# Live-attenuated *Mycobacterium tuberculosis* vaccine, MTBVAC, in adults with or without *M tuberculosis* sensitisation: a single-centre, phase 1b–2a, double-blind, dose-escalation, randomised controlled trial

*Angelique Kany Kany Luabeya*, Virginie Rozot*, Claire Imbratta*,Frances Ratangee, Justin Shenje, Michele Tameris, Simon C Mendelsohn, Hennie Geldenhuys, Michelle Fisher, Munyaradzi Musvosvi, Carly Young, Humphrey Mulenga, Nicole Bilek, Simbarashe Mabwe, Ingrid Murillo Jelsbak, Esteban Rodríguez, Eugenia Puentes, Juana Doce, Nacho Aguilo, Carlos Martin, Cadwill Pillay, Dereck Tait, Marisa Russell, Arrie Van Der Merve, Kathryn Rutkowski, Devin Hunt, Ann Ginsberg, Thomas J Scriba, Mark Hatherill, the A050 team*

## Summary

**Background** An effective adult vaccine is needed to control tuberculosis. We evaluated the safety and immunogenicity of a live-attenuated *Mycobacterium tuberculosis* vaccine (MTBVAC).

**Methods** This single-centre, phase 1b–2a, double-blind, dose-escalation, randomised controlled trial (NCT02933281) enrolled South African adults previously vaccinated with BCG, who were HIV negative and aged 18–50 years, with or without *M tuberculosis* sensitisation assessed by QuantiFERON-tuberculosis Gold-Plus assay (QFT). Participants were recruited from the local community and randomly allocated (2:1) to receive MTBVAC ($5\times10^3$, $5\times10^4$, $5\times10^5$, or $5\times10^6$ colony-forming unit [CFU] doses) or BCG revaccination ($5\times10^5$ CFU dose). The primary outcomes were the occurrence of systemic solicited adverse events within 7 days and unsolicited adverse events within 28 days after vaccination, the occurrence of solicited and unsolicited injection-site reactions within 84 days after vaccination, and the occurrence of serious adverse events (SAEs) until the end of study, 365 days after vaccination. Data were analysed per modified intention to treat. The trial is now complete and closed.

**Findings** Between Jan 15, 2019, and Sept 7, 2020, 485 participants provided consent and were screened. 144 participants were enrolled and 143 (99%) were vaccinated. BCG was administrated to 47 (33%) of 143 and MTBVAC to 96 (67%) of 143. 12 participants with QFT-negative results and 12 with QFT-positive results were randomly allocated to receive each dose of MTBVAC and 24 participants with QFT-negative results and 24 with QFT-positive results were randomly allocated to receive BCG revaccination. Injection-site pain, discharge, erythema, and swelling increased with MTBVAC dose level. MTBVAC $5\times10^5$ CFU recipients reported a similar proportion of related adverse events (23 [96%] of 24) as BCG recipients (45 [96%] of 47). MTBVAC recipients who were QFT positive reported more injection-site reactions (46 [96%] of 48; 95% CI 85·7–99·5) than MTBVAC recipients who were QFT negative (32 [67%] of 48; 51·6–79·6). No vaccine-related SAEs were reported. All doses of MTBVAC were immunogenic; vaccine-induced antigen-specific CD4 T-cell responses peaked 28 days after vaccination. The MTBVAC $5\times10^5$ and $5\times10^6$ CFU doses induced T-helper-cell-1 cytokine-expressing CD4 T-cell responses that exceeded BCG-induced responses in participants who were QFT negative and QFT positive.

**Interpretation** MTBVAC at the $5\times10^5$ dose showed similar safety and reactogenicity and greater immunogenicity when compared to BCG. These results suggest that the $5\times10^5$ dose of MTBVAC could be selected for a subsequent efficacy evaluation.

**Funding** Congressionally Directed Medical Research Programmes and US National Institutes of Health.



*Lancet Glob Health* 2025; 13: e1030–42

Published Online April 15, 2025 https://doi.org/10.1016/S2214-109X(25)00046-4

See Comment page e975

*Contributed equally

**South African Tuberculosis Vaccine Initiative, Institute of Infectious Disease and Molecular Medicine and Department of Pathology, University of Cape Town, Cape Town, South Africa** (A K K Luabeya MSc, V Rozot PhD, C Imbratta PhD, F Ratangee RN, J Shenje MPH, M Tameris MPhil, S C Mendelsohn PhD, H Geldenhuys MD, M Fisher PhD, M Musvosvi PhD, C Young PhD, H Mulenga PhD, N Bikek PhD, S Mabwe MBA, T J Scriba PhD, Prof M Hatherill MD); **Biofabri, Porriño (Pontevedra), Spain** (I M Jelsbak MSc, E Rodríguez BSc, E Puentes PhD, J Doce PhD); **Department of Microbiology Pediatrics, Radiology, and Public Health, Faculty of Medicine, University of Zaragoza, CIBERES, Instituto Salud Carlos III, Madrid, Spain** (N Aguilo PhD, C Martin PhD); **IAVI, New York, NY, USA** (C Pillay BSc, D Tait MMed, M Russell BSc, A Van Der Merve MSc, K Rutkowski MSc, D Hunt MSc, A Ginsberg PhD); **IAVI, Cape Town, South Africa** (C Pillay, D Tait, M Russell, A Van Der Merve, K Rutkowski, D Hunt, A Ginsberg); **Bill & Melinda Gates Foundation, Seattle, WA, USA** (A Ginsberg)

## Introduction

Tuberculosis caused more than 1·25 million deaths in 2023.[1] An effective vaccine to prevent adult pulmonary tuberculosis is crucial to achieve the WHO goal of reducing the number of tuberculosis deaths by 95% and incidence by 90% by 2035.[1] BCG, a live-attenuated vaccine derived from *Mycobacterium bovis*, has saved millions of lives by protecting children against severe and disseminated forms of tuberculosis.[2,3] Unfortunately, BCG has low efficacy in preventing cavitary pulmonary tuberculosis in adolescents and adults, which drives airborne transmission of *M tuberculosis*.[4]

Mathematical modelling suggests that a new effective tuberculosis vaccine that targets adults would have a higher effect on tuberculosis incidence worldwide than newborn vaccination;[5] a vaccine effective in

Correspondence to:
Prof Mark Hatherill,
South African Tuberculosis Vaccine Initiative, Institute of Infectious Disease and Molecular Medicine, Division of Immunology, Department of Pathology, University of Cape Town, Cape Town 7925, South Africa
**mark.hatherill@uct.ac.za**

## Research in context

### Evidence before this study

We searched PubMed by combining the terms "live", "*Mycobacterium tuberculosis*", "human", and "vaccine" for original research articles and systematic reviews published in any language from inception up to March 29, 2023. We identified two published clinical trials of a whole, live-attenuated *Mycobacterium tuberculosis* vaccine (MTBVAC)—by Spertini and colleagues in Swiss adults who were BCG naive (NCT02013245) and by Tameris and colleagues in adults who were BCG vaccinated and newborns who were BCG naive in a tuberculosis-endemic region (NCT02729571). The safety and immunogenicity of MTBVAC were similar to BCG in adults unexposed to *M tuberculosis*. MTBVAC at the $2{\cdot}5 \times 10^5$ colony-forming-unit (CFU) dose was more immunogenic than BCG at the same dose in infants, and was selected for a multicentre, phase 3 trial.

### Added value of this study

A new effective tuberculosis vaccine for adults is needed to prevent pulmonary tuberculosis and halt *M tuberculosis* transmission. Many adults in tuberculosis-endemic regions have been presensitised to *M tuberculosis* during childhood and adolescence. The safety, reactogenicity, and immunogenicity of MTBVAC in adults sensitised to *M tuberculosis*, defined by positive QuantiFERON-tuberculosis Gold-Plus assay (QFT), are unknown. We evaluated vaccination with MTBVAC at escalating $5 \times 10^3$, $5 \times 10^4$, $5 \times 10^5$, and $5 \times 10^6$ CFU doses and BCG revaccination at the $5 \times 10^5$ CFU dose in South African adults with or without previous *M tuberculosis* sensitisation. MTBVAC recipients showed dose-dependent increases in injection site pain, discharge, erythema, and swelling. MTBVAC reactogenicity at the equivalent $5 \times 10^5$ CFU dose was similar to BCG. QFT-positive MTBVAC recipients had a higher incidence of injection site reactions than QFT-negative MTBVAC recipients. The $5 \times 10^5$ and $5 \times 10^6$ CFU MTBVAC doses induced higher T-helper-cell-1 cytokine-expressing CD4 T-cell responses than BCG in both QFT-negative and QFT-positive participants. QFT conversion at day 28 was induced in more than 50% of individuals vaccinated with the three highest doses of MTBVAC, most of whom converted to negative by day 365.

### Implications of all the available evidence

WHO has declared the development of new tuberculosis vaccines for adults and adolescents a global health priority. MTBVAC safety and reactogenicity were similar to BCG revaccination at the same dose. MTBVAC was immunogenic in individuals previously vaccinated with BCG, whether or not previously sensitised to *M tuberculosis*. These findings support the advancement of MTBVAC into efficacy trials, potentially leading to licensure in these populations.

