Clinical Trial Protocol

A randomized, double-blind, dose-escalation clinical trial of the safety, reactogenicity and immunogenicity of MTBVAC compared to BCG Vaccine SSI, in newborns living in a tuberculosis endemic region with a safety arm in adults

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STATEMENT OF COMPLIANCE

The study will be carried out in accordance with this protocol, the guidelines and ethical principles set forth in the Declaration of Helsinki, Good Clinical Practice (ICH-GCP) and SA GCP. Any proposed changes to this protocol, or to informed consent or participant information documents, will be submitted to the Human Research Ethics Committee of the University of Cape Town for approval prior to implementation. Monitoring representatives of the Medicines Control Council of South Africa (MCC), or representatives of the Human Research Ethics Committee of the University of Cape Town, South Africa will have reasonable access to inspect facilities and study records at the study site.

SIGNATURE SECTION

The signature below constitutes the approval of this protocol and the attachments, and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable ICH guidelines.

PRINCIPAL INVESTIGATOR: _____

Date: _____

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LIST OF ABBREVIATIONS AND ACRONYMS

AEs	adverse events
BSL	Biosafety Level
CFU	colony forming units
cGMP	current Good Manufacturing Practice
CHUV	Centre Hospitalier Universitaire Vaudois (Lausanne, Switzerland)
CMO	Contract Manufacturing Organization
CRO	Contract Research Organization
CRP	C-reactive protein
CTA	Clinical Trial Application – to solicit approval for a clinical trial
D	Day or day
DAT	diacyltrehalose (mycobacterial cell-wall lipid)
EU FP5	European Union funded Fifth Framework Programme
EU FP6	European Union funded Sixth Framework Programme
EU FP7	European Union funded Seventh Framework Programme
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practices
HIV	human immunodeficiency virus
Hyg	Hygromycin
hyg^r	Hygromycin resistance marker
ICL	isocitrate lyase (glyoxylate shunt enzyme for anaplerotic utilisation of carbon sources by *M. tuberculosis*; implicated in intracellular persistence of the bacillus)
ICS	Intracellular Cytokine Staining
IFN-γ	interferon gamma
IL	interleukin
kb	kilobase
bp	base pair(s)
nt	nucleotide
Km	Kanamycin
km^r	Kanamycin resistance marker
$\log_{10}$	Base 10 Logarithms
LSM	Local safety monitor
M	Month or month
MDR	multi-drug resistant strains of *M. tuberculosis* (with resistance to, at least, isoniazid and rifampicin)
MDR-TB	TB due to multi-drug resistant strains
MIC	Minimum inhibitory concentration
MOI	Multiplicity of infection
MSL	Master Seed Lot
MTBC	*Mycobacterium tuberculosis* complex
N/A	Not applicable
NRA	National Regulatory Authority
ORF	open reading frame
PAT	Polyacyltrehalose (mycobacterial cell-wall lipid)

PBMCs	Peripheral blood mononuclear cells
PDIM	Phthiocerol dimycocerosate (mycobacterial cell-wall lipid and one of the most important virulence factor determinants)
PCR	Polymerase chain reaction
PhEur	European Pharmacopoeia
EP	European Pharmacopoeia
PhoP	Transcription factor regulator in virulent *M. tuberculosis* strains, encoded by *phoP* gene PhoP-/PDIM-Deficient in PhoP and PDIM
PPD	Purified protein derivative
QFT	QuantiFERON®-TB Gold In-Tube (IT)
RD	Region of difference
SAE(s)	Severe adverse events
SATVI	South African Tuberculosis Vaccine Initiative
SCID	Severe combined immunodeficiency
SHD	Single human dose (also appears on p. 22, line 2)
SNPs	Single-nucleotide polymorphisms
SPF	Specific pathogen free
SRT	Safety Review Team
TB	Tuberculosis
TBVI	Tuberculosis Vaccine Initiative – a European based foundation
TBVI PDT	TBVI Product Development Team
TBVI CDT	TBVI Clinical Development Team *PDT and CDT are TBVI supported team of international experts in vaccine research, development, and clinical evaluation.*
TNF	Tumour necrosis factor
T_H1	Effector CD4+ T helper cell responses characterized by IFN-□ production by $CD4^+$ lymphocytes which activates the bactericidal activities of macrophages, resulting in to cell-mediated immunity. T_H1 responses are more effective against intracellular pathogens
T_H2	Effector CD4+ T helper cell responses characterized by the release of Interleukin 4, which results in the activation of B-cells leading to humoral immunity. T_H2 type response is more effective against extracellular bacteria, parasites and toxins. Like cytotoxic T-cells, most of the $CD4^+$ helper cells will die upon resolution of infection, with a few remaining as $CD4^+$ memory cells.
TU	Tuberculin unit(s)
UNIZAR	University of Zaragoza, Spain
VIC	Vaccine and Immunology Center (Lausanne, Switzerland)
WHO	World Health Organization
WSL	Working Seed Lot
XDR-TB	TB disease due to MDR strains that are also resistant to a fluoroquinolone and at least one second-line injectable agent (amikacin, kanamycin and/or capreomycin)

Protocol summary

Title	**A randomized, double-blind, clinical trial of the safety, reactogenicity and immunogenicity of MTBVAC compared to BCG Vaccine SSI, in newborns living in a tuberculosis endemic region with a safety arm in adults**
Rationale	**Rationale for evaluation of a new infant TB vaccine** Bacille Calmette Gurein (BCG) is the only licensed vaccine against tuberculosis (TB). Whereas BCG given at birth has shown to be efficacious against severe disseminated TB (miliary and meningitic disease) in children, newborn BCG vaccination provides inconsistent protection against pulmonary TB in childhood, and little or no protection against pulmonary TB in adults, who are the source of transmission in the epidemic areas. Although children rarely transmit TB, infants and very young children are most vulnerable to TB, having the highest age-specific incidence and being most at risk of severe TB clinical forms [1, 2]. An improved TB vaccine is needed - one that is effective against all forms of TB disease in infants, children, and adults, and also safe in immune-compromised persons, including those with HIV infection. BCG is a live mycobacterial vaccine derived from the bovine TB pathogen *M. bovis*, which has lost a series of >100 genes when compared to the human TB pathogen *M. tuberculosis*. These genes are thought to be important for the successful interaction of *M. tuberculosis* with the human immune system, and therefore, we hypothesize that a successful vaccine against TB should incorporate several of these *M. tuberculosis* genes. Recent studies have shown that 23% of the known human T-cell epitopes present in *M. tuberculosis* are absent in BCG [3]. MTBVAC is a live-attenuated derivative of a clinical *M. tuberculosis* isolate belonging to the Euro-American lineage, containing all the genes present in BCG, plus the *M. tuberculosis* genes deleted in *M. bovis* and BCG [4] including the human T-cell epitopes lost in BCG [3]. It is our hypothesis that a vaccine such as MTBVAC, based on the human TB pathogen, should be more efficient at inducing specific protective immunity against human TB disease caused by *M. tuberculosis*. **Rationale for evaluating three MTBVAC dose levels** The primary aim of this Phase Ib clinical trial is to evaluate the safety, reactogenicity, and immunogenicity of the candidate TB vaccine MTBVAC in BCG naïve newborns, administered at a single intradermal dose of either 5 x 10^3, 5 x 10^4 or 5 x 10^5 CFU, compared to BCG Vaccine SSI as control. These three dose levels of MTBVAC were tested in a Phase 1a trial in healthy BCG naïve adults and were selected based on doses currently used for BCG vaccine in global immunisation programs. Initially, the two lower dose levels, justified by preclinical data and acceptable safety and immunogenicity data in a current Phase Ia trial in adults, will be compared to BCG at the usual dose of 5 x 10^5 CFU. If safety is demonstrated at lower doses, MTBVAC will

	then be compared at equivalent dose to BCG Vaccine SSI. **Rationale for selection of the study population** Safety, reactogenicity and immunogenicity of MTBVAC have been demonstrated, and compared to BCG Vaccine SSI, in healthy BCG-naïve adults living in a TB non-endemic region. Planning considerations for the optimal clinical development pathway for live mycobacterial vaccines is summarised in the Second Geneva Consensus Statement [5], which recommends swift progression into the target population most likely to benefit from MTBVAC (i.e. BCG-naïve newborn infants in a TB endemic country). Infants in a TB endemic country are more likely to be exposed to *M. tuberculosis,* and non-tuberculous mycobacteria (NTM), after birth, compared to infants in non-endemic countries whose safety, reactogenicity, and immunogenicity profile after MTBVAC might differ. Therefore, following acceptable first-in-human safety data, it is deemed necessary to demonstrate safety in healthy, TB uninfected adults living in a TB endemic country, before progressing directly to newborn infants in that same country. BCG vaccine is administered universally to infants in TB endemic countries, with the result that a representative BCG-naïve indigenous adult study population would be virtually impossible to find. Administration of BCG, itself a live mycobacterial vaccine, to adults previously vaccinated with BCG in infancy is known to be safe. Therefore, we will first demonstrate safety of MTBVAC among a group of previously BCG vaccinated, TB uninfected adults in South Africa, before progressing to newborns. We acknowledge that prior BCG vaccination, potential sub-clinical *M. tuberculosis* exposure, and age-specific immunity, are likely to limit the applicability of immunogenicity data from BCG vaccinated adults to BCG naïve newborns and therefore, we will not test immunogenicity of MTBVAC in this adult study population.
Population	18 healthy, BCG vaccinated, HIV negative, South African adults, aged 18 – 50 years, in whom latent tuberculosis (TB) infection has been excluded by negative QuantiFERON® Gold-In-Tube (QFT) assay (Cellestis; Australia). Thirty-six (36) healthy, BCG naïve, HIV unexposed, South African newborn infants.
Site	Adult participants will be recruited from the community of the Cape Winelands district of the Western Cape, South Africa. Newborn infant participants will be enrolled from birthing clinics in the Cape Winelands district of the Western Cape, South Africa. Infant participants' mothers will be recruited antenatally at clinics in the same area. The study will be conducted at the field site of the South African Tuberculosis Vaccine Initiative (SATVI) located in the town of Worcester, Western Cape, South Africa.
Intervention	BCG Vaccine SSI is a licensed vaccine administered routinely at birth by the intradermal route in South Africa for prevention of tuberculosis. BCG Vaccine SSI is

	licensed for neonatal and adult administration. MTBVAC® is a live, rationally attenuated clinical strain of Mycobacterium tuberculosis Euro-American lineage 4, constructed by two unmarked deletion mutations in the virulence genes phoP and fadD26, without antibiotic resistance markers [4]. MTBVAC is developed as prophylactic vaccine for prevention of TB disease (pulmonary and extrapulmonary) in infants and children, to be given at birth by the intradermal route.
Objectives	Primary objectives: 1) To evaluate safety and reactogenicity of MTBVAC, compared to BCG Vaccine SSI, in healthy, BCG naïve, HIV unexposed, South African newborn infants 2) To evaluate immunogenicity of MTBVAC, compared to BCG Vaccine SSI, in healthy, BCG naïve, HIV unexposed, South African newborn infants Secondary objectives: • To evaluate safety and reactogenicity of MTBVAC, compared to BCG Vaccine SSI, in healthy, BCG vaccinated, HIV negative, QFT negative, South African adults
Study endpoints	**Safety and reactogenicity endpoints (in infants and adults):** **1)** Injection site adverse events, including redness, swelling, induration, tenderness, ulceration, fluctuation, drainage, and scarring. **2)** Systemic and regional adverse events, solicited and unsolicited, including lymphadenopathy and abscess formation, or any other abnormal findings. I**mmunogenicity endpoints (in infants only):** *Primary immunogenicity endpoints* 1) Frequencies and co-expression patterns of CD4 and CD8 T cells expressing IFN-γ, TNF-α, IL-2 and/or IL-17 induced by MTBVAC or BCG, and suitable antigens in healthy, BCG naïve, HIV unexposed, South African newborn infants. *Exploratory immunogenicity endpoints* **1)** Whole blood supernatants samples for further immunogenicity tests will be collected and stored frozen. Exploratory assays will be planned, based on data from the primary immunogenicity analyses.

Table 1. Timepoints and blood volume for the safety and immunogenicity evaluation in the infant trial

Readouts & blood volume per visit (mL)	Time-points D0	D7	D14	D28	D70	D91	D180	D360
Safety (chemistry and haematology)		2mL		2mL				
Immunogenicity (whole blood ICS assay)		0.75mL		2.5mL	2.5mL		2.5mL	
Quantiferon (QFT)							3.0 mL	3.0 mL
Total blood volume per visit		2.75mL		4.5mL	2.5mL		5.5mL	3.0 mL

- Safety and total blood cell count

Phlebotomy for immunogenicity will be evaluated at: D7, D28, D70 and D180 (0.75 ml at D7 and 2.5 mL the rest of the days). A QFT test will be performed at D180 and D360

Narrative for whole blood ICS assay:

Fresh whole heparinized blood will be stimulated immediately with BCG, MTBVAC, newly identified single "mega pool" of mycobacterial peptides, or phytohemagglutinin (PHA) or will be left unstimulated (Nil), for 12 hours at 37^0C. D7 stimulation conditions include half the blood volume [250 µL (0.25 mL)] and only Nil, MTBVAC and BCG. After 7 hours of stimulation, supernatant (for soluble cytokine/chemokine analysis) will be collected from all the conditions, frozen at -80C and stored for shipping to Sponsor for further analysis.

