TB Vaccine R&D Roadmap
Background Document

Version Date: 12.04.2021

This document summarizes the state-of-the-art in research and development for new vaccines for tuberculosis (TB). It is meant as a background document for the TB Vaccine Research & Development Roadmap and support the various consultations that have been held as part of the process of its development.[a]

This background document seeks to provide an overview of the TB vaccine development goals, the current vaccine R&D pipeline, issues in clinical development, (new) directions in discovery and preclinical research, and considerations about moving vaccine candidates through the pipeline. It is a living document, that has been updated as the Roadmap development process went along. It is not meant to be exhaustive but to provide the reader with sufficient background to understand the Roadmap's considerations and recommendations.

For more detail the reader is referred to a number of recent reviews on the topic [1] [2] [3] [4] [5] [6]. The recommendations for TB vaccine R&D recently published by a number of stakeholders in have been added as Annex 1[7].

LIST OF ABBREVIATIONS

BCG	Bacille Calmette-Guérin
CHIM	Controlled human infection model
CI	Confidence interval
CoP	Correlate of protection
DS-TB	Drug-susceptible tuberculosis
IAVI	International AIDS Vaccine Initiative
IGRA	Interferon-gamma release assay
MDR-TB	Multidrug-resistant tuberculosis
MIP	*Mycobacterium indicus pranii*
Mtb	*Mycobacterium tuberculosis*
NHP	Non-human primate
PDP	Product development partnership
PoD	Prevention of disease (clinical endpoint)
PoI	Prevention of infection (clinical endpoint)
PoR	Prevention of recurrence (clinical endpoint)
PPC	Preferred product characteristic
R&D	Research and development
TB	Tuberculosis
TBVI	Tuberculosis Vaccine Initiative
TST	Tuberculin skin test
WHO	World Health Organization

[a] The TB Vaccine R&D Roadmap was developed by the Amsterdam Institute for Global Health and Development, with financial support from the European & Developing Countries Clinical Trials Partnership (EDCTP).

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TB VACCINE DEVELOPMENT GOALS

The WHO has set three development goals for TB vaccines along with Preferred Product Characteristics (PPCs), published in 2018 and 2019. The strategic coals for each are summarized below. The PPCs are shown in Annexes 2, 3 and 4.

1. A safe, effective and affordable TB vaccine for adolescents and adults[b]

Given the central role that adolescents and adults with active pulmonary TB disease play in spreading *Mtb* infection, the prevention of pulmonary TB disease in adolescents and adults is the priority strategic target in TB vaccine development. The vaccine should be protective in people with or without evidence of *Mtb* infection, and prevent progression to TB disease following primary infection, as well as following re-infection(s) and re-activation in subjects with latent infection. Mathematical modelling studies suggest that the ability for vaccines to prevent pulmonary disease in subjects already *Mtb* infected will be a most important driver of impact on incidence in the short term.

2. Affordable TB vaccine for neonates and infants with improved safety and efficacy as compared to BCG[2]

Infants and young children with TB do not represent a major source of *Mtb* transmission, but are an important, vulnerable group. There is a need to improve upon the BCG vaccines currently in use. A new TB vaccine for administration in early life would represent an important public health advance if it:

- Provides superior degree and longer duration of protection as compared to the current BCG vaccines,
- Could be safely administered to infants with HIV infection or other causes of immune suppression,
- And/or has improved manufacturing securing sustainable supply.

Evidence of superiority would likely drive policy change but demonstrating only marginally improved characteristics may not support global implementation as a BCG replacement. BCG boosting strategies are also being considered.

3. **A therapeutic vaccine to improve tuberculosis treatment outcomes[c]**

A therapeutic vaccine for TB patients, administered towards completion of a prescribed course of drug therapy or at certain time(s) during treatment, could improve outcomes through immune-mediated control and even clearance of bacteria, potentially prevent re-infection, and provide an opportunity to shorten and simplify drug treatment regimens. Such a vaccine should:

- Reduce the rate of recurrence following completion of a full course of drug therapy,
- Increase the proportion of patients surviving to cure,
- And/or shorten the duration of drug treatment and/or reducing the number of drugs necessary to affect cure.

Important in this context are identification and quantification of the factors that will drive introduction and scale-up of a TB vaccine once it is licensed. Work is ongoing, among others to establish the full public health value for TB vaccines through impact and health economic modeling. Also of note are the evaluation criteria that Gavi, the main funder of procurement and delivery of vaccines for low- and lower-middle income countries, uses for decision making about adding a vaccine to their investment portfolio (see Annex 5 for the evaluation criteria).

[b] WHO Preferred Product Characteristics for New Tuberculosis Vaccines, Geneva 2018
[c] WHO Preferred Product Characteristics for Therapeutic Vaccines to Improve Tuberculosis Treatment Outcomes, Geneva 2020

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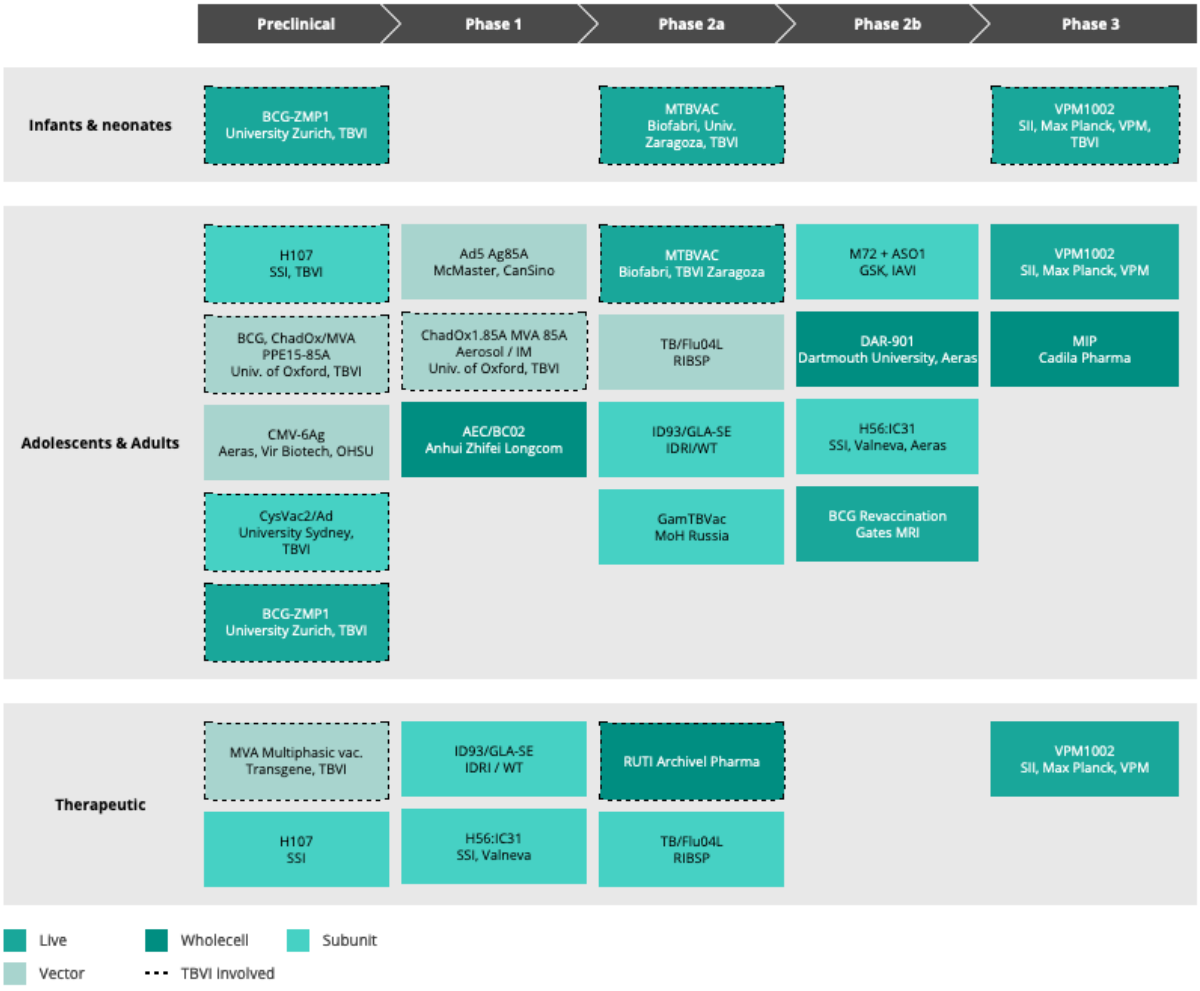
TB VACCINE PIPELINE

TB vaccine pipeline chart

as published by TBVI, updated October 2020[d]



[d] https://www.tbvi.eu/what-we-do/pipeline-of-vaccines/

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Details of vaccine candidates

Vaccine type	Vaccine candidate
Live, attenuated mycobacteria	MTBVAC VPM-1002 BCG revaccination
Killed, whole cell mycobacteria	DAR-901 M. vaccae Mycobacterium indicus pranii (MIP)
Mycobacterial extracts	RUTI
Adjuvanted protein vaccines	M72/AS01E H56:IC31 ID93/GLA-SE
Viral vectored vaccines	Ad5Ag85A ChAdOx185A/MVA85A TB/FLU-04L

Below are the vaccine candidates in clinical development, **phase 2A and beyond**, listed in alphabetical order.
NCT numbers refer to Clinical Trial Registration Numbers[e]
Primary trial endpoints: **PoD** = prevention of disease, **PoI** = prevention of infection, **PoR** = prevention of recurrence.

BCG revaccination	
Sponsor	The Bill & Melinda Gates Medical Research Institute is leading the clinical development programme.
Composition	Bacille Calmette-Guérin.
Indication	Adolescents and adults. BCG revaccination as an option for adolescents and adults emerged from a phase 2b trial for a different candidate.[8]
Specific advantages	Widely available, known safety.
Specific challenges	Risks related to live-attenuated vaccines. Protection may be affected by prior exposure to non-tuberculous mycobacteria.
Completed trials	A trial in South African adolescents BCG-vaccinated at birth of H4:IC31 Vaccine (discontinued) with safety and PoI endpoints showed no efficacy for H4:IC31 but did show reduced sustained IGRA conversion in the BCG revaccination arm compared to the placebo arm (secondary endpoint).[8]
Ongoing/planned trials	Ongoing Phase 2b trial comparing BCG vs placebo among QFT-negative South African adolescents BCG-vaccinated at birth, with sustained IGRA conversion as the primary endpoint (NCT04152161).

