Live-attenuated *Mycobacterium tuberculosis* vaccine MTBVAC versus BCG in adults and neonates: a randomised controlled, double-blind dose-escalation trial

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Summary

Background Infants are a key target population for new tuberculosis vaccines. We assessed the safety and immunogenicity of the live-attenuated *Mycobacterium tuberculosis* vaccine candidate MTBVAC in adults and infants in a region where transmission of tuberculosis is very high.

Methods We did a randomised, double-blind, BCG-controlled, dose-escalation trial at the South African Tuberculosis Vaccine Initiative site near Cape Town, South Africa. Healthy adult community volunteers who were aged 18–50 years, had received BCG vaccination as infants, were HIV negative, had negative interferon-γ release assay (IGRA) results, and had no personal history of tuberculosis or current household contact with someone with tuberculosis were enrolled in a safety cohort. Infants born to HIV-negative women with no personal history of tuberculosis or current household contact with a person with tuberculosis and who were 96 h old or younger, generally healthy, and had not yet received routine BCG vaccination were enrolled in a separate infant cohort. Eligible adults were randomly assigned (1:1) to receive either BCG Vaccine SSI (5×10^5 colony forming units [CFU] of Danish strain 1331 in 0·1 mL diluent) or MTBVAC (5×10^5 CFU in 0·1 mL) intradermally in the deltoid region of the arm. After favourable review of 28-day reactogenicity and safety data in the adult cohort, infants were randomly assigned (1:3) to receive either BCG Vaccine SSI ($2\cdot5\times10^5$ CFU in 0·05 mL diluent) or MTBVAC in three sequential cohorts of increasing MTBVAC dose ($2\cdot5\times10^3$ CFU, $2\cdot5\times10^4$ CFU, and $2\cdot5\times10^5$ CFU in 0·05 mL) intradermally in the deltoid region of the arm. QuantiFERON-TB Gold In-Tube IGRA was done on days 180 and 360. For both randomisations, a pre-prepared block randomisation schedule was used. Participants (and their parents or guardians in the case of infant participants), investigators, and other clinical and laboratory staff were masked to intervention allocation. The primary outcomes, which were all measured in the infant cohort, were solicited and unsolicited local adverse events and serious adverse events until day 360; non-serious systemic adverse events until day 28 and vaccine-specific CD4 and CD8 T-cell responses on days 7, 28, 70, 180, and 360. Secondary outcomes measured in adults were local injection-site and systemic reactions and haematology and biochemistry at study day 7 and 28. Safety analyses and immunogenicity analyses were done in all participants who received a dose of vaccine. This trial is registered with ClinicalTrials.gov, number NCT02729571.

Findings Between Sept 29, 2015, and Nov 16, 2015, 62 adults were screened and 18 were enrolled and randomly assigned, nine each to the BCG and MTBVAC groups. Between Feb 12, 2016, and Sept 21, 2016, 36 infants were randomly assigned—eight to the BCG group, nine to the $2\cdot5\times10^3$ CFU MTBVAC group, nine to the $2\cdot5\times10^4$ CFU group, and ten to the $2\cdot5\times10^5$ CFU group. Mild injection-site reactions occurred only in infants in the BCG and the $2\cdot5\times10^5$ CFU MTBVAC group, with no evidence of local or regional injection-site complications. Systemic adverse events were evenly distributed across BCG and MTBVAC dose groups, and were mostly mild in severity. Eight serious adverse events were reported in seven vaccine recipients (one adult MTBVAC recipient, one infant BCG recipient, one infant in the $2\cdot5\times10^3$ CFU MTBVAC group, two in the $2\cdot5\times10^4$ CFU MTBVAC group, and two in the $2\cdot5\times10^5$ CFU MTBVAC group), including one infant in the $2\cdot5\times10^3$ CFU MTBVAC group treated for unconfirmed tuberculosis and one in the $2\cdot5\times10^5$ CFU MTBVAC group treated for unlikely tuberculosis. One infant died as a result of possible viral pneumonia. Vaccination with all MTBVAC doses induced durable antigen-specific T-helper-1 cytokine-expressing CD4 cell responses in infants that peaked 70 days after vaccination and were detectable 360 days after vaccination. For the highest MTBVAC dose (ie, $2\cdot5\times10^5$ CFU), these responses exceeded responses induced by an equivalent dose of the BCG vaccine up to 360 days after vaccination. Dose-related IGRA conversion was noted in three (38%) of eight infants in the $2\cdot5\times10^3$ CFU MTBVAC group, six (75%) of eight in the $2\cdot5\times10^4$ CFU MTBVAC group, and seven (78%) of nine in the $2\cdot5\times10^5$ CFU MTBVAC group at day 180, compared with none of seven infants in the BCG group. By day 360, IGRA reversion had occurred in all three infants (100%) in the $2\cdot5\times10^3$ CFU MTBVAC group, four (67%) of the six in the $2\cdot5\times10^4$ CFU MTBVAC group, and three (43%) of the seven in the $2\cdot5\times10^5$ CFU MTBVAC group.

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Interpretation **MTBVAC had acceptable reactogenicity, and induced a durable CD4 cell response in infants. The evidence of immunogenicity supports progression of MTBVAC into larger safety and efficacy trials, but also confounds interpretation of tests for *M tuberculosis* infection, highlighting the need for stringent endpoint definition.**

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Introduction

More than 1 million children develop tuberculosis every year, accounting for roughly 10% of the global burden of new disease.[1] Most of these cases occur in countries with universal infant coverage of BCG vaccination.[2] Infant BCG vaccination provides protection against severe disseminated forms of childhood tuberculosis, but efficacy and durability of protection against intrathoracic tuberculosis are highly variable.[3–5] BCG vaccination might also provide some protection against all-cause infant mortality.[6] Infants and children younger than 5 years, who carry the greatest burden of severe morbidity and mortality caused by tuberculosis, are a priority target population for new vaccine development.[7,8] A new vaccine to replace or boost infant BCG vaccination is needed to provide consistent and durable protective immunity against tuberculosis in children and adults.[8]

Childhood vaccines based on live, whole, attenuated viruses or bacteria, such as the smallpox and measles vaccines, induce very long-lived protective immunity.[9] Evidence from a non-human primate model[10] and a meta-analysis[11] of human studies from the pre-antibiotic era suggests that the host immune response induced by latent *Mycobacterium tuberculosis* infection confers protection against reinfection and subsequent active tuberculosis disease, which supports development of a live-attenuated *M tuberculosis* vaccine.

MTBVAC (which was developed at the University of Zaragoza [Zaragoza, Spain] and licensed to Biofabri [Pontevedra, Spain]) is a live, rationally attenuated clinical strain of *M tuberculosis* Euro-American lineage 4 that has been genetically engineered to contain two independent unmarked stable deletion mutations in the virulence genes *phoP* and *fadD26*, without antibiotic resistance markers.[12] The transcription factor PhoP regulates expression of 2% of the *M tuberculosis* genome, including production of immunomodulatory cell-wall lipids and secretion of the immunogenic protein ESAT-6.[13] Deletion of *fadD26* results in abrogation of synthesis of phthiocerol dimycocerosates, which are necessary for *M tuberculosis* virulence.[14] MTBVAC thus has a similar protein, lipid, and carbohydrate antigen repertoire to virulent *M tuberculosis*, excluding those regulated by *PhoP* and coded by *fadD26*. MTBVAC contains all the *M tuberculosis* genes that are in the BCG vaccine, plus genes from *Mycobacterium bovis* that are deleted in the BCG vaccine. Of the 1603 experimentally validated human T-cell epitopes of *M tuberculosis*, 433 (27%) are located in deleted regions that are absent from the BCG vaccine.[12,15,16]

Preclinical studies show comparable biodistribution and equivalent or improved safety and immunogenicity for vaccination with MTBVAC as compared to BCG.[12,17,18]

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 up to Feb 10, 2019. We identified only one published clinical trial of a live whole cell *Mycobacterium tuberculosis* vaccine, MTBVAC, which was done by Spertini and colleagues in BCG-naive Swiss adults. The safety of vaccination with MTBVAC was similar to that of BCG, and MTBVAC was at least as immunogenic as BCG.