*M tuberculosis*-sensitised populations would have a greater effect than one effective only in *M tuberculosis*-unsensitised populations;[6] and a vaccine with only 50% efficacy in adolescents and adults could prevent at least 37 million tuberculosis cases and 4·6 million deaths between 2025 and 2050.[7] The investment case for such a vaccine is compelling, being cost-effective in all countries with a high tuberculosis burden and cost-saving in 58 (55%) of 105 low-income and middle-income countries.[8,9] For these reasons, WHO has prioritised adolescents and adults as the primary strategic targets for a new tuberculosis vaccine.[10]

A novel, live-attenuated *M tuberculosis* vaccine (MTBVAC) candidate has been developed for both infant and adolescent and adult indications. MTBVAC is derived from a clinical isolate of *M tuberculosis* lineage 4, rendered non-virulent by independent stable deletion of the *phoP* and *fadD26* genes.[11] PhoP is a transcription factor that regulates the expression of more than 2% of *M tuberculosis* genes, many of which are implicated in virulence, including secretion of ESAT-6. FadD26 is required for the biosynthesis of phthiocerol dimycocerosates, a major virulence-associated cell-wall lipid of *M tuberculosis*. MTBVAC contains all the genes that were lost during attenuation of *M bovis* to derive BCG by subcultivation, including RD1 genes that encode highly antigenic proteins such as ESAT-6 and CFP10,[12] which were shown to be protective against *M tuberculosis* challenge in mice.[13] MTBVAC thus expresses a broader collection of potentially protective mycobacterial antigens than BCG.

Preclinical studies have demonstrated that MTBVAC offers a similar safety profile to BCG in relevant animal models, including mice, severe combined immunodeficiency mice, guinea pigs, and non-human primates, and superior immunogenicity and efficacy against *M tuberculosis* challenge.[14,15,16] Immunisation of newborns with MTBVAC showed a similar safety profile to BCG, but led to dose-dependent QuantiFERON-tuberculosis Gold-Plus assay (QFT) conversions that confounded the interpretation of *M tuberculosis* sensitisation; most infants reverted to being QFT negative by 1 year.[17]

The majority of adolescents and adults in tuberculosis-endemic regions have been BCG vaccinated at birth and a large proportion show evidence of previous *M tuberculosis* sensitisation.[18,19] The safety, reactogenicity, and immunogenicity of MTBVAC in BCG-vaccinated and previously *M tuberculosis*-sensitised individuals has not been evaluated and will likely differ from infants and adults who are BCG naive in non-endemic countries. The outcomes assessed in this trial are intended to inform the optimal dose of MTBVAC to enter prevention of disease efficacy trials in tuberculosis-endemic countries. This trial evaluated the safety, reactogenicity,

and immunogenicity of MTBVAC at escalating dose levels of $5\times10^3$, $5\times10^4$, $5\times10^5$, and $5\times10^6$ colony-forming units (CFU), compared with BCG revaccination at the standard dose of $5\times10^5$ CFU.

## Methods

### Study design and participants

We did a single-centre, phase 1b–2a, double-blind, dose-escalation, randomised, controlled, safety and immunogenicity study in South Africa, among adults who were healthy, HIV negative, and previously BCG vaccinated, with or without *M tuberculosis* sensitisation, defined by a positive QuantiFERON-tuberculosis Gold-Plus (QFT) result (≥0·35 IU/mL). The University of Cape Town Human Research Ethics Committee and the South African Health Products Regulatory Authority approved the study, registered under the ClinicalTrials.gov identifier NCT02933281.

Participants were recruited from the local community in the Cape Winelands region of the Western Cape province of South Africa, where the tuberculosis notification rate is estimated at 700 in 100 000.[20] Participants provided written, informed consent and were screened for eligibility within 28 days before vaccine administration. Eligible volunteers needed to meet the following criteria: have completed the written informed consent process; be male or female aged 18–50 years on vaccination day; and agree to stay in contact with the clinical trial site and the study area for the duration of the study. Female participants had to avoid pregnancy within 21 days of vaccination and for the duration of the study. Male participants had to use barrier contraception for at least 2 weeks after receiving MTBVAC or BCG. All participants had to be in good health, previously BCG vaccinated, and not have shared an enclosed living or workspace with someone diagnosed with tuberculosis during the 3 months before vaccination (appendix p 2).

Volunteers were excluded if they had any of the following characteristics: acute illness on vaccination day; abnormal laboratory values equivalent to grade 2 or higher toxicity; screening thyroid stimulating hormone greater than the upper limit of normal; suspicion or evidence of active tuberculosis disease or history of treatment for tuberculosis disease; history or evidence of autoimmune disease or immunodeficiency; if they received immunoglobulin or blood products within 42 days or any investigational drug or vaccine within 182 days before vaccination; planned participation in any other investigational study during the study or received an investigational vaccine against tuberculosis at any time before vaccination; presence of allergic disease or reactions likely to be exacerbated by any component of the investigational product; any previous medical history that might compromise the safety of the participant; evidence of any systemic disease or any acute or chronic illness that could interfere with the evaluation of the safety or immunogenicity of the vaccine; and any current medical, psychiatric, occupational, or substance abuse problems that, in the opinion of the investigator, could endanger the participant or make it unlikely that the participant will comply with the protocol. Lactating or nursing female participants were excluded (appendix p 2).

### Randomisation and masking

Eligible participants were randomly assigned 2:1 within each study subgroup, defined by MTBVAC dose and QFT stratum, to receive a single injection of MTBVAC or BCG revaccination administered intradermally on study day 0. Randomisation was achieved by randomly generated number sequences managed by a validated interactive voice web response system (IXRS). The schedule was prepared by a statistician not involved in the study. Study pharmacists were masked to vaccine allocation; clinical and laboratory teams and monitors were masked. Labels accompanying BCG and MTBVAC syringes for administration were identical. Vaccines were administered by a study nurse not involved in participant follow-up or safety evaluation.

### Procedures

MTBVAC vaccine was administered at escalating dose levels in eight subgroups, 1–4 for 72 participants who are QFT negative and 5–8 for 72 who are QFT positive. Subgroups 1 and 5 received $5\times10^3$ CFU, subgroups 2 and 6 received $5\times10^4$ CFU, subgroups 3 and 7 received $5\times10^5$ CFU, and subgroups 4 and 8 received $5\times10^6$ CFU MTBVAC. The comparator vaccine was BCG Japan (0·05 mg, approximately $5\times10^5$ CFU), administered to 24 participants who were QFT negative and 24 who were QFT positive.

### Outcomes

The primary outcome measures were: the occurrence of systemic solicited adverse events within 7 days and unsolicited adverse events within 28 days after vaccination; the occurrence of solicited and unsolicited injection-site reactions within 84 days after vaccination; and the occurrence of serious adverse events (SAEs) through end of study 365 days after vaccination.

See Online for appendix

The secondary outcome was to evaluate the immunogenicity of MTBVAC at escalating doses. The immunogenicity outcomes were frequencies of antigen-specific CD4 T cells expressing any combination of interferon (IFN)g, tumour necrosis factor (TNF), interleukin (IL)-2, IL-17, and IL-22 at study day 0, day 28, day 56, day 182, and day 365, measured by 12-h whole-blood intracellular cytokine staining (WB-ICS) assay, and, as a secondary immunogenicity outcome, serum IgG antibody ELISA at study day 0, day 56, and day 365. We also assessed levels of IFN-γ in plasma from the QFT assay at study days 28, 56, 84, 182, and 365, in those who were QFT negative at screening (subgroups 1–4 and BCG); and at study day 365 in those who were QFT positive at screening (subgroups 5–8 and BCG). The

immunology analytical set consisted of all participants without treatment deviations.

### Safety evaluations: solicited and unsolicited adverse events

Vital signs were measured before and after vaccination. Participants remained under observation for 60 min (SD 10) after vaccination. Investigators classified the severity of adverse events as mild, moderate, or severe and graded adverse events according to the modified final US Food and Drug Administration toxicity table.[21] Solicited systemic adverse events were recorded up until day 7 with diary cards. Solicited systemic adverse events included fever, myalgia, arthralgia, fatigue, headache, anorexia, hives, and chills. Solicited injection-site reaction advserve events included pain, redness, swelling, ulceration, drainage, and regional lymphadenopathy, recorded up until day 84, with diary cards used up until day 14 after vaccination. SAEs were reported throughout the follow-up period. The safety analysis included all participants who received a vaccine.

### Safety monitoring during dose escalation

Participants who were QFT negative were enrolled sequentially, with no safety review between the $5\times10^3$, $5\times10^4$, and $5\times10^5$ CFU MTBVAC doses. The $5\times10^6$ CFU MTBVAC subgroup was enrolled after Data Safety Monitoring Board (DSMB) review.