[e] https://clinicaltrials.gov/

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DAR-901	
Sponsor	Dartmouth-Hitchcock Medical Center in collaboration with AERAS/IAVI.
Composition	*Mycobacterium obuense* in liquid growth.
Indication	Adolescents and adults
Specific advantages	N/A
Specific challenges	N/A
Completed trials	Phase 1, first time in human, safety, immunogenicity and dose-finding study (NCT02063555). Phase 2 proof-of-biological-effect trial (NCT02712424) in Tanzanian adolescents, PoI: showed no significant efficacy for the primary (IGRA conversion) or secondary (sustained IGRA conversion) endpoint.
Ongoing/planned trials	N/A

GAMTBvac	
Sponsor	Sechenov University (Moscow) and Ministry of Health, Russian Federation
Composition	Recombinant subunit vaccine containing dextran-binding domain-modified Ag85a and ESAT6- CFP10 Mtb antigens and CpG ODN adjuvant, formulated with dextrans.
Indication	Adolescents and adults, as booster vaccine.
Specific advantages	N/A
Specific challenges	N/A
Completed trials	Phase 1 trial (NCT03255278) trial showed the vaccine to be safe and immunogenic. Phase 2a trial (NCT03878004) ongoing.
Ongoing/planned trials	N/A

H56:IC31	
Sponsor	Statens Serum Institute (Denmark), with multiple partners including Valneva, GmBH (Austria) and Aeras/IAVI.
Composition	Adjuvanted fusion protein, consisting of three Mtb antigens – Ag85B, ESAT-6 and Rv2660c – and adjuvanted with Valneva IC31 consisting of ODN1a, a TLR9 ligand.
Indication	Immunotherapeutic vaccine (prevention of recurrence); adolescents and adults.
Specific advantages	N/A
Specific challenges	Administered in two doses.

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Completed trials	Phase 1 and phase 2 studies of safety, immunogenicity and dose finding have been completed in IGRA+ and IGRA- adults, IGRA- adolescents, and in adults completing treatment for active TB.
Ongoing/planned trials	Ongoing open label phase 1 trial in Norway is assessing the safety and immunogenicity of H56:IC31 given three months into active TB treatment as adjunctive immunotherapy with and without additional COX2-inhibition (NCT02503839). Ongoing double-blind, randomized, placebo-controlled phase 2 trial of prevention of recurrence (PoR) trial among 900 patients being treated for pulmonary TB in South Africa and Tanzania (NCT03512249). Primary objective is to accelerate the development of H56:IC31 toward a possible phase 3 PoR trial and licensure for this indication.

ID93/GLA-SE	
Sponsor	Infectious Disease Research Institute (Seattle), in collaboration with Aeras/IAVI.
Composition	Fusion protein of four Mtb antigens: Rv1813, Rv2608, Rv3619 and Rv3620. GLA-SE is a synthetic TLR-4 agonist adjuvant formulated in a squalene oil in a water nano-emulsion.
Indication	As immunotherapeutic agent to improve the outcome of drug treatment for active TB, and as prophylactic vaccine to prevent infection with TB in adolescents and adults.
Specific advantages	GLA-SE has been demonstrated to be safe in humans, with thousands of doses delivered, induces a TH1-biasing immunological response, and production is readily scalable.
Specific challenges	Administered in two doses
Completed trials	Two phase 1, and one phase 2a clinical trial in healthy adults in the United States and South Africa have been completed, including persons not vaccinated with BCG, BCG vaccinated individuals, and persons who are IGRA-, IGRA+ and those with active TB disease. A phase 2a trial in which vaccination occurred at the end of TB treatment demonstrated encouraging CD4+ T-cell and antibody responses to vaccination (NCT02465216)
Ongoing/planned trials	Ongoing phase 1 trial of safety and immunogenicity in BCG-vaccinated healthy adolescent (age-de-escalation). Ongoing phase 2a study to evaluate the safety, immunogenicity and preliminary efficacy for preventing TB infection among high-risk health care workers in Korea. In preparation: two Phase 2b clinical trials to evaluate the vaccine as an immunotherapeutic adjunct to TB treatment: one in India (both drug-sensitive and drug-resistant TB), one in South Africa (PoR).

M72/AS01E	
Sponsor	GlaxoSmithKline in collaboration with Aeras/IAVI. The license for M72 was transferred from GlaxoSmithKline to the Bill & Melinda Gates Medical Research Institute in January 2020.

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Composition	Fusion protein expressing Mtb antigens Mtb39A and Mtb32A, combined with the adjuvant system AS01E containing monophosphoryl lipid A (MPL) and QS21 in a liposomal suspension.
Indication	Adolescents and adults.
Specific advantages	N/A
Specific challenges	Administered in a 2-dose regimen 1 to 6 months apart.
Completed trials	Tested for safety and immunogenicity in 12 completed phase 1 and phase 2 studies: adults who were PPD negative, PPD positive, HIV negative, HIV positive on antiretroviral therapy, and HIV positive not receiving ART, adults during or after TB treatment; adult trials have been conducted in nonendemic and endemic TB settings. The vaccine has also been assessed in adolescents in South Africa and in infants in The Gambia. Phase 2b proof-of-concept efficacy study in approximately 3,500 IGRA positive, HIV-negative adults in clinics in South Africa, Kenya and Zambia, followed up for three years for the occurrence of TB disease (NCT01755598), showing 49.7% (95% CI 2.1-74.2) efficacy against TB disease (PoD).
Ongoing/planned trials	Ongoing phase 2a double-blind randomized trial of safety and immunogenicity among 400 virally suppressed people living with HIV aged 16-35 years in South Africa (NCT04556981). Planned phase 3 trial among at least 14,000 subjects in very high incidence settings (multiple continents), to include both IGRA-positive and IGRA-negative individuals, as well as people living with HIV.

MIP	
Sponsor	Various, among others Ministry of Science and Technology, India.
Composition	Heat-killed *Mycobacterium indicus pranii.*
Indication	Immunotherapeutic use in TB and prevention of TB in adolescents and adults.
Specific advantages	N/A
Specific challenges	Multiple doses
Completed trials	A phase 3 trial of MIP compared to placebo in adults with definite or probable tuberculous pericarditis (NCT00810849) showed no reduction in death/tamponade/constrictive pericarditis but a significant increase in (mainly HIV-associated) cancers.[9] A phase 3 trial of pulmonary retreatment TB (NCT00341328) showed no improvement in cure rate but significant reduction in culture conversion at week 4 of treatment.[10] A phase 3 trial of immunotherapeutic effect in new drug susceptible pulmonary TB (NCT00341328) was completed in 2012 but has not been reported.
Ongoing/planned trials	In preparation: phase 3 trial for prevention of TB disease in exposed household contacts in India.

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MTBVAC	
Sponsor	University of Zaragoza and Biofabri in collaboration with TBVI.
Composition	Genetically modified Mtb clinical isolate with deletions of the *phoP* and *fadD26* genes.
Indication	Primarily being developed to replace BCG as a priming immunization against TB, or in populations without prior sensitization to BCG, Mtb or environmental mycobacteria.
Specific advantages	Single dose.
Specific challenges	Risks related to live-attenuated vaccines. Protection may be affected by prior exposure to non-tuberculous mycobacteria.
Completed trials	Phase 1 study in BCG-unvaccinated adults living in an area not endemic for TB (NCT02013245). Further assessed for safety and immunogenicity in newborns in a phase 2a dose escalation study in a TB-endemic region of South Africa, with a safety arm in adults (NCT02729571)
Ongoing/planned trials	Ongoing phase 2a double blind, randomized, BCG-controlled trial for dose-defining, safety and immunogenicity in South African neonates (NCT03536117). Ongoing phase 1/2a, double blind, randomized, BCG-controlled, dose-escalation safety and immunogenicity study in 120 healthy South African adults, ages 18–50 years, with and without LTBI (NCT02933281)

M. vaccae	
Sponsor	Various, among others Anhui Zhifei Longcom, China.
Composition	Heat-killed preparation of *Mycobacterium vaccae.*
Indication	Licensed in China as adjunctive immunotherapy for drug treatment of active TB
Specific advantages	N/A
Specific challenges	Multiple doses.
Completed trials	Meta-analysis of 13 RCTs as adjunct treatment (DR/DS-TB): faster culture conversion, no difference in end-of-treatment outcomes.[11] Placebo-controlled phase 3 trial was completed in 2018 in China to test the Anhui Zhifei Longcom vaccine for prevention of TB disease in 10,000 subjects with LTBI, with a 6-dose vaccination regimen (NCT01979900) (PoD). Data not yet released.
Ongoing/planned trials	Unknown.

RUTI	
Sponsor	Autonomous University of Barcelona and Archival Farma, S.I. Spain.
Composition	Cell wall fragments of Mtb formulated in a liposome suspension.

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Indication	Immunotherapeutic agent for adults, intended to improve the efficacy and shorten the duration of drug treatment for cases of active TB, including drug resistant TB.
Specific advantages	Single dose
Specific challenges	N/A
Completed trials	Two clinical trials have been completed: a phase 1 safety, immunogenicity and dose ranging trial in 24 healthy adults in Spain, and a phase 2a safety, immunogenicity and dose ranging trial in 48 HIV+ and 48 HIV- persons with LTBI in South Africa.
Ongoing/planned trials	Ongoing phase 2a trial to evaluate the safety and immunogenicity in MDR-TB patients was scheduled to begin 2019, with a plan for a subsequent phase 2b/3 pivotal clinical trial in this population (NCT02711735). In preparation: phase 2a trial in patients with drug susceptible TB as well as MDR-TB patients in India.

TB/Flu 04L	
Sponsor	Research Institute for Biological Safety Problems and Research Institute of Influenza, Kazakhstan
Composition	Live recombinant influenza-vectored tuberculosis vaccine expressing antigens Ag85A and ESAT-6.
Indication	Boost vaccine in children and adolescents and adults
Specific advantages	N/A
Specific challenges	N/A
Completed trials	Phase 1: single centre, double-blind, randomized, placebo-controlled trial that explored the safety and immunogenicity of 2 doses (Day 1 and Day 21) TB/FLU-04L tuberculosis vaccine versus matched placebo in BCG-vaccinated healthy adult subjects aged 18-50 years (NCT02501421) (2015)
Ongoing/planned trials	In preparation: Phase 2 trial of prevention of TB disease in individuals with LTBI (PoD)

VPM1002	
Sponsor	Max Planck Institute, licensed to Vakzine Projekt Management and later sublicensed to the Serum Institute of India Pvt. Ltd.
Composition	Recombinant BCG (*BCGΔureC::hly*): a listeriolysin gene has been added to the BCG genome and a urease gene has been deleted.
Indication	Being developed both as a replacement for BCG vaccination in infants and as a TB vaccine in adolescents and adults.
Specific advantages	Single dose. Manufacturing process offers the prospect of avoiding the frequent, global shortages of BCG, as it is manufactured using fermentation media, with a 50-liter batch yielding approximately 5 million doses.
Specific challenges	Risks related to live-attenuated vaccines.

