End-point definition and trial design to advance tuberculosis vaccine development

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Given the substantial resources required for efficacy trials and the limited amount of funding available for TB vaccine development, it is crucial that trial end-points are carefully selected and study designs are as efficient as possible. https://bit.ly/3KmTGgZ

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Abstract

Tuberculosis (TB) remains a leading infectious cause of death worldwide and the coronavirus disease 2019 pandemic has negatively impacted the global TB burden of disease indicators. If the targets of TB mortality and incidence reduction set by the international community are to be met, new more effective adult and adolescent TB vaccines are urgently needed. There are several new vaccine candidates at different stages of clinical development. Given the limited funding for vaccine development, it is crucial that trial designs are as efficient as possible. Prevention of infection (POI) approaches offer an attractive opportunity to accelerate new candidate vaccines to advance into large and expensive prevention of disease (POD) efficacy trials. However, POI approaches are limited by imperfect current tools to measure *Mycobacterium tuberculosis* infection end-points. POD trials need to carefully consider the type and number of microbiological tests that define TB disease and, if efficacy against subclinical (asymptomatic) TB disease is to be tested, POD trials need to explore how best to define and measure this form of TB. Prevention of recurrence trials are an alternative approach to generate proof of concept for efficacy, but optimal timing of vaccination relative to treatment must still be explored. Novel and efficient approaches to efficacy trial design, in addition to an increasing number of candidates entering phase 2–3 trials, would accelerate the long-standing quest for a new TB vaccine.

Introduction

Tuberculosis (TB) remains a leading infectious cause of death worldwide [1]. The bacille Calmette–Guérin (BCG) vaccine, the only available licensed TB vaccine, has shown partial efficacy and effectiveness against childhood TB, but limited protection against adult pulmonary TB disease [2]. Thus, if we are to

meet the targets of TB mortality and incidence reduction set by the global End TB Strategy, new more effective adult and adolescent TB vaccines are urgently needed [3]. There are several new vaccine candidates at different stages of the clinical development pipeline [4]. Given the substantial resources required for efficacy trials and the limited amount of funding available for vaccine development, it is crucial that trial designs are as efficient as possible [5]. The selection of primary and secondary end-points for currently proposed approaches is of paramount importance. Clinically and epidemiologically relevant outcomes and how they relate to the stages of the natural history of TB need to be carefully defined (figure 1). In this article, we discuss possible approaches and knowledge gaps related to end-point selection in pre-licensure TB vaccine efficacy trials.

Prevention of infection (POI) approaches to accelerate candidate vaccines into prevention of disease (POD) efficacy trials

In recent years, there has been renewed interest in POI approaches, partially due to the positive efficacy signal associated with BCG revaccination in adolescence [6], but also due to the potential impact that successful POI candidates could have on POD approaches. Given that *Mycobacterium tuberculosis* (MTB) infections occur much more frequently than TB disease episodes, efficacy signals obtained by POI candidates would require a much lower sample size and a shorter duration of follow-up compared to those candidates tested in POD trials (sample size calculation depends on various parameters; for any set values of expected efficacy, significance level and power, a higher event rate would favour a smaller overall sample size). However, it is possible that a vaccine could have a differential effect in POI compared to POD.

The fundamental premise behind the search for vaccines that can prevent MTB infection is that by preventing infection, subsequent disease would also be prevented. TB progression occurs at its highest rate within 1–2 years after acquisition of MTB infection [7–9] and the lifetime risk of progression into active TB disease is around 10%. Interestingly, an existing MTB infection may protect from re-infection in animal models [10], and humans with immune sensitisation from earlier infection are less likely to progress to TB disease after re-infection [11]. Thus, ideally, a POI vaccine should prevent either all MTB infections (*i.e.* 100% efficacy) or at least all of the subset of MTB infections that would have progressed to TB disease. Although we do not know who among the infected will progress to disease, a POI vaccine with partial efficacy would likely have an important public health impact [12].

Measuring MTB infection as an end-point in clinical trials is complicated by the fact that there are no validated assays that directly measure the presence of viable MTB in healthy individuals. Rather, MTB