Participants who were QFT positive were enrolled into the lowest dose of $5\times10^3$ CFU MTBVAC (subgroup 5), in parallel with those who were QFT negative enrolled in subgroups 1, 2, and 3. DSMB review was triggered when participants who were QFT positive who received $5\times10^3$ CFU and $5\times10^4$ CFU MTBVAC had completed 28 days of

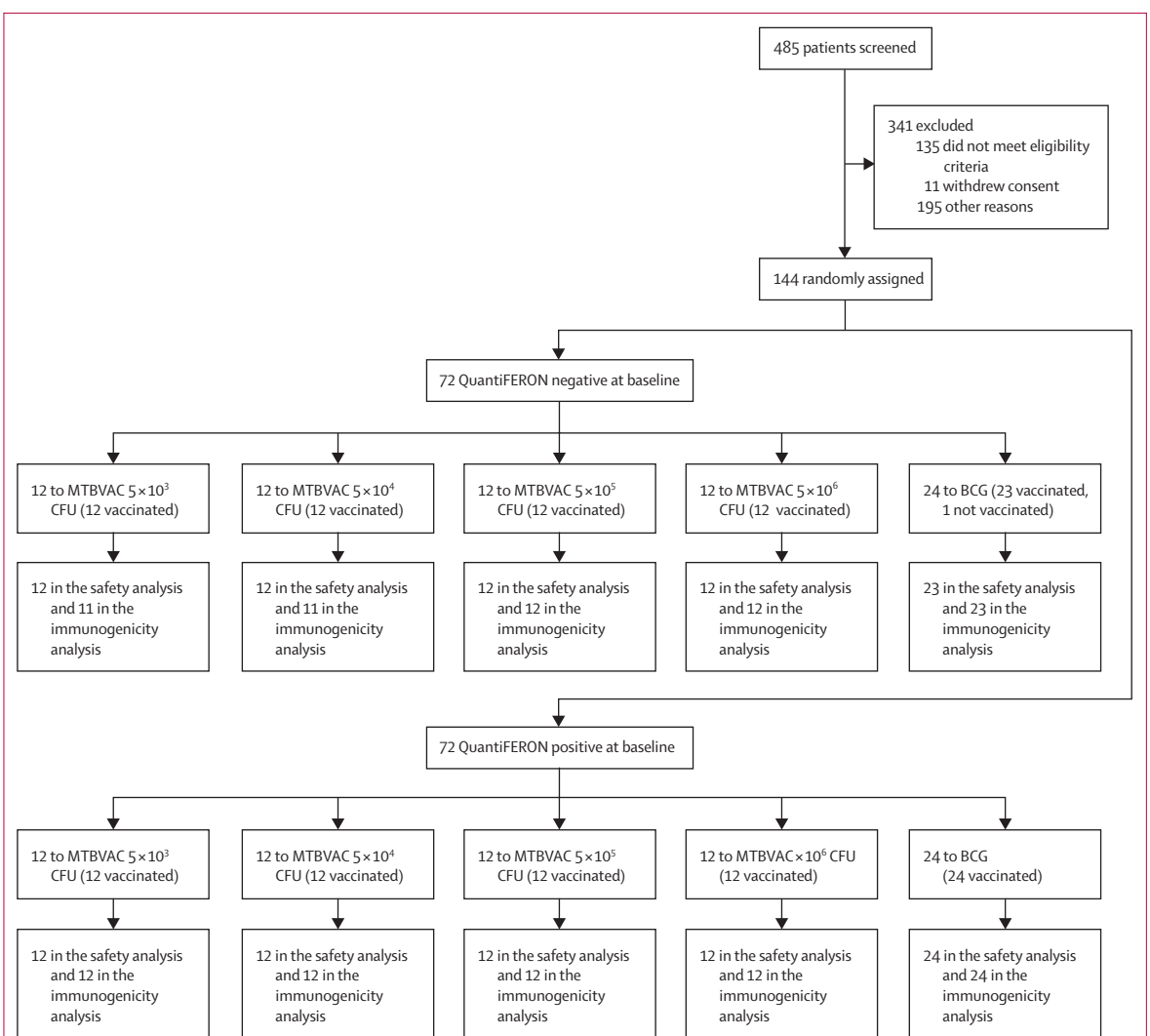



***Figure 1:*** **Trial profile by MTBVAC dose and QFT status**
The safety analysis included all participants who received at least one dose of the study intervention. The immunology analytic set consisted of all participants without treatment deviations. CFU=colony-forming unit. MTBVAC=live-attenuated *Mycobacterium tuberculosis* vaccine. QFT=QuantiFERON-tuberculosis Gold-Plus assay.

follow-up after vaccination. The DSMB reviewed all available safety data of QFT-negative and QFT-positive groups before dose escalation to $5\times10^6$ CFU in QFT-negative groups and at each MTBVAC dose escalation in QFT-positive groups.

During follow-up visits, procedures included medical and medication history updates, blood sampling for whole-blood assays, serum chemistry, haematology, and reviewing diary-card entries.

### Immunogenicity evaluations

WB-ICS was done as previously described.[17] Briefly, whole blood was collected in sodium heparin tubes and stimulated within 75 min with MTBVAC ($10^6$ CFU/mL), BCG ($10^6$ CFU/mL BCG; Pasteur, Aeras, Rockville, MA, USA), phytohemagglutinin (5µg/mL; Thermo Fisher, Waltham, MA, USA), or left unstimulated in the presence of costimulatory antibodies (anti-CD28 and anti-CD49d, at 0·25 µg/mL each; BD, Franklin Lakes, NJ, USA) for 12 h at 37°C. After 7 h of stimulation, brefeldin A (10 mg/mL; Sigma Aldrich, St Louis, MO, USA) was added, and the blood incubated for another 5 h at 37°C. Erythrocytes were lysed and leukocytes fixed using FACS lysing solution (BD). For flow cytometry, leukocytes were stained using a monoclonal antibody panel (appendix p 6). Acquisition on a BD Fortessa flow cytometer was done in batches that included samples collected from the same participant (ie, all timepoints and conditions) to minimise variability. The QuantiFERON-tuberculosis Gold-Plus assay (Qiagen, Venlo, Netherlands) was done according to the manufacturers' protocol. Details about the analyses of the WB-ICS assay and the assay done to measure MTBVAC-specific IgG responses are in the appendix (p 18).

### Statistical analysis

A modified intention-to-treat approach was used to analyse the data. The safety dataset included all participants who received a study vaccine. The immunology analytical set excluded participants with major protocol or treatment deviations. The safety and immunogenicity analyses were done per protocol using SAS version 9.4. Quantitative variables were summarised by the number of participants in each category (n), mean (SD), minimum, median, and maximum, and IQR. Qualitative variables were summarised by percentage in each category. Risk differences and their 95% exact unconditional CIs were computed using the score method from a series of 2×2 contingency tables comparing safety events between the control and active subgroups and between each active subgroup.

### Role of the funding source

IQVIA was the clinical research organisation. The sponsors designed the study in collaboration with the

| | Cohort (n) | QFT IFNγ level (IU/mL) | | Age (years) | | Sex | | Ethnicity | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | Median (IQR) | p value* | Median (minimum, maximum) | Mean (SD) | Female | Male | Black | White | Mixed Cape Ancestry |
| **MTBVAC $5\times10^3$** | | | | | | | | | | |
| Negative | 12 | 0·01 (0–0·04) | <0·0001 | 19·5 (19, 48) | 23·4 (8·51) | 5 (42%) | 7 (58%) | 3 (25%) | 1 (8%) | 8 (67%) |
| Positive | 12 | 2·32 (0·55–10·00) | .. | 29·5 (19, 45) | 29·6 (8·17) | 5 (42%) | 7 (58%) | 4 (33%) | 0 | 8 (67%) |
| **MTBVAC $5\times10^4$** | | | | | | | | | | |
| Negative | 12 | 0·04 (0–0·13) | <0·0001 | 21·5 (18, 42) | 24·5 (6·79) | 2 (17%) | 10 (83%) | 6 (50%) | 0 | 6 (50%) |
| Positive | 12 | 5·01 (0·97–9·92) | .. | 31·0 (19, 47) | 33·8 (10·32) | 5 (42%) | 7 (58%) | 5 (42%) | 0 | 7 (58%) |
| **MTBVAC $5\times10^5$** | | | | | | | | | | |
| Negative | 12 | 0·05 (0–0·09) | <0·0001 | 25·5 (18, 43) | 26·2 (7·88) | 5 (42%) | 7 (58%) | 6 (50%) | 0 | 6 (50%) |
| Positive | 12 | 2·04 (1·18–7·53) | .. | 28·0 (18, 43) | 27·5 (7·4) | 6 (50%) | 6 (50%) | 5 (42%) | 0 | 7 (58·3) |
| **MTBVAC $5\times10^6$** | | | | | | | | | | |
| Negative | 12 | 0·11 (0–0·26) | <0·0001 | 22·0 (18, 43) | 24·1 (6·47) | 7 (58%) | 7 (58%) | 11 (92%) | 0 | 1 (8%) |
| Positive | 12 | 6·04 (1·36–9·82) | .. | 23·0 (20, 47) | 27·1 (8·5) | 1 (8%) | 11 (91%) | 5 (42%) | 0 | 7 (58%) |
| **MTBVAC subtotal** | | | | | | | | | | |
| Negative | 48 | 0·04 (0–0·11) | <0·0001 | 22·0 (18, 48) | 24·5 (7·29) | 19 (40%) | 29 (60%) | 26 (54%) | 1 (2%) | 21 (44%) |
| Positive | 48 | 3·10 (1·10–9·69) | .. | 28·0 (18, 47) | 29·5 (8·81) | 17 (35%) | 31 (65%) | 19 (40%) | 0 | 29 (60%) |
| **Pooled BCG** | | | | | | | | | | |
| Negative | 23 | 0·08 (0–0·17) | <0·0001 | 29·0 (18, 48) | 30·4 (9·22) | 13 (57%) | 10 (43%) | 16 (70%) | 1 (4%) | 6 (26%) |
| Positive | 24 | 3·75 (1·45–10·00) | .. | 25·0 (21, 44) | 26·9 (6·14) | 6 (25%) | 18 (75%) | 9 (38%) | 0 | 15 (63%) |
| **Subtotal** | | | | | | | | | | |
| Negative | 71 | 0·04 (0–0·14) | <0·0001 | 23·0 (18, 48) | 26·4 (8·37) | 32 (45%) | 39 (55%) | 42 (59%) | 2 (3%) | 27 (38%) |
| Positive | 72 | 3·27 (1·14–10·00) | .. | 27·5 (18, 47) | 28·6 (8·07) | 23 (32%) | 49 (68%) | 28 (39%) | 0 | 44 (61%) |

FN=interferon. MTBVAC=live-attenuated *M tuberculosis* vaccine. QFT=QuantiFERON-tuberculosis Gold-Plus assay. *p values calculated using a non-parametric Kruskal-Wallis test.