Following supernatant removal, brefeldin A will be added for the remaining whole blood and tubes incubated for a further 5hrs in a programmable waterbath. The waterbath will switch off after a total of 12 hours of stimulation. The next morning, FACSLysing solution will be added to lyse red cells and fix white cells. Fixed, white cells will then be frozen for later intracellular cytokine staining and flow cytometry. Flow cytometric staining and acquisition will be run in batches at a later time point. Measurement of frequencies and patterns of specific type-1 cytokines and IL-17 by CD4 and CD8 T cells will be assessed.

The timepoints for immunogenicity have been selected on the basis of recent studies conducted by SATVI, which have shown that the peak of the BCG-induced T cell responses in infants is around 6-10 weeks of age [6]. Hence, the primary immunogenicity assays will be performed on day 70 and end of study.

Inclusion and exclusion criteria	**Adult Stage:** Inclusion criteria: **1.** Male or female, age 18 to 50 years. **2.** Written informed consent, including permission for access to medical records and an HIV test. **3.** Available for study follow up and display a willingness and capacity to comply with study procedures. **4.** In good general health, as assessed by medical history and a focused physical examination. **5.** HIV test (rapid test, ELISA, or PCR) negative. **6.** Quantiferon®-TB Gold (Cellestis) test for latent TB infection negative within 3 weeks of enrolment **7.** BCG vaccination at birth as confirmed by history or the presence of a BCG scar **8.** In the case of female participants, a negative urine or serum pregnancy test at enrolment, not lactating, and willingness to use an acceptable method of contraception to avoid pregnancy for the duration of the study Exclusion criteria **1.** A history or evidence of an acute or chronic medical or surgical condition likely to affect the safety, reactogenicity, or immunogenicity of the investigational vaccine 2. Skin condition, bruising or birth mark at the intended injection site. **3.** History or evidence of previous or current active TB disease **4.** History of a household contact with active TB disease who has received less than 2 months of treatment **Infant Stage:** Inclusion criteria: **1.** Male or female neonates within 96 hours of birth. **2.** Written informed parental consent, including permission to access medical records and results of antenatal HIV tests. **3.** Infant participants and their caregivers available for study follow-up and display the willingness and capacity to comply with study procedures. **4.** Neonates must be in good general health during pregnancy and delivery, as assessed by medical history and focused physical examination **5.** Birth weight more than or equal to 2450 grams. **6.** Apgar score at 5 minutes more than or equal to 7. **7.** A maternal HIV test result (rapid test, ELISA or PCR) taken within 30 days of delivery must be available, documented and negative. **8.** Estimated gestational age more than or equal to 37weeks Exclusion criteria: **1.** Infant must not have received routine BCG vaccination prior to enrolment. **2.** Antenatal, intrapartum, or postnatal medical or surgical condition that may

	affect the safety, reactogenicity, or immunogenicity of the investigational vaccine. **3.** Skin condition, bruising or birth mark at the intended injection site. **4.** Maternal HIV test (rapid test, ELISA or PCR) not performed, within 30 days of delivery. HIV test results not available, or HIV test result known positive. **5.** Maternal or other household contact with newly diagnosed or incompletely treated active TB disease.
Study design	Randomized, controlled, double blind clinical trial in 2 stages (adult stage, infant stage). The first stage will include 18 HIV uninfected, QFT negative, BCG vaccinated, adult participants, randomized 1:1 to receive BCG Vaccine SSI or MTBVAC at equivalent dose ($5x10^5$ CFU/0.1mL) (n=9 in each group). The Data Safety Monitoring Board (DSMB) will evaluate the reactogenicity and safety data for all 18 adults up to day 28 after study vaccination. Upon favourable safety review of Day 28 safety data by the DSMB, the second stage will commence in thirty-six (36) HIV unexposed, BCG naïve, newborn infants, randomized 1:3 to receive BCG Vaccine SSI or MTBVAC at one of three different dose levels (n=9 in each group). The Principal Investigator will follow up the Day 7 reactogenicity and safety data for first three infants before enrolment of remaining 9 in Cohort 1. Progression to the next dose level will occur after DSMB review of Day 28 safety data of preceding cohort. After unblinding after D180, BCG Vaccine will be given at approximately D194 to all MTBVAC vaccinated infants
Study duration	Each adult participant will remain active on the study for a period of 6 months after enrolment and vaccination. Each infant participant will remain under active follow-up for a period of 12 months after study vaccination. Thereafter, infant participants will remain under passive surveillance with 3-monthly telephonic contacts, to detect incident TB disease, until 2 years of age.
Study procedures	**Adult Stage:** Eighteen (18) adult participants will be recruited and randomized equally into 1 of 2 study groups (n=9 per group): MTBVAC highest dose group (approx. $5x10^5$ CFU/0.1mL) or BCG SSI standard human dose (approx. $5x10^5$ CFU/0.1mL). Safety assessments will be conducted at D0, D7, D14, D28, D56, D90, and D180 post study vaccination. A diary card will be used to collect solicited local, regional, and systemic adverse event data from D0 through D14. Reactogenicity data will be collected at each study visit. Non-serious adverse events will be collected through D28. Serious adverse events will be collected during the entire study period.

Infant Stage:
Thirty-six (36) infant participants will be recruited, randomized and allocated into 4 groups: BCG (single dose level 2.5 x 10^5 CFU/0.05 mL, n=at least 6)); or MTBVAC at three different dose levels (lowest 2.5x10^3 CFU/0.05mL (n=9), middle 2.5x10^4 CFU/0.05mL (n=9), highest 2.5x10^5 CFU/0.05mL (n=at least 9).

Vaccination of neonates will be staggered to allow gradual evaluation of safety and reactogenicity, as follows:

- Cohort 1: First 3 infants in the first week, each at least 3 days apart. These 3 infants should be followed up for a minimum of 7 days prior to proceeding with further recruitment of remaining 9 infants with a maximum of 1 study vaccination administered per day. (for these 12 infants, 9 will receive the lowest MTBVAC dose level and 3 BCG control)
- Cohort 2: A maximum of 2 study vaccinations per day for the next 12 infants (9 will receive middle MTBVAC dose level and 3 BCG control)
- Cohort 3: Unrestricted enrolment and vaccination of the remaining 12 infants (at least 9 will receive the highest MTBVAC dose level and up to 3 BCG control).

Following the expiry (30 June 16) of the only currently available BCG SSI vaccine, the enrolment into cohort 3 from 1 July 2016 will be unblinded, and only MTBVAC 2.5x10^5 CFU/0.05mL will be administered until cohort n=12

All AEs and biochemical and haematological parameters will be collected up until Day 28 after vaccination. The DSMB will review the group's D28 safety data to authorize progression to the next group.

Safety assessments will be conducted at D0, D7, D14, D28, D70, D90, D180 and D360 post study vaccination. A diary card will be used to collect solicited local, regional, and systemic adverse event data from D0 through D14. Reactogenicity data will be collected at each study visit. Non-serious adverse events will be collected through D28. Serious adverse events will be collected during the entire study period. Unscheduled follow-up face-to-face visits will be performed as needed for safety and adverse event management.

Phlebotomy for immunogenicity will be evaluated at: D7, D28, D70, and D180. 0.75 mL at D7 and 2.5 mL the rest of the days. A QFT test will be performed at D180 and D360.

Swabs at injection site for microbiology analysis will be conducted in all infants with an open injection site event such as a discharge and will be repeated until 2 negative cultures are obtained.

Unblinding by group will occur once all infants in a dose group have had D180 visit, within 2 next weeks (D194 visit approximately), BCG Vaccine will be given to all

	MTBVAC vaccinated infants. The risk of incident TB disease will be minimized by exclusion of TB exposed infants. All infants will be screened actively for possible TB disease at every study visit to detect any incident TB disease early and to allow prompt treatment. Infants with symptoms consistent with suspected TB, including persistent unexplained cough, fever, weight loss, or failure to thrive, will undergo standardized investigation, including chest radiography, and paired induced sputum and gastric lavage for Xpert MTB/Rif and liquid mycobacterial culture. 3 monthly passive surveillance will continue for 12 months (until infant is 2 years old). Infants diagnosed with TB disease will be referred to the public health sector to receive standard of care drug therapy, tailored to drug sensitivity if necessary.

Safety Review by the Data Safety Monitoring Board (DSMB), and other safety provisions	Progression from adults to infants, and enrolment of infants into the higher dose cohort may not commence until a favourable safety review by the DSMB of the D28 data of the preceding cohort. The DSMB may request additional information, including unblinding of the adult data, and may recommend changes to the trial protocol to enhance safety measures. In addition, the DSMB will be convened ad hoc if protocol defined safety pausing rules have been met during the adult or infant stage. In order to enroll as many infants into cohort 3 blinded,(thus receiving either MTBVAC or BCG SSI), before the expiry of BCG SSI and subsequent unblinded completion of enrolment into the cohort, the DSMB will review Day 28 safety data for the first 9 participants of cohort 2 for consideration of advance into cohort 3. Review of the final 3 participants in cohort 2's Day 28 safety data will occur once available. A local medical monitor (LMM) will be assigned by the sponsor to oversee safety in collaboration with the PI. The LMM will be consulted on all SAEs or other safety issues. Safety signals defined by pre-set pausing rules will be reviewed by the DSMB. Further vaccination will be paused, pending review of blinded or unblinded data by the DSMB if a pausing rule is triggered. Study vaccination will only resume upon recommendation by the DSMB, which may include changes to the study protocol.
Statistical analysis	Full details of the statistical analysis will be documented in the statistical analysis plan, drafted by the responsible statistician from Triclinium Clinical Development (Dr J van Tonder). The statistical analysis plan will be finalized prior to database lock.

Section 1: Background information and rationale

1.1 Background

Tuberculosis (TB), caused by the M. tuberculosis organism, is a major disease worldwide, particularly in developing countries. The World Health Organization (WHO) estimates that a third of the world's population is infected with the TB bacillus. In 2014, there were an estimated 10.8 million TB incidence cases, globally equivalent to 133,2 cases per 100, 000 population, and 1.5 million deaths associated with the disease (including 390 000 deaths among HIV-positive people). The TB incidence rate at a country level ranges substantially, with around 1000 or more per 100, 000 people in South Africa and Swaziland, Western Europe with 117 per 100,000 people and fewer than 30 per 100 000 in some parts of the Americas (28), Japan (18), Australia (9) and New Zealand (7,4).

Epidemiological data in areas with the highest incidence of TB, where BCG is routinely used, show that the incidence of TB cases in HIV-negative individuals is highest early in life between 0 and 4 years of age and in adolescents and adults vaccinated with BCG at birth [8-10]. These figures indicate that BCG does not confer protection against TB regardless of age, underlining the need for a new BCG-replacement strategy effective against all forms of the disease.
Efficacy evaluation of a new priming TB vaccine at birth is most feasible because newborns are the only naive age group population that have not been previously vaccinated with BCG or exposed to TB or environmental mycobacteria, which could have "masking" and "blocking" effects on vaccine-induced protection or vaccine take [11-13].

1.2 Expected course of BCG reactogenicity and immunogenicity

There is much experience with the adverse effects of BCG vaccination given via the Mantoux technique [14, 16]. Induration and redness of the skin at the site of intradermal BCG vaccination typically develop within several days and represents a normal response to BCG vaccination [17]. The induration gradually resolves over several days and is followed by a small local superficial ulcer. The ulcer opens and drains for about 4 weeks on average, and spontaneously heals within 2 to 3 months, leaving a small scar. In some cases, no visible scar develops. A brief period of minor enlargement of the regional cervical and axillary lymph nodes (< 1 cm), frequently asymptomatic, is common [17, 18].

Following BCG vaccination, antigen presenting cells prime naive T cells to become BCG-specific. Initially, the BCG-specific T cells expand (expansion stage) and carry out immediate effector functions. Later, the BCG-induced T cell response contracts (contraction stage) leaving a pool of vaccine-specific T cells (central memory). In infants, the vaccine-induced T cell response following administration of BCG at birth peaks at 10 weeks post-vaccination and starts to wane thereafter. The BCG-induced T cell responses show a sharp decline at 14 weeks of age [6].

1.3 Expected course of MTBVAC reactogenicity and immunogenicity

1.3.1. Preclinical data supporting expected reactogenicity and efficacy profile of MTBVAC

Rigorous preclinical studies in mice and guinea pigs to date have demonstrated that MTBVAC presents a very similar safety, biodistribution, persistence/clearance, excretion and shedding profile as compared to BCG when tested by the clinical route and intended dose of administration (intradermal inoculation of $5x10^5$ CFU in 0.1 mL). In terms of local tolerance / skin reactivity MTBVAC appears to be less reactogenic than BCG, as observed in mice and guinea pigs. In continuation is a summary of the preclinical studies conducted to date to assess biodistribution, safety and tolerability and efficacy profile of MTBVAC freeze-dried preparation produced by Biofabri, SL (Spain) in GMP compliance and following European Pharmacopoeia monograph and the WHO recommendations to assure the quality, safety and efficacy of BCG vaccines, see Arbues et al., Vaccine, 2013 [4] and Investigator's Brochure for more details.