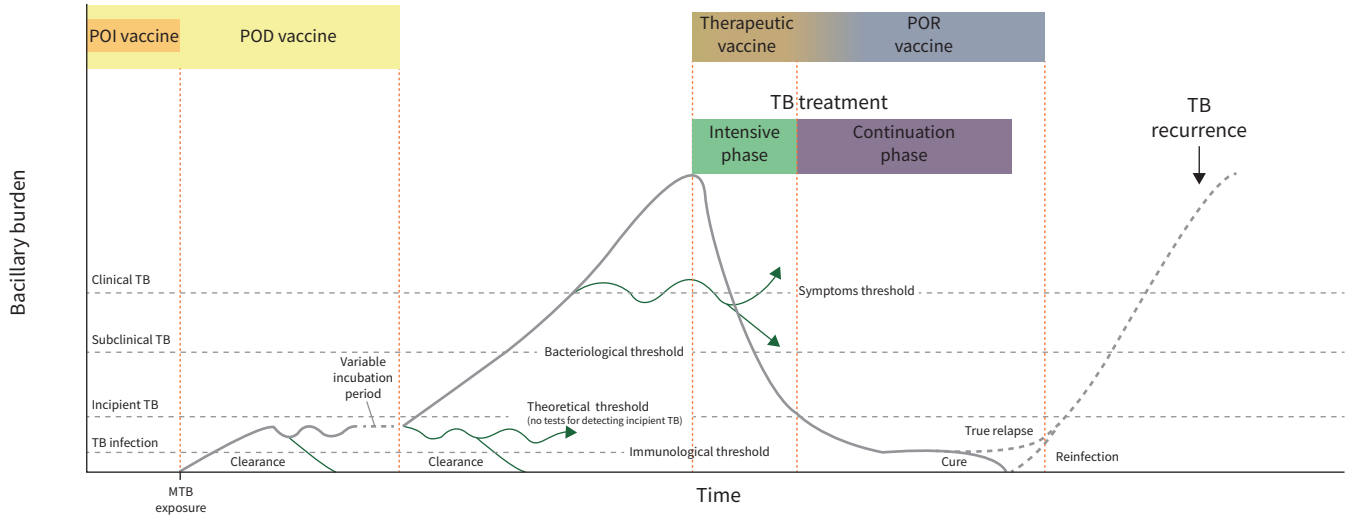

FIGURE 1 Vaccine strategies along the natural history of tuberculosis (TB), according to the expected effect. Vaccines developed with the strategy to prevent infection (POI) are mostly targeted for populations not yet exposed to *Mycobacterium tuberculosis* (*i.e.* pre-infection). Vaccines developed for prevention of disease (POD) could be useful if administered post- or pre-TB infection to prevent development of symptomatic disease. Therapeutic vaccines could have effects both in treatment-shortening and/or prevention of recurrence. Vaccines primarily developed for a prevention of recurrence (POR) strategy could be given with the aim of prevention of either re-infection or recurrence of disease (true relapse).

infection is inferred through indirect assays, such as purified protein derivative (PPD)-based tuberculin skin tests (TSTs) or interferon gamma release assays (IGRAs) [13]. These assays detect the presence of a cellular immune response to MTB-specific antigens, which is indicative of recent or past *in vivo* exposure to MTB [14, 15]. IGRAs have similar sensitivity to TSTs for identifying individuals with TB disease, but higher specificity, as they do not give false-positive results in those with previous BCG vaccination or exposure to environmental mycobacteria. In addition, IGRAs allow a more objective, laboratory-based, assessment in a single visit, whereas a TST needs a return visit to read the result which, in addition, is subject to intra and inter-reader variability [16]. Therefore, although many epidemiological studies employ PPD-based TST due to lower cost, the increased specificity and reduced need for repeat visits makes IGRA the measurement of choice for POI clinical trials.

Although higher levels of interferon-gamma (IFN-γ) detected by IGRA (at the time of IGRA conversion) have been associated with higher risk of TB progression (higher positive predictive value) [14, 15, 17], neither TSTs nor IGRAs accurately identify healthy individuals who will develop disease. Discovery and validation of novel biomarkers (*i.e.* whole-blood RNA transcriptional signatures including several combinations of genes) that predict progression from MTB infection to active TB disease are ongoing [18–20], but they are limited by a short prognostic horizon [21] and their applicability as clinical trial end-points has not been explored. In particular, it is not clear whether such biomarkers predict progression to disease that could be prevented by vaccination.

Most TB vaccine related studies have used an IGRA assay from the different QuantiFERON (QTF) generations (QTF-Gold, QTF-TB Gold in Tube or QTF-TB Gold Plus test, by Qiagen) as an indirect test for TB infection. QTF measures the IFN-γ response in $IU{\cdot}mL^{-1}$. IGRA conversion from a negative to a positive test is currently considered the most robust biomarker of acquisition of MTB infection that is a suitable end-point for clinical trials (table 1). However, serial IGRA testing is associated with substantial variability in the test results, which may result from both biological and technical variability (sample preparation and processing) [13, 17]. To partly address these issues, more stringent end-point definitions can be applied. These include defining "sustained" IGRA conversion as one (or more) negative test followed by at least two positive tests over 6 months. Sustained IGRA conversion has been hypothesised to represent persistent MTB infection, as opposed to a transient conversion to positive, followed by reversion to negative within 6 months. IGRA reversion occurs more frequently with low IFN-γ conversion values just above the assay cut-off [14, 22, 23]. The clinical significance of IGRA reversion remains to be established, but TST reversion has been associated with self-cured TB in humans and sterilised infection in guinea pigs [24]. Alternative cut-offs to interpret IGRA results may also be considered (for research purposes) to define alternative POI end-points, to exclude values falling in the "uncertainty zone" (IFN-γ 0.2–0.7 $IU{\cdot}mL^{-1}$) [17] or enrich for highest risk of TB progression (IFN-γ>4 $IU{\cdot}mL^{-1}$ at conversion) [14, 15], at the expense of lower sensitivity to detect new MTB infections. Table 1 shows the advantages and disadvantages of several possible POI end-points in vaccine efficacy clinical trials, many of them used in ongoing trials).

