapy, neither of which are easily implemented. Children with HIV on stable ART with well-controlled viral load and acceptable immune reconstitution should not be excluded from clinical trials of new TB vaccines. An early study showed that anti-BCG immune responses were restored after a year of ART in children with advanced HIV, supporting studying the benefits of TB vaccines in this population [63].

Inadequate Vaccine Pipeline

As illustrated in Figure 1, the current pipeline of clinical development of TB vaccines for children is fundamentally unbalanced. The overall shift to new trials on adolescents has resulted in inadequate advancement of new infant vaccines; no phase 1 trials targeting infants are currently active. Should MTBVAC and VPM1002 phase 3 trials show no additional benefit over BCG, no other candidates are available for testing in this key population. Despite increased recognition that preventing TB in adolescents is critical to reduce *M. tuberculosis* transmission, only 1 active phase 3 trial includes older children and adolescents and is likely under-powered to assess efficacy among pediatric age groups alone (Figure 2, Table 1). Future plans for TB vaccine development should be more inclusive and specifically: (1) test new vaccine candidates in infants; (2) include adolescents in efficacy trials with sufficient numbers to estimate protection in this key population; and (3) perform safety, immunogenicity, and feasibility trials in pre-adolescents, which would allow wide-scale global programmatic rollout of a new TB vaccine administered in conjunction with human papillomavirus (HPV) vaccine and tetanus booster given around age 10 years.

Trial Design and Longitudinal Follow-up

An ideal TB vaccine would be administered to IGRA-negative children and have long-term efficacy to prevent TB disease through adulthood. Yet, phase 3 trials evaluating the prevention of TB disease in IGRA-negative populations are a high-risk investment, since they require very large sample sizes and prolonged longitudinal follow-up. Innovative trial designs and planning for long-term surveillance could provide valuable insights beyond traditional phase 3 trials. Extended follow-up of historical TB vaccine trials has yielded valuable data; the Karonga trial recently reported modest TB disease protection 30 years after childhood BCG revaccination, and a trial of primary BCG among Native Americans demonstrated 52% efficacy to prevent TB disease after 60 years [64, 65]. Assessment of long-term efficacy through linkage to routinely collected data is more feasible with the expanded use of electronic medical records and notifiable disease registries [66]. Promising vaccine candidates that demonstrate efficacy to prevent *M. tuberculosis* infection could be evaluated head-to-head in cluster randomized community trials measuring TB disease as the primary endpoint, with longer passive follow-up of large numbers of participants to complement the standard phase 3 trial design.

CONCLUSION

Despite over 100 years of research and significant advances over the past decade, major challenges and opportunities remain in developing new, effective TB vaccines for children, who are a critical target population to reduce TB morbidity and mortality and to diminish TB transmission. Acceleration and expansion of the pediatric TB vaccine pipeline are urgently needed to end the TB pandemic.

Notes

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