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	Protection may be affected by prior exposure to non-tuberculous mycobacteria.
Completed trials	Safety and tolerability have been assessed in a recently concluded phase 2 trial of HIV-exposed and HIV unexposed infants in sub-Saharan Africa (NCT02391415)
Ongoing/planned trials	Infants: phase 3 trial, comparing VPM1002 safety and efficacy to BCG in appr. 7000 infants in various African countries started in 2020 (NCT04351685). Efficacy endpoint will be IGRA conversion (PoI). Adults: phase 2b/3, randomized, double blind, placebo-controlled trial to assess VPM1002 vaccine efficacy in preventing recurrence of TB in adults recently treated and cured of active TB is underway in India (NCT03152903), n= 2x1000 (PoR). Phase 3 trial of protection against TB disease among household contacts of persons with active TB is in preparation (PoD).

CLINICAL DEVELOPMENT

Vaccines in phase 2b or phase 3 of clinical development (clinical efficacy endpoint).

Reported phase 2b/3 trial results

BCG revaccination

Phase 2b trial was reported in 2019, sponsors by Aeras and others; ClinicalTrials.gov number, NCT02075203).[8] The trial randomly enrolled adolescents in a high-risk setting who had undergone neonatal BCG vaccination, randomized to receive H4:IC31, BCG revaccination, or placebo. All the participants had negative results on testing for Mtb infection on the QuantiFERON-TB Gold In-tube (QFT) assay and for HIV. The primary outcomes were safety and acquisition of Mtb infection, as defined by initial conversion on QFT that was performed every 6 months during a 2-year period. QFT conversion occurred in 44 of 308 participants (14.3%) in the H4:IC31 group and in 41 of 312 participants (13.1%) in the BCG group, as compared with 49 of 310 participants (15.8%) in the placebo group; the rate of sustained conversion was 8.1% in the H4:IC31 group and 6.7% in the BCG group, as compared with 11.6% in the placebo group. Neither the H4:IC31 vaccine nor the BCG vaccine prevented initial QFT conversion, with efficacy point estimates of 9.4% (95% CI -36.2 to 39.7, P=0.63) and 20.1% (95% CI -21.0 to 47.2, P=0.29), respectively. However, the BCG vaccine reduced the rate of sustained QFT conversion (a secondary outcome), with an efficacy of 45.4% (95% CI 6.4 tot 68.1, P=0.03); the efficacy of the H4:IC31 vaccine was 30.5% (95% CI -15.8 to 58.3, P=0.16).
There were no clinically significant between-group differences in the rates of serious adverse events, although mild-to-moderate injection-site reactions were more common with BCG revaccination.

DAR-901

Phase 2b trial was reported in 2020, sponsors Dartmouth-Hitchcock Medical Center Muhimbili University of Health and Allied Sciences; ClinicalTrials.gov number, NCT02712424).[12] The trial enrolled QFT-negative adolescents aged 13-15 years, BCG-vaccinated at birth, in Tanzania, randomized to receive 3 doses of DAR-901 or placebo. HIV status was not reported. The primary outcomes were safety and acquisition of Mtb infection, as defined by initial conversion on

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QFT performed at 1, 2 and 3 years of follow-up. Secondary efficacy outcome was sustained IGRA conversion based on a positive QFT repeated after 3 months or later and then still positive.
Of 667 enrolled participants, 625 were evaluated for the efficacy outcome, with 559 having completed 3-year follow-up. There were no significant differences in adverse even rate.
Serious adverse events occurred among 6 (2%) DAR-901 recipients and 3 (1%) placebo recipients (p=0.33), none was judged to be related to study treatment.
The primary efficacy endpoint was observed in 19 DAR-901 recipients and 18 placebo recipients (VE 3.2%, 95% CI -13.9 to17.7, p=0.69), and the secondary efficacy endpoint in 10 vs 5 (VE 4.4%, 95% CI -12.1 to 18.5, p=0.58).

M72/AS01E
Phase 2b trial was reported in 2019, sponsors GlaxoSmithKline Biologicals and Aeras (ClinicalTrials.gov number, NCT01755598)[13]. The trial enrolled adults 18-50 years with *M. tuberculosis* infection (defined by positive results on interferon-γ release assay) without evidence of active tuberculosis disease in Kenya, South Africa, and Zambia, randomized to receive two doses of either M72/AS01$_E$ or placebo, administered 1 month apart. Participants were followed for 3 years after the second dose.
Among the 3289 participants in the according-to-protocol efficacy cohort, 13 of the 1626 participants in the M72/AS01$_E$ group, as compared with 26 of the 1663 participants in the placebo group, had bacteriologically confirmed pulmonary tuberculosis (incidence, 0.3 vs. 0.6 cases per 100 person-years). The vaccine efficacy at month 36 was 49.7% (90% CI 12.1 to 71.2; 95% CI 2.1 to 74.2).
Serious adverse events, potential immune-mediated diseases, and deaths occurred with similar frequencies in the two groups.

Ongoing and unreported phase 2b/3 trials

As listed in ClinicalTrials.gov; trials with efficacy endpoints only.

BCG revaccination
Ongoing Phase 2b trial started in 2019, sponsor Gates Medical Research Institute (ClinicalTrials.gov number, NCT04152161). The trial compares BCG vs placebo among 1800 QFT-negative adolescents aged 10-18 years in South Africa BCG-vaccinated at birth, with sustained IGRA conversion as the primary endpoint. The trial has an extensive discovery programme for correlates of protection.

H56:IC31
Phase 2b trial ongoing, trial sponsor Aeras/IAVI, funded by EDCTP (ClinicalTrials.gov number, NCT03512249). The trial enrolls 900 HIV-negative adults with a diagnosis of drug susceptible pulmonary TB in South Africa and Tanzania who are culture-negative at the end of treatment, randomized 1:1 to receive H56:IC31 in two doses at 2 months interval, followed for culture-confirmed TB disease recurrence (relapse or reinfection) over a period of 2 years.
Results expected: 2025.

M. Vaccae
Phase 3 trial completed in 2017, trial sponsor Anhui Zhifei Longcom Biologic Pharmacy Co., Ltd. (ClinicalTrials.gov number, NCT01979900). This trial enrolled 10,000 adults in China aged 15-65 years whose Tuberculin Skin Test was strongly positive, randomized 1:1 to receive Vaccae once every 2 weeks, 6 times in total, or placebo, and followed for incident tuberculosis disease for 2 years. The results have not yet been reported. Results expected: unknown.

VPM1002

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Phase 2b/3 trial ongoing, trial sponsor Serum Institute of India Pvt. Ltd. (ClinicalTrials.gov number, NCT03152903). This trial enrolls 2000 category 1 pulmonary TB patients in India who have successfully completed ATT and declared cured by bacteriological confirmation, randomized 1:1 to receive VPM1002 or placebo, and followed for bacteriologically confirmed or clinically diagnosed TB recurrence for 12 months.
Results expected: 2022.
Phase 2b/3 trial ongoing, trial sponsor Serum Institute of India Pvt. Ltd., funded by EDCTP (ClinicalTrials.gov number, NCT04351685). This trial enrolls 6940 infants (within 14 days of birth) in Gabon, Kenya, South Africa, Tanzania and Uganda, randomized 1:1 to receive VPM1002 or BCG SII, stratified by maternal HIV status. Primary endpoint is incidence of QFT conversion (indicating infection), secondary outcome is incidence of TB disease, over a follow-up period of 12-36 months.
Results expected: 2025.

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STATE OF THE ART

Basic and translational science

Natural history of M. tuberculosis infection and TB disease

Over the past decade the paradigm of a dichotomy of TB disease versus latent TB infection has been challenged. Recent data from studies of TB-exposed cohorts have shown changes in immune markers[14], metabolites[15] and gene transcription profiles[16], as well as lesions on PET CT scans[17], to occur from 6 to 12 months before the onset of clinically apparent TB disease, indicating a clinically silent stage of inflammatory response to multiplying *Mtb* now termed incipient TB[18]. In addition, data from TB prevalence surveys[19], post-mortem studies[20] and observational cohorts[21] suggest the importance of a state in which the patient has chest X-ray abnormalities and positive diagnostic tests but no TB-typical symptoms, or symptoms on and off, known as subclinical TB[18]. Studies using PET CT imaging furthermore suggest marked heterogeneity in the different lesions in the same individual[22].

The drivers of transition between these states, in either direction along the spectrum from true latency (if that exist at all) and clinically apparent TB disease, are only partially known. In addition to predisposing factors for progression that have generally been well characterized (e.g. HIV infection, type 2 diabetes) there may be precipitating factors tipping the balance towards progression that are yet to be defined[23]. These insights have consequences for our understanding of protection against TB disease as well as for TB case definitions in vaccine trials[24].

Clearance (the successful eradication of inhaled *Mtb* before an adaptive immune response develops) may also be more important than previously recognized[25]. Although the concept is still awaiting a clear definition in terms of assay responses, early clearance was associated with a history of BCG (re)vaccination[8] [26] and with increased innate immune responses[27].

Human protective immune response to *M. tuberculosis*

T-helper-1 cell-mediated responses to *Mtb* characterised by IFN-γ and TNF-secreting antigen-specific CD4 T cells are critical for protective immunity in humans. They may however not be sufficient to provide long-term protection against *Mtb* infection[3]. Other potential contributors to a protective immune response include class-I restricted CD8+ T cells[28], IL-17-producing T cells[29] and MAIT cells[30], but also antibody-dependent responses including Fc-mediated effector functions[31]. Reduced levels of BCG-induced, mycobacteria-specific antibodies were associated with increased risk of developing TB disease in infants[32]. Innate immune responses, including unconventionally restricted T cells, may be important in the early clearance of mycobacteria[33]. Furthermore, BCG vaccination has been suggested to modify innate immune responses through epigenetic reprogramming of monocytes ("trained innate immunity")[34], which may contribute to clearance.

Novel platforms

There has been recent attention to cytomegalovirus (CMV) recombinant vaccines. Engineering of the CMV vector leads to constant, low-level replication of the virus, giving sustained antigen expression and long-term immunity. Two Rhesus CMV (RhCMV-)TB vaccines have been created thus far, one expressing nine Mtb proteins across four different RhCMV vectors, the other expressing six Mtb proteins as a single polyprotein from one vector. Pre-clinical studies in NHP, using low-dose challenge, showed significant reduction in TB disease at one year following Mtb challenge. This effect was attenuated if BCG vaccination was given prior to RhCMV-TB vaccination. Moreover, no TB disease was detected via lung CT scans or at necropsy in 13 of 27 RhCMV-TB vaccinated animals, as compared to unvaccinated controls where all demonstrated TB involvement

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of lungs and lung-draining lymph nodes. A phase 1 human trial of a CMV-TB vaccine is being planned.