Three POI trials have been conducted in recent years, using these different end-point definitions as primary and secondary objectives. The first POI phase IIb trial, which assessed efficacy of H4:IC31 and BCG revaccination in adolescents [6], specified one primary end-point and several secondary end-points, all of them based on QFT Gold-in-Tube assay. The trial did not show efficacy for either H4:IC31 or BCG revaccination based on analysis of the primary end-point: QFT conversion from negative at baseline to $\geqslant 0.35\ IU{\cdot}mL^{-1}$ any time during follow-up. However, BCG revaccination showed 45.4% efficacy against the secondary end-point, sustained QFT conversion, defined as the conversion from a negative QFT at baseline to a positive QFT without reversion to negative status at 3 months and 6 months after conversion (*i.e.* three consecutive positive QFT results within 6 months). Upon initial conversion, QFT reversion rates were 46% in BCG and 25% in placebo recipients. A potential explanation could be that some new MTB infections are transient, and the QTF result would correspondingly be transient. This assumption, for which no direct evidence is yet available, would imply that individuals with QFT reversion might have cleared infection and have lower risk of progression to TB disease. Conversely, sustained QFT conversion could be hypothesised to represent persistent MTB infection, which would be associated with a higher risk of progression to TB disease (figure 2 shows the assumed IGRA dynamics after vaccination with a POI candidate). Notably, BCG revaccination also showed 45% efficacy in preventing QFT conversion when defined as $>4\ IU{\cdot}mL^{-1}$ (exploratory end-point).

A new larger phase IIb POI trial of BCG revaccination is currently being conducted in South Africa with sustained QFT conversion as the primary end-point (*i.e.* a prevention of sustained conversion trial) and QFT conversion when defined as $>4\ IU{\cdot}mL^{-1}$ as secondary end-point (NCT04152161). Another POI

TABLE 1 Advantages and disadvantages of possible end-point definitions for tuberculosis (TB) vaccine efficacy trials

	Advantages	Disadvantages
POI		
IGRA (QTF) conversion (>0.35 IU·mL^{-1})	~10-fold more frequent than TB disease Widely used threshold in routine practice Higher sensitivity for detecting MTB infection compared to higher thresholds	Does not detect all true MTB infections IGRA reversion is common, but of unclear clinical significance Significance of protection unclear Test performance in PLHIV, people with immune-mediated inflammatory diseases taking immunosuppressive treatment, and very young children is unclear
IGRA (QTF) conversion (>4.0 IU·mL^{-1})	Higher risk of progression to TB disease than conversion at manufacturer threshold Higher specificity for detecting MTB infection than manufacturer threshold	Less frequent event than conversion at manufacturer threshold Lower sensitivity for detecting MTB infection than manufacturer threshold
Sustained IGRA conversion (6 months)	Might represent sustained (persistent) MTB infection Might be associated with higher risk of TB disease compared to a single conversion Lower risk of false-positive result compared to a single test	Less frequent event than initial IGRA conversion Does not encompass IGRA conversion–reversion–conversion events Need for TB preventive therapy precludes nested POI in POD efficacy trial design
POD		
MTB liquid culture (sputum)	Gold standard (most sensitive) tool Allows genotyping of MTB strain	Need for central laboratory Variable contamination rate (largely laboratory-dependent) Lower yield in pauci-bacillary TB disease: - HIV-associated TB - childhood TB
- One sample	Logistically simple Lower sensitivity	Potential false-positive results
- Two or more separate samples (processed independently)	Higher specificity (if both need to be positive)	Logistically complex Less frequent than single positive sample Decreased sensitivity (if both need to be positive), especially in pauci-bacillary TB disease: - HIV-associated TB - childhood TB
- Before treatment of the TB episode starts	Not affected by effect of vaccination on response to TB therapy	Logistically complex Lower sensitivity than before or after treatment starts
Xpert Ultra (sputum)	Does not need central laboratory Available at district-level hospitals Rapid turnaround	Does not allow to genotype MTB strain Lower sensitivity and specificity than culture (higher false-positive rate, especially among trace results)
MTB liquid culture OR Xpert Ultra (sputum)		
- With symptoms	Protection associated with direct health benefit	No opportunity to assess vaccine efficacy against subclinical disease
- Without symptoms	Allows assessment of vaccine efficacy against subclinical TB disease	Significance of clinical protection unclear Need for TB treatment precludes subsequent assessment of vaccine efficacy against symptomatic TB disease
Digital chest radiograph (with or without CAD)	High sensitivity for TB, widely available and with high added value in populations with paucibacillary disease, such as: - HIV-associated TB - childhood TB	Limited specificity
Urine LAM	High specificity in PLHIV with low CD4 counts	Limited sensitivity with increasing levels of CD4 counts
TB symptoms (clinical diagnosis)	High sensitivity, especially in pulmonary TB among HIV-negative individuals, cheap, no laboratory infrastructure needed Sometimes used to define unconfirmed TB	Limited specificity Subjective interpretation of symptoms
Prevention of recurrence/therapeutic		
M. tuberculosis liquid culture (sputum)	Gold standard Allows genotyping of MTB strain (true relapse *versus* reinfection) if collected before treatment	Need for central laboratory
Xpert Ultra (sputum)	Logistically simple at treatment start	Cannot distinguish viable from non-viable MTB bacilli No opportunity to genotype MTB strain

