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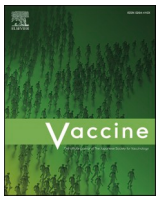

Sample size efficiency of restricting participation in tuberculosis vaccine trials to interferon-gamma release assay-positive participants

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ABSTRACT

Background: A common approach to reducing sample sizes for late-stage tuberculosis vaccine trials is to restrict enrolment to interferon-gamma release assay (IGRA)-positive participants to maximize tuberculosis case accrual. The efficiency gain, if any, from this screening strategy is unknown.

Methods: We estimated the age specific IGRA positivity prevalence for transmission levels generally considered in tuberculosis vaccine trials (annual risk of tuberculosis infection [ARTI] 2–6 %) and calculated the expected tuberculosis incidence at each age by IGRA status, using a difference equation model. We modelled scenarios that assumed constant or increasing ARTI during adolescence and differing levels of partial protection afforded by previous *Mycobacterium tuberculosis* infection. We then estimated sample size requirements for tuberculosis vaccine trials enrolling only IGRA-positive participants or participants without prior IGRA testing ('Mixed' trial). We assumed participants were 15–44 years at enrolment and followed-up for 3 years.

Results: Estimated tuberculosis incidence was 4.7 times higher in IGRA-positive compared to IGRA-negative participants at age 15 years, but 0.9 times lower at age 44 years (assuming ARTI 4 %). This age-cohort effect was exacerbated when assuming partial protection and attenuated when assuming increasing ARTI during adolescence. In a model that included both these assumptions, the sample size required for a Mixed trial compared to that for an IGRA-positive participants-only trial was 124 % larger at 2 % ARTI, 36 % larger at 4 % ARTI but only 8 % larger at 6 % ARTI. Prioritizing enrolment of participants aged 15–29 years improved sample size efficiency for an IGRA-positive participants-only trial. These results were largely unaffected by our model assumptions.

Conclusion: In late-stage tuberculosis vaccine trials among adults and adolescents, pre-enrolment screening by IGRA testing provides a large sample size efficiency when *M. tuberculosis* transmission levels are relatively low, but modest or no sample size benefits at high transmission levels.

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1. Introduction

With 10.8 million people newly affected and 1.25 million deaths in 2023, tuberculosis remains a major global health threat [1]. To curb the epidemic and eliminate tuberculosis, new vaccines that effectively protect adults and adolescents against the disease are an urgent priority [2]. Several tuberculosis vaccine candidates are in late-stage phase 2b or 3 prevention of disease trials, including GamTBVac, Immuvac (MIP), M72/AS01$_E$, MTBVAC and VMP1002, while several more are considered for late-stage trials [3]. Such trials are large, long and expensive due to the low incidence and long incubation period of tuberculosis [4]. Therefore, changes in enrolment strategies, follow-up and endpoints of late-stage tuberculosis vaccine trials are being explored that may improve their efficiency by reducing the required sample size, which would improve their feasibility and reduce their cost [5].

One innovation may be to enroll all eligible participants regardless of their interferon-gamma release assay (IGRA) status [5]. Enrolment into late-stage tuberculosis vaccine trials may be restricted to IGRA-positive participants for two reasons. First, the vaccine candidate may be expected to have better protective efficacy in those already sensitized to *Mycobacterium tuberculosis* (Mtb) by boosting the existing immune response. This has been put forward as the rationale for conducting the phase 3 trial of M72/AS01$_E$, that showed better immunogenicity in IGRA-positive compared IGRA-negative individuals [6], in an IGRA-positive population (NCT06062238). Second, a trial in an IGRA-positive population may be considered more efficient in terms of sample size than one in IGRA-negative or mixed IGRA-positive/negative populations. Past enrolment strategies have focused on IGRA-positive individuals under the assumption that they are more likely to progress to tuberculosis. In a meta-analysis of 33 cohort studies conducted in low, intermediate and high tuberculosis burden countries without age restriction, IGRA-positive individuals had a 9.35 times increased incidence of tuberculosis disease progression over a period of at least 12 months compared to IGRA-negative individuals [7]. In a cohort study in South Africa, the incidence of tuberculosis was 8.54 times higher in QuantiFERON TB Gold In-tube (QFT, a whole blood IGRA) positive compared to QFT-negative adolescents [8].

It has therefore been argued that a tuberculosis vaccine trial done among IGRA-positive individuals can have a much smaller sample size than a trial done among IGRA-negative individuals while accruing the same number of TB disease endpoints [6]. However, those involved in clinical trial design may not be aware that IGRA status is an imperfect surrogate marker of future risk of developing pulmonary TB and that this efficiency gain in terms of required sample size (henceforth called 'sample size efficiency') may be less for an adult trial population than for one of adolescents. Most progression to disease occurs in the first years following Mtb infection established by IGRA or tuberculin skin testing (TST), and the older the trial population, the longer the average period from infection will be [9], although this may in part depend on differences in Mtb infection rates between different age groups [10]. Furthermore, previous Mtb infection may provide protection against disease progression following reinfection [11] [12], potentially reducing the rate of Mtb reinfection and primary progression to tuberculosis among those already IGRA-positive. Finally, the sample size efficiency gain of exclusively enrolling IGRA-positive trial participants may depend on the level of Mtb transmission in the population.