Added value of this study

To our knowledge, ours is the first clinical trial of a live whole cell *M tuberculosis* vaccine to be done in BCG-vaccinated adults in a region where tuberculosis is endemic and in BCG-naive infants in any region. MTBVAC had similar safety and reactogenicity to BCG vaccination in infant participants. The highest MTBVAC dose tested ($2{\cdot}5 \times 10^5$ colony-forming units) induced a response of greater magnitude than the equivalent dose of the BCG vaccine up to 360 days after vaccination. We noted a higher-than-expected frequency of dose-dependent positive interferon-γ release assay (IGRA) results in MTBVAC recipients.

Implications of all the available evidence

Our safety and immunogenicity results support the progression of MTBVAC to larger dose-defining studies and subsequent infant efficacy trials. Although dose-dependent IGRA conversion and reversion is an encouraging sign of immunogenicity, it might complicate future use of IGRA as a diagnostic tool for *M tuberculosis* infection in MTBVAC recipients. Future studies should attempt to differentiate the vaccine-induced response from that of natural *M tuberculosis* infection, define the mechanism and duration of IGRA cross-reactivity with MTBVAC, and apply stringent tuberculosis endpoint definitions independent of IGRA.

Aguilo and colleagues hypothesised that MTBVAC could provide better protection against tuberculosis than the BCG vaccine because it induces immune responses to more *M tuberculosis*-specific antigenic targets.[19] The mechanisms underlying BCG-induced immunological protection are incompletely understood.[20] Although animal models have clearly shown that intact T-helper-1 (Th1) immunity is necessary to control *M tuberculosis*,[20] human studies have not consistently shown an association between Th1 responses and risk of tuberculosis disease. A study[21] of immune responses in infants in the MVA85A phase 2B trial[22] suggested that increased numbers of BCG-reactive cells secreting interferon-γ (IFN-γ), as measured with the ELIspot assay, were associated with a reduced risk of developing tuberculosis. However, although the MVA85A vaccine induced long-lived, polyfunctional Th1 CD4 T cells that co-expressed IFN-γ, tumour necrosis factor α (TNFα), and interleukin 2 (IL-2), no vaccine efficacy was noted.[22] Similarly, in a study[23] of 10-week-old infants who received BCG vaccination at birth, frequencies of BCG-reactive Th1 cells were not associated with subsequent risk of tuberculosis.

Preclinical and clinical development of MTBVAC was guided by the Second Geneva Consensus recommendations for novel live TB vaccines.[24] Infants are the primary target population for MTBVAC and other live mycobacterial vaccines, because, like the BCG vaccine, this class of vaccines has the potential to protect infants and children against both *M tuberculosis* infection and subsequent active tuberculosis disease.[3,25,26] Vaccination of infants soon after birth might also circumvent masking or blocking effects against live-attenuated vaccines by previous mycobacterial sensitisation in older children and adults.[3]

MTBVAC was safe in newborn, SCID, and BALB/c mice and in guinea pigs.[12,27] It was also safe and immunogenic in BCG-naive adults with negative results on a purified protein derivative skin test in Switzerland in three dose cohorts, who were injected with 5×10^3, 5×10^4, and 5×10^5 colony-forming units (CFU) in 0·1 mL, respectively.[28] However, the safety and immunogenicity of tuberculosis vaccines in infants cannot be directly inferred from adult studies, even those done in BCG-naive and *M tuberculosis*-naive populations.[22,29] Infant and adult findings for cross-reactivity between mycobacterial vaccines and common tests for *M tuberculosis* infection could also be discordant.[28,30] Studies in older children and adults in countries where previous *M tuberculosis* exposure is common and BCG vaccination is universal could potentially misdirect assessment of potential harms and immunogenicity in infants. Thus, reliable safety and immunogenicity data to inform clinical development of live mycobacterial vaccines for infants have to be obtained from infant studies in tuberculosis-endemic countries. Here we report the findings of a dose-escalation trial of MTBVAC in South African infants.

Methods

Study design and participants

We did a randomised, double-blind, BCG-controlled, dose-escalation trial of MTBVAC in South African infants, preceded by an adult safety cohort. The trial was done at the South African Tuberculosis Vaccine Initiative site near Cape Town, South Africa, where transmission of *M tuberculosis* is very high.[26,31,32] Healthy adult community volunteers were enrolled in the adult safety cohort if they were aged 18–50 years, had received BCG vaccination as infants, were HIV negative (as measured with the Uni-Gold Recombigen HIV-1 and HIV-2 rapid test (Trinity Biotech, Bray, Ireland), with positive results confirmed by ELISA), had negative IFN-γ release assay (IGRA) results (measured with the Quantiferon-TB Gold In-Tube [QFT; Qiagen, Hilden, Germany] according to manufacturers' instructions), and had no personal history of tuberculosis or current household contact with someone with tuberculosis. For the infant cohort, we approached HIV-negative women with no personal history of tuberculosis or current household contact with a person with tuberculosis at public antenatal clinics during their third trimester of pregnancy. Infants born to consenting women were eligible for inclusion in the trial if they were 96 h old or younger, generally healthy, weighed at least 2·45 kg, had 5-min Apgar scores of at least 7, were of 38 weeks' gestation or longer, and had not yet received routine BCG vaccination. A full list of inclusion and exclusion criteria is available in the trial protocol.

For the **trial protocol** see https://zivahub.uct.ac.za/s/ee212125b49b1982588a

The trial protocol was approved by the South African Health Products Regulatory Authority and the University of Cape Town Human Research Ethics Committee. Written informed consent was obtained from adult participants before any study procedures, and from the mothers of infant participants before the onset of labour.

Randomisation and masking

Adults judged by investigators to be eligible for inclusion in the trial were enrolled and immediately randomly assigned (1:1) to receive BCG Vaccine SSI (Statens Serum Institut, Copenhagen, Denmark) or MTBVAC (Biofabri, Pontevedra, Spain). The study team used a pre-prepared block randomisation schedule that was generated off-site by the clinical research organisation TCD (Centurion, South Africa) in SAS (version 9.4), which linked treatment numbers to the allocated treatment. Study pharmacists allocated a treatment number (and corresponding treatment) in ascending order sequentially as participants were enrolled.

After favourable review of 28-day reactogenicity and safety data in the adult cohort by an independent data and safety monitoring board, eligible infants were randomly assigned (1:3) to receive either BCG Vaccine SSI or MTBVAC in three sequential cohorts of increasing MTBVAC dose. Dose escalation between infant cohorts was dependent upon favourable review of 28-day data for