CAD: computer-aided detection; IGRA: interferon gamma release assay; LAM: lipoarabinomannan MTB: *Mycobacterium tuberculosis*; PLHIV: people living with HIV; POD: prevention of disease; POI: prevention of infection; QFT: QuantiFERON-TB.

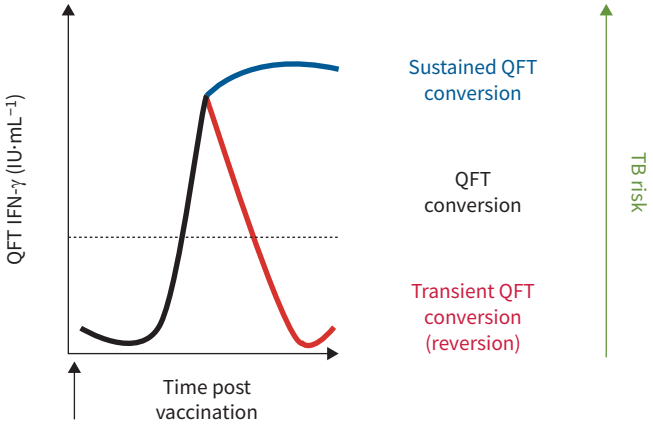

FIGURE 2 Hypothesised interferon gamma (IFN-γ) release assay (IGRA) dynamics after vaccination with a prevention of infection candidate. QuantiFERON-TB (QFT) negative participants receiving vaccine or placebo. Rates of acquisition of *Mycobacterium tuberculosis* infection (QFT conversion), established infection (sustained QFT conversion through 6 months post conversion) and transient infection (QFT reversion within 6 months post conversion) are compared between the study arms. Participants with sustained QFT conversion and QFT >4 IU·mL^{-1} are considered at higher risk of tuberculosis compared to non-converters and reverters. The dotted line represents the standard QFT cut-off at 0.35 IU·mL^{-1}.

candidate, the inactivated *Mycobacterium obuense* vaccine DAR-901, was recently tested in a phase IIb POI trial in Tanzanian adolescents with a lower force of MTB infection than that observed in South Africa (NCT02712424). DAR-901 did not show efficacy against either initial or sustained IGRA conversion in this trial [25].

Besides representing indirect evidence of MTB infection, IGRAs and TSTs have other limitations. Some people with culture-confirmed TB disease are QFT-negative, suggesting that there are individuals who do not convert their QFT at the time of infection (or that they reverted their QFT status but nonetheless progressed to disease). For example, it has been shown that 5–10% of heavily exposed TB contacts show persistent QFT-negative results, despite having evidence of TB exposure through humoral response against different MTB antigens [26, 27]. Therefore, the true MTB infection status in these individuals is uncertain and they would not be identified by IGRAs as infected, highlighting the need to explore other immunological assays to ascertain TB infection status. In addition, people living with HIV (PLHIV) who are immunosuppressed or people with immune-mediated inflammatory diseases taking tumour necrosis factor-α inhibitors might also have a poorer IFN-γ response, affecting the interpretation of IGRA results. Conversely, evidence suggests that adaptive immune responses (positive IGRA or positive TST) have been shown to persist for long periods in individuals who have very likely cleared the MTB infection and who have very low risk of progression to TB [28]. Modelling analyses suggest that the number of people harbouring viable MTB at any given time worldwide might be much lower than previously estimated by the number of IGRA-/TST-positive individuals [29, 30]. Therefore, while detection of IGRA conversion is considered a biomarker for acquisition of MTB infection, it is possible that some individuals with sustained conversion may also clear infection [31].