*Table 1:* **Participant baseline characteristics by vaccine type, dose, and QFT status**

investigators and oversaw monitoring, data collection, data analysis, data interpretation, writing of the clinical study report, and reviewed the manuscript before submission.

## Results

Between Jan 15, 2019, and Sept 7, 2020, 485 individuals provided consent and were screened. 144 enrolled participants were randomly assigned; 143 (99%) of 144 were vaccinated and 133 (93%) of 143 completed the study. One participant was not vaccinated because of a randomisation error (figure 1). Demographic and baseline characteristics were similar across subgroups (table 1). Median age was 24 years (range 18–48). More male participants (88 [62%] of 143) were vaccinated than female participants (55 [38%] of 143). Most participants reported either Black African (70 [49%] of 144) or Cape Mixed ancestry (71 [50%] of 144).

A dose-dependent increase in injection-site adverse events was observed among MTBVAC recipients. At least one related injection-site reaction was reported among 11 (46%) of 24 MTBVAC recipients in the $5\times10^3$ CFU subgroup, 18 (75%) in the $5\times10^4$ CFU subgroup, 21 (88%) in the $5\times10^5$ CFU subgroup, and 23 (96%) in the $5\times10^6$ CFU subgroup (table 2; appendix p 3).

At least one related solicited adverse event was reported in 86 (90%) of 96 MTBVAC recipients and 45 (96%) of 47 BCG recipients (table 2). Fewer MTBVAC $5\times10^3$ CFU and $5\times10^4$ CFU recipients reported injection-site reactions than BCG recipients, including erythema pain, swelling, ulceration, and discharge (figure 2A–B). MTBVAC $5\times10^5$ CFU recipients had no statistically significant difference in risk of injection-site reactions compared with BCG recipients (figure 2C). By contrast, MTBVAC $5\times10^6$ CFU recipients had a higher risk of injection-site pain and swelling than BCG recipients (figure 2D).

Injection-site ulceration was reported in 103 (72%) of 143 participants. Ulceration was reported more frequently in BCG recipients than in MTBVAC recipients (table 2). BCG was cultured from three participants with injection-site discharge; *M tuberculosis* complex, which was not definitively strain typed but is presumed to be MTBVAC,

| | MTBVAC $5\times10^3$ CFU (n=24) | MTBVAC $5\times10^4$ CFU (n=24) | MTBVAC $5\times10^5$ CFU (n=24) | MTBVAC $5\times10^6$ CFU (n=24) | MTBVAC subtotal (n=96) | Pooled BCG (n=47) |
|---|---|---|---|---|---|---|
| Participants with at least one related adverse reaction | 19 (79%) | 24 (100%) | 23 (96%) | 24 (100%) | 90 (94%) | 46 (98%) |
| Participants with at least one related unsolicited adverse reaction | 13 (54%) | 18 (75%) | 12 (50%) | 21 (88%) | 64 (67%) | 34 (72%) |
| Participants with at least one related solicited adverse reaction | 18 (75%) | 21 (88%) | 23 (96%) | 24 (100%) | 86 (90%) | 45 (96%) |
| Participants with at least one related injection-site adverse reaction | 11 (46%) | 18 (75.0%) | 21 (88%) | 23 (96%) | 73 (76%) | 41 (87%) |
| Injection-site ulcer | 9 (38%) | 15 (63%) | 20 (83%) | 22 (92%) | 66 (69%) | 37 (79%) |
| Injection-site scab | 10 (42%) | 15 (63%) | 10 (42%) | 16 (67%) | 51 (53%) | 27 (57%) |
| Injection-site pain | 5 (21%) | 12 (50%) | 14 (58%) | 20 (83%) | 51 (53%) | 26 (55%) |
| Injection-site discharge | 2 (8%) | 3 (13%) | 13 (54%) | 19 (79%) | 37 (39%) | 30 (64%) |
| Injection-site erythema | 1 (4%) | 2 (8%) | 10 (42%) | 13 (54%) | 26 (27%) | 17 (36%) |
| Injection-site swelling | 0 | 0 | 5 (21%) | 16 (67%) | 21 (22%) | 10 (21%) |
| Injection-site exfoliation | 1 (4%) | 3 (13%) | 1 (4%) | 3 (13%) | 8 (8%) | 5 (11%) |
| Injection-site induration | 0 | 0 | 0 | 2 (8%) | 2 (2%) | 1 (2%) |
| Injection-site pruritus | 1 (4%) | 1 (4%) | 0 | 0 | 2 (2%) | 1 (2%) |
| Injection-site rash | 1 (4%) | 1 (4%) | 0 | 0 | 2 (2%) | 1 (2%) |
| Injection-site vesicles | 0 | 0 | 0 | 2 (8%) | 2 (2%) | 0 |
| Injection-site scar | 0 | 0 | 0 | 1 (4%) | 1 (1%) | 1 (2%) |
| Injection-site papule | 0 | 0 | 0 | 0 | 0 | 1 (2%) |
| Injection-site abscess | 0 | 0 | 0 | 1 (4%) | 0 | 1 (2%) |
| Myalgia | 4 (17%) | 10 (42%) | 8 (33%) | 11 (46%) | 33 (34%) | 12 (26%) |
| Arthralgia | 2 (8%) | 6 (25%) | 4 (17%) | 6 (25%) | 18 (19%) | 8 (17%) |
| Musculoskeletal pain | 0 | 1 (4%) | 0 | 0 | 1 (1%) | 0 |
| Headache | 10 (42%) | 8 (33%) | 4 (17%) | 7 (29%) | 29 (30%) | 14 (30%) |
| Dizziness | 0 | 0 | 1 (4%) | 0 | 1 (1%) | 1 (2%) |
| Fatigue | 6 (25%) | 10 (42%) | 8 (33%) | 10 (42%) | 34 (35%) | 12 (26%) |
| Chills | 3 (13%) | 2 (8%) | 3 (13%) | 4 (17%) | 12 (13%) | 3 (6%) |
| Pyrexia | 1 (4%) | 1 (4%) | 1 (4%) | 2 (8%) | 5 (5%) | 1 (2%) |

CFU=colony-forming units. MTBVAC=live-attenuated *M tuberculosis* vaccine. QFT=QuantiFERON-tuberculosis Gold-Plus assay.

***Table 2:*** **Participants with related solicited and unsolicited adverse events by MTBVAC dose and BCG, independent of QFT status**

was cultured from one participant with injection-site discharge.

Injection-site abscess was reported in one BCG recipient who was QFT negative and in one MTBVAC $5\times10^6$ CFU recipient who was QFT positive. A pus culture from the abscess of the BCG recipient was positive for BCG and *Staphylococcus aureus*; a pus swab from the abscess of the MTBVAC recipient was positive for the MTB complex. An injection error, intramuscular instead of intradermal administration, had previously been reported for the MTBVAC recipient, which might have contributed to abscess formation. Both abscesses resolved without specific anti-mycobacterial therapy.

The proportion of participants reporting at least one related unsolicited adverse events was similar between BCG and all MTBVAC doses (table 2). Solicited systemic adverse events related to the vaccine were less frequent in MTBVAC $5\times10^3$ CFU recipients and more frequent with higher MTBVAC doses and in BCG recipients (table 2).

Reactogenicity per QFT status showed at least one related solicited injection-site reaction among 43 (90%) of 48 MTBVAC recipients who were QFT positive and 30 (62%) of 48 of those who were QFT negative (appendix p 3). A higher risk of injection-site reactions was observed in MTBVAC recipients who were QFT positive than in those who were QFT negative, including erythema in 18 (37%) of 48 versus eight (17%) of 48 participants, and ulceration in 39 (81%) of 48 versus 27 (56%) of 48 participants (appendix p 3).

An MTBVAC dose-dependent increase in the rate of injection-site ulceration was observed in participants who were QFT positive and QFT negative (appendix p 3). Higher ulceration risk was reported among MTBVAC recipients in the QFT-positive group than the QFT-negative group (appendix p 3). A severe injection-site ulceration was observed in two QFT-positive recipients (one MTBVAC $5\times10^6$ CFU recipient and one BCG recipient; appendix p 5).

The proportion of participants reporting related solicited systemic adverse events was similar in MTBVAC and BCG recipients who were QFT positive and QFT negative. Myalgia, headache, and fatigue were similar among MTBVAC recipients who were QFT positive and QFT negative (appendix p 3).