New approaches to antigen discovery

Recent data suggest that Mtb has distinct phases of growth, which may be associated with active mycobacterial replication, persistence and dormancy. Antigens used extensively in TB vaccine development included the early secreted antigens, such as the Ag85 family, ESAT-6 and CFP-10, as they are highly immunogenic and have shown protection in animal models. These antigens are associated with active bacterial replication. Other antigens to consider are in vivo expressed antigens and those in the DosR regulon that are associated with dormancy, as vaccines based on these antigens may more specifically target latent TB infection[35].

Alternative routes for TB vaccine delivery

Alternative routes include mucosal delivery via aerosolization, and intravenous (IV) delivery. As suggested by animal models, delivering a vaccine by aerosol directly to the respiratory mucosa may provide better protection than intradermal or intramuscular administration. Aerosolized BCG protected against TB infection and disease in non-human primates using a repeated limiting dose *Mtb* challenge model[36]. In humans, a phase 1 trial of aerosol inhaled MVA85A in healthy BCG-vaccinated adults showed this administration route to be well tolerated and immunogenic[37]. Similarly, alternating aerosol and intradermal vaccination routes for Ag85A showed that aerosol vaccination induced potent cellular Ag85A-specific mucosal and systemic immune responses. However, while the intradermal-aerosol vaccination regimen resulted in modest, significant boosting of the cell-mediated immune response to Ag85A, intradermal prime-aerosol boost resulted in transient but significant respiratory AEs[38]. Several more trials are underway.
IV BCG was recently shown to be strongly protective against *Mtb* infection and disease in non-human primates compared to intradermal or aerosol delivery[39]. The feasibility of this delivery route in humans requires further study.

Controlled human infection model

A controlled human infection model (CHIM) with engineered Mtb or an Mtb surrogate could be used for vaccine selection as well as for immunobiology studies to inform basic knowledge gaps in TB vaccine development. Major question facing TB vaccine CHIM developers is whether Mtb can be manipulated to be safe enough to administer to volunteers. An additional question is whether BCG could be used either as the challenge organism or at least as an agent that would permit further clinical development of the clinical challenge model.
There are two key elements to developing a human challenge strain of Mtb for a CHIM: developing a control system to elicit bacterial death and developing a system to detect viable Mtb in the days and weeks following challenge[7]. Given many uncertainties, a different CHIM strategy, based on a human intradermal (ID) BCG challenge, is being developed in parallel to CHIMs based on intrapulmonary Mtb administration. Efforts are currently underway to develop a human aerosol BCG challenge model, with regulatory discussions ongoing.

Animal models

Small animal models

Mice and guinea pigs have been the main small animal species utilized for evaluating TB vaccine candidates. Both models provide the opportunity to compare vaccine efficacy by assessing bacterial load and survival following *Mtb* challenge. Mice are inbred, easily manipulated, inexpensive, and there are extensive reagents for immunological studies. Guinea pigs are susceptible to very low dose *Mtb* challenge and can be used for natural transmission or repeated low- and ultralow-exposure models. The pulmonary pathology of Mtb infection in guinea pigs is similar to that of

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human primary TB. Standard animal challenge models of Mtb infection differ from natural infection in a number of ways, including challenge with a higher Mtb exposure than the very low exposures that characterize natural infection; a single challenge as compared to the repeated exposures associated with natural infection; and exposure to a naturally occurring form of Mtb rather than to laboratory-grown strains.
Small animal models are being used to (down-)select vaccine candidates for their ability to prevent TB disease. An Mtb challenge in vaccinated small animals provides a direct measure of an anti-mycobacterial host response as well as an opportunity to detect pathological outcomes. The optimal outcome in a small animal would be to reduce the bacterial burden below the level of detection. Assessment of immunotherapeutic effects of vaccines can also be performed in small animals, but due to the variability in outcomes, large numbers of mice are required. A prevention if infection vaccine model is not currently optimized for small animals, although studies of guinea pigs in a natural exposure environment could provide proof of concept.

Non-human primate models

Non-human primates (NHPs) demonstrate the full spectrum of TB, including active disease, latent infection and reactivation. TB-induced pathology in NHPs reflects human pathology, including caseating granulomas and other granuloma types, as well as cavitary lung disease. Additionally, unconventional T-cell subsets and delayed type hypersensitivity responses to BCG and Mtb in NHPs are immunologically closer to those of humans than in other animal models. Rhesus macaques, mainly of Indian origin, and cynomolgous macaques currently are the most common NHP species used to model Mtb infection and TB disease. There are extensive differences in susceptibility and responses between animal subspecies. Major advances include non-invasive imaging techniques to serially track disease progression in a sensitive and quantifiable manner (PET-CT); low-dose Mtb challenge (<25 CFU) and very low-dose Mtb challenge (<10 CFU), doses that more closely approximate natural human exposure to Mtb than did the 500–3,000 CFU exposures previously used; and advanced tools for studying immune responses, permitting detailed studies of adaptive immune responses as well as assessments of diversified components of the rhesus immune response. The Collaboration for TB Vaccine Development (CTVD) has published recommendations on designing NHP-based studies for TB vaccine development[40].
Despite these advances, NHP models have hardly been validated for protective responses in humans due to absence of protection signals in clinical studies. A recent study of a prime-boost strategy in NHP in which animals were given BCG followed by intramuscular M72/AS01E failed to show enhanced protection, despite the protective signal seen for M72/AS01E in BCG-vaccinated, TB infected individuals[41].

Stage gates – from preclinical to clinical studies

Although advancing candidates for preclinical to clinical development is influenced by various considerations (such as commercial, manufacturer, IP), animal models play an important role in these decisions. There is currently however no single, harmonized animal model that could be used for clear 'go/no-go' decisions for candidate TB vaccines, even though there is greater confidence in data that show the efficacy of a candidate in multiple in vivo systems from independent laboratories. The NHP model is considered the most reliable for selecting TB vaccine candidates for clinical testing. However, demonstrating statistically robust efficacy in NHP is costly and difficult due to limitations of space and animal availability and must be balanced against the cost of collecting data in humans. Since resources to do human efficacy trials are limited, the way candidates are selected for moving to the next stage in the pipeline is being made more systematic by defining an agreed set of stage gates. These stage gates specify the criteria for progression at each stage of TB vaccine development, from discovery through to licensure[42], and have recently been updated by Aeras/IAVI and TBVI as part of the TB Vaccine Development pathway.[f] The revised stage gate criteria make experiments in small animals an explicit part of TB vaccine

[f] https://www.tbvacpathway.com/

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candidate development. In this context, protection is defined as being reproducibly and statistically better at preventing TB disease than BCG or a relevant benchmark. Currently the revised stage gates are being piloted for reference and potential use in studies comparing different preclinical candidates head-to-head in different animal models in independent labs.

Clinical trials

Diagnosis of disease and infection

Diagnostics for TB disease

The gold standard for bacteriological confirmation is mycobacterial culture. Liquid media culture has higher sensitivity than solid media culture, but also higher contamination rates. The number of sputum specimens that need to be cultured to achieve maximum sensitivity for pulmonary TB is unknown, probably because of day-to-day variation in sputum bacterial load in sputum. Molecular diagnostics are an alternative to culture[43]. Most used are the within-cartridge real-time PCR GeneXpert assays, Xpert MTB/RIF and Xpert Ultra. Ultra has highest sensitivity, similar to that of liquid culture when used on sputum, but lower specificity due to 'trace calls'. False-positive trace calls may occur in patients who have been recently treated for TB disease[44]. The sensitivity of culture and molecular methods tends to be lower in extrapulmonary disease, in children and in HIV-infected patients with low CD4 counts. Children, the elderly and very sick patients may not be able to expectorate sputum, requiring more invasive sampling such as sputum induction or bronchoalveolar lavage. Recent studies suggest that wearing a filter-containing face mask may be highly sensitive alternatives for sampling Mtb from the lungs.[45]

Diagnostics for TB infection

TB infection has in the past been diagnosed by the tuberculin skin test (TST). In recent years the standard has become interferon-gamma release assays (IGRA) that have superior specificity because they show no cross-reactivity following BCG vaccination and limited cross-reactivity with non-tuberculous mycobacterial infections[46]. IGRA require 24H stimulation, the two platforms (whole blood stimulation and ELISPOT) have largely similar results. Irrespective of possible cross-reactions there are several unsolved questions with regard to the interpretation of IGRA results for diagnosing TB infection. (1) Conversion from negative (not infected) to positive (infected) is not well defined. Manufacturer-defined cut-offs may provide false-positive conversions. Using higher cut-offs, although these may be associated with higher incidence of subsequent TB disease, may underestimate the true conversion rate. (2) Reversions occur frequently. Whether these can be interpreted as early self-clearance of an initial Mtb infection, and if so at which cut-offs, is yet unknown. Whether "sustained IGRA conversion" (conversion not followed by reversion) is a valid and clinically meaningful PoI endpoint remains to be proven.

Clinical endpoints

Prevention of Disease

The clinical endpoint most relevant for licensure and scale-up is prevention of (pulmonary) TB disease (PoD), which requires measuring the incidence of clinical, ideally microbiologically confirmed, TB in the vaccine and placebo arms. Since the estimated incidence of TB disease in most high-incidence countries is not above 400/100,000 per year, very large sample sizes are needed in order to measure protective efficacy with sufficient precision. In addition, TB disease, a chronic condition with often insidious onset, is difficult to diagnose (even more so in infants), altogether making efficacy trials with a PoD endpoint extremely expensive. For licensure as a

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vaccine for adolescents/adults or for neonates/infants, phase 3 trials with a PoD endpoint will be inevitable. They are feasible if the finding can be made available.
In order to accelerate clinical development, alternative endpoints are being used for phase 2, proof-of-concept trials. These are prevention of infection (PoI) and prevention of recurrence (PoR).

Prevention of Infection
PoI is established by measuring in the vaccine and placebo arms the incidence of new Mtb infection. The advantage of this endpoint is that the incidence of TB infection is 10-20 times higher than the incidence of TB disease, so that much smaller sample sizes are needed. However, for lack of a gold standard test for (latent) TB infection, there is no consensus about how incident infection should be measured. Conversion from negative to positive of the IGRA response has been generally used in observational studies, and in children high levels of interferon-gamma response correlated with the risk of developing TB disease.[47] Reversions of IGRA responses occur as well[48] and have been proposed as early clearance of an Mtb infection[8], while others have argued that early clearance reflects increased innate rather than transient adaptive responses.[27] A recent phase 2b trial among adolescents showed that BCG revaccination prevented infection when measured as sustained IGRA conversion (a secondary outcome) but not when measured as IGRA conversion as such (the primary outcome).[8] This trial is currently being repeated with sustained IGRA conversion as the primary outcome. Furthermore, measurement of infection will be hampered by possible cross-detection between the vaccine antigen(s) and the antigen(s) used in the diagnostic test.
Another challenge is the clinical significance of a PoI endpoint. Unless cellular immunity is affected, most Mtb infections are contained by the host response and only 5-10% of Mtb infections progress to TB disease. A vaccine may effectively reinforce containment and thereby prevent disease but not infection. Failure to prevent infection may therefore not imply failure to prevent disease, thereby limiting the utility of this endpoint for down-selecting vaccine candidates early in the clinical development pathway. Conversely, it is unknown whether the infections prevented by a vaccine are amongst those 5-10%. Unless the protective efficacy for infection is very high it may thus be that prevention of infection does not translate into a similar, or even any, level of PoD. PoI is therefore generally not considered an endpoint for vaccine licensure.