We modelled the sample size required for a tuberculosis vaccine trial among adults and adolescents that only enrolls IGRA-positive individuals, compared to a trial that enrolls both IGRA-positive and IGRA-negative individuals ('Mixed') in the same population. We further investigated and quantified the effects of variation in assumptions – specifically, an increase in infection rate during adolescence, disease protection conferred by previous infection, level of transmission in the population and age at trial enrolment – on the efficiency and sample size required from these trials.

2. Methods

We used a difference equation model (MS-Excel, Microsoft Corp, USA) to create hypothetical populations in which we calculated the IGRA-positive prevalence per one-year age band from birth for a given annual risk of infection (ARTI). Difference equations model changes in discrete intervals, in this case one year, where all changes between intervals are assumed to occur at once [13]. We defined the ARTI as the number of new Mtb infections leading to IGRA conversion or reinfections per 100 person-years and assumed no deaths and no in- or outmigration. We then calculated the expected incidence of new pulmonary tuberculosis at each age as the sum of incidences for the current and the preceding nine 1-year bands. We assumed that IGRA-positive *Mtb* infections progress to tuberculosis in the first 10 years after IGRA conversion at a rate that follows a specified distribution over this period (Table1, Fig. S1) [11,14]. Cumulative progression risk was assumed to be 10 % based on historical cohorts using TST [9], broadly equivalent to the progression risk for the manufacturer-recommended cut-off for QuantiFERON (0.35 IU/ml) [7,15]. Age-specific incidences of tuberculosis in one-year age groups were calculated for individuals with a positive IGRA test, for those with a negative IGRA test, and for the entire modelled population regardless of the IGRA results. Tuberculosis vaccine trials and cohort studies in sites developed for such trials showed ARTI estimates between 2 and 3 % in Kenya and Tanzania [16,17], 4 % in Uganda [18], and 6 to 7 % in extremely high incidence settings in Western Cape, South Africa [8,19]. We therefore used 2–6 % as the range of ARTI values with 4 % as our primary value.

From these incidences we calculated the expected number of tuberculosis endpoints in a hypothetical vaccine trial that only enrolled IGRA-positive participants, versus a Mixed trial that enrolled both IGRA-positive and IGRA-negative individuals without prior IGRA screening. We assumed that the trial enrolled adolescents and adults aged 15 to 44 years, typical of efficacy trials, with equal numbers for each of the thirty 1-year age groups, and the follow-up duration was 36 months for all participants with no deaths or losses to follow-up. In the Mixed trial, the age-specific proportions IGRA-positive and negative were assumed to reflect those of the population, i.e., enrolment was population representative. Participants were censored when diagnosed with tuberculosis which happened at 12-, 24- or 36-months post-enrolment. We then compared the sample sizes needed to accrue 50 TB disease endpoints in the placebo arm for each trial, i.e., without making assumptions about the protective efficacy of the vaccine candidate. This model was run with different sets of assumptions (Table 1).

The Base Model assumed that the overall ARTI was constant over time (i.e., a long-term endemic situation), that all infections that could result in disease progression lead to IGRA conversion, that disease progression rates were independent of age, and that IGRA reversions only occurred within the first year after conversion. Since our difference equation model had a one-year time step, IGRA reversions were not explicitly modelled. The Base Model in addition assumed the ARTI was constant with age and reinfections occurred at the same rate of progression as first infections.

Our Age Varied ARTI Model differed from the Base Model by assuming the ARTI was lower in children than in adults and that the ARTI increased linearly between 13 and 18 years of age [20] [21]. The Partial Protection Model differed from the Base Model by assuming prior IGRA conversion conferred protection against disease progression resulting from reinfection, irrespective of the number or timing of previous (re-)infections [12].

Our Combined Model included the assumptions of both the Age Varied ARTI Model and the Partial Protection Model. Using this model, we furthermore explored the effect on required sample sizes of varying the age distribution of trial enrollment from a 3-times higher enrollment in age group 15–29 years than in age group 30–44 years, to 3-times higher enrollment in age group 30–44 years than in age group 15–29 years.

Table 1
Model assumptions.

Parameter	Model default value	Sensitivity analysis values low - high	References
All models			
ARTI at adult age[i]	0.04	0.02–0.06	[10,15,16,8,18,17]
TB disease progression rate following IGRA conversion (/year)	0.10	0.05–0.20	[9]
Period post-IGRA conversion during which disease progression occurred (years)	10		
Proportion of progression that occurs in the:	0.580	0.474–0.859	[14,11,21,22]
1st year	0.238	0.179–0.112	
2nd year	0.182	0.347–0.028	
3rd-10th year following IGRA conversion[ii]			
Partial protection models			
Proportion TB disease progression risk upon reinfection	0.21	0.105–0.42	[12]
Age-dependent models			
Proportionally lower childhood ARTI compared to adult ARTI	0.50	0.25–0.75	[21,19,20,27,33,28]
Relative increase in ARTI during ages 13–18 years (proportion/year of age)	0.10		[19]

i: Likely range of adult ARTI values in populations selected for phase 3 tuberculosis vaccine trials. ii: See Fig. S1. iii: 95 % confidence interval for the estimate from the systematic review of direct observational studies was 0.14–0.30. ARTI: annual risk of tuberculosis infection.