In mice, MTBVAC has shown to be more immunogenic and more protective than BCG. However, the current immunogenicity assays used in preclinical and clinical studies do not correlate with protective efficacy induced by BCG or other new candidates.

Summary of preclinical study results to date

(see Arbues et al., Vaccine, 2013 [4] and Investigator's Brochure for more details)

MTBVAC exhibits comparable safety profile to reference BCG SSI

- Inoculation of 50 standard adult human doses of BCG SSI or MTBVAC (equivalent to 2.5×10^7 CFU) in immunocompromised mice (IFN γ-knockout or TNFalpha-knockout) with severe-combined immunodeficiency (SCID) (lacking adaptive immune responses, B and T cells) and in guinea pigs showed that both vaccines show comparable safety profile, as measured by survival, TB lesions and bacterial in target organs.
- A single dose of MTBVAC is as safe as BCG SSI and safer than BCG Pasteur in SCID mice.

MTBVAC appears to be less reactogenic than BCG in mice and guinea pigs.

- Fifty-day intradermal toxicity study in mice showed no evidence of systemic toxicity following vaccination with MTBVAC, and the intradermal injection of MTBVAC on 2 occasions over 3 weeks to BALB/c mice was only associated with local inflammation at the site of administration, noted in-life and histologically, and a higher number of circulating white blood cells. These findings were also observed with BCG. The character of the inflammatory response noted histologically was similar for BCG and MTBVAC, but at Day 4 and Day 25, tended to have a higher severity grade in animals that received BCG (CRL_520026).
- Unlike the BCG vaccine, no significant reaction was observed at the site of intradermal injection over a period of 7 weeks in MTBVAC vaccinated guinea

pigs. A vaccine site 'opening event' was observed only in BCG vaccinated animals between 20 and 26 days post-vaccination (HPA_4401).

- The final lots MTBVAC 110142 and 110380 comply with Eur. Ph. dermal reactivity test (01/2008:0163) for BCG. MTBVAC appears to be less reactogenic than BCG Danish SSI in guinea pigs (MTBVAC_LTRD_3)

Protective efficacy (and immunogenicity) relevant to BCG

In the current relevant animal models (mice, guinea pigs, non-human primates) what protection actually means is an improvement in a disease-related readout, be it bacterial load (expressed as colony forming units or CFU) at a fixed time point post challenge, long-term survival, or pathology score, compared with BCG. The level of improvement seen to date with all TB vaccine candidates has been a reduction in bacterial burden of 0.5-1 log CFU. Below is a summary of the animal models in which the vaccine prototype of MTBVAC, named SO2, has shown improved protection over BCG.

- *Protective efficacy comparing MTBVAC prototype SO2 and BCG SSI in Rhesus macaques*, BCG vaccination revealed an average reduction of lung bacillary burden of 0.43 logs, whereas SO2 vaccination revealed a reduction of 1.2 logs in lung. SO2 but not BCG vaccination revealed significant body weight gain and marked reduction of lung pathology when compared to unvaccinated control[s.]
- *Long-term protective efficacy study in guinea pigs* demonstrated that the protection conferred by MTBVAC prototype SO2 after high-dose (100-500CFU) aerosol challenge is superior to that conferred by BCG Danish in not only enhancing survival of the animals (100% vs 33%), but also in providing reduction in the severity of disease in lungs and dissemination of infection to spleen after high-dose aerosol challenge (Martin et al Vaccine 2006). *In guinea pigs, the most sensitive animal model for evaluation of TB pathogenesis, MTBVAC and BCG show similar protection against aerosol challenge with low-dose (10-50 CFU) of virulent M. tuberculosis H37Rv.*
- MTBVAC prototype SO2 was able to induce a higher expansion and differentiation of antigen-specific $CD4^+$ T cells into central memory T cells as compared to BCG in vaccinated mice correlating with a longer duration of protective efficacy in this model.

1.3.2. Clinical data to date

Phase 1a trial in healthy adults in Lausanne, Switzerland

This report has been written by Dr R. Chakour, Dr O. Karoui and Dr V. Steiner-Monard based on the paper CRF (source documents).

The MTBVAC study is a Phase I, double blind, dose-escalating trial to evaluate the safety, reactogenicity and immunogenicity of live vaccine MTBVAC when given as primary vaccination to BCG naive, ELISPOT Tb (ESAT-6, CFP10) and HIV-negative adults aged 18 to 45 years.

The vaccination (MTBVAC or BCG) was very well tolerated. We observed mild local and systemic AEs, mostly grade 1 AEs during the first month after vaccination. Local related AEs (erythema, swelling) were more frequent (in all the participants) than systemic ones (tiredness, headache).

No clinically significant changes in vital signs or in the lab tests were noticed except a case of microcytic hypochromic anaemia noted since V5 in a participant who had an iron deficiency anaemia even before vaccination.

Ten participants developed a local discharge. The secretions of three participants remained serous and light, the seven others were suppurative. Interestingly, five of these ten participants presented with elevated Elispot PPD values at screening.

No participant throughout the study had positive values for ESAT-6. Three participants had positive values for CFP-10 at V6 that were under the threshold at V10. Three participants had positive values for PPD at V6 that were under the threshold at V10. Only one participant had a positive Elispot value at V 10 (vol. #015, cohort 1, with a PPD of 112 SFU/mio).

The mycobacterial cultures done on cutaneous swabs became positive for *Mycobacterium spp*. in 5 participants: one in Cohort 1 (at V6), one in Cohort 2 (at D9), and three participants in Cohort 3 (two positive cases detected at V6 and one case at V7). The subsequent cultures were negative in these participants. A molecular study will determine the nature of the mycobacteria at the end of the study in order to avoid unblinding of the investigators. The mycobacterial cultures on stool and urine were negative in all participants.

Results of this clinical trial have been published in "Lancet Respir Med 2015". Published Online. November 16, 2015

1.4 Rationale for the planed Phase 1b clinical trial in healthy newborns in South Africa

The current Phase 1b trial is designed to test the hypothesis that MTBVAC is as safe as BCG SSI in healthy newborns in a high-burden country and that there is a

measurable vaccine take in this age-group.

MTBVAC is developed as a preventive TB vaccine for use at birth. If it shows improved efficacy and a safety profile that is at least comparable to BCG, MTBVAC could eventually replace BCG. The final target age group population for MTBVAC to show improved efficacy is healthy newborns in highly endemic countries. The aim is to accelerate clinical development of MTBVAC as safely and efficiently as possible from healthy adults in non-endemic areas to healthy newborns in TB endemic countries. To this aim, with external expertise from clinical expert advisors from TBVI, the current clinical development plan of MTBVAC is designed in the following way (**Figure 1**):

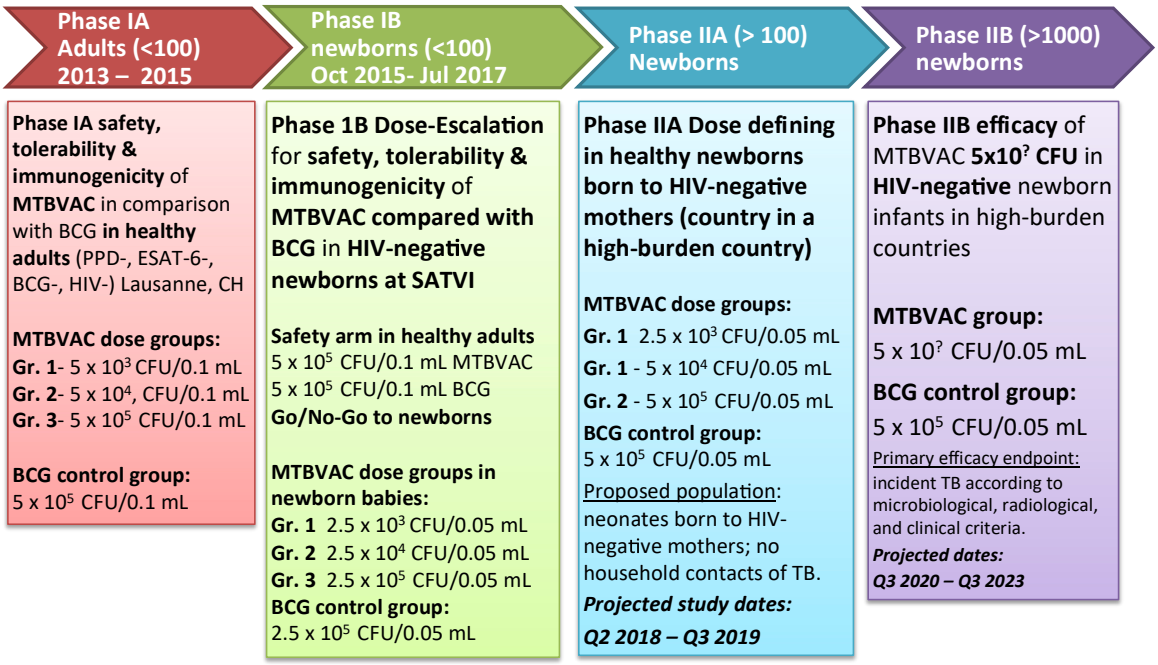

Figure 1. Proposed product development plan of MTBVAC to Phase 2b efficacy evaluation.

The rationale for moving directly to newborns from healthy adults follows the second Geneva Consensus document generated following a meeting of international group of TB vaccine experts, regulators, and vaccine developers and manufacturers held in Geneva on 7 and 8 April 2009. The meeting was jointly organised by the European funded Tuberculosis Vaccine Consortium (TB VAC), the AERAS Global TB Vaccine Foundation and the World Health Organization under the auspices of the Global Partnership to Stop TB [5].

> *"In studies where the target product profile is to replace the current BCG vaccine then neonatal or young children will be the final target population; if acting as a booster, children or possible young adults may become part of the target population. This raises questions as to the relevance and necessity of performing early clinical studies in healthy adult males and also the strategy of age de-escalation and movement towards cohorts of increased "risk" or cohorts closer to the final target population. In addition, some pragmatic considerations relating to cohort access need to be addressed, for*

example, in many developing countries where TB is endemic, the possibility of recruiting young healthy adults who are PPD negative or indeed BCG negative may be, for all practical purposes, impossible."

Before we address the question of impact of a new vaccine on TB control, it is essential to know if the vaccine is more efficacious with respect to BCG. The more reliable way of knowing whether a new vaccine works better than BCG is by conducting an efficacy trial in a naive population without previous environmental sensitization (e.g., previous BCG vaccination, mycobacterial infection and/or TB contact) in order to avoid possible effects of masking (which provide some protective immunity against TB) or blocking (which prevent a new vaccine from providing protection) [12, 19, 20].
Most recently published data from BCG efficacy studies in Brazil by Barreto, M., et al, [12] and meta-analysis findings [11, 13] add further support to existing evidence from human and animal studies that prior nontuberculous mycobacteria (NTM) exposure interferes with the efficacy of BCG, and that BCG is most effective in mycobacterially naive hosts. In her editorial comment to Mangtami et al manuscript [13], Prof. Helen McShane states "If this theory is correct, We should optimize deployment of BCG to administration as close to birth as possible" [21].

1.5 Potential risks and benefits

1.5.1 Potential complications of BCG administration in neonates

Complications associated with BCG administration are uncommon. Injection site and local complications such as extensive local ulceration, local subcutaneous abscesses, and suppurative lymphadenitis occur in less than 1 per 1,000-10,000 [17].

Systemic adverse events from BCG occur very infrequently. Fever, headache and non-injection site cutaneous manifestations occur in less than 1% of those vaccinated. Severe systemic adverse events such as osteitis and disseminated BCG infection are rare (about 1 per 5 million vaccinations). When disseminated BCG infection does occur it is most often in immunocompromised infants e.g. HIV positive infants [17]. Infants of HIV-positive mothers will be excluded in this trial. Therefore, the risk of disseminated BCG disease in trial participants is very low.

1.5.2 Potential complications of MTBVAC administration in neonates

Given the satisfactory safety and immunogenicity data from the Phase 1a clinical trial of MTBVAC in adults in Lausanne, showing comparable safety and tolerability profile of MTBVAC and BCG, and the exhaustive preclinical studies to date from mice to non-human primates, we expect that MTBVAC will act very similarly to BCG in newborns. All potential complications of BCG administration in newborns could be expected for MTBVAC.

1.5.3. Contingency Plan for MTBVAC

Antibiotic sensitivity of MTBVAC and BCG strains

(See Investigator's Brochure for more information)

MTBVAC appears to be more sensitive to isoniazid and ethambutol than BCG SSI as determined by the resazurin microtiter assay. MTBVAC is considered fully susceptible to the front-line antibiotics for TB treatment in the clinic. In the hypothetical case of infection with MTBVAC, treatment would be possible.