Prevention of recurrence
Another alternative endpoint is PoR, by comparing in the vaccine and placebo arms the incidence of recurrent TB disease among patients who successfully completed treatment for TB disease. The advantage of the PoR endpoint is that the incidence of recurrent TB disease is 5-10 times higher than the incidence of new TB disease, thus also requiring smaller sample sizes. Cases of recurrent disease after treatment completion are a mixture of true relapses (recurrence with the same Mtb strain) and reinfections with different Mtb strains. The relative contribution of each depends on factors that affect relapse rates (e.g. pretreatment extent of disease, drug resistance, duration of treatment and drug regimen used), background incidence of TB infection in the population and duration of follow-up[49]. Also for the PoR endpoint its translation to PoD is challenging. The efficacy for preventing true relapse (probably an immunotherapeutic effect) may be higher or lower than for new disease, and high rates of reinfection after completion of treatment[50] [51] suggest that following TB treatment there may be a suppressed protective response, which may affect vaccine efficacy. Therefore, failure to prevent recurrence may also not imply failure to prevent disease, again limiting the utility of the endpoint for down-selection. Contrary to PoI however, PoR has clinical significance and may be a licensure endpoint by itself.

Post-exposure versus pre-exposure protection
Another approach that trials have used is a PoD endpoint in those latently infected, by randomizing individuals with a positive IGRA response only and comparing the incidence of TB disease between the vaccine and placebo arms. An example is the phase 2b trial of M72/AS01E that showed a PoD efficacy signal[13].

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The protection measured in this way is regarded as "post-exposure" or "post-infection" protection, in contrast with "pre-exposure" or "pre-infection" protection that is measured in individuals with a negative IGRA response. The advantage of this endpoint is that the incidence of TB disease among those infected is also higher than the incidence of TB disease in the population at large, again requiring smaller sample sizes. Since most TB disease occurs shortly following Mtb infection or reinfection[52], the incidence among individuals with a positive IGRA response depends on various factors including background force-of-infection and, since IGRA responses probably measure cumulative infection over time, possibly historical force-of-infection over several years to decades. This approach also has challenges. Whether and to what extent post-exposure PoD translates into pre-exposure PoD is unknown. Therefore, unless the vaccine is only given to individuals how have latent TB infection (which requires large-scale pre-immunization IGRA testing), it is unclear how protective efficacy shown against disease in latently infected individuals would translate into public health impact and cost-effectiveness when the vaccine is given in the population at large. Modeling studies suggest that a vaccine efficacious for prevention of disease in post-infection populations would have greatest impact, but vaccines efficacious for prevention of infection or disease in pre-infection populations have increasing impact in higher transmission settings[53].

Heterogeneity in clinical protection and safety, including HIV

New TB vaccines may need to be evaluated for efficacy and safety in various specific populations. People living with HIV will be an essential one, in addition to others in which lower efficacy may be expected such as the elderly and individuals with type-2 diabetes.

There is a clear need for TB vaccines that have protective efficacy in people living with HIV. TB causes one-in-three HIV deaths globally, and HIV co-infection is a major driver of TB especially in Africa. In HIV infection immune responses are deficient, even with stable antiretroviral treatment: the risk for HIV-associated TB is maximal in the period prior to immune reconstitution but TB incidence during HIV infection, even after immune recovery and virologic suppression, remains higher than in the general population. However, protection afforded by a TB vaccine may be reduced in people living with HIV. In addition, vaccine safety may be compromised, especially with live attenuated vaccines. Modeling studies indicate the impact of a new TB vaccine would be reduced if the vaccine were contraindicated in HIV+ persons.[53] Inclusion of people living with HIV in clinical trials will therefore be important to establish at minimum safety and possibly also efficacy in this important population.

Geographical diversity in trials may be important as well. BCG has shown heterogeneous protection by vaccine substrain and manufacturer, but also by geographic latitude, possibly related to different background prevalence of non-tuberculous mycobacterial infections that may affect the immunogenicity of live-attenuated TB vaccines[54]. Mtb genotype may also affect the protective efficacy, as has been suggested for the Beijing genotype family that is highly prevalent in East Asia[55].

Correlates of protection[g]

Use of Correlates of Protection for regulatory approval

Correlates of protection (CoP) will be highly important to accelerate clinical development, as they allow much smaller trials of shorter duration to (down-)select candidates in phase 2 for subsequent phase 3 trials with clinical endpoints. Currently no agreed CoPs for TB exist.

In general, a CoP would be an immunological biomarker, or a combination of biomarkers (biomarker signature), measured in a validated assay, that reliably predicts vaccine efficacy (VE) against a disease endpoint, supported by a variety of data, including correlates analyses in one or

[g] Several sections of this chapter, including Annex 6 and 7, were adapted from information generously provided by Peter Gilbert and Andrew Fiore-Gartland, 30 July 2020. were also kindly provided by

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more phase 3 trials that demonstrated success on clinical vaccine efficacy (e.g., 95% lower confidence bound for vaccine efficacy > 30%).
A widely accepted hierarchy of trial outcome measures is that proposed by Fleming and Powers[56]: Level 1- true clinical efficacy measure, Level 2 - validated surrogate (for a specific disease setting and class of interventions), Level 3 - non-validated surrogate, yet one established to be 'reasonably likely to predict clinical benefit' (for a specific disease setting and class of interventions), Level 4 – a correlate that is a measure of biological activity, but not established to be at a higher level.
The licensure implications for a CoP would depend on the type of regulatory approval that is sought. For *traditional regulatory approval* if the biomarker or biomarker signature is *scientifically well established to reliably predict vaccine efficacy*, then subsequent phase 3 efficacy trials may use the biomarker (signature) as the primary endpoint. Examples of such application of CoPs would be phase 3 trials of the same vaccine in different populations, or possibly new vaccines in the same class for the same or different populations.
In the event that there is no well accepted correlate of protection, in the U.S. regulatory system, it may be possible to seek approval using the accelerated approval pathway (21 CFR 601.40, 601.41).[h] The accelerated approval pathway requires a determination that the product has an effect on a "surrogate endpoint that is reasonably likely ….to predict clinical benefit, or on the basis of an effect on a clinical endpoint other than survival or irreversible morbidity." Accelerated approval is reserved for products that are intended to treat a serious condition and that provide a meaningful benefit over existing therapy. If approval is granted based on a surrogate endpoint, post-marketing confirmatory clinical trials have been required to verify and describe the clinical benefit. In determining whether an endpoint is reasonably likely to predict clinical benefit regulatory, agencies may consider evidence derived from a number of sources, including epidemiologic or pathophysiologic data.
Regulators generally do not have predefined goalposts for accepting a surrogate endpoint; in practice one seeks evidence from all angles and the particular synthesis case is reviewed.

Current approaches to discovery of CoP for TB vaccines

Targeted approaches for identifying potential COP for TB vaccines include detection of cellular mycobacteria-specific responses by ELISpot and multiparametric flow cytometry; of humoral immune responses by antigen-specific antibody or predefined analyte assays; and of B-cell memory responses by cultured ELISpot in combination with flow cytometry[57]. Unbiased approaches include transcriptional profiling of blood cells and mycobacterial growth inhibition assays (MGIAs). Recently, several RNA transcriptional signatures have been identified as correlates of risk for TB disease among infected individuals[58]. MGIAs measure the ability of cells to inhibit in vitro mycobacterial growth and have potential utility in vaccine evaluation as they measure the collective effect of a wide range of immune mechanisms and their complex interactions[59].

Analytical approaches to assessing CoPs in phase 3 vaccine efficacy trials

CoPs, measured at a given post-vaccination time point, that can be assessed in each individual phase 3 trial include the following (see Annex 6 for details).

1. Correlates of VE in vaccine recipients: VE across subgroups of vaccine recipients defined by biomarker (signature) level in vaccine recipients. Analytically this comes down to effect modification / principal stratification.
2. Mediators of VE: the proportion of VE mediated by a biomarker (signature). This would look at the biomarker (signature) as representing a mechanism of protection; analytically it represents quantifying natural direct and indirect effects in a causal analysis framework.
3. Stochastic-intervention effects on the VE: how much would disease risk be lowered by shifting the biomarker distribution upwards[60].

[h] The European Medicines Agency has a similar pathway.

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4. Surrogate/replacement endpoint evaluation approaches, e.g. by analysing the strength of the association of individual-level causal effects on the biomarker (signature) and on the clinical endpoint[61].

In addition, meta-analysis of multiple phase 3 vaccine efficacy trials can provide powerful additional assessment of CoPs.

Evidence to support a biomarker as a Correlate of Protection

Annex 7 summarizes potential lines of evidence building a case for an immunological biomarker (measured with a validated assay) at a given post vaccination time point to be accepted as a surrogate endpoint for TB disease or post-treatment disease recurrence, for a defined population. For simplicity, Annex 7 focuses on a single biomarker measured with a single validated assay. However, the concepts/approaches apply similarly if the biomarker is an aggregate/synthesis of multiple biomarkers measured using multiple validated assays.

Note that correlates of risk (CoRs) (i.e., disease incidence biomarkers) are also critical to assess, as an intermediate step toward a CoP that is ultimately needed. While a CoR may generally fail to be a CoP, evidence for a very strong CoR plus additional evidence on mechanism of protection may imply the CoR is likely to be a CoP.

Epidemiology

Burden and incidence of TB disease

Estimates of the burden and incidence of TB disease in all countries in the world are published annually by the WHO. Estimated incidence of TB disease varies widely between countries from less than five to more than 500 new cases per 100,000 population per year, the global average being around 130/100,000[62]. Within countries TB incidences may vary, tending to be particularly increased in people living with HIV. Whereas for high-income countries with advanced surveillance systems these estimates are generally considered accurate, estimates for low- and middle-income countries (LMIC) are uncertain. Here they are based on notification of TB patients starting on treatment, and indirect estimation of the numbers of patients with TB disease who are not notified or not diagnosed – quantities that are essentially unknown. Over the past two decades, several LMIC have conducted nationwide surveys of TB prevalence. These prevalence estimates have helped reduce the uncertainty around the estimates, but not solved the problem that we do not know the duration of unnotified and undiagnosed disease, and thereby cannot translate prevalence into incidence of TB disease. Direct estimation of TB incidence at the population level requires intensive epidemiological investigation and has been attempted in only a limited number of areas. More extensive data exist on TB incidence in specific populations and settings that are easier to follow over time, such as household members of infectious TB patients, people on antiretroviral treatment, occupational hazard groups such as health care workers and miners, and prisoners.