2.1. Secondary analyses

We performed secondary analyses by changing the parameter values for the ARTI, the proportion protection conferred by earlier IGRA conversion, the overall rate of progression to tuberculosis, and the difference between childhood ARTI and adult ARTI (Table 1). We also added a secondary analysis in which we assumed that the progression rates were higher or lower in the first two years post-IGRA conversion compared to what was assumed in the models [22] [23]. Finally, in order to allow comparison with previously published cohort studies of tuberculosis incidence by IGRA status among adolescents we used our model to calculate the expected tuberculosis incidence rate ratio (IRR) for IGRA-positive versus IGRA-negative participants enrolled at different ages in simulated cohorts with 2-year follow-up at different levels of ARTI.

3. Results

In the Base Model, the required sample size to accrue 50 tuberculosis endpoints in the placebo arm declined with increasing ARTI, but much less for the IGRA-positive participants-only trial than for the Mixed trial (Fig. 1A, Tables S1, S2). At 2 % ARTI, a Mixed trial needed to be 2.33 times larger than an IGRA-positive participants-only trial, whereas this was only 1.23 times at 6 % ARTI. Adding age varied ARTI to the model, whereby children up to 12 years had only half the infection rate of that for adults 18 years and above, reduced the required sample size to some extent for an IGRA-positive participants-only trial but with overall limited effect on either the ARTI pattern or the ratios between the required sample sizes (Aged Varied ARTI Model, Fig. 1B). However, assuming 79 % partial protection afforded by previous Mtb infection resulted in a strong increase in required sample size compared to the Base Model (Partial Protection Model, Fig. 1C). This increase was larger for an IGRA-positive participants-only trial (2.07 times at 2 % ARTI, 2.83 times at 4 % ARTI and 3.64 times at 6 % ARTI) than for a Mixed trial (1.49, 2.07 and 2.63 times, respectively). The required sample size decreased with increasing ARTI for a Mixed trial and increased for an

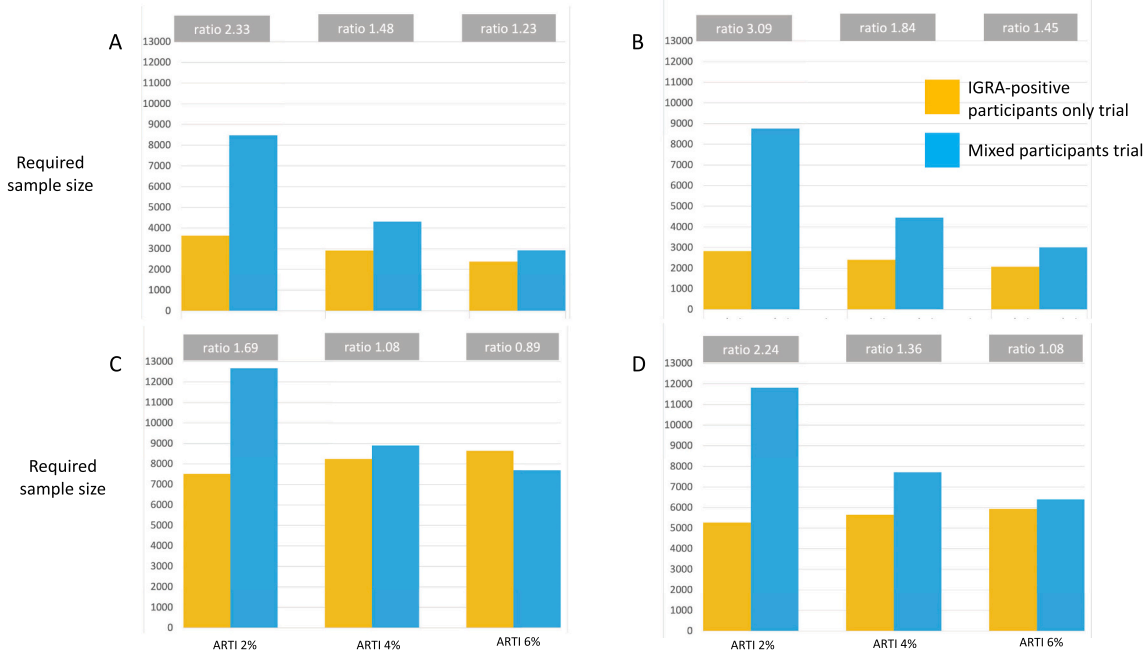

Fig. 1. Estimated sample sizes required to accrue 50 tuberculosis disease endpoints comparing a vaccine trial conducted among IGRA-positive participants only, with a Mixed trial enrolling participants in the same population regardless of IGRA testing, by model and annual risk of tuberculosis infection. Panel A: Base Model. Panel B: Age Varied ARTI Model assuming 50 % lower ARTI in children compared to adults. Panel C: Partial Protection Model assuming 21 % relative progression rate due to previous infection. Panel D: Combined Model, assumptions as for panels B and C. Ratios in grey shaded areas represent the sample size for the Mixed trial divided by the sample size for the IGRA-positive participants-only trial. ARTI: annual risk of tuberculosis infection. IGRA: interferon-gamma release assay.