The majority of adverse events were graded mild among both MTBVAC and BCG recipients

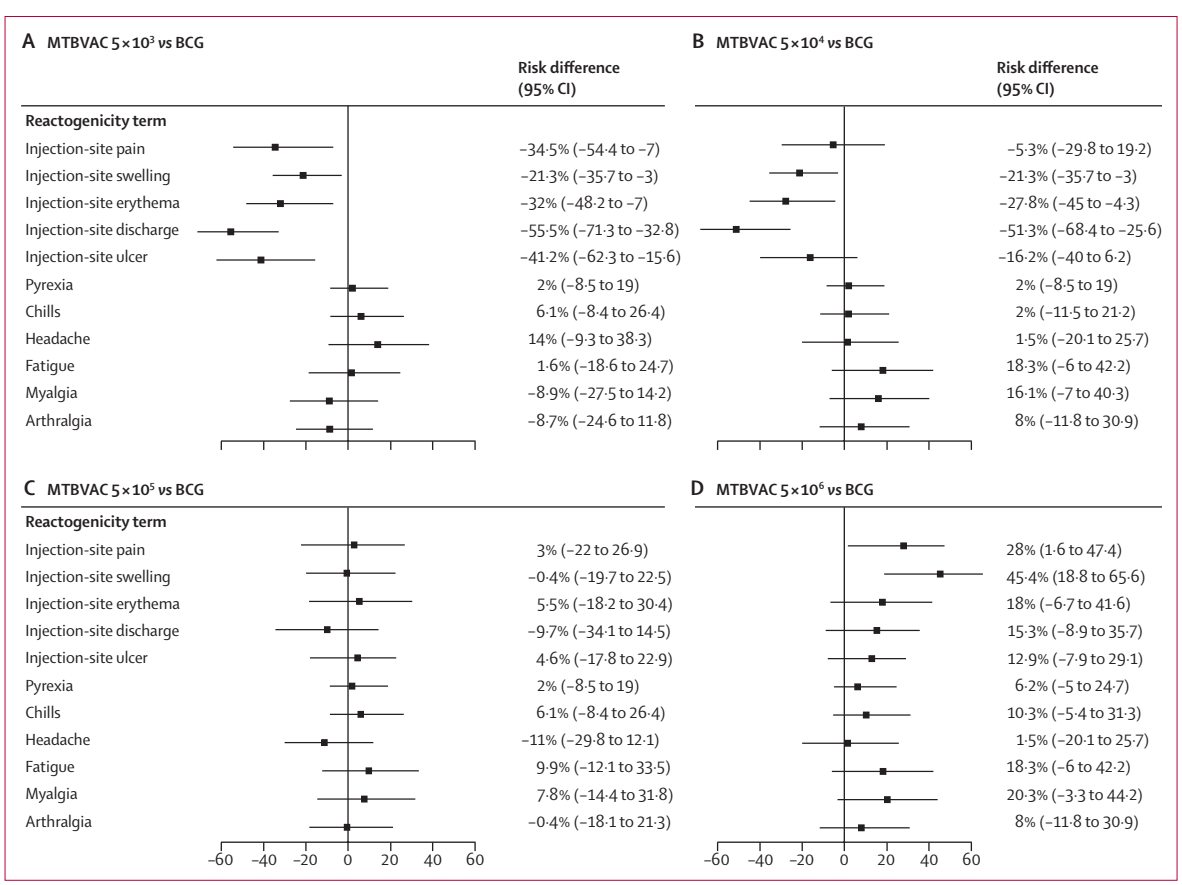
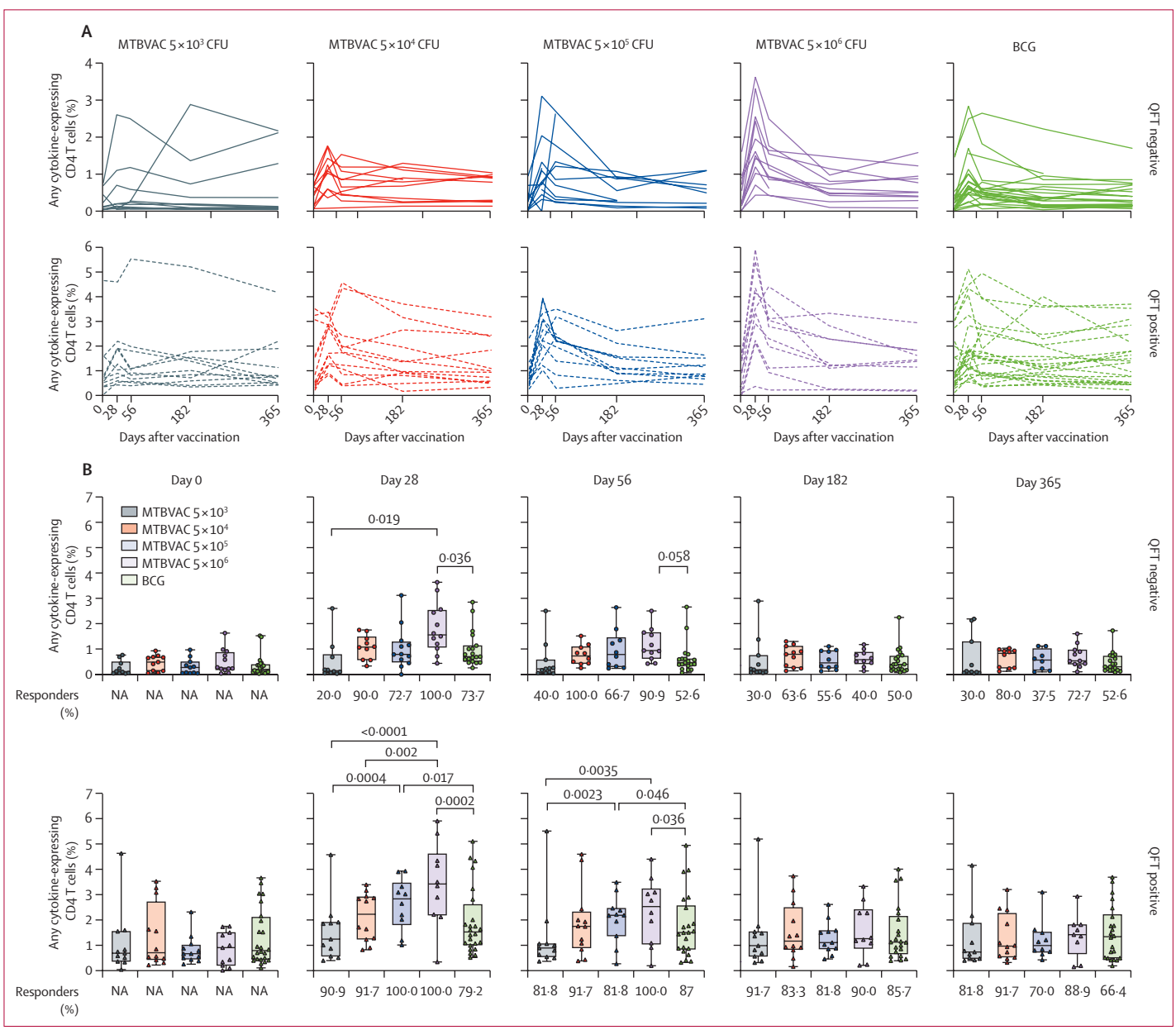



***Figure 2:*** **Risk difference in local and systemic adverse events**
Differences in the proportions of participants between MTBVAC dose cohorts who reported the listed local and systemic adverse events and BCG recipients. Shifts to the right indicate a greater risk in the higher MTBVAC dose cohort than BCG. CIs that do not include zero are considered statistically significant.
MTBVAC=live-attenuated *M tuberculosis* vaccine.

(appendix p 10). One severe reactogenicity event was observed across all MTBVAC dose subgroups and one in BCG recipients (appendix p 5). One severe systemic reaction was reported in one participant in the MTBVAC $5\times10^3$ CFU subgroup (appendix p 5). No participants discontinued the study because of an adverse event.

No relevant differences were noted for laboratory measurements, vital signs, or physical examination findings. Two MTBVAC recipients who were QFT negative developed incident HIV infection. 14 participants were investigated for tuberculosis, but no tuberculosis cases were diagnosed. No deaths and three unrelated SAEs, due to trauma or sepsis, were reported.

Frequencies of MTBVAC-specific CD4 T cells coexpressing IFN-γ, TNF, IL-2, IL-17, and, or IL-22 were measured by WB-ICS. Most participants had detectable prevaccination (day 0) frequencies of MTBVAC-specific CD4 T cells (figure 3); these responses were generally lower in the QFT-negative MTBVAC subgroups (1–4) and the QFT-negative BCG group (appendix p 11) than the QFT-positive MTBVAC subgroups (5–8) and the QFT-positive BCG group; figure 3A–C; appendix p 11). Increases above these prevaccination MTBVAC-specific CD4 T-cell responses, measured after stimulation with MTBVAC, were observed in all MTBVAC and BCG-vaccinated subgroups (figure 3A–B). In most participants,




(Figure 3 continues on next page)

these CD4 T-cell responses peaked at day 28 after vaccination and waned over the 1-year follow-up period (figure 3A–C). The same patterns were observed for BCG-specific cytokine-expressing CD4 T-cell responses, measured by blood stimulation with BCG (appendix p 12). An MTBVAC dose-dependent response was observed in QFT-negative and QFT-positive groups; frequencies of MTBVAC-specific CD4 T-cell responses were higher at day 28 and day 56 in participants who received the highest MTBVAC doses ($5\times10^6$ CFU) than those who received the $5\times10^3$ CFU dose (figure 3). Responses in the two highest MTBVAC dose subgroups also exceeded those in BCG recipients at day 28 and day 56 for the QFT-positive group (figure 3).

We also computed the proportions of responders at each timepoint by quantifying cell-subset-level MTBVAC-specific CD4 T-cell responses above those observed before vaccination using COMPASS (appendix p 14).[22] Higher proportions of positive responders were observed in subgroups that received the higher MTBVAC doses ($5\times10^4$, $5\times10^5$, or $5\times10^6$ CFU) than those that received the lowest $5\times10^3$ CFU dose (figure 3A–B). Similar responder proportion patterns were observed for BCG-specific (blood stimulated with BCG) CD4 T-cell responses (appendix p 15) than MTBVAC-specific responses. Vaccine-induced MTBVAC and BCG-specific CD4 T-cell responses, expressed as cell-subset-level posterior probabilities using COMPASS, were predominantly comprised of cell subsets that coexpressed IFN-γ, TNF, and IL-2, while some low-frequency CD4 T cells also coexpressed IL-22. IL-17A-expressing T cells were not commonly detected (figure 3D; appendix p 12). Frequencies of cytokine-expressing CD8 T cells were also low or not detected in all subgroups, irrespective of the stimulation antigen (except PHA; figure 3D; appendix p 12). We also assessed boosting of MTBVAC-specific CD4 T cells over and above prevaccination responses by computing COMPASS functionality scores at each timepoint after vaccination. Higher functionality scores were observed in QFT-positive versus QFT-negative groups (appendix p 14), suggesting greater boosting in those with *M tuberculosis* sensitisation. This analysis also showed that functionality scores at the visits after vaccination exceeded prevaccination responses up to day 365, providing evidence of long-lasting CD4 T-cell memory responses (appendix p 14).