Burden and incidence of Mtb infection

In the past several countries in Asia and Africa have indirectly estimated their burden and incidence of TB infection at the population level through tuberculin surveys. These surveys measured the age-specific prevalence of positive TST among primary school children and from that the annual risk of infection (ARI), under the assumption that the ARI was uniform across ages and constant over time. For most high TB incidence populations this annual risk was 1-3%, with the exception of very high incidence settings where it could be up to 5%. Studies from South Africa in addition showed that the ARI increases from the age of 12-14 years[63]. Adolescent (12-18 years) LTBI prevalence was 40-50% in South Africa depending on the method used[64], but clearly lower in Kenya (32%)[65] and rural Uganda (16%)[66]. Few cohort studies have been done to directly estimate the incidence of TB infection among children and adolescents using IGRA. A large study among

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South African adolescents showed the annual risk of infection to be 7%, but twice as large when reversions were taken into account[48].
Several of these and other studies have quantified the association between incidence of TB infection and incidence of TB disease at the population level. While these have generally shown consistent relationships, these data do not provide direct information on which infections will lead to disease at the individual level, which would be important to understand how well PoI as a vaccine trial endpoint translates into PoD.

TB recurrence

Recurrent TB, i.e. TB disease among previously treated individuals, constitutes 5-30% of the TB burden, with higher proportions found in high-prevalence settings. Recurrence may be due to endogenous relapse or exogenous reinfection; the two can be distinguished by genotyping of the paired Mtb strains in the same patient. In high-prevalence settings reinfection is thought to drive the higher proportion of retreatment due to higher transmission rates, in particular in people living with HIV who have increased risk of reinfection disease. Population genotyping data have suggested that the risk of recurrence due to reinfection is increased after a previous period of TB disease[50], and analyses of notification indicate that the risk of recurrence increases with each subsequent disease episode[67]. Patients who completed TB treatment may thus be at increased risk for exposure to Mtb, increased risk of reinfection disease when exposed, or both; the latter may reflect altered local or systemic immune responses.

Role of genotype

Genotyping of Mtb strains globally has identified 7 distinct lineages with geographically diverse distributions. Lineage 2, in particular the "modern Beijing genotype" has been consistently associated with drug resistance, increased relapse rates in humans and increased virulence in animal models[68]. It's global spread in the last decades has raised the hypothesis that this genotype is an escape variant of BCG vaccination. Indeed, a recent study from Indonesia found that a history of BCG vaccination was associated with lower infection rates among household contacts if the index cases was infected with a non-Beijing strain, but not if the index cases was infected with a Beijing strain[55].

Implementation

Needs and preferences for TB vaccines

There has been limited research to define needs and preferences for TB vaccines among country policy makers and other stakeholders. The WHO Preferred Product Characteristics featured in this Roadmap have been a first systematic approach to defining these needs and preferences.

Vaccine delivery in adolescents and adults

A TB vaccine for adolescents and adults requires delivery strategies that are quite different from neonatal and childhood vaccination. Experience with large-scale preventive vaccination at adolescent age mainly comes from vaccination against human papilloma virus (HPV), hepatitis B virus (HBV) and meningococci. A recent meta-analysis of implementation trials mainly from high-income countries showed that HPV vaccination uptake was enhanced by health education and financial incentives, while mandatory vaccination enhanced uptake of HBV vaccination. Provider prompts had little effect; most of the evidence was considered to have low to moderate certainty[69].
In a comparison of European country policies, high vaccination coverage rates were associated with delivery through school health services, and invitations and reminders to attend for vaccination[70].
In observational studies, completion of multidose vaccination schedules in adolescence was

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negatively associated with minority racial or ethnic groups and inadequate health insurance coverage, and enhanced by parental healthcare seeking behavior[71].
With regard to adult vaccination, experience is largely limited to seasonal influenza vaccination (SIV) of the elderly. Uptake of SIV among the elderly generally remains suboptimal. In Europe uptake was lower among in immigrants and in more deprived areas[72], while in the US and Canada high uptake was associated with being older and white, and with having higher income and health insurance[73]. In a meta-analysis of trials from high-income countries SIV uptake was clearly enhanced by patient outreach, personal invitations, pharmacy-based vaccination, and free delivery on the patient side, and physicians reminders and chart reviews plus benchmarking on the provider side[74].
It is expected that many countries will soon gain experience with vaccination against SARS-CoV-2, the causative agent of COVID-19, which will also be targeted at adults. This experience will be valuable for designing TB vaccination strategies in various settings.

Vaccine acceptance and hesitancy

Low vaccine acceptance ("vaccine hesitancy") has been identified as one of the major threats to global health. Among the complex reasons for vaccine hesitancy are lack of confidence in vaccine safety, driven by concerns about adverse events[75]. This applies to childhood vaccination as well as to vaccination of adolescents and adults. Acceptance of HPV vaccination for adolescent girls and young women is strongly governed by financial considerations, social norms and values relating to sexual activity, and by trust in vaccination programmes and healthcare providers[76]. Deterrents for SIV uptake in Asia vary according to population groups but include concerns with vaccine safety and efficacy[77]. There is a vast literature on approaches to improve vaccine acceptance, but limited work has yet been done specifically for (new) TB vaccines[75].

TB-associated stigma

Stigma is well known to affect uptake of TB services and TB treatment outcomes in both high- and low- and middle-income countries[78] [79], and may have important cultural variation[80]. TB-related stigma remains highly understudied[81]. A systematic review of interventions to address stigma showed that knowledge-shaping and attitude-changing interventions aimed at the public, patients and their families were effective in reducing anticipated stigma associated with TB. Home visits and support groups were effective in reducing both anticipated and internalized stigma. However, studies were few and of poor quality[82]. There are no data on stigma associated with TB vaccination (e.g. of high-risk groups) or on TB-associated stigma affecting participation in TB vaccine trials.

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Annex 1. Conclusions and recommendations from review/viewpoint paper by major TB vaccine R&D stakeholders

Taken from [7]

Box 2. Conclusions and recommendations

TB vaccine technologies

- The global TB vaccine community should unite to maintain a dynamic vaccine candidate pipeline from discovery to late stage.
- There are currently no unanimously agreed criteria for advancing vaccine candidates.

TB vaccine research and development

- Future discovery efforts should include investigation of immune quality and vaccine efficacy in response to combinations of vaccine platform, antigen and adjuvant.
- The evaluation of host factors impacting protection should be included in future scientific investigation.
- Whole cell mycobacterial vaccines should continue to be central to TB vaccine development.

The role of animal models

- Animal models and clinical studies should progress in parallel and may offer opportunities for cross-validation.
- The most appropriate animal models should be selected based on evidence and the underlying question(s) to be answered.
- The use of multiple different animal models can have a cumulative value in assessing vaccine candidates or answering pathogenesis questions.
- There is an opportunity for the funders to encourage further standardization of models.
- An obligation to publish animal studies regardless of the outcome (as it is the case for clinical trials) should be encouraged and would facilitate vaccine development.

Biomarkers, Systems Biology and immune correlates

- The approach to biomarkers should remain broad, looking at correlates of safety, risk of stable infection or disease, and vaccine efficacy.
- Observational studies will help to identify biomarkers of risk of infection or disease.
- Interventional (vaccine) studies and observational studies should be used to create and expand biobank repositories.

Experimental medicine and human challenge

- A space for clinical research studies needs to be maintained and expanded.
- A favourable regulatory environment is critical for the conduct of clinical research studies and should be advocated for.
- Investment into the establishment of controlled human TB challenge models needs to continue. Learnings from the malaria field should be integrated in this process and synergies with biomarker research needs to be created.

Clinical and late stage development

- We need to keep (pipeline) diversity at all levels since we still wait for a clear efficacy signal.
- De-risking candidates through gating criteria does not mean being risk-adverse.
- We need to evaluate and accept some risk but prepare carefully and perform high quality studies which can advance the field even in the absence of an efficacy signal.

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Annex 2. Preferred Product Characteristic for a new TB vaccine to be used in adolescents and adults

Adapted from [83]

Parameter	Preferred Characteristic	Comments
Indication	Immunization for prevention of active pulmonary TB disease	
Target population	Adolescents and adults	Adolescents and adults with TB disease represent the most common sources of *Mtb* spread and are therefore the WHO priority target for TB vaccine development. Demographic changes in some high endemicity countries justify inclusion of older adults in the target population. The optimal timing for paediatric evaluation should be discussed with regulators and policy makers but a paediatric clinical development program should certainly be considered when proof of concept is established in adolescents and adults.
Outcome measure and efficacy	50% or greater efficacy in preventing confirmed pulmonary TB.	A vaccine with lesser vaccine efficacy against confirmed TB in adolescents and adults, if widely used in areas of high TB endemicity, may still prove valuable and contribute to reducing the spread of *Mtb* in a cost-effective way, but this would fall short of the requirements necessary to meet the End TB goals by the 2035 target date. A bacteriologically confirmed case of TB is the preferred endpoint for TB vaccine efficacy assessment. Preferred endpoint case definitions have been published. A bacteriologically confirmed case is one from whom a biological specimen is confirmed positive by culture or WHO-approved rapid diagnostic method. A clinically diagnosed TB case is one who does not fulfil the criteria for bacteriological confirmation but has been diagnosed with active TB by a clinician or other medical practitioner who has decided to give the patient a full course of TB treatment. The vaccine should be protective in both subjects with and without evidence of latent *Mtb* infection, in different geographical regions and latitudes, irrespective of environmental exposure to mycobacteria. In high endemicity countries, rapid population-level vaccine impact will be derived

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		mostly from prevention of TB disease in subjects with latent *Mtb* infection. Vaccine efficacy against recurrent TB (PoR) should be characterized. Preferences regarding efficacy outcome measures are similar to those used in PoD studies when considering prevention of recurrent TB disease. It is anticipated that vaccines protecting against drug-sensitive TB would also protect against drug-resistant TB. Over the long term, the impact of vaccine use on the incidence of drug-resistant *Mtb* cases should be assessed to confirm this effect, given the enormous public health benefit that would accrue from a reduction in cases of drug-resistant *Mtb*.
Duration of protection	Ten years or more of protection should be conferred after primary immunization	Demonstrated efficacy over at least 2 years after completion of the primary immunization regimen to support initial policy decisions. Longer-term follow-up studies, possibly after initial vaccine introduction, will be important in informing duration of protection and possible booster requirements.
Safety	Safety and reactogenicity profile should be favourable, similar to other current WHO-recommended routine vaccines for use in adolescents and adults.	Safety profile demonstrates no or only mild, transient reactogenicity and no serious adverse events related to vaccination. Considering the severity and public health concern associated to the target disease, mitigations may need to be considered for mild reactions or very rare events. Safety should be favourable in particular risk groups, such as individuals living with HIV/AIDS and other causes of immuno-deficiencies, the elderly, pregnant and lactating women. Careful investigations will be required for live platform vaccine candidates.
Schedule	A minimal number of doses and boosters required.	A requirement for more than three doses to achieve primary immunization would not be desirable due to logistical and cost concerns. While complexity should be avoided if possible, heterologous prime boost regimes, are being considered. Long term follow-up studies, possibly after receiving initial marketing approval, should determine the requirement for booster dose(s). If a booster is required, administration 10 years or more after completion of the primary immunization series would be preferred. A requirement for boosters to be administered more than every five years will likely be associated with delivery challenges.