Modified IGRAs may also be needed to evaluate MTB infection in study populations who have received novel TB vaccines expressing antigens that are also included in currently available commercial IGRA. For example, ESAT6- and CFP10-free IGRAs may be needed to evaluate MTB infection in individuals who have received H56:IC31 or MTBVAC, respectively, for which traditional IGRAs would not distinguish between vaccination and MTB infection. A promising ESAT6-free assay has recently been developed with similar accuracy as QFT for detecting established MTB infection [32].

Optimising TB disease end-points for future efficacy trials

POD

In addition to utilising POI trials to accelerate and streamline down-selection of candidates to progress to further stages of development, there is a need to optimise the selection of efficacy end-points in POD trials. For POD studies, a foundational question is “what is the definition of TB disease?”. The answer to

this question helps to guide the screening procedures necessary at baseline to rule out prevalent disease prior to vaccination, and to guide the tests used in follow-up to ascertain incident disease outcomes as a function of vaccination status and thus to estimate vaccine efficacy. Broadly speaking, there are two potential angles to the definition of TB disease – one pertaining to illness regardless of transmissibility and a public health perspective that puts an emphasis on potential for transmission. While it is clear that an individual could be both symptomatic and contagious, the growing literature around subclinical tuberculosis [33], in which an individual is asymptomatic but is found to have positive microbiologic tests for MTB, allows further profiling of TB disease based upon expected transmissibility.

Regardless of the TB case definition, it is paramount that prevalent cases at baseline, which could not be prevented by vaccination, are identified and excluded. Simple symptom screening would overlook individuals with existing subclinical TB disease, who might progress to symptomatic disease during the trial period, and in consequence underestimate the true vaccine efficacy. For this reason, it would be important to perform microbiological assessments at enrolment on all subjects.

A phase 2b M72/AS01$_E$ prevention of disease study (NCT01755598) [34] excluded randomisation individuals who either had symptoms of TB at screening or were Xpert-positive at screening (Xpert is a rapid molecular test based on detection of MTB nucleic acid). No significant vaccine efficacy was demonstrated during the first year after vaccination, whereas considerable efficacy against disease was reported in the second and third year of the trial. Therefore, it might be hypothesised that despite efforts to exclude participants with active tuberculosis, incipient or subclinical TB cases might have been missed in this study, and these were the cases seen in the first 9–12 months, which occurred at similar rates in the vaccine and placebo arms, before incidence in the vaccine arm began to decline. A recent national TB prevalence survey from South Africa demonstrated that among 234 survey cases (220 culture-positive and 14 culture-negative), 57.7% of the individuals had abnormal chest radiography (CXR) with no clinical symptoms and only 6% of cases with abnormal CXR were negative by culture, suggesting screening radiology could be useful to exclude asymptomatic individuals with TB from clinical trials [35]. The recent World Health Organization (WHO) systematic screening guidelines for TB disease also recommend CXR in those populations in which TB screening is indicated, especially in PLHIV, in whom CXR improves the sensitivity of the WHO-recommended four-symptom screen in those on antiretroviral therapy (ART) [36]. However, CXR reading requires either human resources, with unavoidable inter-rater variability, or computer-aided detection (CAD) software, for which the pooled specificity ranges from 54 to 60% when benchmarked at 90% sensitivity [37]. This significant limitation applies to both baseline CXR screening to determine eligibility and as a component of algorithms to define unconfirmed TB disease.

Obtaining samples from study participants to ascertain disease end-points might not always be feasible. Indeed, not all participants, even those with TB, are able to produce an expectorated sputum sample for microbiological confirmation. In those unable to produce sputum spontaneously (sputum unproductive), induction with hypertonic saline followed by two or three samples collected on the same day has been recommended for clinical purposes, avoiding the complex logistics of requesting three samples on three separate days [38].

Selection of the efficacy end-point will depend on the primary goal of the trial. If the goal is to reduce the rate of incident symptomatic TB by vaccination, the presence of symptoms and a positive microbiological test during follow-up will suffice to ascertain incident TB cases. If, however, the goal is to reduce the development of any MTB-culture positivity, then serial sputum tests would be needed to look for all new potentially contagious cases that might impact MTB transmission, including subclinical disease. Of note, nucleic acid amplification tests would not be a substitute for culture in patients with recent previously treated TB since the technology cannot discern live from dead tubercle bacilli. It should also be noted that regular microbiologic screening to detect subclinical or asymptomatic TB would not allow subsequent progression to symptomatic disease, as all individuals with microbiologically confirmed TB would be referred for treatment, halting further disease progression. Thus, it might not be possible to properly ascertain both subclinical and symptomatic TB disease end-points in the same trial. One alternative approach would be microbiologic screening of all participants at the end of the study, regardless of presence or absence of symptoms. Collecting sputum samples in all participants at different time points for retrospective microbiological testing at the end of the study could also shed light on the efficacy against subclinical disease.