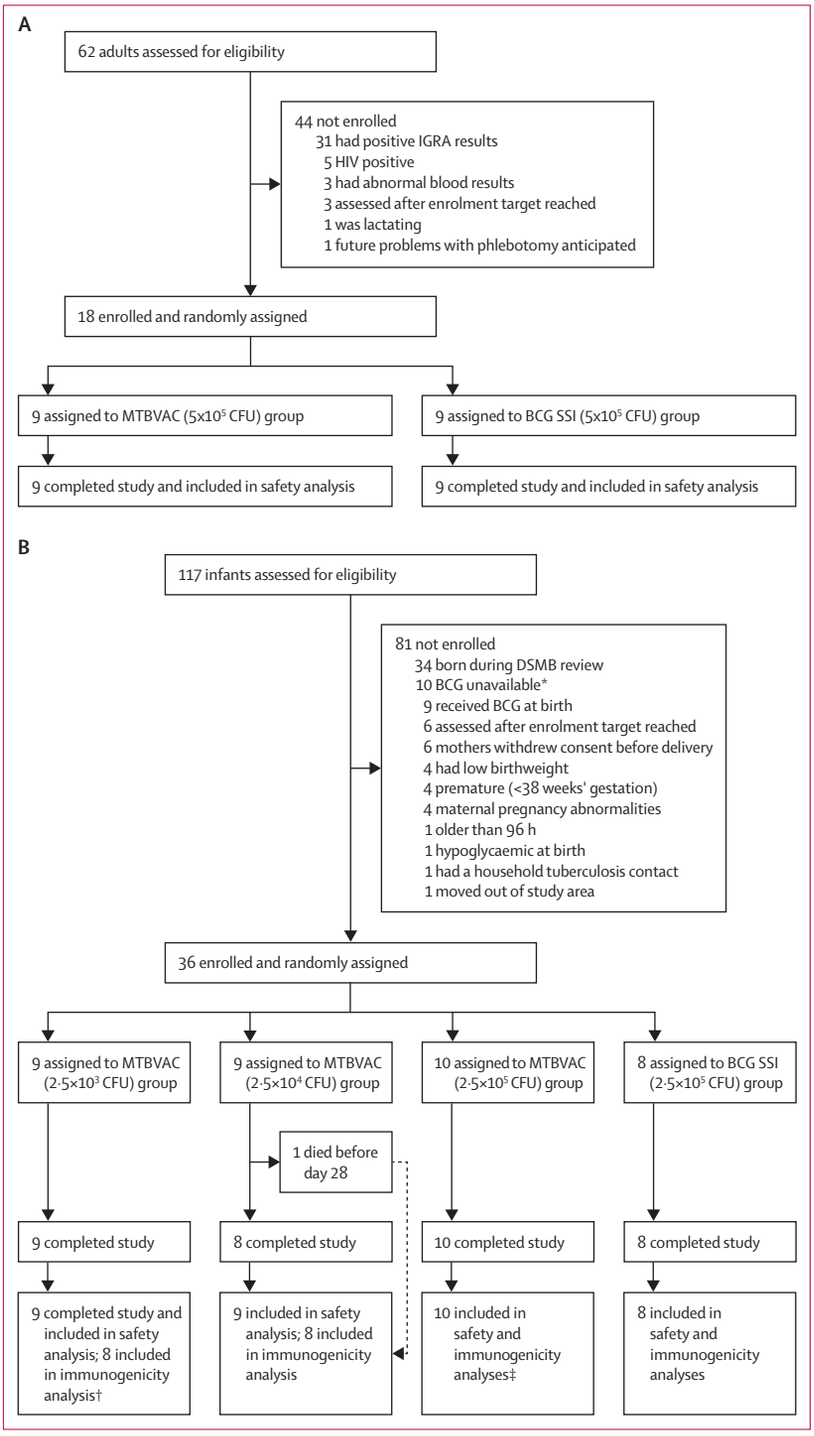


Figure 1: **Trial profiles for the adult (A) and infant (B) cohorts**
Safety monitoring continued for all infants until the end of the trial. IGRA=interferon-γ release assay. CFU=colony-forming units. DSMB=data and safety monitoring board. *Babies were born during a shortage of BCG, and thus study enrolment was paused. †One parent refused to allow blood to be drawn at all timepoints, and a parent of a second infant refused to allow blood to be drawn after day 7 (although the initial samples taken were included in immunogenicity analyses). ‡A parent of one infant refused to allow any further blood to be drawn after day 28.

reactogenicity, adverse events, and serious adverse events by the data and safety monitoring board at the preceding MTBVAC dose level. The randomisation process in the infant cohort was the same that was used for the adult cohort.

Participants and parents or carers were masked to group assignment. All vaccine syringes were prepared by the study pharmacist, masked by semitransparent tape, and administered by a vaccination nurse who was not involved in study safety assessments. Investigators and other clinical and laboratory staff who were involved in assessing outcomes and analysing data remained masked to adult intervention allocation until study completion. Clinical staff were unmasked to infant group allocation after 180 days' follow-up, so that BCG vaccination could be offered to MTBVAC recipients per protocol. Laboratory staff remained masked to infant intervention assignment until all immunology data had been analysed.

Procedures

Adults were administered either BCG Vaccine SSI (5×10^5 CFU of Danish strain 1331 in 0·1 mL diluent) or MTBVAC (5×10^5 CFU in 0·1 mL) intradermally on study day 0 in the deltoid region of their non-dominant arm. Infants were administered either the BCG vaccine ($2\cdot5\times10^5$ CFU in 0·05 mL diluent) or MTBVAC ($2\cdot5\times10^3$ CFU, $2\cdot5\times10^4$ CFU, or $2\cdot5\times10^5$ CFU in 0·05 mL) intradermally on day 0 in the deltoid region of their left arm. Follow-up visits were scheduled on study days 7, 14, 28, 56, 90, and 180 in the adult cohort, and on days 7, 14, 28, 70, 91, 180, and 360 in the infant cohort. In both groups, injection-site reactions were assessed at all visits. In the adult cohort, all adverse events were recorded until day 90 on diary cards and at follow-up visits. In the infant cohort, all adverse events were recorded until day 360 on diary cards, at follow-up visits and during any other contact with caregivers between follow-up visits. Haematological (full blood count, differential white cell count, and platelets) and biochemical (liver and renal function) analyses were done on days 7 and 28 in both cohorts. QFT IGRA was done on day 180 in both cohorts, and also on day 360 in the infant cohort.

Venous blood for immunogenicity outcomes was collected on days 7, 28, 70, 180, and 360 in the infant cohort. Immunogenicity of MTBVAC was assessed in infants by measuring frequencies of CD4 and CD8 T cells expressing antigen-specific IFN-γ, TNFα, IL-2, interleukin 17 (IL-17), or IL-22. Longitudinal T-cell response magnitudes after MTBVAC or BCG vaccination were measured in each infant by computing the total area under the response curve (AUC), allowing comparison between groups. We compared the change in antigen-specific response magnitude from baseline (day 7) to peak (day 70) and from baseline to day 360, the latter of which we defined as the memory response. We also measured vaccine-induced

immune responses on days 180 and 360 with an IGRA incorporating the *M tuberculosis*-specific antigens ESAT-6 and CFP-10.

All infants were screened actively for risk factors and symptoms compatible with tuberculosis disease at each visit. Infants with new symptoms, household tuberculosis contacts, or positive IGRA test conversion (which was defined as >0·35 international units IFN-γ per mL) were referred to the clinic for assessment of possible tuberculosis disease and provision of free curative treatment or isoniazid preventive therapy as indicated. After unblinding at day 180, infant MTBVAC recipients were offered catch-up BCG vaccination. Active tuberculosis surveillance ended at the day 360 visit, after which passive tuberculosis surveillance by telephone contact continued until infants were aged 2 years.

Outcomes

The primary aims of the trial were to assess the safety, reactogenicity, and immunogenicity of three different doses of MTBVAC compared with BCG in infants. The primary safety and reactogenicity outcomes were solicited and unsolicited local adverse events and serious adverse events until day 360, and non-serious systemic adverse events until day 28. These outcomes were assessed by recording local injection-site reactions (erythema, ulceration, induration, swelling, tenderness, drainage, scarring, axillary or cervical lymphadenopathy, and abscess formation), solicited systemic adverse events (fever, irritability, myalgia, headache, changes in feeding patterns, lethargy, skin rash, weight loss, and failure to thrive), and other unsolicited systemic adverse events up to day 360. Adverse events and serious adverse events are reported by study group and MTBVAC dose group, and were classified by severity and relationship to the intervention.

Primary immunogenicity outcomes were antigen-specific CD4 and CD8 T-cell responses on days 7, 28, 70, 180 and 360, which were measured by whole blood intracellular cytokine staining as previously described,[33] after stimulation with MTBVAC (1×10^6 CFU per mL blood), a pool of 122 peptides of immunodominant *M tuberculosis* epitopes (MegaPool [Genscript, Piscataway, NJ, USA];[34] 1 μg/mL per peptide), phytohaemagglutinin (R30852801 [Thermo Fisher Scientific, Waltham MA, USA; 5 μg/mL), or media (unstimulated control). Details of the assay procedures are in the appendix (pp 1–3).

Safety and reactogenicity in adults, which were assessed before infant recruitment, were secondary outcomes, and were assessed by recording local injection-site and systemic reactions (including malaise, fever, myalgia, headache, and skin rash). Haematological and biochemical parameters at day 7 and 28 were compared with baseline in adults and infants.

The protocol was amended on Dec 23, 2016, to allow for assessment of the potential for MTBVAC to cross-react with the *M tuberculosis* antigens ESAT-6 and CFP-10 as a secondary outcome, which was measured by IGRA in both age groups. A full list of all outcomes is provided in the trial protocol. The protocol also details procedures for grading the severity of adverse events.

	BCG group (5×10^5 colony-forming units; n=9)	MTBVAC group (5×10^5 colony-forming units; n=9)
Age, years	28·4 (8·1)	29·4 (8·2)
Height, cm	160·8 (5·5)	162·0 (7·1)
Weight, kg	63·9 (19·6)	85·2 (26·0)
BMI, kg/m²	24·7 (7·3)	32·3 (9·2)
Sex		
Female	7 (78%)	8 (89%)
Male	2 (22)	1 (11%)
Race		
Black	3 (33%)	3 (33%)
Cape mixed ancestry	6 (67%)	6 (67%)
Current smoker	5 (56%)	1 (11%)

Data are mean (SD) or n (%).