MTBVAC-specific IgG antibody responses at the prevaccination (day 0) timepoint were highly variable between participants (appendix p 16). However, significant boosting of MTBVAC-specific IgG responses

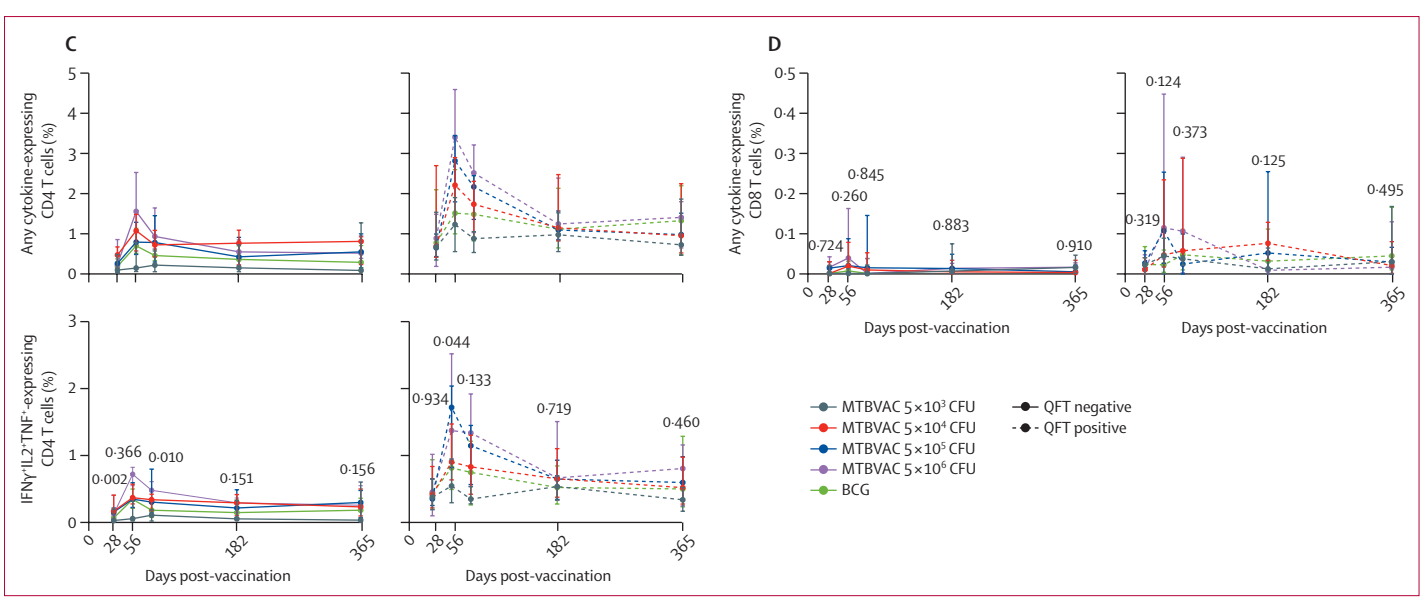



***Figure 3*: Kinetics of cytokine-expressing MTBVAC-specific CD4 or CD8 T-cell responses in individuals vaccinated with different doses of MTBVAC or BCG and stratified by QFT status at screening**
Frequencies of MTBVAC-specific T cells expressing any combination of IFN-γ, IL-2, TNF, IL-17A, or IL-22 (any cytokine-expressing CD4 T cells) measured after MTBVAC stimulation by whole-blood intracellular cytokine staining assay in MTBVAC or BCG vaccinees stratified by QFT status at screening. (A) Individual longitudinal trajectories of CD4 T-cell responses in participants who were QFT negative (top panels) or QFT positive (bottom panels) at screening, stratified by treatment group and dose at the indicated timepoints. Each line represents one participant. (B) Frequencies of CD4 T-cell responses in participants who were QFT negative (top panels) or QFT positive (bottom panels) at screening. Horizontal lines represent medians, boxes the IQR, and whiskers the range. p values were computed by generalised estimating equation (GEE) linear regression assessing the difference between each timepoint after vaccination relative to before vaccination (day 0). Values were adjusted for multiple testing using the Holm method. Only p values lower than 0·1 are shown. The percentage of responders (bottom of the graphs) at each timepoint was computed using COMPASS, relative to baseline to take into account the fact that populations were non-naive at baseline because of either previous BCG vaccination at birth or previous *Mycobacterium tuberculosis* exposure for QFT-positive groups (appendix p 14). (C, D) Median longitudinal trajectories of CD4 (C) and CD8 (D) T-cell frequencies expressing any combination of IFN-γ, IL-2, TNF, IL-17A, or IL-22 (C top panels and D) or co-expressing IFN-γ, IL-2, and TNF (C, bottom panels) in participants who were QFT negative (left) and QFT positive (right), stratified by treatment group and dose at the indicated timepoints. p values between cohorts were calculated using the Kruskal-Wallis test. Error bars denote IQRs. Frequencies of cytokine-positive CD4 T cells in the unstimulated control were subtracted from MTBVAC-stimulated frequencies. CFU=colony-forming unit. IFN=interferon. IL=interleukin. MTBVAC=live-attenuated *M tuberculosis* vaccine. NA=not applicable. QFT=QuantiFERON-tuberculosis Gold-Plus assay. TNF=tumour necrosis factor.

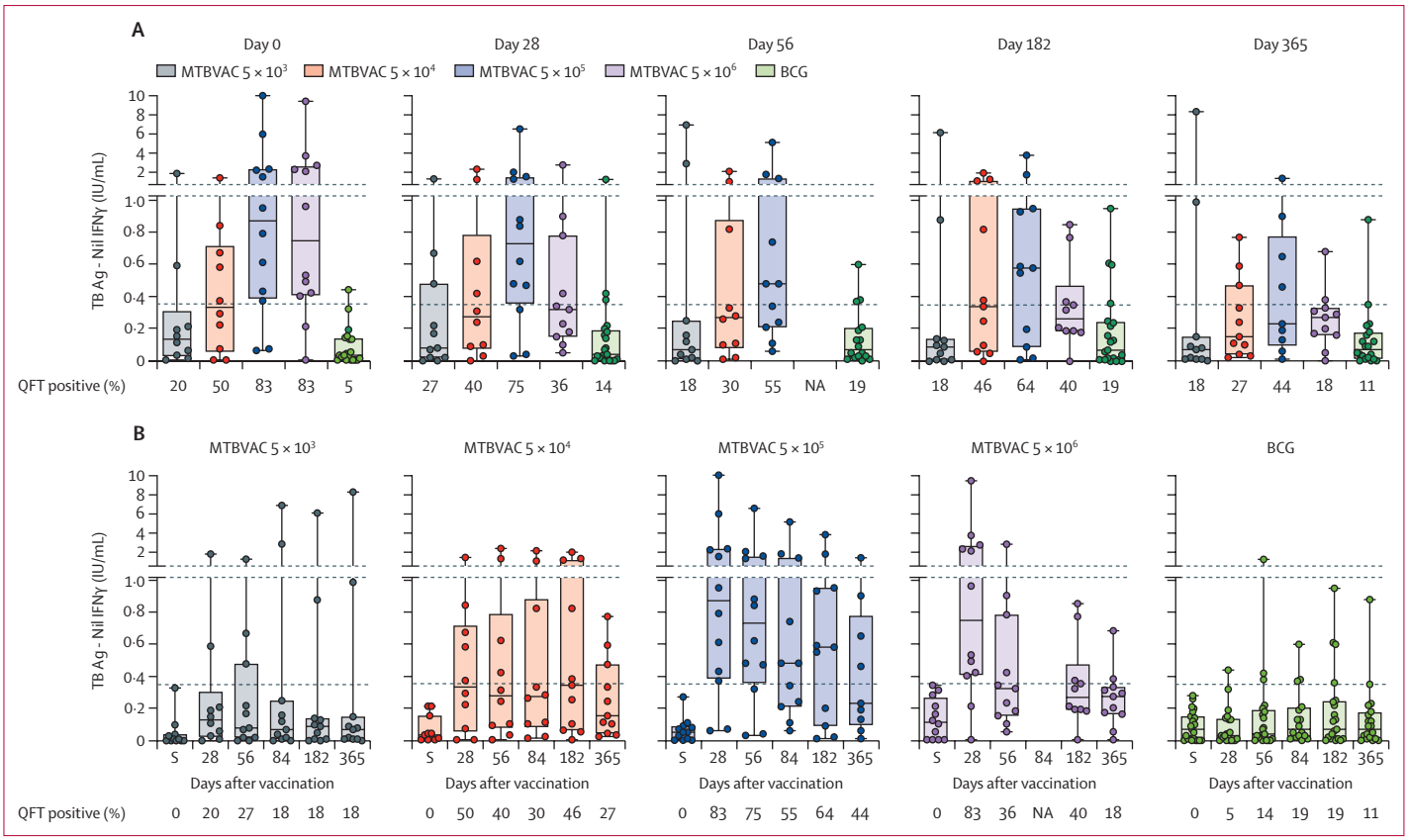



***Figure 4:*** **IFN-γ responses measured by QFT in individuals vaccinated with different doses of MTBVAC or BCG who were QFT negative at screening**
IFN-γ concentrations (IU/mL) measured by QFT, either stratified by timepoints (A) or vaccine group (MTBVAC dose or BCG) at the indicated timepoints after vaccination (B). The higher IFN-γ concentration between TB1 and TB2, after subtracting the Nil condition, is shown. The dotted horizontal lines indicate the QFT positivity cutoff (0·35 IU/mL). Horizontal lines represent medians, boxes the IQR, and whiskers the range. The percentage of participants who were QFN positive at each timepoint for each cohort is indicated below each graph. All day 84 visits in the $5\times10^6$ CFU MTBVAC group were missed because of the COVID-19 lockdown in South Africa. IFN=interferon. MTBVAC=live-attenuated *M tuberculosis* vaccine. QFT=QuantiFERON-tuberculosis Gold-Plus assay.

was observed in some of the QFT-negative and QFT-positive groups at day 56, particularly in the QFT-positive groups who received the higher MTBVAC doses. These responses persisted at levels exceeding day 0 up to day 365 in the MTBVAC $5\times10^5$ CFU-vaccinated group.