For BCG SSI, the MIC of isoniazid is 0.4 mg/L as determined by Bactec 460. By the Resazurin Microtiter assay, BCG SSI has MIC for isoniazid of 1 mg/L and the MIC for MTBVAC is in the range of 0.5 - 0.125 mg/L, as shown in Table 1 below with the minimum inhibitory concentrations (MIC) for currently employed anti-tuberculous drugs in clinic towards the BCG SSI and MTBVAC (as determined by the Resazurin Microtiter Assay).

Table 1. Drug Minimum Inhibitory Concentration (MIC) by the Resazurin Microtiter Assay

	Drug MICs (µg/ml)	
	MTBVAC	BCG SSI
Isoniazid	< 0.5	1
Ethambutol	1-0.5	4
Streptomycin	< 0.125	0.5
Rifampicin	< 0.002	<0.002

1.6. Potential complications of BCG revaccination in adult

Adults in this trial will have received BCG vaccination at birth, but will be free of latent TB infection. TB infection will be excluded by means of a negative Quantiferon®-TB Gold (Cellestis) test. BCG administration in these participants can therefore be considered BCG revaccination.

Some previous studies indicate a possibility of increased reactogenicity from BCG in adults

Previous data have suggested an increased frequency of local injection site reactions after BCG in older age groups. In a prospective national study conducted in Australia of adverse reactions among 918 participants aged 1 to 54 years of age, it was reported that local reactions such as lymphadenitis, injection-site abscesses and more severe local reactions occurred less frequently in participants younger than 6 months of age [22]. Some data have suggested an increase in injection site reactogenicity such as duration of ulceration associated with an increased baseline lymphoproliferative activity associated with environmental mycobacterial exposure [23]. It should be noted however, that the frequency of systemic, major or serious adverse events reported from BCG, including in adults, remains very low.

Other studies suggest that BCG revaccination is safe
In a large population-based trial of BCG revaccination in adolescents (7-14 years) in Brazil, only 25 of the 71341 participants had adverse reactions and no deaths or cases of disseminated BCG disease were reported. In that trial BCG was administered to all participants, 83% of whom had a BCG scar indicative of previous BCG vaccination during the neonatal period [24]. New data in the intended adult study population shows that BCG revaccination is safe [25].

Preclinical revaccination studies with MTBVAC and BCG in guinea pigs.
Revaccination with MTBVAC of previously immunized guinea pigs with BCG (by the same route and same dose of administration for BCG in adult humans) showed no signs of disease in vivo and at autopsy. All guinea pigs gained weight, showed normal behaviour and survived until fixed endpoint of study as observed to saline (non-vaccinated control group). We expect that vaccination with MTBVAC of healthy adults immunized with BCG at birth could show comparable safety profile as that observed following revaccination with BCG. We consider MTBVAC to be biologically similar to BCG in terms of safety.

1.6.1 Other risks and measures to minimize risks

Risk to participants from adverse events will be minimized in this trial through intensive post-vaccination follow-up and appropriate management and referral by the Principal Investigator (PI) or sub-Investigator. If necessary, participants will be referred for further medical management to the appropriate facility, or medical practitioner. All adverse events (AEs) and Serious Adverse Events (SAE) will be recorded, monitored and reported. A local medical monitor will oversee the safety of participants.

The risk of incident TB disease will be minimized by exclusion of TB exposed infants. All infants will be screened actively for possible TB disease at every study visit to detect any incident TB disease early and to allow prompt treatment. Infants with symptoms consistent with suspected TB, including persistent unexplained cough, fever, weight loss, or failure to thrive, will undergo standardized investigation, including chest radiography, and paired induced sputum and gastric lavage for Xpert MTB/Rif and liquid mycobacterial culture. Infants diagnosed with TB disease will be referred to the public health facility and receive standard of care drug therapy, tailored to drug sensitivity if necessary Three-monthly passive surveillance will continue after study completion for 12 months i.e. until infants are 24 months of age.

Risk of phlebotomy
There are minor risks to phlebotomy including phlebotomy site complications such as hematoma formation. Circulatory blood volume loss and anemia due to repeated phlebotomy is an uncommon but potential side-effect. In order to minimize risks, experienced and qualified phlebotomists will be used in this trial. Prescribed maximum blood volumes for infants will be adhered to according to accepted international guidelines.[26], as summarized in Appendix 1. No more than 6 milliliters of blood will be taken per infant participant at a single visit.

Required volumes will be minimized by limiting phlebotomy points to 5 visits during the year on the study; by making use of assays that require very small amounts of blood; and by limiting the volume of blood that can be taken at each visit. Blood will be taken for safety and immunology testing only. No more than two (2) attempts at phlebotomy will be performed at a single visit per infant participant.

Risk of disclosure of confidential information and participant confidentiality

Disclosure of HIV infection status, or other medical information, may be associated with social stigmatization in some South African communities and with breach of confidentiality. The utmost effort will be made to avoid breach of participant confidentiality, in all discussions with study staff, handling of documentation, and management of the study database. Access to records will be restricted to study staff only, participant folders will be stored on-site in lockable, fire-proof cabinets, and access to computer records will be password-restricted. Participant and laboratory data with potential identifiers will be recorded on study data capture forms and the database using a unique and coded participant identification number only. Access to study files, the study database, and participant identifiers, will be restricted to the Principal Investigator, sub-Investigators, the clinical trial staff, and the SATVI Data Manager.

Protection of participant rights

The study will be conducted in accordance with the Declaration of Helsinki (2013 version), South African Good Clinical Practice Guidelines (2006), and the regulations of the University of Cape Town Human Research Ethics Committee (UCT HREC) and Medicine Control Council of South Africa (MCC). All potential adult participants and the mothers of potential infant participants will be required to sign an Informed Consent Form (ICF) prior to enrolment, and prior to any private medical information being made available to the study team. Prior to signing of the ICF, the study team will provide the participant or the participant's mother with adequate information about the trial and trial procedures. He or she will be given adequate time to consider the information, ask questions, and consult with friends or family members, if required. Informed consent will be provided in the language of choice of the participant (English, Afrikaans or isiXhosa). The adult participant or the infant participant's legal guardian will be free to withdraw themselves or their child from trial participation at any time without prejudice. If a participant's mother declines to give consent for trial participation, her infant will be provided the opportunity to receive the routine BCG vaccination as offered by the state healthcare service free of charge. BCG vaccination is not standard of practice for adults in South Africa and so no alternative needs to be offered to adult participants declining consent.

All study team staff will provide proof of completion of a certified GCP course. The study will be monitored by a local medical monitor and an external monitor, who will have access to the study site and all study documentation. The investigators will abide by any decision of the UCT HREC, sponsor or regulatory authorities in event that the UCT HREC, sponsor, or authorities decide to interrupt the study.

Risk of inconvenience and financial loss to participants

It is expected that participants and their caregivers will experience some inconvenience in attending the study visits. Participants will be compensated for their inconvenience,

time, and possible financial loss, in the amount of ZAR 150 (approximately $10 USD) per study visit. This amount is consistent with the external recommendations of the South African Medicines Control Council and with SATVI internal recommendations for participant compensation in clinical studies. Where necessary, transport will be provided to participants by the study team.

1.6.2 Potential benefits of this trial

Potential benefits in newborns

The administration of BCG to newborns is standard of care in South Africa. When administered at birth, BCG provides consistent protection against TB. The recent BCG REVAC trial in two different sites in Brazil, Salvador and Manaus, considered to differ in varying environmental mycobacterial sensitization, showed that when BCG is given at birth protection is conferred and it is comparable (40% Salvador and 36% Manaus) [12]. These results indicate that there is room for testing improved BCG efficacy in newborns with a new candidate vaccine (e.g., MTBVAC or an equivalent) and the anticipated incremental effect that could be ascribed to a new more effective vaccine that shows at least 60% efficacy as compared to BCG would be significant.

MTBVAC is a live-attenuated vaccine from human origin of the *M. tuberculosis* Euro-American lineage (commonly transmitted between humans by the aerosol route) containing all the genes present in BCG plus the genes deleted in *M. bovis* and BCG [4]. Recent studies have shown that 23% of the known human T-cell epitopes present in *M. tuberculosis* are absent in BCG [3]. This makes MTBVAC able to present a wider collection of mycobacterial antigens to the host immune system, a highly desirable feature for a vaccine [27]. Moreover, the close genealogy of *M. tuberculosis* and *M. bovis* suggests that live-attenuated *M. tuberculosis*-based vaccines could share the live-saving benefits observed with BCG, and could be confirmed in large efficacy or post-licensing trials [28].

Thirteen years of preclinical studies with MTBVAC have demonstrated that this candidate is at least as safe as BCG and confers better protection against TB in different relevant animal models. MTBVAC Phase 1a trial in healthy adults has shown an excellent safety profile. After showing safety in newborns, the fastest way to show improved efficacy compared to BCG would be in a Phase 2b in non-exposed newborns.

Another argument to be considered for MTBVAC is that all current boosting strategies, which are based on a limited number of *M. tuberculosis*-specific antigens, are designed for use after BCG immunization at birth, whereas, if MTBVAC confers improved protection as compared to BCG at birth, it may also provide a more effective alternative for boosting strategies at later ages.

Potential benefits in adults

We think that given that MTBVAC expresses all the antigens in BCG and those deleted in M. bovis compared to M. tuberculosis, vaccination with MTBVAC would show improved efficacy compared with BCG in adults. However, the current Phase 1b trial only evaluates safety and reactogenicity in adults. Moreover, the effects of masking (environmental sensitization due to previous exposure to BCG, NTM and M.

tuberculosis provide some protective immunity against TB) and blocking (previous environmental sensitization prevents a new vaccine from providing protection) would make it difficult to obtain concluding data about potential benefits of vaccinating adults with BCG or MTBVAC. The recent BCG REVAC trial in Brazil showed that when the first dose of BCG was given at school age, protection was highly variable depending on previous environmental sensitization, 34 % in Salvador, where sensitization is considered lower, and 8% in Manaus, which close to the equator and sensitization is much higher [12]. Importantly, when a second dose of BCG was given in school-aged children (first dose neonatal), the observed protection was much lower, 19 % and 1%, respectively. Supporting these data, recent meta-analysis studies show that "Absence of prior M. tuberculosis infection or sensitization with environmental mycobacteria is associated with higher efficacy of BCG against pulmonary tuberculosis and possibly against miliary and meningeal tuberculosis. Evaluations of new tuberculosis vaccines should account for the possibility that prior infection may mask or block their effects" [13].

BCG revaccination was common historical practice in older children, adolescents, and adults. The World Health Organization (WHO) recommended against BCG revaccination in national vaccination programs in 2004 because mass BCG revaccination campaigns did not offer clear benefit to the population as a whole [29].

We would postulate that the potential for BCG revaccination benefit in non TB latently infected, HIV uninfected, young adults in a high-burden population is at least equal to, or greater than, any potential for harm

1.6.3 Risk and benefits to rescue dose of BCG in infants

Within 2 weeks of unblinding by dose group (once all infants in that group have completed D180 visit), infants who received MTBVAC will be given a rescue dose of BCG.

Delayed primary BCG vaccination in the absence of BCG administration at birth has been shown to induce a good immune response [30]. In order to minimize a potential increased risk for developing TB disease prior to the rescue dose of BCG, participants with potential household TB exposure will be excluded from participating and participants will undergo active surveillance for signs or symptoms of TB throughout follow-up. After study completion a further 12 months of 3 monthly passive surveillance will take place, consisting of home visits or telephone calls by a field worker with targeted questions on the symptoms and signs of TB and body weight evaluation for failure to thrive.

Section 2: Objectives

Primary objectives:

1) To evaluate safety and reactogenicity of MTBVAC, compared to BCG Vaccine SSI, in healthy, BCG naïve, HIV unexposed, South African newborn infants

2) To evaluate immunogenicity of MTBVAC, compared to BCG Vaccine SSI, in healthy, BCG naïve, HIV unexposed, South African newborn infants

Secondary objectives:

To evaluate safety and reactogenicity of MTBVAC, compared to BCG Vaccine SSI, in healthy, BCG vaccinated, HIV negative, QFT negative, South African adults.

Immunogenicity endpoints (in infants only):

Primary immunogenicity endpoints

Frequencies and co-expression patterns of CD4 and CD8 T cells expressing IFN-γ, TNF-α, IL-2 and/or IL-17 induced by MTBVAC or BCG, and suitable antigens in healthy, BCG naïve, HIV unexposed, South African newborn infants.

Exploratory immunogenicity endpoints

Whole blood supernatant samples for further immunogenicity tests will be collected and stored frozen. Exploratory assays will be planned, based on data from the primary immunogenicity analyses.

Section 3: Study design and setting

3.1 Study design

This is a randomized, doubled-blinded, dose-escalation clinical trial in 2 stages (adult stage, newborn infant stage) conducted at a single site.