Table 1: **Baseline demographics in the adult safety cohort**

	BCG group (5×10^5 colony-forming units; n=9)	MTBVAC group (5×10^5 colony-forming units; n=9)
Participants with at least one adverse event	9 (100%)	9 (100%)
Adverse events	97	93
Solicited adverse events	65	60
Severity*		
Mild	90 (93%)	78 (84%)
Moderate	7 (7%)	13 (14%)
Severe	0	2 (2%)
Relationship to vaccine*		
Not related	5 (5%)	10 (11%)
Related	92 (95%)	83 (89%)
Serious adverse events	0	1 (11%)
Local solicited adverse events*		
Erythema	9 (100%)	7 (78%)
Injection-site swelling	9 (100%)	9 (100%)
Injection-site exfoliation	5 (56%)	8 (89%)
Systemic solicited adverse events*		
Headache	5 (56%)	4 (44%)
Malaise	3 (33%)	1 (11%)
Myalgia	2 (22%)	2 (22%)
Unsolicited adverse events		
Fatigue	3 (33%)	2 (22%)
Injection-site scarring	9 (100%)	8 (89%)

Data are n or n (%). This table lists common adverse events of interest and summarises severity, relationship to vaccine and seriousness of adverse events. A table showing all adverse events is in the appendix (p 4). *Participants were counted once at the highest severity level in each dose group; percentages are calculated on the basis of the total number of adverse events.

Table 2: **Adverse events in the adult safety cohort**

See Online for appendix

	Demographics	Dose cohort	Time since vaccination	History and diagnosis	Special investigations	Relatedness to study vaccine	Course and outcome
A014	28-year-old woman	MTBVAC (5×10^5 CFU)	100 days	History of headache, nausea, and photophobia. Aseptic meningitis (possible tuberculous meningitis) diagnosed (HIV seroconversion syndrome and Enterovirus meningitis were differential diagnoses)	Positive HIV ELISA, $4{\cdot}1\times10^9$ white cells per L, CRP 4 mg/L, IGRA at day 180 negative, chest radiograph normal. CSF contained 119 mmol/L chloride, 2·8 mmol/L glucose, 0·64 g/L protein, six polymorphonuclear lymphocytes per µL, and 64 lymphocytes per µL; repeat CSF sample taken 3 days later contained 118 mmol/L chloride, 2·4 mmol/L glucose, 1·35 g/L protein, and no cells; original CSF was India ink stain negative, both samples were Gram stain negative and neither showed bacterial growth after 2 days; initial CSF Xpert MTB/RIF negative, CSF MGIT culture insufficient	Unlikely	Patient was discharged after 5 days when asymptomatic and with no neurological sequelae. She received a standard tuberculosis regimen for 6 months, and then started antiretroviral therapy. The patient was well at the end of the study, with no sequelae and an undetectable HIV viral load.
B036 (event 1)	10-day-old girl (birthweight 2675 g)	MTBVAC ($2{\cdot}5\times10^4$ CFU)	9 days	History of jaundice. Neonatal jaundice diagnosed	Total serum bilirubin 326 µmol/L	Unrelated	Jaundice resolved with phototherapy, but participant died age 29 days because of possible viral pneumonia
B036 (event 2)	29-day-old girl (birthweight 2675 g)	MTBVAC ($2{\cdot}5\times10^4$ CFU)	27 days	Short history of runny nose, lethargy,and poor feeding; unresponsive, apneic, and declared dead on arrival at hospital. Possible viral pneumonia diagnosed	An academic autopsy revealed no evidence of mycobacterial infection (no injection-site or local lymph node reactions, and no foamy macrophages, granulomas, or caseous necrosis on microscopy; Ziehl Neelsen staining of lung, liver, and spleen samples were negative for acid-fast bacilli)	Unlikely	Death
B012	1-year-old boy (birthweight 3560 g)	MTBVAC $2{\cdot}5\times10^3$ CFU)	365 days	History of recurrent pneumonia that did not resolve with intravenous antibiotics, and failure to thrive; household tuberculosis contact. Aspiration pneumonia and gastro-oesphophagal reflux disease provisionally diagnosed; classified as unconfirmed tuberculosis	$23{\cdot}5\times10^9$ white cells per L, IGRA at days 180 and 360 negative, chest radiograph showed perihilar opacification, right middle lobe infiltrates, and honeycombing, bronchoscopic bronchoalveolar lavage contained no organisms (auramine stain negative) and 18·5% lipid-laden macrophages. Two gastric washings and two induced sputum samples were taken on consecutive days: all samples negative on auramine staining, Xpert MTB/RIF, and MGIT culture	Unrelated	Tuberculosis treatment was started (standard regimen), but the infant was subsequently treated for two episodes of pneumonia with no improvement in appearance of chest radiographs. Tuberculosis treatment was continued, and patient was well after 6 months; failure to thrive resolved during the next year
B090	6-month-old girl (birthweight 3460 g)	MTBVAC ($2{\cdot}5\times10^5$ CFU)	6 months	History of diarrhoea, pyrexia, and dehydration; no symptoms of tuberculosis. Dysentery diagnosed; classified as unlikely tuberculosis	IGRA positive at day 180 but negative at day 360, gastric washing samples negative on auramine staining, Xpert MTB/RIF, and MGIT culture, chest radiograph showed patchy infiltrates in the right lung and perihilar lymphadenopathy suggestive of tuberculosis.	Unrelated	Dysentery responded rapidly to rehydration and intravenous antibiotics. Tuberculosis treatment was started on the basis of chest radiograph but mother stopped giving infant her tuberculosis treatment after 2 weeks; infant was healthy and thriving off treatment for the next 18 months
B042	9-day-old boy (birthweight 3065 g)	BCG	2 days	History of diffuse pustular rash. Diffuse staphylococcal rash diagnosed	IGRA negative at days 180 and 360, light growth of *Staphylococcus aureus* in pus swab, Gram positive cocci in blood culture	Unrelated	Thriving at end of study
B049	6-week-old girl (birthweight 3220 g)	MTBVAC ($2{\cdot}5\times10^4$ CFU)	40 days	History of cough and tachypnoea. Right bronchopneumonia diagnosed	CRP 55 mg/L, $7{\cdot}9\times10^9$ white cells per L, IGRA negative at days 180 and 360, chest radiograph showed right-sided infiltrates	Unrelated	Responded rapidly to intravenous antibiotics; well at end of study
B082	10-week-old girl (birthweight 3220 g)	MTBVAC ($2{\cdot}5\times10^5$ CFU)	69 days	History of cough, bronchospasm, and tachypnoea. Left bronchopneumonia diagnosed	CRP 1mg/L, $16{\cdot}9\times10^9$ white cells per L, IGRA negative at days 180 and 360, Gram positive cocci in blood culture, chest radiograph showed bronchopneumonia and increased patchiness in left upper lobe	Unrelated	Responded well to intravenous antibiotics; well at end of study

CFU=colony-forming units. CRP=C-reactive protein. IGRA=interferon γ release assay. MGIT=Mycobacteria Growth Indicator Tube.

Table 3: **Serious adverse events and immediately reportable events in the adult and infant cohorts, by participant identifier**

Statistical analysis

The sample size was selected for preliminary assessment of the safety profile of MTBVAC in infants. Sample size selection was based on similar trials designed for preliminary assessment of the type and frequency of adverse events in vaccine recipients, and the trial was not

powered to detect rare or uncommon adverse events, or to define the frequency of adverse events precisely by study arm or MTBVAC dose. Safety and immunogenicity analyses were done in all participants who received a dose of vaccine according to a prespecified statistical analysis plan). This plan contains details of all statistical calculations done for this trial.

The data and safety monitoring board, operating by charter, considered comparative frequencies and relative risk of serious adverse events, grade 3 or 4 adverse events for reactogenicity and systemic adverse events, and trends in adverse events within and between study groups. It also met ad hoc to review any safety issues of concern, including possible cases of tuberculosis, infant deaths, and IGRA conversion data.

We used Prism (version 8) or R (version 4.3) for all analyses. The trial was registered at ClinicalTrials.gov, number NCT02729571.