Immunogenicity was also assessed using the QFT assay in groups that tested QFT negative at screening (subgroups 1–4 and the QFT-negative BCG group). We did not assess participants who were QFT positive after screening since an accurate assessment of MTBVAC-induced immune responses using the QFT assay was not plausible (data not shown). A dose-dependent increase in IFN-γ levels was observed after MTBVAC vaccination in subgroups 1–4 (figure 4A). The highest responses were observed in vaccinees who received the highest MTBVAC doses ($5\times10^5$ CFU and $5\times10^6$ CFU), while BCG recipients showed no increases in IFN-γ levels (figure 4A–B). As observed for responses measured by ICS assay, QFT-measured IFN-γ levels in the highest MTBVAC dose subgroups peaked at day 28 and thereafter waned up to 1 year after vaccination (figure 4B). These data illustrate that MTBVAC was immunogenic in individuals who were QFT negative and QFT positive and that the $5\times10^5$ and $5\times10^6$ CFU doses were the most immunogenic.

## Discussion

We showed that the live-attenuated tuberculosis vaccine MTBVAC, given at escalating doses ranging from $5\times10^3$ to $5\times10^6$ CFU in adults with and without evidence of previous *M tuberculosis* sensitisation, had a similar tolerability and safety profile to BCG revaccination at the standard $5\times10^5$ CFU dose. The $5\times10^5$ and $5\times10^6$ CFU MTBVAC doses were highly immunogenic in adults, irrespective of baseline QFT status, inducing or boosting antigen-specific T-helper-cell-1 cytokine-expressing CD4 T-cell responses that peaked around 28 days, and which persisted at frequencies that exceeded prevaccination levels up to 1 year after vaccination. Our results suggest that the $5\times10^5$ CFU MTBVAC dose had a similar safety profile to BCG revaccination at the same dose, and that the $5\times10^5$ and $5\times10^6$ CFU MTBVAC doses were more immunogenic than BCG. These findings also suggest no masking or blocking effect on the MTBVAC-induced

immune response when vaccinating individuals previously sensitised by *M tuberculosis*.

MTBVAC appeared safe and tolerable at all doses. MTBVAC at the $5\times10^3$ and $5\times10^4$ CFU doses showed a lower incidence of injection-site reactions than BCG revaccination. We observed an increase in the frequencies of non-severe injection-site pain, discharge, erythema, and swelling as the MTBVAC dose increased. The incidence of grade 3 solicited systemic adverse events was low across all subgroups. The highest MTBVAC dose, $5\times10^6$ CFU, induced significantly higher injection-site pain and swelling than BCG revaccination, whereas the reactogenicity and safety profile of the $5\times10^5$ CFU MTBVAC dose was similar to BCG. These findings are consistent with those reported in the study of MTBVAC administered to adults who were BCG naive and *M tuberculosis* unsensitised in Switzerland.[23]

Culture of both BCG and MTBVAC from injection-site discharge has previously been reported.[23] Local injection-site mycobacterial abscesses were observed in two participants in this study and demonstrate that, like BCG, MTBVAC administration might also be associated with vaccine strain abscess formation at the injection site, possibly associated with error of intradermal vaccination technique.[17,24] Notably, both abscesses resolved without sequelae following aspiration and did not require specific anti-mycobacterial therapy.

Previous *M tuberculosis* sensitisation affected the reactogenicity of MTBVAC in this study. Significantly higher rates of injection-site erythema and ulceration were observed in QFT-positive compared with QFT-negative MTBVAC recipients at all dose levels except $5\times10^5$ CFU, whereas injection-site ulceration risk was similar in QFT-negative and QFT-positive BCG recipients. Ulcerations were not reported previously among neonates who were BCG naive vaccinated with MTBVAC, nor in Swiss adults who were BCG naive and *M tuberculosis* unexposed who received either MTBVAC or BCG.[23] We infer that pre-existing mycobacteria-specific T-cell responses or ongoing *M tuberculosis* infection could result in greater reactogenicity of MTBVAC. No adverse reactions suggestive of the Koch phenomenon or incident tuberculosis cases were reported, and no serious adverse events related to MTBVAC administration were observed. Thus, MTBVAC appears safe in adults sensitised to *M tuberculosis* at all doses investigated. Assessing whether the boosted MTBVAC immune response observed in this study is associated with protection in future efficacy trials will be important.

Our results suggest that the $5\times10^5$ and $5\times10^6$ CFU MTBVAC doses were highly immunogenic and induced durable T-helper-cell-1 cytokine-expressing CD4 T-cell responses that exceeded those induced by BCG revaccination. A key finding was that the $5\times10^5$ and $5\times10^6$ CFU doses were highly immunogenic in adults with a history of previous BCG vaccination, including participants with a positive QFT at the time of MTBVAC administration. Greater boosting of MTBVAC-specific CD4 T cells above the prevaccination responses was observed in the QFT-positive groups compared with the QFT-negative groups. A study of immune responses to BCG revaccination in India also demonstrated boosting of BCG-specific CD4 T-cell responses in adults who were inferferon-gamma release assay (IGRA) negative and IGRA positive.[13] These results are somewhat surprising, given the evidence from several clinical trials[25–30] that vaccination of older children and adults who were previously *M tuberculosis* sensitised with the live-attenuated BCG vaccine was associated with poor or no efficacy against tuberculosis.[2] However, the measured responses might not reflect immune correlates of vaccine-induced protection against tuberculosis, which are yet to be discovered.

Our results expand on those from previous trials, which demonstrated that MTBVAC was highly immunogenic, inducing long-lived antigen-specific T-helper-cell-1 responses in individuals with minimal immunological sensitisation to mycobacterial antigens in Swiss adults with no previous history of tuberculosis or BCG vaccination[23] and South African infants.[17] Participants who were QFT negative and QFT positive in this trial had readily detectable mycobacteria-specific T-helper-cell-1 cytokine-expressing CD4 T-cell responses before vaccination, confirming the high levels of immunological sensitisation in this South African population, as previously observed.[19,31]

Further evidence of promising immunogenicity comes from the finding that MTBVAC induced ESAT-6 or CFP10-specific T-cell responses in the QFT-negative groups, particularly in those who received the $5\times10^5$ and $5\times10^6$ CFU MTBVAC doses. These responses, detected using the QFT assay as significant increases in IFN-γ levels and conversion to QFT-positive status, were transient, peaking at day 28 after vaccination for participants receiving $5\times10^5$ CFU MTBVAC, and reverting in most participants to QFT-negative levels by day 365. QFT conversion was reported previously in two of nine adult MTBVAC recipients and in most neonates who received a $2{\cdot}5\times10^5$ CFU dose of MTBVAC in the previous trial in South Africa.[17]

QFT conversion following MTBVAC administration, which is analogous to a false-positive tuberculin skin-test result induced after BCG vaccination, could be an encouraging signal of potential vaccine efficacy. Preclinical studies of MTBVAC in mice and non-human primates showed that vaccine-induced ESAT-6-specific or CFP10-specific T-cell responses correlated with enhanced vaccine-induced protection against the *M tuberculosis* challenge.[13,16] We recently also found that T-cell responses to CFP10 were enriched in individuals infected with *M tuberculosis* who remained tuberculosis-free relative to those who progressed to tuberculosis.[32] However, it remains unknown whether these antigen-specific responses will contribute to protection against

tuberculosis in humans. The results of this trial provide useful information about the dynamics and duration of MTBVAC-induced QFT conversion. However, they also show that MTBVAC-induced ESAT-6 and CFP10-specific responses after vaccination confound the interpretation of subsequent QFT tests to identify individuals with *M tuberculosis* sensitisation who are at risk of tuberculosis and might benefit from tuberculosis preventive therapy. This limitation is not insurmountable given that national programmatic tuberculosis control guidelines in high-burden countries require a known tuberculosis contact to trigger the provision of tuberculosis preventive therapy, rather than the result of IGRA testing (South African guidelines). However, an alternative test for *M tuberculosis* sensitisation independent of MTBVAC vaccination might facilitate acceptance by tuberculosis programme staff and policy makers. Such companion diagnostics, such as the ESAT-6-free IGRA, are under development for ESAT-6-containing subunit vaccines such as H56:IC31 and H107:CAF10b.[33,34] A CFP10-free IGRA is being developed as a companion assay for MTBVAC (data not shown).