One question that arises is whether an isolated positive test is sufficient to define disease for the detection of incident TB cases. Based on published literature indicating that the rate of false-positivity is 1/50 to

1/100 [39, 40] (Xpert MTB/RIF or culture, respectively), it is unlikely that a single positive test would falsely assign any one individual. However, multiple false-positives in both arms of a trial could have the effect of decreasing effect size, *i.e.* biasing the efficacy estimate for an effective vaccine towards zero. Given that the goal of the baseline test is to screen out individuals who may develop symptomatic disease due to already existing subclinical TB, it would be reasonable to use a single positive result for this purpose. However, since ascertainment of TB disease end-points after the vaccine intervention should be highly specific, two or more tests should ideally be used to define the primary outcome. Table 1 shows the advantages and disadvantages of several possible POD end-points in vaccine efficacy trials, many of them used in ongoing trials.

Two special situations require additional consideration: paediatric TB and extrapulmonary TB (EPTB). In high-burden TB settings, there is a bimodal curve of paediatric TB disease incidence with a peak <2 years, a trough from age 5–10 years, and a second upward trend starting in adolescence. The first peak in very young children is associated with a high risk for morbidity and mortality due to disseminated and severe forms of TB disease. Children under age 5 years may be investigated by gastric lavage or induced sputum and nasopharyngeal aspirate after hypertonic saline nebulisation, but diagnostic yield seldom exceeds 30%, even in hospitalised children [41, 42]. Since true TB disease in young children may be missed by a case definition requiring microbiologic confirmation, a composite end-point including evidence of MTB exposure and/or infection, presence of symptoms, and radiologic evidence of intrathoracic TB may be used [43]. However, definition of MTB infection may not even be possible in trials of vaccines that cross-react with IGRA without the development of new tools. While such composite end-points are less specific than one requiring microbiologic confirmation, specificity may be improved by applying stringent definitions for exposure and compatible symptoms, and by applying a standardised approach to radiologic evaluation by an expert panel. Unfortunately, CAD solutions for paediatric TB are still under evaluation.

EPTB disease has also been excluded from the primary end-point of the M72 efficacy trial (conducted among adults aged 18–50 years) and it has not been included as a standalone end-point amongst the secondary end-points [34]. Likely reasons are the lower bacteriological confirmation rate found in EPTB patients, the lower frequency of EPTB events overall, and the possibility of different vaccine-induced immune responses against pulmonary TB and EPTB, which would hinder the efficacy assessment of the most common TB form, pulmonary TB. A joint secondary end-point including pulmonary and extrapulmonary tuberculosis disease should be explored in future POD trials. EPTB in children is an important outcome and one of the reasons why BCG is given at birth is to prevent miliary and meningeal TB. However, the number of such cases in modern BCG clinical trials has been remarkably small [44], and most data have been generated in case-control studies [45, 46]. For future trials, an optimal diagnosis of EPTB (for children and adults) should include microbiologic assessment (single positive nucleic acid amplification test or culture) and/or histopathologic evidence of necrotising granulomas with acid-fast bacilli, but the number of end-points is likely to be too small to provide a precise estimate of protective efficacy for EPTB alone.

Prevention of recurrence (POR) and therapeutic approaches

In this section we include considerations for both classical POR trials, in which vaccination occurs towards the end or at the end of TB treatment and can only affect post-treatment outcomes, and therapeutic trials, in which vaccination occurs during TB treatment and may affect both on- and post-treatment outcomes (figure 1) [47]. It is thus apparent that timing of vaccination is likely critical to the magnitude and scope of potential benefit to TB patients. Interest in clinical trials of POR approaches has been growing, driven in part by the potential to fast-track vaccine development by conducting small, less costly trials in TB patient populations, in whom recurrent TB end-points accrue several times faster than TB disease end-points in the general population [48]. A study from Cape Town showed that 18% of patients with a first TB episode in 2013 had at least one further episode by 2016 [49]. Therapeutic vaccine approaches for TB patients, in whom treatment failure is likewise more common than incident TB in the general population, also have potential to directly reduce immediate post-treatment pulmonary morbidity and mortality in patients treated for either drug-sensitive TB (DS-TB) or drug-resistant TB (DR-TB).