	BCG group ($2{\cdot}5\times10^5$ colony-forming units; n=8)	MTBVAC groups		
		$2{\cdot}5\times10^3$ colony-forming units (n=9)	$2{\cdot}5\times10^4$ colony-forming units (n=9)	$2{\cdot}5\times10^5$ colony-forming units (n=10)
Age, days	1·9 (1·1)	2·2 (1·3)	1·6 (0·7)	1·5 (0·7)
Length, cm	49·0 (3·0)	49·4 (1·6)	48·3 (3·3)	47·6 (1·7)
Weight, kg	3·21 (0·49)	2·98 (0·23)	3·06 (0·57)	2·95 (0·37)
Sex				
Female	5 (63%)	4 (44%)	5 (56%)	9 (90%)
Male	3 (38%)	5 (56%)	4 (44%)	1 (10%)
Race				
Black	2 (25%)	1 (11%)	3 (33%)	5 (50%)
Cape mixed ancestry	6 (75%)	8 (89%)	6 (67%)	5 (50%)

Data are mean (SD) or n (%).

Table 4: **Baseline demographics in the infant cohort**

Role of the funding source

Biofabri had roles in study design, data interpretation, and writing of the report, but not in data collection or data analysis. The Norwegian Agency for Development Cooperation had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Between Sept 29, 2015, and Nov 16, 2015, 62 adults were screened and 18 were enrolled and randomly assigned, nine each to the BCG and MTBVAC groups (figure 1A). Both groups had similar demographics at baseline, but body-mass index was higher in the MTBVAC group than in the BCG group (table 1). All 18 participants completed all study visits. The frequency, severity, and type of adverse events were similar between the two groups (table 2). The most frequent local injection-site reactions were swelling, erythema, and injection-site scarring, most of which were mild (table 2; appendix p 4). Systemic reactions were mostly mild to moderate (appendix p 4), with headache, malaise, and myalgia the most common solicited events (table 2). No vaccine-related severe adverse events or serious adverse events were recorded.

One serious adverse event occurred in an adult MTBVAC recipient but was judged to be unrelated to the vaccine. She was diagnosed with newly acquired HIV infection (with 378 CD4 cells per µL) and aseptic meningitis 3 months after vaccination (table 3). She rapidly and completely recovered from the aseptic meningitis after treatment with empirical broad-spectrum antibiotics and anti-tuberculous therapy for possible tuberculous meningitis, and was discharged from hospital after 5 days with no neurological sequelae typical of tuberculous meningitis.[35,36] She had negative IGRA results on day 180, but completed 6 months' treatment for possible tuberculous meningitis before starting antiretroviral therapy, and remains well with undetectable HIV viral load. Two (22%) adult MTBVAC recipients had positive IGRA at day 180, but were asymptomatic and had no known tuberculosis contacts. A favourable review of 28-day adult safety data by the data and safety monitoring board enabled progression to infant recruitment.

For the **statistical analysis plan** see https://zivahub.uct.ac.za/s/992494159c4f55dd4d92

Between Feb 12, 2016, and Sept 21, 2016, 117 pregnant women consented to partake in the trial, and 36 infants were enrolled (figure 1B, table 4). Eight (22%) infants were assigned to the BCG group and 28 (78%) were assigned to the MTBVAC groups – nine (25%) each to the $2{\cdot}5\times10^3$ CFU and $2{\cdot}5\times10^4$ CFU doses, and ten (28%) to the $2{\cdot}5\times10^5$ CFU dose. A worldwide distribution shortage of the BCG Vaccine SSI necessitated a protocol amendment (on Dec 23, 2016) and revision of the informed consent document so that the final six infants enrolled in the third dose cohort (ie, $2{\cdot}5\times10^5$ CFU) could receive open-label MTBVAC, which resulted in the slight deviation from the randomisation ratio in group numbers. Follow-up was completed on Sept 11, 2017, with 35 infants completing the study (one infant died).

Local injection-site reactions, most frequently swelling, erythema, and scarring, were recorded only in the BCG and high-dose MTBVAC (ie, $2{\cdot}5\times10^5$ CFU) groups, and were mild in severity (table 5; appendix pp 5–6). No local or regional lymphadenopathy or ulceration were reported. Systemic adverse events were evenly distributed across all four study groups (table 5), and were mostly mild in severity (appendix pp 5–6). The most frequent solicited systemic adverse events were failure to thrive, irritability, and pyrexia (table 5). The most common unsolicited systemic adverse event was upper respiratory tract infection (table 5). Seven

	BCG group ($2·5 \times 10^5$ CFU; n=8)	MTBVAC groups		
		$2·5 \times 10^3$ colony-forming units (n=9)	$2·5 \times 10^4$ colony-forming units (n=9)	$2·5 \times 10^5$ colony-forming units (n=9)
Participants with at least one adverse event	8 (100%)	9 (100%)	9 (100%)	10 (100%)
Adverse events	67	44	33	67
Solicited adverse events	26	2	5	13
Severity*				
Mild	59 (88%)	34 (77%)	27 (82%)	62 (93%)
Moderate	5 (7%)	7 (16%)	5 (15%)	5 (7%)
Severe	3 (4%)	3 (7%)	1 (3%)	0
Relationship to vaccine*				
Not related	29 (43%)	37 (84%)	32 (97%)	44 (66%)
Related	38 (57%)	7 (16%)	1 (3%)	23 (34%)
Serious adverse events	1 (1%)	1 (2%)	3 (9%)	2 (3%)
Local solicited adverse events				
Injection-site erythema	4 (50%)	0	0	1 (10%)
Injection-site swelling	6 (75%)	0	0	8 (80%)
Injection-site pustule	3 (38%)	0	0	0
Systemic solicited adverse events				
Failure to thrive	1 (13%)	2 (22%)	2 (22%)	1 (10%)
Irritability	3 (38%)	0	2 (22%)	0
Pyrexia	1 (13%)	0	1 (11%)	0
Unsolicited adverse events				
Upper respiratory tract infection	3 (38%)	6 (67%)	3 (33%)	6 (60%)
Papular rash	3 (38%)	2 (22%)	3 (33%)	5 (50%)
Increased alkaline phosphatase concentrations	1 (13%)	2 (22%)	4 (44%)	5 (50%)
Diaper dermatitis	3 (38%)	2 (22%)	3 (33%)	4 (40%)
Injection-site scarring	7 (88%)	0	0	5 (50%)

Data are n or n (%). This table lists common adverse events of interest and summarises severity, relationship to vaccine, and seriousness of adverse events, by study groups. A table showing all adverse events is in the appendix (pp 5–6).
*Participants were counted once at the highest severity level in each dose group; percentages are calculated on the basis of the total number of adverse events.

Table 5: **Adverse events in the infant cohort**

serious adverse events were reported, including one death (in an MTBVAC recipient at age 4 weeks), but none of these events were judged to be related to study treatment. An autopsy showed changes suggestive of viral pneumonia and possible liver disease with cholestasis, and the pathologist concluded that the cause of death was not related to mycobacterial infection (table 3).

One infant was treated for unlikely tuberculosis and one for unconfirmed tuberculosis (as defined by Graham and colleagues[37]) during active study follow-up (ie, up to day 360; table 3). No children were treated for tuberculosis during the second year of passive follow-up.