IGRA-positive participants-only trial. This resulted in a sample size ratio between the two trials that was only 1.08 at 4 % ARTI and 0.89 at 6 % ARTI. Combining these assumptions resulted in a similar pattern: a decreasing sample size with increasing ARTI for a Mixed trial but increasing sample size for an IGRA-positive participants-only trial (ratios 1.36 at 4 % ARTI and 1.08 at 6 % ARTI). The required sample sizes were overall larger than in the Base Model but smaller than in the Partial Protection Model (Fig. 1D).

These observed patterns reflect a strongly influential effect of the ARTI in the trial population on the required sample size for IGRA-positive participants-only versus Mixed trials. While under the Combined Model the sample size needed to accrue 50 tuberculosis cases in the placebo arm declined from around 20,000 at an ARTI of 1 % to just over 5000 for an ARTI of 10 %, it remained stable for an IGRA-positive participants-only trial at 5000–6000 (Fig. S2).

Trials that preferentially enrolled younger (15–29 years) over older participants (30–44 years) required smaller sample sizes than trials that enrolled participants equally across ages, whereas trials that enrolled more older than younger participants required larger sample sizes (Fig. 2, Table S3). This effect was much more pronounced for IGRA-positive participants-only trials than for Mixed trials. Under the Combined Model, assuming 4 % ARTI and enrolment in age group 30–44 years being three times that in age group 15–29 years, an IGRA-positive participants-only trial and a Mixed trial needed to have a 74 % and 19 % larger sample size, respectively, compared to when enrollment in age group 15–29 years was three times that in age group 30–44 years. At higher levels of ARTI, enrolling a higher proportion of older participants made a Mixed trial almost as efficient (4 % ARTI: ratio 1.09) or more efficient (6 % ARTI: ratio 0.89) in terms of sample size than an IGRA-positive participants-only trial.

The sample size efficiency of an IGRA-positive participants-only trial versus a Mixed trial did not appreciably vary across plausible value ranges for most parameters (Fig. 3). Based on the combined model at 4 % ARTI where this ratio was 1.36, the ratio stayed between 1.23 and 1.52 for changes in assumptions about the level of partial protection afforded by previous *Mtb* infection or in the magnitude of increase in ARTI during adolescence. Varying the cumulative proportion of IGRA-positive individuals that progressed to disease had almost zero effect on the ratio estimate. However, the ratio was quite sensitive to variation in time pattern of disease progression: the ratio decreased to 1.09 (i.e., Mixed trial is almost as sample size efficient as IGRA-positive participants-only trial) if the proportion of all disease progression occurring in the first two years following re-infection was 97.2 % compared to 81.8 % in the primary analyses. This effect was due to larger required sample sizes for IGRA-positive participants-only trials as the proportion disease progression in the first 2 years post-infection got closer to 100 % while the sample size for Mixed trials was not affected (Fig. S3).

When using these models to simulate cohorts with baseline IGRA testing and 2-year follow-up, the IRR of tuberculosis among those with positive IGRA versus those with negative IGRA strongly declined with age at enrolment (Fig. 4). In the Base Model, at 4 % ARTI the IRR fell from 4.66 at age 15 years to 0.90 (i.e., incidence among IGRA-positive participants was lower compared to IGRA-negative participants) at age 44 years. In the Combined Model these IRRs were 7.08 and 1.18, respectively; at 2 % ARTI it remained relatively high (3.34 at 44 years) while at 6 % ARTI it fell below 1 from age 34 years onward. The IRR was lowest in the Partial Protection Model at 6 % ARTI, where it fell below 1 from age 25 onward.

4. Discussion

Our model shows that the decrease in required sample size for a tuberculosis vaccine trial decreases with increasing level of Mtb transmission in the population, much more for a Mixed trial without IGRA pre-enrolment screening than for a trial enrolling IGRA-positive participants only. At high transmission levels (ARTI >4 %), the required sample size for a Mixed trial thereby approaches that for an IGRA-positive participants-only trial. This effect is more pronounced if partial protection by previous infection is considered and attenuated when the ARTI is assumed to increase during adolescence. As a result, an IGRA-positive participants-only trial can have a one third smaller sample size than a Mixed trial in settings with an ARTI of 4 % but needs to have a similar sample size in very high incidence populations (ARTI 6 % or above). The required sample size, in particular for an IGRA-positive participants-only trial, is reduced by enrolling proportionally younger participants within an enrolment age range 15–44 years.