POR approaches, in which vaccination occurs at the end of treatment when bacillary and antigen load is low, may be the preferred trial design for candidate vaccines with an unknown safety profile in TB patient populations. It is likely that the potential for harm, in the form of excessive systemic or local inflammatory reaction to vaccination, including the so-called Koch phenomenon [50], is reduced as the vaccination time point shifts further from the start of TB treatment and bacillary burden decreases. It would also be expected that for trials including PLHIV, the potential immune dysregulation of therapeutic vaccines is minimised as the time to vaccination increases in relation to ART initiation. Conversely, the potential for

on-treatment benefit would increase as the vaccination time point shifts closest to the start of treatment. Since there is also potential for overlapping drug/vaccine toxicities early during the treatment phase, a time de-escalation approach could be used to select the optimal time point for therapeutic vaccination [47], in which vaccination shifts iteratively towards treatment start in the absence of safety signals from prior cohorts. In addition to injection site reactogenicity, safety end-point evaluation might be enhanced by comparison of lung inflammation measured by positron emission tomography or computer tomography [51, 52]. This approach would allow time de-escalation for evaluation of safety to occur in real time, without waiting for immunogenicity end-point analyses.

The primary efficacy end-point for both POR and therapeutic TB vaccine trials is microbiologically confirmed pulmonary TB, occurring in the post-treatment period and/or at the end of treatment, respectively [53]. An efficacy signal from a POR or therapeutic vaccine trial might be used to green-light a conventional POD trial in the general population. However, it must be acknowledged that the mechanism of vaccine-mediated eradication of live bacilli in the context of ongoing or recent disease may differ from that required to prevent progression to disease, whether in uninfected individuals or those with asymptomatic MTB infection. Further, it would be ideal to understand whether a POR vaccine protects against post-treatment true relapse (endogenous reactivation of bacilli not cleared by TB treatment), reinfection, or both. For this, enrolment at the start of treatment may be required to obtain the MTB genotype, and an increased sample size would be required to power efficacy estimates for each end-point separately. In addition, a particular candidate could hypothetically protect against reinfection, but not against true relapse, or *vice versa*. In both situations, the observed vaccine efficacy would underestimate the true vaccine efficacy specifically for a particular protective mechanism, while the extent of underestimation depends on the incidence of the phenomena against which no protection is afforded.

The potential for therapeutic vaccination to improve treatment outcomes for both DS- and DR-TB hinges not only on the vaccine-specific immune response, but also on the potential for vaccine-mediated improvement in reducing treatment duration, adverse effects, and outcome, which may be specific to a drug regimen. Therapeutic vaccination could have a significant impact in DR-TB patients, as their treatment has traditionally been lengthy, toxic, poorly tolerated, with low rates of cure and high rates of mortality. DR-TB patients demonstrated a lower risk of post-treatment recurrence than DS-TB patients [54], either because many patients with DR-TB did not survive to cure or because of the long treatment duration. However, treatment outcomes for DR-TB using new regimens are comparable to DS-TB [55]. Patients with moderate and hard-to-treat TB, both DS- and DR-TB, might benefit from a POR vaccine to reduce the risk of recurrence. In both DS- and DR-TB, a therapeutic TB vaccine may further contribute to treatment-shortening and reducing post-TB treatment morbidity and mortality, and towards preventing resistance. Consequently, the inclusion of post-TB lung function-related secondary end-points (measured at baseline and after treatment) could provide additional information on vaccine capacity to prevent lingering sequelae.

It is possible, if not likely, that the magnitude of a POR or therapeutic vaccine efficacy signal would be specific to a particular drug regimen, due to the interaction of the therapeutic effect on antigen load and potential vaccine-mediated immune response. Given the progress in new shorter effective regimens for both DS- and DR-TB, POR or therapeutic vaccines should be evaluated against different treatment regimens, not only the current standard of care. This is particularly true if vaccine-mediated reductions in the rates of adverse treatment outcomes, including pulmonary morbidity, treatment failure, mortality and recurrence, are to be parlayed into further reductions in treatment regimen duration or complexity. The primary challenge to this approach is that TB-recurrence end-points accrue slowly and late in trial follow-up, too late to allow adaptive designs to advance optimal vaccine-regimen combinations or conversely to halt suboptimal vaccine-regimen combinations. It seems that implementation of a TB treatment-shortening strategy using adjunctive therapeutic vaccination would require multiple, sequential trials, first incorporating vaccination at different time points and thereafter incorporating different treatment regimens for both DS- and DR-TB.

End-point definition in vaccine trials including PLHIV

MTB-infected PLHIV are at higher risk of progression to TB disease and of worse treatment outcomes [54]. Thus, PLHIV are a priority group for TB-preventive strategies (preventive treatments or vaccines) and to improve treatment outcomes (therapeutic and POR vaccines) [55]. Safety end-points are of special importance in this population and safety-related concerns have been key arguments precluding the inclusion of PLHIV in certain TB vaccine clinical trials. In order to progress into clinical development, vaccines need to show an appropriate safety profile in early stages, and this would be of even greater importance in trials involving live attenuated vaccines that included untreated or immunocompromised PLHIV.

However, modelling has demonstrated the potentially important population-level impact of protection of PLHIV through TB vaccination in high HIV prevalence settings [12]; so non-immunosuppressed PLHIV should be included in trials where possible, ideally in pre-licensure trials so that this population can be included in the indication at launch.