3.2 Study setting

The trial will be conducted at the field site of the South African Tuberculosis Vaccine Initiative (SATVI) in the Cape Winelands East district of the Western Cape of South Africa. SATVI is a clinical research unit within the Institute of Infectious Disease and Molecular Medicine (IDM) of the University of Cape Town. SATVI has an established field site in the Cape Winelands district and has been conducting epidemiological and clinical trial research in this area for over 13 years (http://www.satvi.uct.ac.za/).
Recruitment of adults will be done in the community using direct approach and word of mouth referrals. Recruitment of pregnant women will take place at the public healthcare antenatal clinics in the area. Screening, vaccination and follow up of adults will take place on the SATVI field site premises. Pregnant women will be consented in the privacy of their homes or at a location of their choice and where possible this will be followed by at least one contact visit with SATVI study staff during the pregnancy to confirm participation and to obtain background information about mother and her pregnancy. Vaccination of infants will take place at the SATVI site with follow up visits at site or at home as per protocol. All study procedures, including vaccination, will be performed by SATVI study staff.

Section 4: Study population

Healthy, HIV-negative, QFT-negative, BCG vaccinated adults and healthy, HIV-unexposed TB-unexposed, BCG naive neonates less than 96 hours old will be enrolled into the trial. Adult participants with HIV and infant participants with HIV positive mothers will be excluded

4.1 Inclusion and Exclusion Criteria

4.1.1. Adult Stage

Inclusion criteria (adults):

1. Male or female, age 18 to 50 years
2. Written informed consent, including permission for access to medical records and an HIV test.
3. Available for study follow up and display a willingness and capacity to comply to study procedures.
4. In good general health, as assessed by medical history and a focused physical examination.
5. HIV test (rapid test, ELISA, or PCR) negative

6. Quantiferon®-TB Gold (Cellestis) test for latent TB infection negative within 3 weeks of enrolment
7. BCG vaccination at birth as confirmed by history or the presence of a BCG scar
8. In the case of female participants, a negative urine or serum pregnancy test at enrolment, not lactating, and willingness to use an acceptable method of contraception to avoid pregnancy for the duration of the study

Exclusion criteria (adults):

1. A history or evidence of an acute or chronic medical or surgical condition likely to affect the safety, reactogenicity, or immunogenicity of the investigational vaccine
2. Skin condition, bruising or birth mark at the intended injection site
3. History or evidence of previous or current active TB disease
4. History of a household contact with active TB disease who has received less than 2 months treatment

4.1.2 Neonate/Infant Stage

Inclusion criteria (infants):

1. Male or female neonates within 96 hours of birth.
2. Written informed consent, including permission to access medical records and results of maternal antenatal HIV tests.
3. Infant participants and their caregivers available for study follow-up and display the willingness and capacity to comply with study procedures.
4. Neonates must be in good general health during pregnancy and delivery, as assessed by medical history and focused physical examination.
5. Birth weight more than or equal to 2450 grams.
6. Apgar score at 5 minutes more than or equal to 7.
7. A maternal HIV test result (rapid test, ELISA or PCR) taken within 30 days of delivery must be available, documented and negative.
8. Estimated gestational age more than or equal to 37 weeks

Exclusion criteria (infants):

1. Participant must not have received BCG vaccination prior to enrolment.
2. Significant antenatal or intrapartum complications that may affect the health of the neonate.
3. Skin condition, bruising or birth mark at the intended injection site
4. Maternal HIV test (rapid test, ELISA or PCR) not performed within 30 days of delivery, HIV test results not available, or HIV test result known positive.
5. Maternal history of current active TB, or other household contact with known active TB disease who has received less than 2 months of treatment.

Section 5: Methodology and study procedures

5.1 Recruitment and enrolment

5.1.1. Adult stage

Adult participant's resident in the study area will be approached by a member of the SATVI study team. They will be informed about the study in general and if interested will be given an appointment to sign the informed consent document and then attend the screening visit at the SATVI site. Potential participants will be identified in the general community via word of mouth, or from a database of potential participants maintained by SATVI.
The Informed Consent and screening session for adult participants will mostly take place at the SATVI field site. During this visit the participant will undergo an informed consent session in the language of their choice and asked to sign an Informed Consent Form (ICF). Informed consent sessions will be conducted in a private space conducive to confidential discussion at the SATVI site. In some cases, consenting may be done at the participant's home or before the screening visit date. The informed consent session must be done within 14 days of the screening visit. Participants will be given adequate time after the information session to consider participation and to formulate any questions they may have. If necessary, participants will be offered the opportunity to return on a different day after the informed consent session for the screening visit, if they wish to consider the information before signing the ICF. Pre-and post-test HIV counseling will be provided by study team members. Study team members are experienced HIV counselors and have received specific training on HIV counseling. If a participant is diagnosed with HIV, he or she will be referred to the routine healthcare services (national treatment programme). In this area, established and dedicated services exist within the clinic and hospital referral centers for the evaluation, monitoring and treatment of HIV. ARVs, supportive care, counseling, testing, diagnostics, and monitoring are available per South African national treatment guidelines.

Volunteers will undergo the screening visit during which time screening procedures will be performed. Screening test results (Quantiferon®-TB Gold (Cellestis) test) will be evaluated as they become available. If the results are favourable and the participant is eligible for enrolment, an enrolment and vaccination visit will be scheduled with the participant. The enrolment visit must be performed within 14 days of the screening visit. If the participant is not eligible due to unfavourable screening results, the reasons will be fully discussed with the participant and if necessary he or she will be referred to the appropriate healthcare facility for appropriate treatment.

5.1.2. Newborn infant stage

Pregnant women will be approached during the third trimester of their pregnancy at the time of their antenatal visits to the state public healthcare clinics. Information on the trial will be presented to them, and if interested, they will be invited to undergo an

informed consent session in their language of choice and asked to sign an Informed Consent Form (ICF). Informed consent sessions will be conducted in a private space conducive to confidential discussion, at the clinic, at the participant's home, or at the SATVI site. Where feasible, at least 1 further antenatal contact will be made by the study team with the mother-to-be to remind her of the study procedures and to obtain background information about the mother and her pregnancy. Clinic and antenatal clinic cards will be marked with an easily identifiable sticker or marking to enable the birthing unit staff to identify potential study participants.

All women are offered HIV testing per standard of care at the antenatal clinics. After signing the ICF, study staff will request access to the HIV result as recorded in the clinic notes, as well as other relevant medical history from medical records. If the pregnant woman has declined to be tested or if the result is unavailable, or if the result is positive, her newborn will not be enrolled into the study. Counseling and treatment of HIV positive mothers will be provided per standard of care by the state healthcare system according to national guidelines. The ICF also includes as separate documents permission to photograph abnormal injection site reactions and to perform investigations for TB when considered necessary by an investigator.

When these women present in labour to the birthing unit, the study team will be notified by the mother –to –be on a dedicated SATVI telephone number provided to her antenatally, as well as by the birthing unit staff when they identify her as a study participant from information on her antenatal record. After delivery a study research nurse will go to the birthing unit in person and will confirm with the woman that she still wishes to enroll her child into the trial and will apply the inclusion and exclusion criteria and ensure that routine BCG administration by the clinic staff is postponed.

5.2 Randomization

Participants will be randomized into 1 of the 2 study arms for each of the stages by using a pre-prepared block randomization schedule linked to the study enrolment number. Triclinium, will generate four randomisation lists, two for the adult stage and two for the infant stage of the trial. Two lists are required for each stage, since participants may be replaced on this trial. Each list will indicate the treatment number, the treatment associated with that treatment number, room to add the participants primary identifier, and a space for the signature of the responsible pharmacist following treatment allocation.
Randomisation lists are to be used during randomisation / treatment assignment.

If enrolment into infant cohort 3 is not yet complete at the time of BCG expiry, enrolment will continue unblinded using MTBVAC alone until the cohort is complete, n=12

Triclinium will also generate separate, individually-sealed code break envelopes for each trial participant that will be used in an emergency to break the blind for individual participants, if necessary. Code break envelopes will use treatment numbers to identify

a particular participant, since the screening number is not known prior to randomisation.

Code break envelopes are to be used to only for breaking the blind, if required.
(Refer to Appendix 7)

5.2.1. Adult Stage

Eighteen (18) adult participants will be recruited and randomized equally into 1 of 2 study groups (n=9 per group): MTBVAC highest dose group (approx. $5x10^5$ CFU/0.1mL) or BCG SSI standard human dose (approx. $5x10^5$ CFU/0.1mL).

Safety assessments will be conducted at D0, D7, D14, D28, D56, D90, and D180 post study vaccination. A diary card will be used to collect solicited local, regional, and systemic adverse event data from D0 through D14. Reactogenicity data will be collected at each study visit. Non-serious adverse events will be collected through D28. Serious adverse events will be collected during the entire study period.

5.2.2. Infant Stage

Thirty-six (36) infant participants will be recruited, randomized and allocated into 4 groups: BCG (single dose level 2.5 x 10^5 CFU/0.05 mL, n-= at least 6)); or MTBVAC at three different dose levels (lowest $2.5x10^3$ CFU/0.05mL n=9, middle $2.5x10^4$ CFU/0.05mL n=9, highest $2.5x10^5$ CFU/0.05mL n= at least 9).

Vaccination of neonates will be staggered to allow gradual evaluation of safety and reactogenicity, as follows:

- Cohort 1: First 3 infants in the first week, each at least 3 days apart. These 3 infants should be followed up for 7 days prior to proceeding with any further recruitment. If there are no safety concerns, a further 9 infants will be recruited and a maximum of 1 study vaccination administered per day. (for these 12 infants, 9 will receive the lowest MTBVAC dose level and 3 BCG control)
- Cohort 2: A maximum of 2 study vaccinations per day for the next 12 infants (9 will receive middle MTBVAC dose level and 3 BCG control)
- Cohort 3: Unrestricted enrolment and vaccination of the remaining 12 infants (at least 9 will receive the highest MTBVAC dose level and up to 3 BCG control)

Progression from each dose level to the next will require that no pausing rules have been observed within 28 days after vaccination of the last infant at the previous dose level and favourable review of the D28 safety data by the DSMB. Cohort 2 safety review will occur after Day 28 for the first 9 participants. This review has been brought forward to allow for blinded enrolment of as many of cohort 3 infants as possible before expiry of the only available BCG SSI on 30 June 2016. Following BCG SSI expiry enrolment will continue unblinded with MTBVAC only until cohort is complete , n=12

5.3 Blinding

5.3.1. Method of blinding and breaking the study blind

Data pertaining to the MTBVAC vaccine and to BCG control will be collected in an observer-blinded manner. By observer blinded, we mean that during the course of the study the vaccine recipient and those responsible for the evaluation of safety and reactogenicity study parameters, will all be unaware of which vaccine preparation was administered to a particular participant. To do so, the MTBVAC vaccine and BCG control will be prepared and blinded by the pharmacist, whereas, the vaccination will be administered by the Vaccination Nurse, a research nurse not involved in follow up and safety evaluations of participants. The principal investigator and the sub-investigators will be responsible for assessing safety and reactogenicity. Blinding will be maintained throughout the vaccination and follow-up portions of the vaccine trial. Unblinding by group will be performed after D180 visit is completed and BCG vaccine will be offered to all MTBVAC vaccinated infants within the next 2 weeks (D194 approximately).

Following expiry of the only available BCG SSI on 30 June 2016 and if enrolment is not yet complete, enrolment into Cohort 3 will continue unblinded with MTBVAC only until the cohort is complete, n=12

No set of individual codes will be held at Biofabri's Headquarters. Biofabri's Headquarters will be able to access the individual randomization code from the SATVI Pharmacy randomization register. The code will be broken by the SATVI Pharmacist (Study Contact for Emergency Code Break) only in the case of medical events that the investigator/physician in charge of the participant feels cannot be treated without knowing the identity of the study vaccine(s).
Biofabri' policy (incorporating ICH E2A guidance, EU Clinical Trial Directive) is to unblind any serious adverse event (SAE) report associated with the use of the investigational product, which is unexpected and attributable/suspected, prior to regulatory reporting. The SATVI Pharmacist is responsible for unblinding the treatment assignment in accordance with specified time frames for expedited reporting of SAEs (Refer to Section10.9).

The statistician who will perform the analysis will have access to the randomisation codes at the time of analysis and will be able to break the codes to perform the analysis.

5.4 Follow-up

Follow-up of trial participants will occur at the SATVI field site. Follow-up visits will be scheduled in consultation with the participant, or in the case of infants, with the participant's caregiver, according to the protocol guidelines and allowable window periods. The participant or his/her caregiver will be asked to complete a diary card to report on adverse events. The contact telephone number of the trial team will be

provided to the participant or participant's caregiver to ask questions, raise concerns, or report major adverse events outside of the scheduled visits.

In summary, adult participants in stage 1 will be scheduled to attend face-to-face visits 1 week, 2 weeks, 1 month, and 3 and 6 months after vaccination. For the infant participants in study stage 2, scheduled face-to-face study visits will occur at D0, 1 week, 2 weeks, 1 month (4weeks), week 10, week 13, week 26 (month 6) and week 52 (month 12) with a home or telephonic visit at month 9.