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Co-administration	Demonstration of favourable safety and absence of immunologic interference with other vaccines recommended for use in the same target population.	In the absence of established correlates of protection, the impact of co-administration on markers of immune 'take' should be characterized and interpreted accordingly.
Immuno-genicity	Identification of a correlate/ surrogate of protection, using a validated assay.	No confirmed correlate/surrogate of clinical efficacy of a TB vaccine currently exists. The identification of immune correlates/surrogates of protection should be included as immunogenicity objectives in TB vaccine efficacy studies. In the absence of established correlates of protection, markers of immune 'take' should be characterized. The conservation of biological specimen for future use upon advances in technology and knowledge is encouraged.
Programmatic suitability and prequalifica-tion	General guidance from WHO expectations about clinical evaluation of vaccines should be followed. The WHO defined criteria for programmatic suitability of vaccines should be met, following guidance on vaccine presentation, packaging, thermostability, formulation and disposal. The vaccine should be prequalified to support purchasing by United Nations agencies.	Beyond the minimum requirements for WHO prequalification, innovation related to programmatic suitability, such as ease of administration and thermostability, would lead to major public health benefits and is strongly encouraged.
Value proposition	Dosage, regimen, and cost of goods should be amenable to affordable supply. Favourable cost-effectiveness should be established and price should not be a barrier to access, including in low and middle income countries.	Modelling the impact of TB vaccines with various characteristics on the TB epidemic in general, and on drug-resistant TB specifically, as well as on TB-associated anti-mycobacterial drug use would be valuable. The vaccine impact on health systems (such as reduction of TB-related medical attendance and hospitalization) and other aspects of implementation science should be evaluated in both modelling and real vaccine use studies.

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Annex 3. Preferred Product Characteristic for a new TB vaccine to be used in neonates and infants

Adapted from [83]

Parameter	Preferred Characteristic	Comments
Indication	Prevention of TB disease, including severe, disseminated TB, TB meningitis and pulmonary TB, in infants and young children.	
Target population	Neonates and infants, in co-administration with other existing vaccines from the Expanded Program on Immunization.	
Outcome measure and efficacy	Equal to or greater than 80% vaccine efficacy as compared to baseline incidence, or superior efficacy as compared to BCG, in preventing TB disease, including severe, disseminated TB, TB meningitis and pulmonary TB, in infants and young children.	Considering existing recommendations and inclusion of neonatal BCG vaccination in standards of care based on the demonstrated efficacy profile of BCG, a new vaccine developed for BCG replacement should be compared to BCG in a randomized controlled study, powered to show superior vaccine efficacy as compared to BCG. Ethics committee(s) of record should confirm that the accrued evidence about the new candidate vaccines justify the absence or delay of BCG vaccination in at least one trial arm, if such a trial design is proposed. BCG provides partial protection against leprosy. Policy decisions related to BCG replacement will also give due consideration, where relevant, to available evidence about the effects against leprosy and Buruli ulcer, as well as evidence established in carefully designed prospective BCG trials with pre-defined endpoints about the possible role of 'non-specific effects' of BCG. Characterisation of vaccine efficacy against paediatric TB mortality and all-cause mortality is desirable, possibly in large-scale pilot introduction studies. BCG boosting strategies are also being considered.
Duration of protection	Ten years or more of protection should be conferred after primary immunization	Demonstrated efficacy over 2 or more years after completion of the primary immunization regimen to support initial policy decision.

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		Longer-term follow-up studies will be important to inform the duration of protection and possible booster requirements.
Safety	Improved safety as compared to current BCG.	Safety should be favourable in HIV-infected subjects. In countries with high HIV endemicity, many HIV-infected neonates and infants are vaccinated with BCG, which may result in severe local and/or regional BCG reactions or disseminated BCG infection, sometimes fatal. A new TB vaccine safe enough to be administered to neonates and infants with innate or acquired immunodeficiency, including HIV infected infants, would represent an important public health advance. Careful investigations will be required for live platform vaccine candidates. Safety profile demonstrates no or only mild, transient reactogenicity and no serious adverse events related to vaccination. Injection site swelling, pain, drainage, and scarring, and local lymphadenopathy, are common adverse events associated with BCG infection. Reduction in the frequency and severity of these and related outcomes would represent welcomed improvement over BCG. The absence of vaccine-related immune activation syndrome upon initiation of antiretroviral therapy in HIV-infected children should be demonstrated. Efforts aimed at minimizing pain at the site of administration are strongly encouraged.
Schedule	A minimal number of doses and boosters required.	A requirement for more than three doses to achieve primary immunization would not be desirable due to logistical and cost concerns. While complexity should be avoided if possible, heterologous prime boost regimens, including those including neonatal BCG, are being considered. Long term follow-up studies, possibly post initial introduction, should determine the requirement for booster dose(s). If a booster is required, administration 10 years or more after completion of the primary immunization series would be preferred. A requirement for boosters to be administered more frequently than every five years will likely be associated with delivery challenges.
Co-administration	Demonstration of favourable safety and immunologic non-interference upon co-	In the absence of established correlates of protection, the impact of co-administration on

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	administration of other vaccines recommended for use in EPI	markers of immune 'take' should be characterized and interpreted accordingly.
Immuno-genicity	Identification of a correlate/ surrogate of protection, utilizing a validated assay.	No confirmed correlate/surrogate of clinical efficacy of a TB vaccine currently exists. The identification of immune correlates/surrogates of protection should be included as immunogenicity objectives in TB vaccine efficacy studies. In the absence of established correlates of protection, markers of immune 'take' should be characterized. The conservation of biological specimen for future use upon advances in technology and knowledge is encouraged.
Programmatic suitability and prequalifica-tion	General guidance from WHO on expectations about clinical evaluation of vaccines should be followed. The WHO defined criteria for programmatic suitability of vaccines should be met, following guidance on vaccine presentation, packaging, thermostability, formula-tion and disposal. The vaccine should be prequal-ified to support purchasing by United Nations agen-cies. An improved production process relative to current BCG, contribut-ing to ensuring affordable supply and avoid shortages, would be valuable.	Beyond the minimum requirements for WHO prequalification, innovation related to programmatic suitability, such as ease of administration and thermostability, would lead to major public health benefits and is strongly encouraged.
Value proposition	Dosage, regimen, and cost of goods should be amena-ble to affordable supply. The vaccine should be cost-effective and price should not be a barrier to access, including in low and middle income countries.	Modelling the impact of TB vaccines with various characteristics on the TB epidemics in general, and on drug-resistant TB specifically, as well as on TB-associated anti-mycobacterial drug use would be valuable. The vaccine impact on health systems (such as reduction of TB-related medical attendance and hospitalization) and other aspects of implementation science should be evaluated in both modelling and real vaccine use studies.

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Annex 4. Preferred Product Characteristic for a new TB vaccine to improve tuberculosis treatment outcomes

Adapted from [84]

Parameter	Preferred Characteristic	Comments
Indication	Protection against TB recurrence, following initial cure. Increase the proportion of cure at end of drug treatment.	Reducing the cumulative incidence of recurrence after initial, drug-mediated cure, and increasing the proportion of cure at the end of drug treatment represent the highest priorities to demonstrate in clinical studies. The possibility of reducing the number of drugs and treatment duration are essential long-term goals. Ethical standards would require that initial proof-of-concept be established while TB patients receive a standard recommended treatment regimen. Changes to the treatment regimen could be investigated after an initial demonstration of efficacy.
Target population	All persons being treated for active TB, both drug-sensitive and drug-resistant Mtb strains	Initial proof-of-concept might best be established in subjects with no specific, increased risk of adverse outcome with drug-sensitive TB. After initial demonstration of efficacy and safety, investigations in cases of MDR-TB and XDR-TB, as well as studies in special populations (children, pregnant women, subjects with HIV/AIDS and other high-risk individuals), should be started promptly.
Outcome measure and efficacy	50% or greater efficacy in reducing the rate of recurrent TB after a standard course of drug treatment, and/or 50% or greater reduction in treatment failure at the end of drug treatment.	These preference levels are selected by analogy with other severe diseases and expert opinion. Further research is required on the correspondence between vaccine efficacy and Mtb transmission and the TB epidemic as a whole including the spread of drug-resistant TB. Further research is also required to define strategy and targets for reduction in the number of drugs needed for treatment, and the duration of drug therapy, as long-term objectives. See panel for case definitions. Impact on long term pulmonary function should be evaluated.
Duration of protection against recurrence	Initial proof-of-concept for prevention of recurrence could be demonstrated on the basis of one year of follow-up.	Recurrence can result from both reactivation and reinfection. Most reactivations occur in the first year post treatment.
Safety	A safety and reactogenicity profile similar to other	The benefit/risk balance will need to be considered. Given the severity and public health concern associated to the target disease, mitigations may be considered for mild

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Parameter	Preferred Characteristic	Comments
	current WHO-recommended routine vaccines for use in adolescents and adults would be preferred	reactions or rare events. The risk of delayed-type hypersensitivity reactions, pulmonary and systemic inflammatory reactions should be carefully considered. Respiratory function tests can help monitor lung safety. Safety should be favorable in particular risk groups, such as individuals living with HIV/AIDS and other causes of immunodeficiencies, the elderly, pregnant and lactating women. In patients with immunosuppression, specific risk management plans will be required for live vaccine candidate platforms.
Schedule	A minimal number of doses required	A requirement for more than three doses to achieve immunization would not be desirable due to logistical and cost concerns.
Co-administration	N/A	Issues generally relevant to co-administration of a preventive vaccine with other vaccines are not applicable here given the intended administration of this therapeutic vaccine to individuals with active TB disease. The potential for adverse interactions with drugs administered for treatment of TB or comorbidities should be considered.
Immuno-genicity	Identification of a correlate/ surrogate of protection, using a validated assay	Little is presently known about immune determinants of protection, and no confirmed correlate/surrogate of clinical efficacy of a TB vaccine currently exists. The identification of marker of risk of recurrence and immune correlates/ surrogates of protection should be included as immunogenicity objectives in TB vaccine efficacy studies. In the absence of established correlates of protection, markers of immune 'take' should be characterized. The conservation of biological specimens for future use upon advances in technology and knowledge is encouraged.
Programmatic suitability and prequalifica-tion	General guidance from WHO expectations about clinical evaluation of vaccines should be followed. The WHO defined criteria for programmatic suitability of vaccines should be met, following guidance on vaccine presentation, packaging, thermostability, formulation and disposal. The vaccine should be prequalified to support purchasing by United Nations agencies.	Beyond the minimum requirements for WHO prequalification, innovation related to programmatic suitability, such as ease of administration and thermostability, would lead to major public health benefits and is strongly encouraged.