The incidence of tuberculosis among IGRA-positive compared to IGRA-negative participants decreased with age regardless of the model. This reflects an age-cohort effect by which the proportion of IGRA-positives that was recently infected and thereby at risk for disease progression becomes smaller with age. This is consistent with the finding in a phase 2b vaccine trial of a tuberculosis incidence rate in the placebo

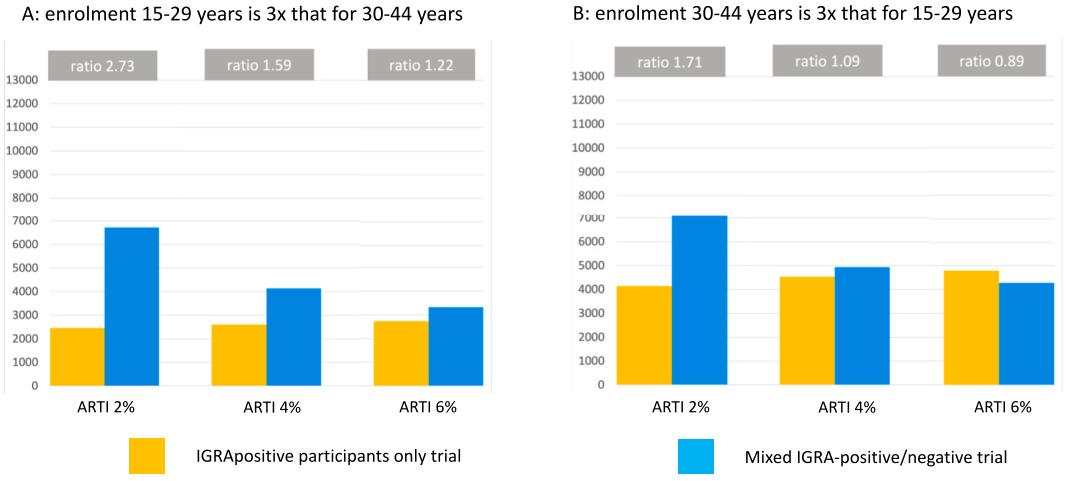

Fig. 2. Estimated sample size required to accrue 50 tuberculosis disease endpoints comparing a vaccine trial conducted among IGRA-positive participants-only with a Mixed trial enrolling participants in the same population regardless of IGRA testing, for different age distributions of enrollment. Panel A: Enrollment rate 3 times higher in age group 15–29 years than in age group 30–44 years. Panel B: Enrollment rate 3 times higher in age group 30–44 years than in age group 15–29 years. Both based on the Combined Model, assuming 50 % lower ARTI in children compared to adults and 21 % relative progression rate due to previous infection. Both models assume equal enrolment across age groups. Ratios in grey shaded areas represent the sample size for the Mixed trial divided by the sample size for the IGRA-positive participants-only trial. ARTI: annual risk of tuberculosis infection. IGRA: interferon-gamma release assay.