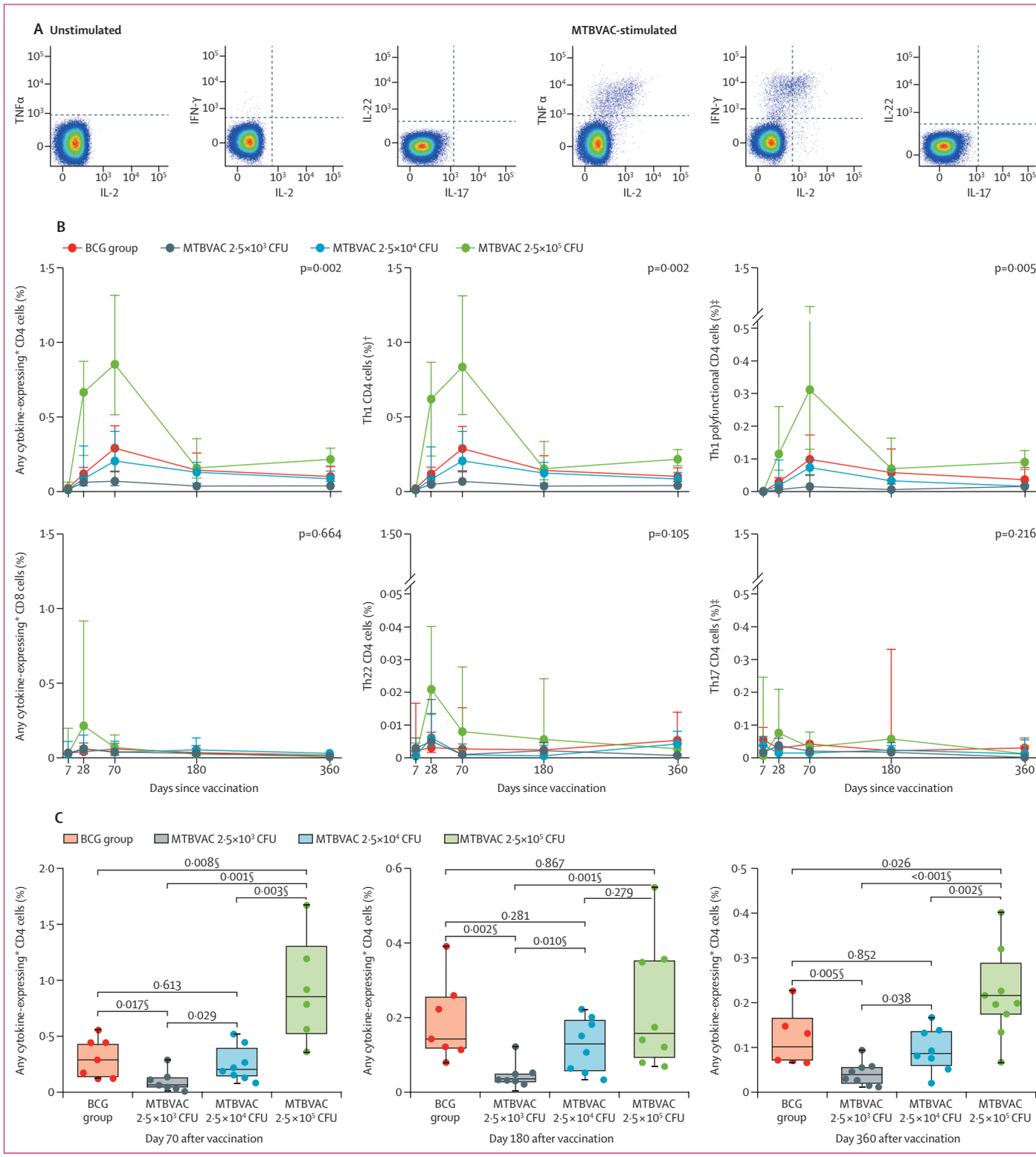
Immunology analyses were completed in 26 of the 28 infants in the MTBVAC group. One infant, who received the lowest dose of MTBVAC, was not included because their parents withdrew consent for blood sampling. One infant died and thus was not included. Figure 2 shows the frequencies of CD4 and CD8 T cells expressing antigen-specific IFN-γ, TNFα, IL-2, IL-17, or IL-22 (appendix pp 7–12). Vaccination with all doses of MTBVAC and with BCG vaccine, primed a CD4 T-cell response comprising Th1 cells that primarily expressed IFN-γ, TNF-α, or IL-2, or a combination thereof, and peaked 70 days after vaccination in all groups (figure 2B; appendix pp 7–12). MTBVAC-specific T-helper-22 (Th22) CD4 cells (producing IL-22), T-helper-17 (Th17) CD4 cells (producing IL-17), and cytokine-expressing CD8 cells were low or undetectable in most participants (figure 2B; appendix pp 7–12). Thus, we focused on CD4 cell co-expression patterns of IFN-γ, TNF-α, and IL-2 in subsequent analyses.

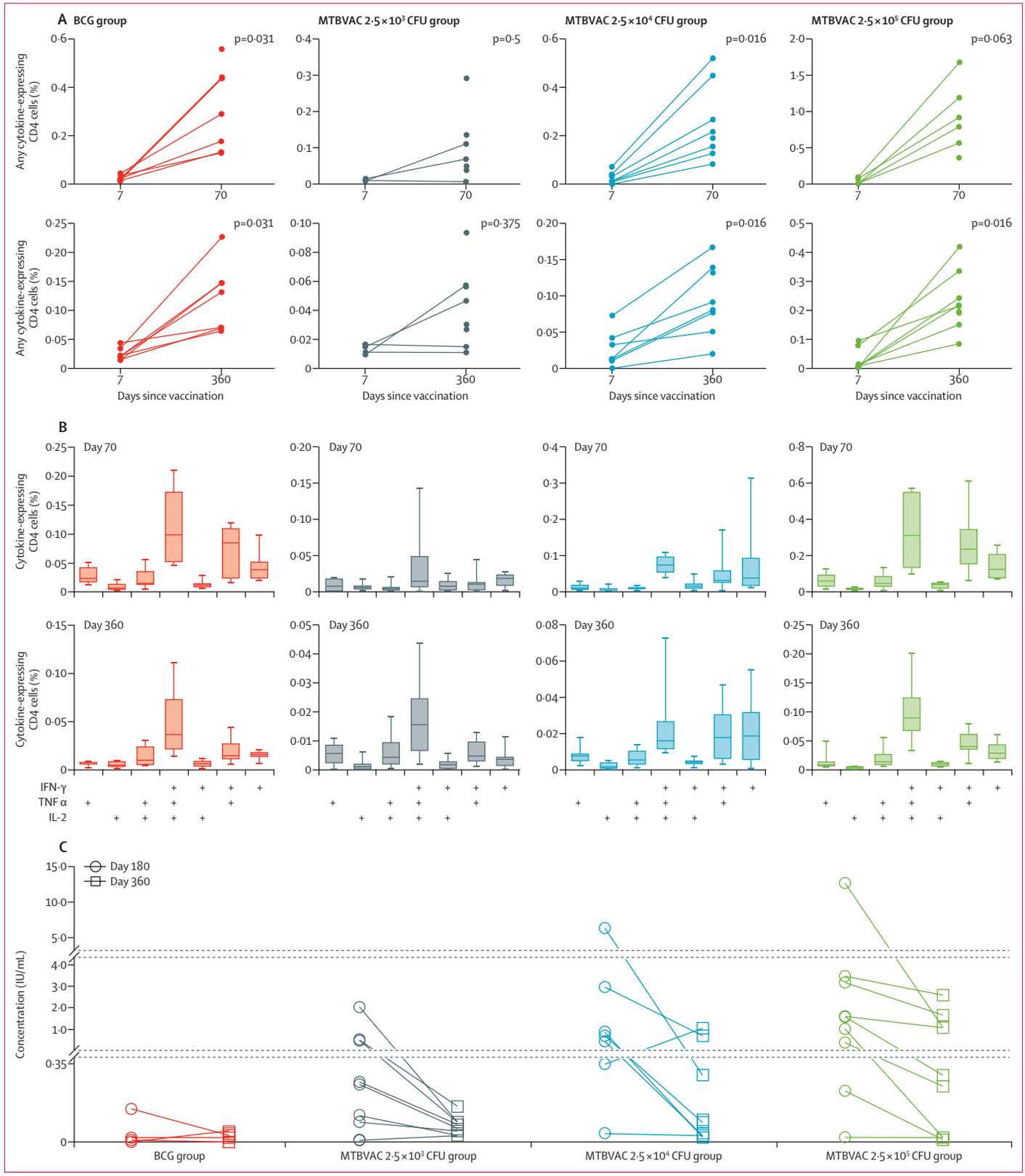
AUCs for MTBVAC-specific CD4 cells expressing any cytokine, Th1-cytokine-positive CD4 cells, and poly-functional Th1 CD4 cells suggested a significant difference between the four groups, whereas AUCs for MTBVAC-specific Th22 CD4 cells, Th17 CD4 cells, and cytokine-expressing CD8 T cells did not differ significantly between groups (figure 2B). Direct comparison of MTBVAC-specific response magnitudes between dose groups showed a dose-dependent increase in cytokine-expressing CD4 cells at days 70, 180, and 360 (figure 2C). Vaccination with the highest dose of MTBVAC also induced significantly higher frequencies of MTBVAC-specific CD4 T cells at days 70 and day 360 than the equivalent dose of BCG (figure 2C).