The findings of this trial are pivotal for selecting the optimal MTBVAC dose to proceed into phase 2b–3 trials. On balance, the $5\times10^5$ CFU MTBVAC dose could be selected for efficacy evaluation. Our results suggest that MTBVAC at this dose would be safe, tolerable, and immunogenic in adults with prior BCG vaccination and with and without sensitisation to *M tuberculosis*. These results pave the way for these populations to be included in a planned efficacy trial of MTBVAC.

#### Contributors

ALKK, VR, CI, TS, and MH contributed to data analysis, interpretation, and writing. ALKK, VR, TS, and MH contributed to writing the original draft. CM, NA, MR, DT, DH, KR, and LS contributed to the review and editing. ALKK, MT, FR, SCM, JS, and HG contributed to the data collection and safety monitoring. CM, NA, and IM contributed to the conceptualisation, study design, methodology, data analysis, and interpretation. HM, NB, and SM contributed to the data curation. DH, NA, CI, MM, VR, and TS contributed to the formal analysis. IM and MR contributed to the funding acquisition. IM, MR, CP, DT, EP, ES, JD, and MF contributed to the project administration and resources. ALKK, VR, and CI contributed equally. MH and TS contributed equally. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

#### Declaration of interests

We declare no competing interests.

#### Data sharing

The study protocol, de-identified safety, immunology, and clinical metadata for all participants will be available on Zivahub (https://zivahub.uct.ac.za/) from Oct 5, 2024. Zivahub is an open-access data repository hosted by the University of Cape Town's data repository powered by Figshare for institutions.

#### Acknowledgments

We gratefully acknowledge the study participants and funders. Aeras and, subsequently, IAVI sponsored the trial through CDMRP (W81XWH-17-1-0656) and NIH funding (U01AI131861). During the preparation of this work, AKKL used Grammarly to improve the language and readability. After using this tool and service, AKKL reviewed and edited the content as needed and took full responsibility for the publication's content.

#### References

1 WHO. Global TB report 2024. World Health Organization, 2024.
2 Mangtani P, Abubakar I, Ariti C, et al. Protection by BCG vaccine against tuberculosis: a systematic review of randomized controlled trials. *Clin Infect Dis* 2014; **58:** 470–80.
3 Trunz BB, Fine P, Dye C. Effect of BCG vaccination on childhood tuberculous meningitis and miliary tuberculosis worldwide: a meta-analysis and assessment of cost-effectiveness. *Lancet* 2006; **367:** 1173–80.
4 Dockrell HM, Smith SG. What have we learnt about BCG vaccination in the last 20 years? *Front Immunol* 2017; **8:** 1134.
5 Harris RC, Sumner T, Knight GM, White RG. Systematic review of mathematical models exploring the epidemiological impact of future TB vaccines. *Hum Vaccin Immunother* 2016; **12:** 2813–32.
6 Harris RC, Sumner T, Knight GM, Zhang H, White RG. Potential impact of tuberculosis vaccines in China, South Africa, and India. *Sci Transl Med* 2020; **12:** eaax4607.
7 Clark RA, Mukandavire C, Portnoy A, et al. The impact of alternative delivery strategies for novel tuberculosis vaccines in low-income and middle-income countries: a modelling study. *Lancet Glob Health* 2023; **11:** e546–55.
8 Portnoy A, Clark RA, Quaife M, et al. The cost and cost-effectiveness of novel tuberculosis vaccines in low- and middle-income countries: a modeling study. *PLoS Med* 2023; **20:** e1004155.
9 WHO. An investment case for new tuberculosis vaccines. World Health Organization, 2020.
10 WHO. Preferred product characteristics for new tuberculosis vaccines. World Health Organization, 2018.
11 Arbues A, Aguilo JI, Gonzalo-Asensio J, et al. Construction, characterization and preclinical evaluation of MTBVAC, the first live-attenuated *M tuberculosis*-based vaccine to enter clinical trials. *Vaccine* 2013; **31:** 4867–73.
12 Gonzalo-Asensio J, Marinova D, Martin C, Aguilo N. MTBVAC: attenuating the human pathogen of tuberculosis (TB) toward a promising vaccine against the TB epidemic. *Front Immunol* 2017; **8:** 1803.
13 Aguilo N, Gonzalo-Asensio J, Alvarez-Arguedas S, et al. Reactogenicity to major tuberculosis antigens absent in BCG is linked to improved protection against *Mycobacterium tuberculosis*. *Nat Commun* 2017; **8:** 16085.
14 Aguilo N, Uranga S, Marinova D, Monzon M, Badiola J, Martin C. MTBVAC vaccine is safe, immunogenic and confers protective efficacy against Mycobacterium tuberculosis in newborn mice. *Tuberculosis* 2016; **96:** 71–74.
15 Martín C, Marinova D, Aguiló N, Gonzalo-Asensio J. MTBVAC, a live TB vaccine poised to initiate efficacy trials 100 years after BCG. *Vaccine* 2021; **39:** 7277–85.
16 White AD, Sibley L, Sarfas C, et al. MTBVAC vaccination protects rhesus macaques against aerosol challenge with M. tuberculosis and induces immune signatures analogous to those observed in clinical studies. *NPJ Vaccines* 2021; **6:** 4.
17 Tameris M, Mearns H, Penn-Nicholson A, et al, and the MTBVAC Clinical Trial Team. Live-attenuated *Mycobacterium tuberculosis* vaccine MTBVAC versus BCG in adults and neonates: a randomised controlled, double-blind dose-escalation trial. *Lancet Respir Med* 2019; **7:** 757–70.
18 Mahomed H, Hughes EJ, Hawkridge T, et al. Comparison of mantoux skin test with three generations of a whole blood IFN-gamma assay for tuberculosis infection. *Int J Tuberc Lung Dis* 2006; **10:** 310–16.
19 Bunyasi EW, Geldenhuys H, Mulenga H, et al. Temporal trends in the prevalence of *Mycobacterium tuberculosis* infection in South African adolescents. *Int J Tuberc Lung Dis* 2019; **23:** 571–78.
20 Western Cape Government. Department of Health and Wellness. Provincial TB dashboard. Breede Valley total TB notifications 2022. https://www.westerncape.gov.za/health-wellness/provincial-tb-dashboard (accessed March 25, 2025).
21 US Food and Drug Administration. Modified final US FDA toxicity table for healthy and adolescents subjects enrolled in preventive vaccine. Toxicity grading scale for healthy adult and adolescent volunteers enrolled in preventive vaccine clinical trials. https://www.fda.gov/regulatory-information/search-fda-guidance-documents/toxicity-grading-scale-healthy-adult-and-adolescent-volunteers-enrolled-preventive-vaccine-clinical (accessed March 26, 2025).

22 Lin L, Finak G, Ushey K, et al. COMPASS identifies T-cell subsets correlated with clinical outcomes. *Nat Biotechnol* 2015; **33:** 610–16.

23 Spertini F, Audran R, Chakour R, et al. Safety of human immunisation with a live-attenuated *Mycobacterium tuberculosis* vaccine: a randomised, double-blind, controlled phase I trial. *Lancet Respir Med* 2015; **3:** 953–62.

24 Villanueva P, Wadia U, Crawford N, et al. Revaccination with bacille Calmette-Guérin (BCG) is associated with an increased risk of abscess and lymphadenopathy. *NPJ Vaccines* 2022; **7:** 6.

25 Karonga Prevention Trial Group. Randomised controlled trial of single BCG, repeated BCG, or combined BCG and killed *Mycobacterium leprae* vaccine for prevention of leprosy and tuberculosis in Malawi. *Lancet* 1996; **9019:** 17–24.

26 Barreto ML, Cunha SS, Pereira SM, et al. Neonatal BCG protection against tuberculosis lasts for 20 years in Brazil. *Int J Tuberc Lung Dis* 2005*;* **9:** 1171–73.

27 Hawkridge A, Hatherill M, Little F, et al. Efficacy of percutaneous versus intradermal BCG in the prevention of tuberculosis in South African infants: randomised trial. *BMJ* 2008; **337:** a2052.

28 Tuberculosis Research Centre. Fifteen year follow up of trial of BCG vaccines in south India for tuberculosis prevention. Tuberculosis Research Centre (ICMR), Chennai. *Indian J Med Res* 1999; **110:** 56–69.

29 Aronson NE, Santosham M, Comstock GW, et al. Long-term efficacy of BCG vaccine in American Indians and Alaska Natives: a 60-year follow-up study. *Vaccine* 2004; **291:** 2086–91.

30 Roth AE, Benn CS, Ravn H, et al. Effect of revaccination with BCG in early childhood on mortality: randomised trial in Guinea-Bissau. *BMJ* 2010; **340:** c671.

31 Mahomed H, Hawkridge T, Verver S, et al. The tuberculin skin test versus QuantiFERON TB Gold in predicting tuberculosis disease in an adolescent cohort study in South Africa. *PLoS One* 2011; **6:** e17984.

32 Musvosvi M, Huang H, Wang C, et al. T cell receptor repertoires associated with control and disease progression following *Mycobacterium tuberculosis* infection. *Nat Med* 2023; **29:** 258–69.

33 Ruhwald M, de Thurah L, Kuchaka D, et al. Introducing the ESAT-6 free IGRA, a companion diagnostic for TB vaccines based on ESAT-6. *Sci Rep* 2017; **7:** 45969.

34 Nemes E, Abrahams D, Scriba TJ, et al. Diagnostic accuracy of early secretory antigenic target-6-free interferon-gamma release assay compared to QuantiFERON-TB Gold in-tube. *Clin Infect Dis* 2019; **69:** 1724–30.