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Parameter	Preferred Characteristic	Comments
Value proposition	Dosage, regimen, and cost of goods should be amenable to affordable supply. Favorable cost-effectiveness should be established and price should not be a barrier to access, including in low-and middle-income countries.	Modelling public health value and impact of TB vaccines with various characteristics on the TB epidemic in general, and on drug-resistant TB specifically, as well as on TB associated anti-mycobacterial drug use would be valuable. The vaccine impact on health systems (such as reduction of TB-related medical attendance and hospitalization) and other aspects of implementation science should be evaluated in both modelling and real vaccine use studies.

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Annex 5: GAVI evaluation criteria for vaccines for endemic disease prevention through routine immunisation

WWW.gavi.org

	Criteria	Indicators
Ranking criteria:	Health impact	Total future deaths averted in the 2020-2035 period, and per 100,000 vaccinated Total future cases averted in the 2020-2035 period, and per 100,000 vaccinated
	Value for money	Vaccine procurement cost per death averted Vaccine procurement cost per case averted
	Equity and social protection impact	Disproportionate impact of disease on vulnerable groups Special benefits of vaccination for women and girls
	Economic impact	Direct medical costs averted Indirect costs averted
	Global health security impact	Epidemic potential of disease Impact of vaccination on antimicrobial resistance (AMR)
Secondary criteria:	Other impact	Total under-five deaths averted in the 2020-2035 period, and per 100,000 vaccinated Total DALYs averted in the 2020-2035 period, and per 100,000 vaccinated Vaccine procurement cost per disability-adjusted life year (DALY) averted
	Gavi comparative advantage	Degree of vaccine market challenges Potential for Gavi support to catalyse additional investment
	Implementation feasibility	Ease of supply chain integration Need for healthcare worker behaviour change Feasibility of vaccination time point Acceptability in target population Long-term financial implications
	Alternative interventions	Optimal use of current and future alternative interventions (prevention and treatment)
	Broader health system benefits	*No specific indicator – evaluated on a case-by-case basis*
Financial implications:	Vaccine cost	Total procurement cost to Gavi and countries, 2020-2035
	Operational cost	Incremental in-country operational costs per vaccinated person
	Additional implementation costs	Additional costs for introduction

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Annex 6. Statistical Approaches to Evaluating Immunological Biomarker Correlates in Phase 3 Prevention of Disease Vaccine Efficacy Trials, in Three Tiers of Increasing Levels of Rigor and Utility*

Courtesy of Dr. Peter Gilbert and Dr. Andrew Fiore-Gartland, 30 July 2020

Notes:
*The same correlates types can be assessed in prevention of recurrence of disease vaccine efficacy trials.
**Analyses of CoRs and all types of specific CoPs should include all baseline participant characteristics that predict both the TB disease study endpoint and the immunological biomarker endpoint. For CoR analyses, controlling for baseline exposure variables provides interpretation of results as biomarkers association with susceptibility to endpoint acquisition. For CoP analyses, controlling for confounders is needed to obtain valid/unbiased estimates of the parameters of interest that quantify the quality of the CoP.

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	Term	Definition (for an immunological biomarker measured at a given time point after vaccine or placebo administration (e.g., two weeks post))	Statistical Framework for Evaluation**
Tier 1	Correlate of Risk	An immunological biomarker that correlates with subsequent occurrence of the TB disease endpoint, in a defined cohort such as vaccine or placebo recipients at-risk for TB disease at the time the biomarker is measured	Regression, e.g. Cox or logistic regression for low-dimensional biomarkers; ensemble machine super-learning for high-dimensional biomarkers. CoR results are expressed as estimates of relative and absolute risk by biomarker level. CoR quality is measured by strength and precision of association parameters (e.g., point and confidence interval estimates) and by degree of accuracy to predict TB disease (e.g., cross-validated area under the ROC curve).
Tier 2	Specific Correlate of Protection (several types)	1. (Correlate of VE) A biomarker in vaccine recipients that correlates with VE against TB disease, which evaluates how the level of VE varies over subgroups defined by the level of the biomarker 2. (Mediator of VE) A biomarker that mediates a large proportion of the VE against TB disease, which evaluates a pathway/mechanism of VE 3. (Stochastic intervention effects on VE) A biomarker such that increases in its distribution (e.g., with a refined vaccine regimen) would translate to improved VE, with application to bridging predictions of VE 4. (Surrogate/replacement endpoint) A biomarker such that the vaccine effect on the biomarker can be used to reliably predict VE	1. Principal stratification analysis (estimate VE over biomarker-defined subgroups and quantify strength of VE moderation) 2. Natural direct and indirect effects mediation analysis (estimate the proportion of VE mediated by the biomarker) 3. Controlled stochastic effects analysis (estimate shift in VE resulting from a shift in the biomarker distribution) 4. Prentice framework surrogate endpoint evaluation methods, which apply if the biomarker varies widely in the placebo arm. If not, other approaches are needed, e.g., that evaluate the strength of association of individual-level causal vaccine effects on the biomarker with individual-level causal VE.
Tier 3	Generalized CoP	A specific CoP that generalizes to be a CoP across different settings (e.g., vaccine lots, vaccine formulations, human populations).	Meta-analysis of multiple efficacy trials that evaluates the association of vaccine effects on the biomarker with VE. Each of the statistical frameworks for evaluation of specific CoPs can also apply to evaluate generalized CoPs, combining individual-level data sets from multiple vaccine efficacy trials.

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Annex 7 Types of studies contributing evidence supporting use of a biomarker (measured with a validated assay) as a surrogate endpoint for TB disease

Courtesy of Dr. Peter Gilbert and Dr. Andrew Fiore-Gartland, 30 July 2020

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Study Type	Remarks
1. Natural history studies to evaluate immunological markers as correlates of risk (CoRs) for TB disease and recurrence of disease: • Cohort = Mtb. infected, uninfected or mixed: TB disease endpoints • Cohort = TB recovered (post-treatment): TB disease recurrence endpoints	• Mtb., BCG and non-tuberculin mycobacteria (NTM) exposures impact risk of disease and recurrence: existing studies have helped generate many transcriptomic and immunological markers of TB disease risk • TB disease endpoint must be defined by biomarker with high specificity and sensitivity for clinical disease for reliable correlates analysis (e.g., microbiologically confirmed by MGIT culture or GeneXpert in two independent samples) • Pre-existing Mtb. immunity is of interest for CoR investigation, as some TB antigen-specific markers will be constant/zero in the absence of Mtb infection, while others may vary due to BCG or NTM exposure • Markers with variability at baseline enable a subset of CoP methods (see Table 2). Understanding and adjusting vaccine CoR/CoP analyses for baseline immunity can guide interpretation and improve precision. • Baseline immunity may modify vaccine response or a vaccine CoR, and should therefore be assessed in all vaccine studies
2. Vaccine challenge studies in animals to evaluate vaccine efficacy against TB disease and to evaluate immunological markers as CoRs and CoPs for these endpoints (see Table 2 for definition of various types of CoPs)	• Strength of evidence will depend on animal model (e.g., cynomolgus vs. rhesus macaques vs. mouse) • Depending on model, endpoints could include sustained Mtb. infection, TB burden (e.g., lung CFUs) or lung pathology (e.g., PET/CT or necropsy scores)
3. Evidence for a biomarker as a mechanistic CoP, i.e., mechanism of protection (e.g., based on challenge models or passive transfer studies in animals or humans)	• Knowledge that a biomarker is connected to a mechanism of protection can be paramount for acceptance of a surrogate endpoint; such knowledge cannot be gained directly from Phase 2b/3 field efficacy trials, as a design with more experimental manipulation (e.g., immune response deletion) is required. • Passive transfer studies in humans or animals could be important (e.g., demonstration of a protective threshold CoP for a monoclonal antibody). Currently not aware of plans to study passive protection modalities in humans.
4. Phase 2b prevention-of-infection (POI) vaccine efficacy trial in Mtb. uninfected persons Evaluate CoRs and CoPs of biomarkers of Mtb. infection (e.g., sustained IGRA conversion or other assay when it becomes available)	• Surrogate markers of Mtb. infection may provide supportive but not sufficient evidence, to establish surrogate of disease. • As for study type 1, Mtb., BCG and NTM exposure impact the set of biomarkers that are relevant for certain correlates investigations. • Immunological biomarkers of interest are likely specific to Mtb. antigens represented in the vaccine • For BCG re-vaccination, many vaccine-specific biomarkers vary in both the vaccine and control groups (i.e., vary at baseline), such that CoRs can be assessed in both groups, and CoP methods requiring dual variability apply (see Table 2). • For non-BCG vaccination (e.g., subunit), many biomarkers will only vary in the vaccine group; such biomarkers are only assessed as CoRs in the vaccine group, and a subset of CoP methods no longer apply.
5. Phase 2b prevention-of-recurrence (POR) following TB treatment Evaluate CoRs and CoPs of biomarkers of recurrent TB disease	• Immunological biomarkers of interest are likely specific to TB antigens represented in the vaccine • With patients recently completing treatment, a large set of immunological biomarkers likely vary enough in each of the vaccine and control groups to assess CoRs in each group and to apply CoP methods requiring dual variability. Yet, the antigen content of the vaccine impacts whether certain biomarkers are only assessed as CoRs in the vaccine group and impact the set of applicable CoP analyses.
5. Phase 3 prevention of disease (POD) vaccine efficacy trial Evaluate CoRs and CoPs of biomarkers of TB disease	• Same remarks as for study type 4. or 5., for a POD trial in a Mtb. infected or heterogeneously exposed population.
7. Temporal ordering support of the above results, e.g., CoRs and CoPs are stronger for endpoint occurrence more proximal to vaccinations than distal, synchronized with the pattern of immunological biomarker waning	• Part of the Bradford-Hill criteria for inferring causality

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