Antigen-specific responses measured at days 70 and 360 were not higher than baseline responses in infants

Figure 2: **Kinetics of total T-cell responses induced by vaccination**
(A) Representative flow cytometry dot plots showing cytokine production by CD4 cells in unstimulated and MTBVAC-stimulated blood collected at day 70 from a representative infant vaccinated with $2·5 \times 10^5$ CFU of MTBVAC (appendix pp 7–12). Blood was stimulated with MTBVAC and frequencies of cytokine-expressing cells from the unstimulated control were subtracted from antigen-specific frequencies. (B) Longitudinal kinetics of antigen-specific CD4 or CD8 cells expressing the indicated cytokine responses in participants after vaccination and measured by whole blood intracellular cytokine staining assay. The lines depict median frequencies of cytokine-expressing CD4 or CD8 cells; error bars depict IQRs for each dose group. p values are for inter-group comparisons (with the Kruskal-Wallis test) of the area under the response curve, which was calculated for each infant. Blood was stimulated with MTBVAC and frequencies of cytokine-expressing cells from the unstimulated control were subtracted from antigen-specific frequencies. (C) Frequencies of antigen-specific CD4 cell responses on days 70, 180, and 360 (end of study). The horizontal lines represent medians, boxes show the IQR, and error bars show the range. p values for between-group comparisons were computed with the Mann-Whitney *U* test. Individual participant responses and antigen-specific responses to stimulation with a peptide pool of 122 *Mycobacterium tuberculosis* immunodominant epitopes are in the appendix (pp 7–12). CFU=colony-forming units. TNFα=tumour necrosis factor α. IL-2=interleukin 2. IFN-γ=interferon-γ. IL-17=interleukin 17. IL-22=interleukin 22. Th1=T-helper-1. Th22=T-helper-22. Th17=T-helper-17. *Co-expression of any combination of IFN-γ, TNFα, IL-2, IL-17, and IL-22. †Expression of any combination of IFN-γ, TNFα, and IL-2. ‡Co-expression of IFN-γ, TNF-α, and IL-2. §p values <0·05 for which the corresponding q value, as calculated with the Benjamini and Hochberg false discovery rate method, was also <0·05 (see appendix p 11 for actual p values).

who received the $2{\cdot}5\times10^3$ CFU dose of MTBVAC (figure 3A). By contrast, the $2{\cdot}5\times10^4$ CFU and $2{\cdot}5\times10^5$ CFU doses induced increased responses at day 360 compared with baseline (figure 3A; appendix p 12). Cytokine co-expression patterns of MTBVAC-specific Th1 CD4 cells showed that the response was





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predominantly polyfunctional (figure 3B; appendix p 12).

Dose-related IGRA conversion was noted in three (38%) of eight infants in the $2·5×10^3$ CFU MTBVAC dose group, six (75%) of eight in the $2·5×10^4$ CFU MTBVAC dose group, and seven (78%) of nine in the $2·5×10^5$ CFU MTBVAC dose group at day 180, whereas IGRA conversion was noted in none of the seven infants in the BCG group who were tested (figure 3C). A known new household tuberculosis contact was reported by three MTBVAC recipients, one of whom became IGRA positive at 6 months. All infants who tested IGRA positive were referred for tuberculosis investigation and possible isoniazid preventive therapy as soon as conversion was detected. 16 of the 17 infants who were referred received isoniazid preventive therapy. By day 360, IGRA reversion had occurred in all three infants (100%) in the low-dose group, four (67%) of the six in the medium-dose group, and three (43%) of the seven in the high-dose group (42·8%) in whom IGRA conversion had been recorded. No new IGRA conversions were noted at day 360 (Figure 3C).

Discussion

We showed that the live-attenuated *M tuberculosis* vaccine MTBVAC had an acceptable reactogenicity profile at doses ranging from $2·5×10^3$ to $2·5×10^5$ CFU in both *M-tuberculosis*-uninfected adults and newborn infants in a setting with a high tuberculosis burden. Local and systemic adverse events occurred in the highest dose ($2·5×10^5$ CFU) group at a similar frequency as in the BCG vaccination group. There was no evidence of local or regional injection-site complications. MTBVAC induced antigen-specific Th1 immune responses that peaked around 10 weeks and established a durable memory response that persisted 1 year after vaccination, and at a magnitude greater than the equivalent dose of BCG. An unexpected dose-related effect of MTBVAC on IGRA conversion and subsequent reversion was also noted, and occurred at a higher frequency than was noted previously in BCG-naive Swiss adults[28] and in the BCG-vaccinated South African adults in this trial. This observation, although providing supportive evidence that MTBVAC induces durable T-cell responses, confounds interpretation of IGRA as a test for *M tuberculosis* infection and as evidence of *M tuberculosis* exposure that would typically be used to support clinical diagnosis of tuberculosis disease in children.

This trial was not designed to test efficacy of MTBVAC, but given that the effect of MTBVAC on the risk of tuberculosis disease is unknown, any possible cases are of interest. In our trial, an adult MTBVAC recipient was diagnosed with new HIV infection and aseptic meningitis consistent with possible tuberculous meningitis.[36] However, she had negative IGRA and CSF Xpert MTB/RIF results and a normal chest radiograph, and recovered completely and rapidly without typical neurological sequelae, findings that do not support this diagnosis. Differential diagnoses included HIV seroconversion syndrome, consistent with her aseptic CSF profile. Two infant MTBVAC recipients were also treated, one for unlikely tuberculosis and one for unconfirmed tuberculosis, by the public health services,[37] but neither diagnosis was convincing. The proportion of infant MTBVAC recipients with clinically diagnosed but unconfirmed tuberculosis in our trial (ie, two [7%] out of 28) was similar to that in a previous trial in this community (9%) in which more than 90% of these diagnoses were unconfirmed.[22,39]

Trials of live attenuated mycobacterial vaccines such as MTBVAC present a unique challenge in that the investigational product and the *M tuberculosis* pathogen are very similar strains, and specific molecular methods would be necessary to differentiate the two if samples from a site of disease were cultured. Clinicians in countries with high burdens of tuberculosis have a high index of suspicion for clinical tuberculosis diagnosis and a low threshold for starting treatment in children. The obvious dilemma for safety reporting, attribution of causality, and tuberculosis case ascertainment is that it is impossible to prove that MTBVAC is not the causative agent of unconfirmed disease in the absence of a speciated, cultured isolate. Thus, vaccine strain disease cannot be conclusively disproven. This challenge is magnified in paediatric trials, in which most childhood tuberculosis cases are not microbiologically confirmed.[22,39]

Because MTBVAC is a live attenuated vaccine, there is a theoretical risk of strain dissemination from the site of injection, however unlikely, in any participant with mycobacterial disease. This possibility has been tested in preclinical studies in immunocompetent and immunosuppressed animal models. In immunocompetent Balb/C mice intradermally inoculated with MTBVAC, no toxic effects were noted, organ biodistribution and

***Figure 3*: Magnitude, durability, and quality of antigen-specific T-cell responses**

(A) Frequencies of antigen-specific CD4 T cells producing any combination of IFN-γ, TNFα, IL-2, IL-17, or IL-22 detected after MTBVAC stimulation of blood from each infant at day 7 (ie, baseline), day 70 (top panels), and day 360 (bottom panels). Each set of dots connected by a line represents an infant. p values are for a comparison of baseline to day 70 or day 360, and were calculated with the Wilcoxon matched-pairs signed rank test. (B) CD4 cell co-expression patterns of IFN-γ, TNFα, and IL-2 at day 70 (peak response) and day 360 (end of study), arranged from left to right by the degree of T-cell differentiation according to Seder and colleagues.[38] The horizontal lines represent medians, boxes show the IQR, and error bars show the range. No statistical analysis was done. The appendix (p 9) shows the same analysis detected after stimulation of blood with a peptide pool of 122 *Mycobacterium tuberculosis* immunodominant epitopes. (C) IFN-γ concentrations at days 180 and 360, as measured by the QuantiFERON-TB-Gold Gold In-Tube (Qiagen, Hilden, Germany) IFN-γ release assay. Each line represents data for one participant. IFN-γ=interferon-γ. TNFα=tumour necrosis factor α. IL-2=interleukin 2. IL-17=interleukin 17. IL-22=interleukin 22. CFU=colony-forming units.

persistence were limited, and the vaccine strain was untraceable in urine and stools up to 6 months after vaccination, similar to the profile of BCG Vaccine SSI in Balb/C mice.[12] The robust safety and attenuation profile of the doubly attenuated *phoP*-deficient and *PDIM*-deficient phenotype has also been shown in two SCID mouse models with the MTBVAC prototype vaccine SO2.[12,17]

The BCG vaccine is a live attenuated mycobacterial vaccine that was originally derived from pathogenic *M bovis*, which causes tuberculosis disease in cattle. Autopsy studies show that BCG vaccination can be accompanied by asymptomatic bacteraemia.[40,41] Distant and disseminated BCG disease that mainly affects immunocompromised infants and infants with HIV is rare but has been well described.[40,42,43] It is estimated to affect two of every million vaccinees,[44] and as many as 992 of every 100 000 vaccinated immunocompromised infants.[44] BCG disease is usually characterised by injection-site abscess and ipsilateral lymph node enlargement or lymphadenitis, and dissemination to distant organs is associated with high mortality in infants with HIV.[42] Historical reports of the so-called Lubeck disaster (1929–33),[45] when infants were given a BCG vaccine preparation that was accidentally contaminated with virulent wild-type *M tuberculosis*, also describe disseminated disease that was usually related to the route of vaccine administration.[45] Safety studies have not been done to establish whether MTBVAC can also cause vaccine strain bacteraemia, but the known risk factors for, and clinical presentation of, BCG disease are useful to inform surveillance for the possibility of MTBVAC vaccine strain disease. We might expect vaccine strain disease to be rare in immunocompetent infants, to usually involve local and regional spread from the injection site to ipsilateral lymph nodes and systemic dissemination to bone marrow, lungs, and other organs (which would be associated with high mortality).