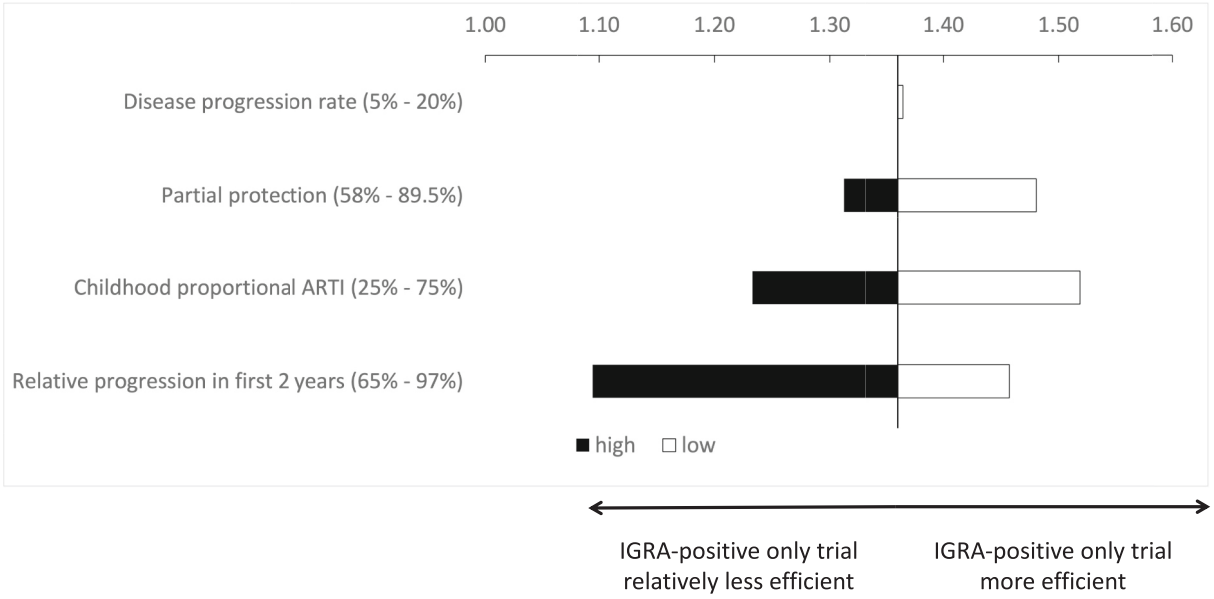

Fig. 3. Sensitivity of the relative sample size needed for a trial enrolling only IGRA-positive participants compared to that for a Mixed trial enrolling both IGRA-positive and IGRA-negative participants to selected model assumptions. Tornado plot showing the deviations of the ratio between the sample size needed for a Mixed trial versus an IGRA-positive participants-only trial when selected parameter values are changed from their assumed value to their lowest boundary (open bars) or to their highest boundary (closed bars), respectively, based on the Combined Model at 4 % ARTI assuming age-equal enrollment across the eligible age group of 15 to 44 years (ratio 1.36). Ratios above 1 reflect higher efficiency of an IGRA-positive trial; at a ratio of 1 both trials are equally efficient in terms of sample size. Disease progression rate: cumulative proportion of IGRA-positive individuals that progress to tuberculosis over the entire period that progression can occur post-IGRA conversion. Partial protection: proportion of reinfections that progress to tuberculosis relative to the progression rate of primary infection. Childhood proportional ARTI: ARTI at ages 0–12 years relative to that at ages 18 years and above. Relative progression in first 2 years: proportion of all disease progression occurring over 10 years that occurred in the first two years post-IGRA conversion. ARTI: annual risk of tuberculosis infection. IGRA: interferon-gamma release assay.

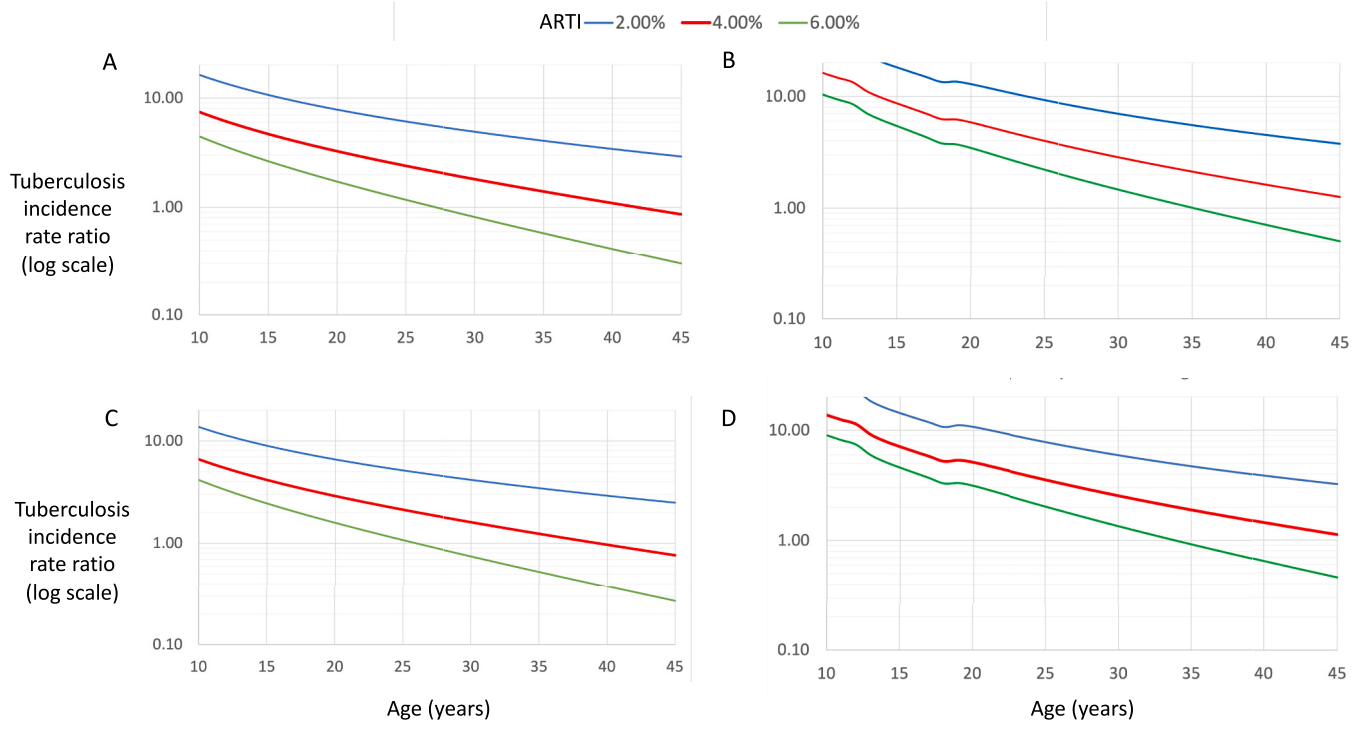

Fig. 4. Incidence rate ratios of tuberculosis among cohort participants who are IGRA-positive versus IGRA-negative at enrolment, in a cohort of 2 years follow-up, by age and ARTI. Incidence rate ratios are expressed on a logarithmic scale; at a rate ratio of 1.00 both incidences are equal. Middle (red) line: annual risk of tuberculosis infection (ARTI) 4 %. Upper (blue) line: ARTI 2 %. Lower (green) line: ARTI 6 %. Panels A: Base Model. Panel B: Age Varied ARTI Model. Panel C: Partial Protection Model. Panel D: Combined Model. IGRA: interferon-gamma release assay. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