5.5 Discontinuation criteria and lost-to-follow up

All participants enrolled and vaccinated will be remain in follow-up per the visit schedule for safety monitoring purposes. It is possible that in some cases misbleeds or missed visits may occur, but this will not influence follow-up or safety monitoring per protocol. Participants who are unavailable for some or all scheduled visits will not be designated lost to follow-up until their final study visit date has passed. Repeated attempts will be made by study staff to locate such a participant and gather relevant data, unless the participant or participant's guardian has withdrawn consent.

5.6 Surveillance

Active and passive surveillance on all trial participants will be performed for the duration of the study in order to identify the occurrence of adverse events (AEs) and serious adverse events (SAEs). Adult participants and caregivers of infant participants will be asked to report side-effects, hospital admissions or major medical events to study staff as they occur, between or on scheduled study visits. A contact telephone number will be provided to participants. Hospital admission lists and TB registers will be monitored actively by the SATVI surveillance team to identify participants who have been admitted to hospital or commenced TB treatment or been a household contact of someone who has.

5.7. Duration of follow-up

Each adult participant will remain active on the study for a period of 6 months after enrolment and vaccination. Each infant participant will remain active in the study for a period of 12 months with an additional 12 months of passive surveillance thereafter.

5.8. Rescue dose of BCG minimising risk of TB disease

5.8.1. Provision for rescue dose of BCG

Once all participants in an infant dose cohort have completed D180, unblinding of the cohort will occur and within the next 2 weeks, approximately at D194, a rescue dose of BCG will be given to infants who received MTBVAC. The injection will be performed in the opposite arm to the previous MTBVAC vaccine. Delayed primary BCG vaccination in the absence of BCG administration at birth has been shown to induce a good immune response [30]. In order to minimize a potential increased risk for developing TB disease prior to the rescue dose of BCG, participants with potential household TB

exposure will be excluded and participants will undergo active surveillance for signs or symptoms of TB throughout follow-up using targeted questions on the symptoms and signs of TB, and body weight evaluation for failure to thrive.

5.8.2. Surveillance for possible incident TB disease

Surveillance will be performed on all infant participants allocated to either study arm in order to maintain blinding. This surveillance will be active, and will take the form of home visits or visits at the site during which a symptom assessment is made by a field worker, and the participant is weighed. If signs or symptoms of TB are detected the infant will undergo standardized investigation for TB and possible referral for TB treatment if necessary. In addition to this active surveillance, passive surveillance will be maintained by asking caregivers to self-report symptoms to the study team. An additional 12 months follow up post study completion consisting of 3 monthly home visits or telephone calls and asking about household contacts, signs or symptoms of TB will occur. All infants who are Quantiferon positive at Day 180 will have the Month 9 visit and all visits of additional 12 months follow up performed face-to-face and will include monitoring the child's weight gain.

SECTION 6: Study measures and endpoints

Data for endpoints will be collected for safety, injection site, measures of intradermal vaccine delivery, and immunogenicity parameters.

6.1 Injection site adverse events

Immediately after vaccination and at each study visit, the injection site will be inspected by the study team and assessed for diameter of redness in millimeters (mm), presence and diameter of ulceration, diameter of induration and swelling in mm, presence and severity of tenderness, fluctuation indicative of abscess formation, drainage from the site, lymphadenopathy, presence and diameter of scarring, or any other abnormal signs. Participants and caregivers of infant participants will also be asked to keep a diary after vaccination to record these same parameters.

6.2 Regional and systemic adverse events

After vaccination and at each study visit, participants will be assessed for symptoms of lethargy, fever measured with a thermometer, disrupted feeding patterns, or any other symptoms or signs. Adult participants will also be assessed for complaints of myalgia, headache or any other systemic symptoms. Adult participants and caregivers of infant participants will also be asked to keep a diary after vaccination to record these same parameters. A targeted physical examination will be performed at each visit to assess signs of lymphadenopathy (presence, size, tenderness, and suppuration of palpable lymph nodes), rash and other cutaneous manifestations, or any other physical abnormalities.

6.3 Immunogenicity endpoints

6.3.1. Rationale for no immunogenicity testing in the adult stage

The immune response to BCG vaccination in adults is likely to be different from that in neonates. Even in an adult study population of non TB latently infected participants, the BCG-specific immune response is likely to be different due to maturation of the immune system and background exposure to environmental mycobacteria. There is therefore limited utility for extrapolating immunogenicity data from adults to neonates. It is proposed therefore, that safety and reactogenicity data be collected from adults and infants, but that immunogenicity data is collected from infants only.

6.3.2. Analysis and storage of blood samples

Blood samples will be processed at the SATVI peripheral laboratory on site, and transported after processing to the SATVI Cape Town laboratory for further analysis. Samples will be stored under controlled conditions per laboratory standard operating procedures (SOPs) in a specially designated area in the SATVI laboratory. Specimens will be labeled with study numbers and study stage, and may additionally be labeled

with other details pertaining to collection and processing dates and particulars of the assay to be performed. Participant identifiers will not be displayed on samples, sample labels, or in laboratory documentation. Documentation will kept per laboratory SOPs to denote storage location of samples for later retrieval. Laboratory staff will remain blinded to study arm allocation and participant identity. Blood collected for storage may be stored for future use for additional tests after the completion of this study. Samples from infants and from adults will be used and stored in the same way, and identified only by anonymous study number and stage of the study without link to participant identifiers. Storage of blood samples will be discussed in the informed consent form (ICF) and the participant will be given the option to have the infant participant's blood samples destroyed after study completion. A decision not to allow storage of blood samples will not influence participation in the main study and will not prejudice the participant in any way. A log will be maintained by the clinical study team of all participants who have elected not to have their specimens stored for future use. After the analysis of all samples is complete for the primary study endpoints, this log will be provided to the laboratory staff that will destroy these samples and document this process. Future use of these stored samples will be participant to participant to approval in UCT HREC approved protocols.

Samples will be stored in fridges and freezers and liquid nitrogen containers that are subject to continuous monitoring for temperature and storage conditions. SATVI laboratory equipment and storage facilities are ISO 15189 compliant and subject to external review by the South African accreditation system. Samples are stored in an environment controlled for access by specified staff security identification swipe cards, which are updated regularly and subject to University of Cape Town institutional security checks.

Short and long term oversight of sample storage and handling will be the responsibility of the principal investigator, laboratory manager, and post-doctoral scientist assigned to this study. If any of these individuals were to leave SATVI, chain of custody responsibility will be maintained and documented by assigning a responsible successor. Documentation of stored samples will be maintained according to laboratory SOPs within the centralized documentation system of the SATVI laboratory. All samples will be kept for a maximum of 15years, after which they will be destroyed.

6.3.3. Blood collection volumes

Required blood volumes and compliance to blood volume guidelines are summarized in appendix 1. Required volumes will be minimized by making use of assays that require very small amounts of blood, and by limiting the volume of blood that can be taken at each visit.

6.3.4. Immunology tests

Immunogenicity endpoints (in infants only):

Primary immunogenicity endpoints

Frequencies and co-expression patterns of CD4 and CD8 T cells expressing IFN-γ, TNF-α, IL-2 and/or IL-17 induced by MTBVAC or BCG in healthy, BCG naïve and suitable antigens, HIV unexposed, South African newborn infants.

Exploratory immunogenicity endpoints

Samples of whole blood supernatants will be kept frozen for future exploratory immunogenicity studies. Exploratory assays will be planned, based on data from the primary immunogenicity analyses.

Phlebotomy for immunogenicity will be evaluated at: D7, D28, D70 and D180; 0.75 mL of blood will be extracted at D7 and 2.5 mL the rest of the days. A QFT test will be performed at D180 and D360

Narrative for whole blood ICS assay:

Fresh whole heparinized blood will be stimulated immediately with BCG, MTBVAC, newly identified single "mega pool" of mycobacterial peptides, or phytohemagglutinin (PHA) or will be left unstimulated (Nil), for 12 hours at 37^0C. D7 stimulation conditions include half the blood volume [250 µL (0.25 mL)] and only Nil, MTBVAC and BCG. After 7 hours of stimulation, supernatant (for soluble cytokine/chemokine analysis) will be collected from all the conditions, frozen at -80C and stored for shipping to Sponsor for further analysis.

Following supernatant removal, brefeldin A will be added for the remaining whole blood and tubes incubated for a further 5hrs in a programmable waterbath. The waterbath will switch off after a total of 12 hours of stimulation. The next morning, FACSLysing solution will be added to lyse red cells and fix white cells. Fixed, white cells will then be frozen for later intracellular cytokine staining and flow cytometry. Flow cytometric staining and acquisition will be run in batches at a later time point. Measurement of frequencies and patterns of specific type-1 cytokines and IL-17 by CD4 and CD8 T cells will be assessed.

The timepoints for immunogenicity have been selected on the basis of recent studies conducted by SATVI, which have shown that the peak of the BCG-induced T cell responses in infants is around 6-10 weeks of age [6]. Hence, the primary immunogenicity assays will be performed on day 70 and end of study.

Note: Phlebotomy of newborns at birth is extremely challenging and it is likely that sampled blood volumes would not be sufficient to measure day 0 baseline immune responses.

Section 7: Visit schedule

7.1 Adult stage (stage 1)

7.1.1 Adult Pre-recruitment visit (any time prior to informed consent and screening visit)

Potential participant contacted telephonically or in person and invited to attend the informed consent and screening visit on a scheduled date.

7.1.2 Visit 1 (Adult Informed Consent and Screening [Day -21 to Day)

The purpose and nature of the study, as well as all procedures will be explained to and discussed with each potential participant in the participant's language of choice. If the participant chooses he or she can take the informed consent form home to discuss and deliberate, and schedule a follow- up appointment with the study staff to sign the consent form. The informed consent session may take place off- site, e.g. at the participant's home. Potential participants must provide written informed consent for participation in the trial, prior to any other screening assessments or procedures being performed.
After informed consent was obtained, the following assessment will be performed at this visit:

- Demographic data will be recorded
- Height and weight, will be recorded
- Medical and surgical history will be recorded
- Previous medications will be recorded
- Vital sign data will be recorded (after resting for at least five minutes in a sitting position)
- An abbreviated physical examination will be performed
- Proposed injection site inspection
- HIV rapid test
- Blood will be drawn for the following:
    - HIV ELISA test (if positive rapid test)
    - Quantiferon®-TB Gold (Cellestis) test
    - Serum pregnancy (women of child-bearing potential only)
    - Haematology
    - Clinical chemistry

The investigator will establish each participant's eligibility prior to enrolment based on the stipulated inclusion/exclusion criteria. If a participant is ineligible, the participant will be informed telephonically or in person of result. If necessary, the participant will be seen by a medical professional and referred to the appropriate healthcare facility for further treatment if indicated. If eligible, an appointment will be made with the participant to attend the subsequent visit.

7.1.3 Visit 2 (Adult Enrolment/Randomisation and Vaccination [Day 0]

Procedures performed ***prior to vaccination***:

The investigator will confirm each participant's eligibility by reviewing the inclusion/exclusion criteria prior to enrolment. If the participant remains eligible, the following procedures will be performed:

- An abbreviated physical examination by the investigator
- Medical and surgical history as obtained by the investigator
- Previous medications taken since Screening will be recorded
- Vital signs (pulse rate, systolic and diastolic blood pressure, body temperature)
- Urine pregnancy (women of child-bearing potential only)
- Injection site assessment (baseline measurement for reactogenicity parameters)
- If eligible, the investigator will enrol the participant, who will be randomised into a study arm by the responsible pharmacist, according to the next available treatment number in the final randomisation schedule
- The trial pharmacist will prepare and dispense investigational product to the clinical study team

Procedures performed ***60 minutes after vaccination***:

Following vaccination (***non-dominant arm, except if a skin condition indicates that the dominant arm should be used***), participants will remain at the trial site for at least 60 minutes during which they will be closely observed by the clinical study team for any local or regional/systemic adverse events. Close attention will be paid to the following manifestations:

Local reactions:

- Presence and diameter of erythema (measured in millimetres)
- Presence and diameter of ulceration (measured in millimetres)
- Presence and diameter of swelling (measured in millimetres)
- Presence and diameter of induration (measured in millimetres)
- Presence and diameter of abscess formation (measured in millimetres)
- Presence and severity of tenderness
- Fluctuation
- Drainage from the site
- Lymphadenopathy
- Any other local abnormalities

Regional/systemic reactions:

- Malaise
- Fever (measured with a thermometer)
- Myalgia

- Headache
- Skin rash
- Any other systemic symptoms

At the end of the 60 minutes observation period the following additional assessments will be performed:

- Vital signs (after resting for at least five minutes in a sitting position)
- A diary card will dispense to the participant and the use thereof will be explained
- Participant contact details will be gathered and an appointment will be scheduled for the next study visit

7.1.4. Visits 3 – 7 (Follow-up visits)

Visits 3 - 7 will take place at the following time points (± window period):

- Visit 3 - Day 7 ± 3 days
- Visit 4 - D14 ± 3 days
- Visit 5 - D28 ± 3days
- Visit 6 - D56 ± 7 days
- Visit 7 - D90 ± 7days

At these follow-up visits the following assessments will be performed:

- Concomitant medications will be captured
- Abbreviated physical examination by the investigator (Visits 3 and 4, thereafter only on report of any abnormality)
- Vital signs (after resting for at least five minutes in a sitting position)
- The injection site will be evaluated for local reactions (as described for Visit 2)
- Participants will be evaluated for regional/systemic reaction (as described for Visit 2) (only at Visit 3)
- Any other AEs will be recorded
- Review of completed diary card (Visits 3 and 4 only)
- Blood will be drawn (Visits 3 and 5 only) for the following:
    - Haematology
    - Clinical chemistry

7.1.5 Visit 8 (Trial completion [Day 180 ± 7days])

Adults will be followed-up for six months. At the trial completion visit the following assessments will be performed:

- Concomitant medications will be captured
- Abbreviated physical examination by the investigator, only if any report of an abnormality
- Vital signs (after resting for at least five minutes in a sitting position)
- The injection site will be evaluated for local reactions (as described for Visit 2)
- Participants will be evaluated for regional/systemic reaction (as described for Visit 2)

- Any other AEs will be recorded
- Blood will be drawn for the Quantiferon®-TB Gold (Cellestis) test

7.1.6 Adult Unscheduled visits

An unscheduled visit can be performed at any time at the clinic or at participant's home or place of work, for adverse event investigation, if the participant reports not understanding completion of the diary card or for any other reason deemed necessary by the investigator. **(Figure 2)**

Adult stage global injection schedule and safety follow-up

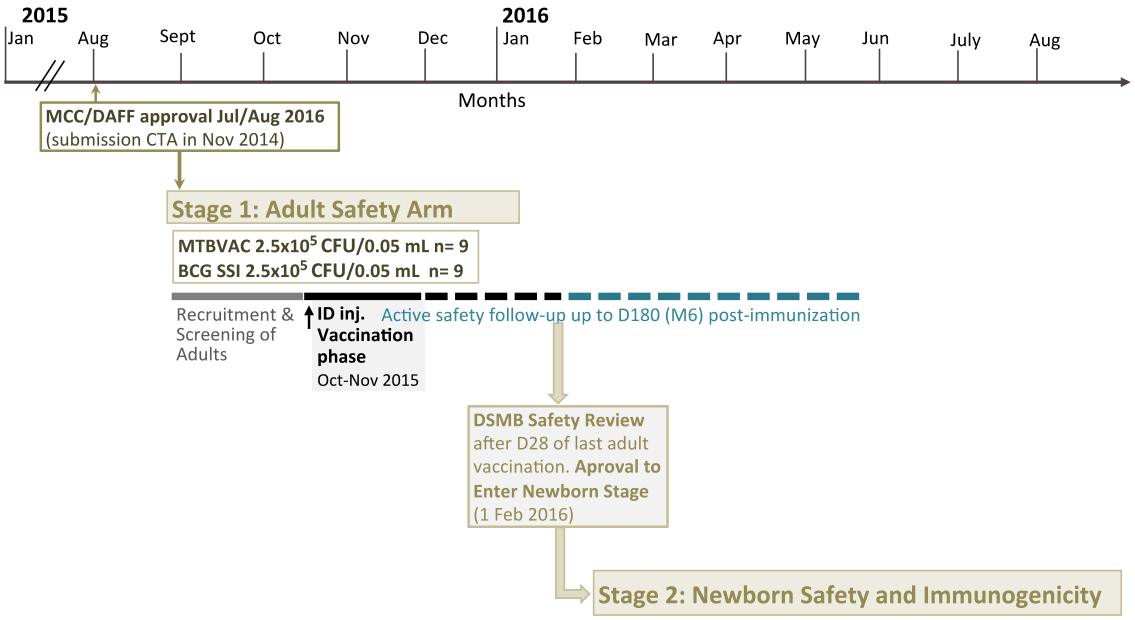

Figure 2. Adult safety arm global injection schedule and safety follow-up. Eighteen (18) adult participants will be recruited and randomized equally into 1 of 2 study groups (n=9 per group): MTBVAC highest dose group (approx. $5x10^5$ CFU/0.1mL) or BCG SSI standard human dose (approx. $5x10^5$ CFU/0.1mL). Safety assessments will be conducted at D0, D7, D14, D28, D56, D90, and D180 post study vaccination. Following data safety review by DSMB of all safety data up to Day 28 (D28) of last study arm vaccinee, DSMB will provide a go/no-go decision to proceed to the newborn vaccination stage. D means Day, M means Month, CFU means colony-forming units.

7.1.7. Study variables

7.1.7.1 Reactogenicity

Injection site assessments

- Presence and diameter of erythema (measured in millimetres)
- Presence and diameter of ulceration (measured in millimetres)
- Presence and diameter of swelling (measured in millimetres)
- Presence and diameter of induration (measured in millimetres)
- Presence and diameter of abscess formation (measured in millimetres)

Presence and severity of tenderness

- Fluctuation
- Drainage from the site
- Lymphadenopathy
- Any other local abnormalities
- Crusting, with forming and falling dates
- Presence and diameter of scarring (measured in millimetres)

Regional and systemic AEs

- Malaise
- Fever (measured with a thermometer)
- Myalgia
- Headache
- Skin rash
- Any other systemic symptoms

7.1.7.2 General safety

Vital signs

The vital sign data to be recorded include:

- Pulse rate
- Systolic blood pressure
- Diastolic blood pressure
- Body temperature

Haematology

The haematology data to be recorded include:

- Erythrocyte count
- Reticulocyte count
- Haemoglobin
- Packed cell volume / haematocrit
- Mean corpuscular volume
- Mean corpuscular haemoglobin
- Mean corpuscular haemoglobin concentration
- Leucocyte count
- Neutrophil count
- Lymphocyte count
- Monocyte count
- Eosinophil count
- Basophil count
- Platelet count

Clinical chemistry

The clinical chemistry data to be recorded include:

- Alanine transaminase
- Aspartate transaminase
- Alkaline phosphatase

- Bilirubin (total)
- Bilirubin (conjugated)
- Albumin
- Creatinine
- Urea

Urinalysis

Urinalysis will only be performed if clinically indicated and documented as part of the applicable adverse event.

7.1.7.3 Other study variables

Demographics

Demographic data to be recorded include:

- Date of birth
- Age
- Gender
- Self-reported race, as follows
    - Asian
    - Black
    - Caucasian
    - Mixed race / Cape coloured
    - Other
- Current smoking patterns and smoking history
- Current alcohol use

Previous and concomitant medications

Previous medications will be collected up to 21 days prior to enrolment.

Physical characteristics

Physical characteristic data to be recorded include:

- Height
- Weight
- BMI (calculated automatically)

7.2. Infant stage (stage 2)

7.2.1 Visit -1: Infant Recruitment visit (≥ 32 weeks of pregnancy duration)

The purpose and nature of the study, as well as all procedures will be explained to and discussed with each potential maternal participant in the participant's language of choice. If the participant chooses, she can take the informed consent form home to discuss and deliberate, and schedule a follow-up appointment with the study staff to sign the consent form. The informed consent session may take place off- site, e.g. at the participant's home. Potential participants must provide written informed consent for participation in the trial, prior to any other screening assessments or procedures being performed.

After signing the informed consent form, mothers will be asked to answer questions about her medical history with emphasis on TB disease history and exposure. The maternal HIV test result will be obtained from clinic records. This must have been performed within 4 weeks of delivery.

7.2.2 Infant Pre-enrolment contact visit (> 32 weeks of pregnancy, after signing of informed consent, prior to delivery)

Telephonic or face-to-face contact by study team member with maternal participant to remind her of the study, confirm continued consent, and remind her to contact the study team when she is in labour and to remind the birthing unit's staff that she is a trial participant

7.2.3 Birth visit (before discharge from birthing unit)

Birthing unit staff identifies the woman in labour as the mother of the potential participant and/or participant herself notifies the study staff
Study nurse visits the woman after delivery, before routine BCG vaccination
Study nurse identifies the participant, and confirms continued informed consent
Study nurse reviews the medical chart, including antepartum, intrapartum, and neonatal history and makes a copy/ takes a photograph of the most recent HIV test result.
Study nurse ensures that routine BCG vaccination is postponed by clinic staff if eligible, or given if ineligible.

7.2.4 Visit 0: Infant vaccination visit (within 96 hours of delivery)

The following assessments will be performed at this visit:

Procedures performed ***prior to vaccination***:

- Demographic data (Date of Birth, Gender, Race, Birth weight, Apgar score, head circumference)
- Medical and surgical history
- Physical examination
- Injection site assessment
- Pre-vaccination Vital Signs (Recumbent length, body weight, heart rate, respiratory rate, body temperature)
- If eligible, the investigator will enrol the participant, who will be randomised into a study arm by the responsible pharmacist, according to the next available treatment number in the final randomisation schedule
- The trial pharmacist will prepare and dispense investigational product to the clinical study team

Procedures performed ***immediately after vaccination***:

- Vaccination site inspection (Bleb, dampness, immediate hypersensitive reaction)

Procedures performed ***60 minutes after vaccination***:

Following vaccination, the infants will remain at the trial site for at least 60 minutes during which they will be closely observed by the clinical study team for any local or regional/systemic adverse events. Close attention will be paid to the following manifestations:

Local reactions:

- Presence and diameter of erythema (measured in millimetres)
- Presence and diameter of ulceration (measured in millimetres)
- Presence and diameter of swelling (measured in millimetres)
- Presence and diameter of induration (measured in millimetres)
- Presence and diameter of abscess formation (measured in millimetres)
- Presence and severity of tenderness
- Fluctuation
- Drainage from the site
- Lymphadenopathy
- Any other local abnormalities

Regional/systemic reactions:

- Irritability
- Fever (measured with a thermometer)
- Lethargy
- Skin rash
- Any other systemic symptoms

At the end of the 60 minutes observation period the following additional assessments will be performed:

- Vital signs (heart rate, respiratory rate, body temperature)

Day 3 Post-vaccination (Infant Telephonic follow-up visits)

Study team member contacts the participant's caregiver telephonically. If she cannot be contacted by telephone, or if an unscheduled face-to-face visit is necessary, a home visit or visit at the research site is performed. Data is gathered and recorded for injection site, systemic, solicited and unsolicited adverse events. Use and understanding of the diary is discussed with the participant caregiver.

7.2.5 Visits 1 - 6 (Infant face-to-face clinic visits)

Visits 1 - 6 will take place at the following time points (± window period):

- Visit 1 - Day 7 ± 3 days

- Visit 2 - D14 ± 3 days
- Visit 3 - D28 ± 3days
- Visit 4 - D70 ± 14 days
- Visit 5 - D90 ± 14 days
- Visit 6 - D180 ± 14 days

At these follow-up visits the following assessments will be performed:

- Concomitant medications will be captured
- The injection site will be evaluated for local reactions (as described for Day 0) and photographed with maternal consent if considered necessary
- Participants will be evaluated for regional/systemic reaction up to and including D180 (as described for Day 0)
- Any other AEs will be recorded
- Abbreviated physical examination by the investigator (D7, D14 and D28, thereafter only on report of any abnormality)
- Vital signs (Recumbent length, body weight, heart rate, respiratory rate, body temperature)
- Review of completed diary card (D7 and D14 only)
- Swabs at injection site for microbiology analysis will be conducted in all infants with a discharging lesion and will be repeated until 2 negative cultures are obtained.
- Blood will be drawn for the following (the breast-feeding status will be confirmed at each visit, to enable an accurate assessment of the results):
    - Haematology (D7 and D28)
    - Clinical chemistry (D7 and D28)
    - Immunogenicity (D7, D28, D70 and D180)
    - QFT test at D180.
- Unblinding will be performed after D180.

7.2.6 Visit 7: BCG rescue dose within two weeks post unblinding

Within 2 weeks after cohort (D 194 approximately), unblinding a rescue dose with BCG Vaccine will be given to all MTBVAC vaccinated infants. The injection will be performed in the opposite arm to the previous MTBVAC vaccine. Follow up visit at site 2 weeks later to check BCG response will be carried out.

7.2.7 Visit 8: Follow-up Visit (Day 360 ± 14days)

This is the Last Study End Visit. At this day, the active FUP period finishes. At this follow-up visit the following assessments will be performed:

- Abbreviated physical examination by the investigator
- Vital signs (Recumbent length, body weight, heart rate, respiratory rate, body temperature)
- Any AE and SAE will be recorded
- Blood will be drawn for a second QFT test

7.2.8 Infant face-to-face telephone or home visits (M9, M 15, M18, M21, M24)

Three- monthly telephone or home visits will be done by the study team or SATVI surveillance team on all infants regardless of study arm, to enquire about infant's wellbeing, any acute illnesses including hospital admissions, symptoms of TB or history of TB contact. This will be performed by completing a standardized interview schedule. If signs or symptoms of TB are detected, the participant will be referred to the local TB clinic for further assessment. If the infant was Quantiferon positive at Day 180 these visits will be performed face-to- face and include monitoring the infant's weight.