None of the MTBVAC recipients in this trial showed evidence of pathology at the site of injection or regional lymph nodes. The MTBVAC strain grows in standard liquid Mycobacteria Growth Indicator Tube culture and solid mycobacterial culture media, including Lowenstein-Jensen medium, and is susceptible to first-line tuberculosis drugs, including isoniazid, ethambutol, streptomycin, and rifampicin.[12] All PCR-based diagnostic methods, including Xpert MTB/RIF, that detect *M tuberculosis* on the basis of IS*6110* or 16RNA should give a positive result for MTBVAC. However, none of the MTBVAC recipients treated for tuberculosis in this trial tested positive by mycobacterial culture or Xpert MTB/RIF. Therefore, in the absence of clinical features consistent with local, regional, or systemic dissemination, the mild clinical course of participants diagnosed with unconfirmed tuberculosis, the diagnoses of other illnesses in these patients, and the absence of laboratory evidence for mycobacterial disease of any kind, including wild-type *M tuberculosis*, we do not consider MTBVAC vaccine strain dissemination to be a reasonable possibility in this trial.

We observed a dose-dependent Th1 CD4 cell response to MTBVAC, which persisted to the end of active follow-up. In recipients of the $2 \cdot 5 \times 10^5$ CFU dose, the peak response to MTBVAC (ie, on day 70) was higher than that to the BCG vaccine, and responses on day 360 were higher for MTBVAC than BCG. Day 70 and day 360 Th1 CD4 cell responses to the low dose ($2 \cdot 5 \times 10^3$ CFU) of MTBVAC were significantly lower than responses to BCG or the high MTBVAC dose. On the basis of these immunogenicity results, the low MTBVAC dose could be omitted from future studies. Although our immunogenicity results are encouraging and warrant further testing of MTBVAC, interpretation of our findings is limited by the small sample size (ie, eight to ten individuals per dose). In view of the dose-dependent response and acceptable safety profile, higher doses of MTBVAC could be explored in future studies. Two expanded dose-finding trials of MTBVAC, one in adults (NCT02933281) and one in infants (NCT03536117), are enroling participants.

We also noted an unexpectedly high frequency of dose-related IGRA conversion in infant MTBVAC recipients, suggesting induction of T-cell responses to the antigens ESAT-6 or CFP-10, or both. MTBVAC expresses both of these antigens, but only CFP-10 is secreted.[19] Transient, low-level CFP-10 responses were detected by IFN-γ ELISpot assay in three (11%) of 27 BCG-naive Swiss adults who received MTBVAC in a previous trial,[28] and IGRA conversion was noted in two (22%) of nine previously BCG-vaccinated adult MTBVAC recipients in our trial. This occurrence, which is analogous to BCG causing false-positive tuberculin skin test results, could be interpreted as an encouraging sign of the immunogenicity of MTBVAC in BCG-naive and *M tuberculosis*-unexposed infants.

A study[19] in mice suggested that the MTBVAC-induced CFP-10-specific response might be necessary for vaccine-induced protection against *M tuberculosis* challenge. However, because IGRA is a diagnostic test for *M tuberculosis* infection, it is crucial that the dynamics and duration of MTBVAC-induced IGRA conversion are explored fully and that vaccine-induced conversion can be differentiated from *M tuberculosis*-induced conversion. In this trial, IGRA conversion in infants who received MTBVAC prompted prescription of isoniazid preventive therapy because natural *M tuberculosis* infection could not be excluded. The high frequency of IGRA conversion noted in infants who received high MTBVAC doses was not expected on the basis of the adult data.[28] Based on these results, a positive IGRA result soon after MTBVAC administration should not be used to diagnose *M tuberculosis* infection or as the basis for prescription of isoniazid preventive therapy, unless the positive IGRA result occurs for the

first time after the peak post-vaccination response has waned—ie, after 70 days.

MTBVAC is a unique candidate in the clinical development pipeline because it is the only live tuberculosis vaccine that contains the whole antigen repertoire of *M tuberculosis*, including the major antigens ESAT-6 (produced but not secreted in MTBVAC because of the *phoP* deletion) and CFP10 (produced and secreted in MTBVAC; unaffected by the *phoP* deletion).[19] Individuals with latent *M tuberculosis* infection who are reactive to CFP-10 and ESAT-6 stimulation seem to be more protected against reinfection with tuberculosis disease than individuals who have never been infected with *M tuberculosis*.[11]

The safety and immunogenicity data that we report here support the further clinical development of MTBVAC, through dose-ranging studies in infant and adult populations, in preparation for efficacy trials in countries where tuberculosis is endemic. Future clinical trials of MTBVAC should incorporate careful clinical assessment of injection-site complications, including swabs of any discharge, and sampling from other sites of suspected pathology for mycobacterial culture and differentiation of wild-type *M tuberculosis* and MTBVAC. The deletions in *phoP* and *fadD26* in MTBVAC can be detected with a reverse transcriptase PCR presence–absence approach designed for the differentiation of MTBVAC from wild-type *M tuberculosis* isolates. Vaccine-induced IGRA conversion, analogous to the vaccine-induced seroconversion that occurs in HIV vaccine trials, will further complicate the inherent difficulty of childhood tuberculosis diagnosis. Enhanced tuberculosis screening and stringent endpoint determination, independent of the current generation of IGRAs, will be crucial for future efficacy trials of MTBVAC in infants.[22] Equally, infants with vaccine-induced IGRA conversion must be protected against unnecessary TB investigations and therapy. Infants who test IGRA positive soon after vaccination with MTBVAC in contexts when natural *M tuberculosis* infection is unlikely should receive a card at the end of study stating that they have received an experimental vaccine that could interfere with interpretation of future IGRA and tuberculin skin tests.

Clearly, if MTBVAC is shown to offer better protection than the BCG vaccine against tuberculosis disease in efficacy trials, as suggested by preclinical studies,[19,27] the data from our trial will be important to support development of alternative diagnostic tests for *M tuberculosis* infection that are not cross-reactive with MTBVAC. For example, a CFP-10-free IGRA would be a useful diagnostic for MTBVAC vaccinees.

Contributors

MT, HM, AP-N, IM, JD, NA, DM, EP, BF, JT, CM, TJS, and MH conceived the study design. MT, YG, HG, JS, and AKKL recruited participants and collected data. HM, AP-N, NB, SM, TJS, and MH collected and analysed data. MT, HM, DM, CM, TJS, and MH contributed to interpretation of results and wrote the first draft of the Article. All authors had full access to the data, and reviewed, revised, and approved the manuscript before submission.

Declaration of interests

NA, DM, EP, ER, JG-A, and CM are co-inventors on a patent entitled tuberculosis vaccine held by the University of Zaragoza and Biofabri. IM, JD, EP, and ER are employees of Biofabri. JT and BF are affiliated with the TuBerculosis Vaccine Initiative. All other authors declare no competing interests.

Data sharing

Individual participant data that underlie the results reported in this Article will be available upon request, after de-identification and publication, on the FigShare platform. The full study protocol (DOI:10.25375/uct.7970186), and clinical (DOI:10.25375/uct.7970177) and immunological (DOI:10.25375/uct.7970198) statistical analysis plans are available on FigShare.

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