group of 0.8/100 person-years (90 % CI: 0.6–1.3) among participants 25 years or younger compared to only 0.4/100 person-years (90 % CI: 0.2–0.7) among older participants [24]. This age-cohort effect also explains the sensitivity of our estimates to the time pattern of disease progression: the larger the proportion of progression that occurs soon after infection, the less efficient in terms of sample size an IGRA-positive participants-only trial becomes. This finding implies that differences in tuberculosis incidence observed between IGRA-positive and IGRA-negative adolescents cannot be extrapolated to older ages [25].

Including age varied ARTI in the model had limited effect on the required sample size but did improve the sample size efficiency of an IGRA-positive participants-only versus a Mixed trial. This effect is explained by attenuation of the age cohort effect by which a lower proportion of young adult IGRA-positive participants are recently infected and thereby at high risk of disease progression. Most cross-sectional studies of tuberculin or IGRA responses among adolescents in high-incidence settings show an increase in ARTI during adolescence, be with varying steepness [16,20] [26] [27]. Assuming a two-fold less steep or two-fold steeper increase in ARTI however had only limited effect on the relative sample size efficiency.

Partial protection by previous Mtb infection is generally considered to occur. A systematic review and meta-analysis of cohort studies that reported tuberculosis incidence among occupationally exposed Mtb infected and uninfected individuals showed a summary incidence rate ratio of 0.21 (95 % CI: 0.14–0.30), translating in a level of protection of 79 % (95 % CI: 0.70–0.86) [12], although it has been argued that the reduced progression rate among individuals that have been previously infected reflects selection of those that are better capable of containing Mtb infection rather than a causal effect [28]. Including 79 % protection in the model strongly increased the required sample size, because now reinfections resulted in fewer tuberculosis cases. Since all new infections in IGRA-positive participants were reinfections, whereas this was the case for only a subset of new infections in IGRA-negative participants, the increase in sample size was more pronounced for an IGRA-positive participants-only trial than for a Mixed trial. Our estimate of the relative sample size efficiency of both trial types was affected to only limited extent by assuming lower partial protection down to 58 %, reflective of a smaller protective effect found by some modelling studies [11] [29].

We considered ARTI levels that are generally seen in adult and adolescent populations selected for tuberculosis vaccine trials. As expected, the ARTI affected the required sample size: the higher the ARTI, the smaller a vaccine trial could be. An important insight however is that this differs by trial type: while in the Combined Model (that included partial protection and age varied ARTI), the required sample size approximately halved across the 2–6 % ARTI range for a Mixed trial, it showed very little change for an IGRA-positive participants-only trial. This implies that from a sample size efficiency perspective, for the 15–44 age range, Mixed trials may be the most efficient option at high ARTI levels such as in South Africa, whereas at relatively low ARTI levels such as in Kenya or Tanzania, IGRA-positive participants-only trials could be preferred.

Due to the age-cohort effect, tuberculosis vaccine trials can have smaller sample sizes when the trial population is younger within 15–44 years. Although representativeness may require that a minimum number of older participants be enrolled, prioritizing enrolment of younger participants improves sample size efficiency. This also implies that enrolling, within the trial's enrolment age range, relatively older participants may lead to an underpowered trial. It is important to note that this age-enrolment effect is much more pronounced for IGRA-positive participants-only than for Mixed trials, such that with over-enrolment of relatively old participants the required sample sizes for both trials approach each other, particularly in high-incidence populations. In addition, the younger the population that is screened for enrolment in an IGRA-positive participants-only trial, the lower the prevalence of Mtb infection and the larger the number that need to be IGRA tested to reach the sample size. The analysis of vaccine trials will generally be triggered by accruing a predefined number of endpoints, such that in practice the precision of the efficacy estimate will not be affected if the trial was underpowered. However, in such case enrolment or follow-up needs to be extended to accrue the number of events that triggers the analysis, which may have major budgetary and operational implications.

Our results were sensitive to the assumed proportion of Mtb infections that progress to tuberculosis within two years. The estimate for our primary analyses (81.8 %) was derived from the placebo arm of a BCG vaccination trial among 35,000 adolescents [11,14]. We based the lower boundary (65.3 %) for our secondary analysis on a nationwide molecular study from The Netherlands where disease incidence over time was derived from the serial interval for pairs of tuberculosis cases linked by DNA fingerprinting and epidemiological data over a 12-year period [22]. While it provides an estimate that is independent of IGRA or TST testing, the DNA fingerprinting method used (*IS6110* RFLP) had relatively low specificity for transmission chains. Moreover, timepoints of infection were based on duration of symptoms which will have missed episodes of asymptomatic, infectious tuberculosis [30]. Both may have resulted in overestimating disease progression rates after the first 2 years following infection. The higher boundary (97.2 %) was sourced from another study from The Netherlands where IGRA or TST testing records were linked to tuberculosis notification data over a 10 year period [23]. In this study, the majority of tuberculosis cases were identified at the time of infection testing (co-prevalent tuberculosis), while those without co-prevalent tuberculosis were offered preventive therapy. This may have resulted in underestimating the proportion diagnosed after the first year post-IGRA conversion. Thereby both boundary values are probably extremes.