POI trials including PLHIV face added challenges regarding the measurement of efficacy end-points, since current tools to measure TB infection are even less optimal in PLHIV than in people without HIV infection. TST sensitivity is lower among PLHIV [56]. Evidence on the diagnostic performance of IGRA to detect recent MTB infection among PLHIV with low CD4 counts is limited. In addition, the predictive value of a given IGRA conversion threshold to develop TB is still uncertain in HIV-uninfected individuals. It would seem reasonable to include PLHIV on ART in POI trials, but not immunosuppressed individuals, due to the uncertainty about the diagnostic performance and interpretation of MTB infection tests and the likely robust response required to show vaccine efficacy.

Efficacy end-points in phase IIb/III POD trials including PLHIV follow similar arguments as for HIV-negative individuals. Capturing subclinical TB as an end-point (or screening for subclinical TB at enrolment) might have the same relevance for PLHIV, as this phenotype seems to be similarly prevalent irrespective of HIV status [35, 57]. However, traditional sputum-based diagnostic tools might be less sensitive for TB disease in PLHIV, and the inclusion of urine-based diagnostics (TB- lipoarabinomannan (LAM), urine Xpert) might be considered [58, 59]. However, although these tools, including the promising FujiLAM [60], may be sensitive for TB diagnosis among PLHIV with low CD4 counts, they lack specificity. EPTB is also more frequent in PLHIV, and this form of TB may need organ-specific diagnostic approaches [61].

The potential inclusion of less stringent end-points for any unconfirmed TB case definition for PLHIV, including the use of digital CXR and CAD, epidemiological linkage, and symptom screening could be explored, as has been done for preventive therapy trials among PLHIV and children [62, 63]. However, it must be acknowledged that such approaches unavoidably decrease specificity and would be a major challenge for inclusion of PLHIV in POD efficacy trials, since the TB disease end-point should be identical for all participants, with and without HIV infection. A need for specially designed TB end-points for immunosuppressed PLHIV might preclude participation in critically important licensure studies. Therefore, until new and better diagnostic tools are developed, the TB end-point definition for inclusive vaccine efficacy trials might not be optimally sensitive for TB disease in immunosuppressed PLHIV. However, PLHIV should be included in efficacy trials for safety and immunogenicity assessments, even if they are not powered to demonstrate vaccine efficacy for POD in this subgroup. If shown to be safe and immunogenic in phase 3 trials, vaccine could potentially be recommended for use in PLHIV once marketing approval is granted.

Conclusions

Recent advances in proof-of-concept efficacy trials have renewed hope for the development of a successful candidate vaccine that could accelerate efforts to decrease the unacceptable global burden of TB. POI approaches offer an attractive opportunity to green-light and accelerate new candidate vaccines to advance into large and expensive POD efficacy trials. However, POI approaches are limited by imperfect current tools to measure MTB infection end-points and uncertainty around the significance of POI for protection against progression to TB disease. Therefore, new and better tests to determine established MTB infection are needed, as well as establishing the clinical significance of IGRA reversions.

The potential contribution of undiagnosed, subclinical TB disease to the epidemic has received increasing attention. However, although subclinical TB may or may not be an important component of MTB transmission, the extent to which subclinical disease progresses to symptomatic TB disease, and thus its importance for individual morbidity and mortality, needs to be elucidated. For this reason, and given that parallel surveillance for both subclinical and symptomatic TB in a clinical trial setting does not seem feasible, the primary end-point for POD efficacy trials that include participants with and without HIV infection is likely to remain symptomatic microbiologically confirmed TB disease. However, it might be feasible to include subclinical TB at end of follow-up as a secondary end-point, which would not affect the ability to demonstrate POD efficacy for symptomatic TB cases during the trial.

The design of therapeutic and POR trials to test new TB vaccine candidates is primarily affected by the timing of vaccination relative to the end of TB treatment. Crucial outstanding questions include whether vaccination close to the start of treatment is safe and can offer an immune-mediated benefit for treatment outcomes. In addition, the extent to which the vaccine efficacy of POR or therapeutic trials can be

extrapolated to direct protection against progression to TB disease needs to be elucidated, given the different immunological status of recipients and the potentially different mechanisms of vaccine-mediated protection. Regardless, demonstration of efficacy for the therapeutic or POR indication would allow incorporation of TB vaccines into trials of shorter and simpler therapeutic regimens.

Improved diagnostic tools and strategies for both MTB infection and TB disease are being developed, which may be useful in the design and selection of end-points for TB vaccine trials.

Efficient approaches to efficacy trial design, in addition to an increasing number of candidates entering phase 2–3 trials, would contribute to acceleration of the long-standing quest for a new TB vaccine.

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