Our study had limitations. We applied a deterministic model with various assumptions, including closed populations and constant ARTIs over time (but not age). We assumed all Mtb infections were captured by IGRA, while false-negative IGRA results may occur. The model did not consider potential gender differences in infection and disease progression rates [11], nor did it consider HIV infection or other known risk factors for tuberculosis such as undernutrition, smoking, diabetes or low socio-economic status [31]. These risk factors may be frequent in tuberculosis high burden settings and affect parameters around infection and progression to disease. We did not include IGRA reversion in the model, implicitly assuming all IGRA reversions occurred within one-year post-conversion. Long-term follow-up data on IGRA reversion in high-incidence settings are scarce and inconclusive as to whether reversion occurs shortly after conversion or gradually over the years [32]. Incorporating an annual reversion rate over multiple years post-conversion would have implied that the ARTI in our model would be higher than what would be observed at adult age, and there are currently no data on which such adjustment of the ARTI could be based. The effects of this simplification on our estimates are thereby difficult to predict. Finally, our model did not take account of all uncertainties; subsequent simulations are needed to estimate sample sizes for specific vaccine trials that do have uncertainty boundaries.

Our study was about TB vaccine trials done in the general population aged 15–44 years. Our findings can only be interpreted in that context and cannot be generalized to vaccine trials in other populations where infection dynamics or rates of disease progression may be different, such as household contacts of infectious TB patients, or imprisoned or elderly populations.

The results from this modelling study are only relevant for trials of vaccine candidates of which the protective efficacy is assumed to be independent of IGRA status. If the efficacy is lower in IGRA-negative compared to IGRA-positive individuals (either through preventing fewer (re-)infections or through preventing fewer infections from progressing to disease), or the other way around, this will affect the precision for a given sample size for a Mixed trial. Late-stage vaccine trials may in practice have variable rather than fixed duration of follow-up. Since the disease progression rate declines with time after infection, the tuberculosis incidence will decrease over time in participants who

are IGRA positive at enrolment but remain the same in participants who are IGRA negative at enrolment. Therefore, the expected effect of extending follow-up beyond a minimum of 3 years per participant in our modelled trials will be more reduction in sample size for a Mixed than for an IGRA-positive participants-only trial.

Furthermore, our results need to be considered in perspective of the reality of tuberculosis vaccine trials. We assessed efficiency in terms of sample size and did not consider other aspects of a trial that may affect its cost, such as the number of potential participants that need to be screened pre-enrolment or the level of effort needed to enroll and retain participants, which may differ between age groups. Population-representative enrolment as we assumed in our model may be unrealistic because of inclusion criteria and trial site strategies of enrolment based on local efficiency (e.g., acceptability of participation, ease of follow-up, transmission hotspots). There may be an ethical duty to provide tuberculosis preventive therapy to those screening IGRA positive, with additional implications for the required sample size [33]. Finally, as phase 3 tuberculosis vaccine trials need to generate generalisable results, they are ideally conducted in multiple countries across a range of Mtb transmission levels, which may complicate comparing sample size requirements for the two types of trial. Therefore, our modelling results should be considered a simplification meant to show patterns of dependence of sample size efficiency on restriction to IGRA-positive participants, local transmission level and participant age, rather than practical guidance to making choices about designs and sampling sizes.

In conclusion, restricting enrolment in late-stage tuberculosis vaccine trials to IGRA-positive adults and adolescents has appreciable efficiency gains in sample size when the ARTI is relatively low but not when the ARTI is relatively high. Therefore, in terms of sample size efficiency, Mixed trials may be the most efficient option at high ARTI levels such as in South Africa, whereas at relatively low ARTI levels such as in Kenya or Tanzania, IGRA-positive participants-only trials could be preferred.

At high ARTI levels it may be cost-effective to omit pre-enrolment IGRA screening, while enrolled trial participants may still be IGRA-tested to explore relationships between infection status and vaccine efficacy.

CRediT authorship contribution statement

Frank Cobelens: Writing – review & editing, Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Conceptualization. **Puck T. Pelzer:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Gavin J. Churchyard:** Writing – review & editing, Methodology, Conceptualization. **Alberto Garcia-Basteiro:** Writing – review & editing, Methodology, Conceptualization. **Mark Hatherill:** Writing – review & editing, Methodology, Conceptualization. **Philip C. Hill:** Writing – review & editing, Methodology, Conceptualization. **Leonardo Martinez:** Writing – review & editing, Methodology, Conceptualization. **Richard G. White:** Writing – review & editing, Methodology, Investigation, Formal analysis, Conceptualization.

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Declaration of competing interest

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2025.127301.

Data availability

The model is made available as supplemental file.

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