7.2.9 Infant unscheduled visit

An unscheduled visit can be performed at any time, at the clinic or at the participant's home, for adverse event investigation, if the participant is unavailable by telephone (for a telephonic follow-up visit), if the participant caregiver reports not understanding completion of the diary card, or for any other reason deemed necessary by the study team. **(Figure 3)**

Infant stage global injection schedule and safety follow-up

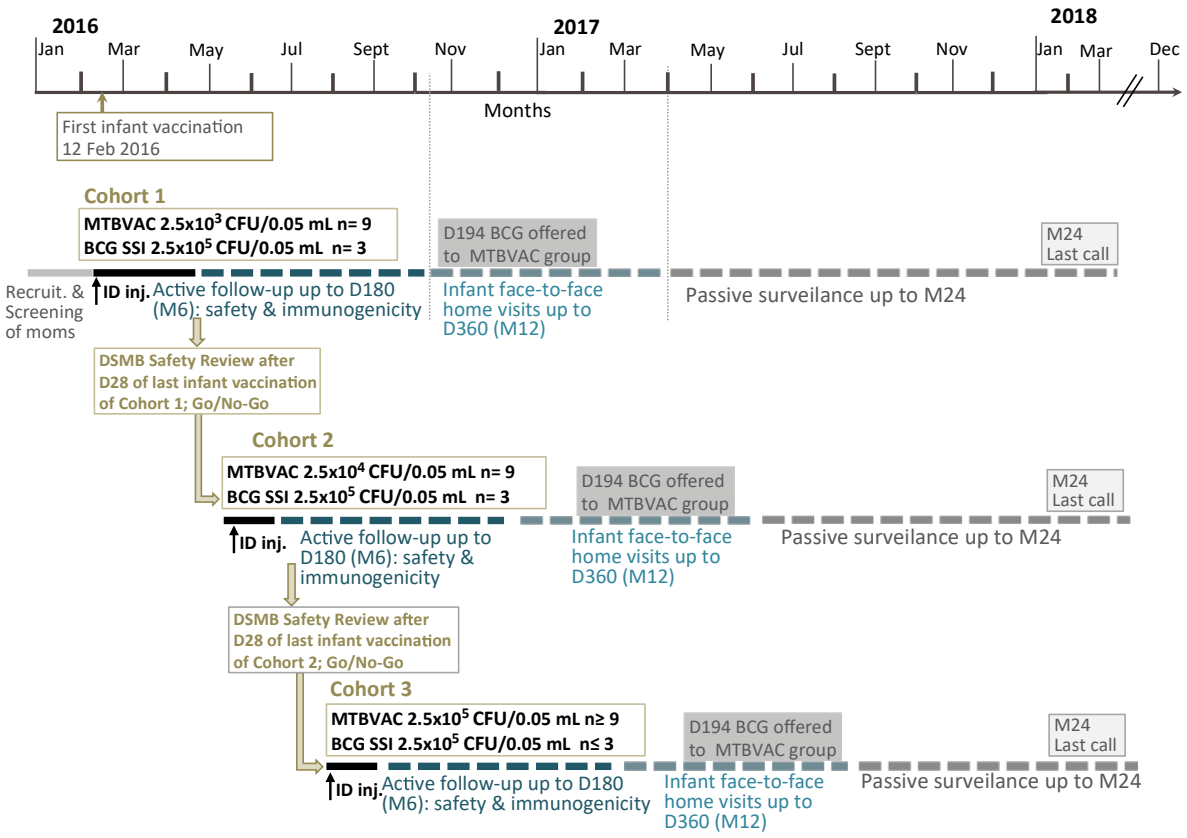

Figure 3. Infant stage vaccination schedule and follow-up. Vaccination of neonates will be staggered by cohorts to allow gradual evaluation of safety and reactogenicity. Cohort 1 and 2 comprise 9 MTBVAC volunteers and 3 BCG and within each cohort neonates will be randomized 3 verum: 1 control to receive either the study vaccine MTBVAC or BCG Danish 1331, respectively. Cohort 3 will commence enrolling blinded, aiming for a 3:1 enrolment as per cohorts 1 and 2 with total of 12 participants, but after expiry of BCG SSI if enrolment is not complete will continue enrolment unblinded until cohort complete, n=12. A single intradermal administration at month zero will be used. In Cohort 1, vaccination of the first three newborn infants is planned in the first week, each at least 3 days apart. If there are no safety concerns

after 7 days safety follow-up, a further 9 infants will be recruited and a maximum of 1 study vaccination will be administered per day. For Cohort 2, a maximum of 2 study vaccinations will be scheduled per day, whereas for Cohort 3, unrestricted enrolment and vaccination of the remaining 12 infants is planned. D means Day, M means Month, CFU means colony-forming units.

7.2.10 Study Variables

7.2.10.1 Reactogenicity

Injection site assessments

- Presence and diameter of erythema (measured in millimetres)
- Presence and diameter of ulceration (measured in millimetres)
- Presence and diameter of swelling (measured in millimetres)
- Presence and diameter of induration (measured in millimetres)
- Presence and diameter of abscess formation (measured in millimetres)
- Presence and severity of tenderness
- Fluctuation
- Drainage from the site
- Lymphadenopathy
- Any other local abnormalities
- Crusting, with forming and falling dates
- Presence and diameter of scarring (measured in millimetres)

Regional and systemic AEs

- Irritability
- Fever (measured with a thermometer)
- Disrupted feeding patterns (only from visit 1 onwards)
- Loss of weight (only from visit 1 onwards)
- Failure to thrive (only from visit 1 onwards)
- Lethargy
- Skin rash
- Any other systemic symptoms

7.2.10.2 General safety

Vital signs

The vital sign data to be recorded include:

- Respiratory rate
- Pulse rate
- Body temperature
- Weight

- Recumbent length
- Head circumference (Day 0 only)

Haematology

The haematology data to be recorded include:

- Haemoglobin
- Haematocrit
- Mean corpuscular volume
- Mean corpuscular haemoglobin
- Mean corpuscular haemoglobin concentration
- Leucocyte count
- Neutrophil count
- Lymphocyte count
- Monocyte count
- Eosinophil count
- Basophil count
- Platelet count

Clinical chemistry

The clinical chemistry data to be recorded include:

- Alanine transaminase
- Aspartate transaminase
- Alkaline phosphatase
- Bilirubin (total)
- Bilirubin (conjugated)
- Urea

7.2.10.3 Other study variables

Demographics

Maternal demographic data to be recorded include:

- Date of birth
- Age in years (calculated)
- Self-reported race, as follows
    - Asian
    - Black
    - Caucasian
    - Mixed race / Cape coloured
    - Other

Infant demographic data to be recorded include:

- Date of birth
- Age in days (calculated)
- Gender
- Race:
    - Asian

- Black
- Caucasian
- Mixed race / Cape coloured
- Other

Physical characteristics

Infant's physical characteristic data to be recorded include:

- Recumbent length
- Body weight
- Head circumference (Day 0 only)
- Apgar score

Previous and concomitant medications

Relevant previous and concomitant medications will be collected for infants. No maternal previous medications will be collected.

TB symptoms and/or exposure

Maternal history of current active TB, or other household contact with known active TB disease who has received less than 2 months of treatment will be checked. Any finding will be recorded.

Section 8: Study product and investigational device

8.1. MTBVAC vaccine

The MTBVAC vaccine has been released by BIOFABRI, Porriño, Spain.
The Quality Control Standards and Requirements for the candidate vaccine are described in separate release protocols and the required approvals have been obtained.
All MTBVAC vials should be stored between -20°C and -40ºC.

Table 2. Composition of MTBVAC study vaccine and BCG control

Vaccine	Formulation (approximately per standard dose)	Presentation	Volume
MTBVAC	$1.5 – 8.5 \times 10^5$ CFU Sucrose 4.16 mg Sodium glutamate 0.33 mg	Lyophilised pellet in vials (20 doses)	0.05 mL/dose[a]
BCG SSI	$1\text{-}4 \times 10^5$ CFU	Lyophilised pellet in vials (20 doses)	0.05 mL/dose[a]

[a]After reconstitution with sterilised water for injections

8.1.1. Dosage and administration (infants or adults)

Participants will be administered one dose of the MTBVAC vaccine or BCG control according to vaccination schedule. The vaccine dose will be administered intradermally in the deltoid area (in the non-dominant arm in adults, left arm for infants). Opsite plaster (breathable) will be used to cover the site of injection. The plaster will be changed at the first visit provided there are no signs of leakage upon examination by study team nurse or investigator. Participants will be instructed to protect the plaster and site of vaccination from water. Participants will be provided with a waterproof shoulder sleeve that can be used in the shower to protect the vaccination site from getting wet. In the event that the plaster became loose or at risk of falling off, participants will be provided with a plastic sample bag and a spare plaster with clear instructions on how to replace the plaster and retain the used plaster in the provided plastic bag and return it to clinic for destruction.

Participants will be observed closely for at least 60 minutes following the administration of vaccines, with appropriate medical treatment readily available in case of a rare anaphylactic reaction following the administration of vaccines.

The vaccines will be administered at the Vaccination clinic, SATVI, under the supervision of investigators and nurses trained in the management of anaphylactic reactions.

8.1.2. Reconstitution of the vaccines

See Vaccine Management manual

8.1.3. Storage of MTBVAC

All vaccines must be stored in a safe and locked place with no access for unauthorized personnel (SATVI Pharmacy). They must be kept in the freezer between -20°C and -40ºC. Storage temperature should be monitored and documented at least once per day, using a calibrated min-max thermometer. It is advisable to have a back-up refrigerator/freezer in case of power failure/ breakdown. Procedures must be in place to ensure that the vaccines are kept at the indicated temperature range at all times.
The study monitor must be contacted, as soon as possible, if the cold chain is broken (e.g. Freezer fails).
Storage conditions for transport of vaccines from country medical department or dispatch centre to study sites or between sites are described in Appendix 5.

8.2 BCG

BCG, an attenuated, live culture of the bacillus Calmette-Guérin, was originally attenuated between 1906 and 1919 by serial passage of an M. bovis strain. The BCG vaccine currently registered for use in South Africa which will be used as control for this trial is BCG Vaccine SSI manufactured by Statens Serum Institut, Copenhagen, Denmark (http://www.ssi.dk/sw10375.asp). The Statens Serum Institut derives this vaccine from the Danish BCG strain 1331.

The vaccine is preapproved by the World Health Organization (WHO) and is registered in 39 countries as a preventive vaccine against tuberculosis. It is also distributed by WHO-based organizations and used in 30 other countries. The vaccine is a freeze dried vaccine that must be transported and stored at 2 to 8°C. Its shelf life is 18 months. It is reconstituted before use with sterile buffered Sauton diluent supplied by the manufacturer and must be used within 4 hours after reconstitution.

Intradermal BCG vaccination shortly after birth is routine practice in South Africa. BCG vaccine coverage in South Africa is high (99% in the Western Cape region and 94% in the rest of South Africa)[31, 15] and is part of the national immunization schedule and the Expanded Programme on Immunization (EPI). BCG is a licensed vaccine in South Africa. See appendix for the BCG package insert.

BCG Vaccine will be purchased from the registered local supplier e.g. Biovac. For both neonates and adults, the BCG is the same product with the same package insert.

Standard vaccine reconstitution, storage and preparation guidelines will be followed per routine protocol and the package insert. BCG preparation will be performed by the SATVI pharmacist as described in the package insert.

8.2.1 BCG supply, storage, and preparation

Since BCG is not routinely administered to adults and adults will be vaccinated at the premises of the SATVI field site, BCG for the adult stage of the study BCG will be supplied by Biofabri and, stored and prepared by the SATVI pharmacy team or study research nurse.
BCG will be transported and stored at the manufacturer's recommended temperature range of 2-8 ˚C. Upon receipt of the product, the SATVI pharmacist will store the BCG in a refrigerator in the SATVI pharmacy, which is set to maintain the desired temperature. This refrigerator 's temperature will be monitored at least daily and preferably continuously, and a back-up power supply in the form of a generator will be available.
Upon confirming eligibility for BCG for an enrolled participant, the SATVI pharmacist or research nurse will prepare the BCG for administration.
In summary, preparation of BCG is performed as follows:

- BCG vaccine is prepared from multi-dose vials according to the package insert using aseptic technique
- Each vial of SSI BCG will be reconstituted with diluted Sauton SSI as specified in the package insert. BCG vaccine will be reconstituted and diluted by gentle mixing, taking care not to contaminate or introduce air into the preparation, which could promote clumping of the live vaccine organisms.
- Reconstituted vaccine will be administered within 4 hours after reconstitution. The vaccine should be used within 4 hours after reconstitution and any reconstituted vaccine not used within 4 hours must be discarded.
- The reconstituted vaccine should be maintained at 4-8 °C. At no time should the freeze-dried or reconstituted vaccine be exposed to sunlight, direct or indirect. Exposure to artificial light should be kept to a minimum. Unused portions of reconstituted vaccine vial will be discarded after use.

The package insert requires that reconstituted BCG is used within 4 hours and is supplied in a multi-dose (10 doses) vial. All study participants eligible for vaccination within this 4 hour window will receive BCG from this multi-dose vial up to the maximum 10 doses available. At all times the cold chain will be maintained by study staff using study fridges and vaccine cooler box containers with cooler packs. Reconstituted and unused vaccine will be returned to the SATVI pharmacy for accountability purposes but will be quarantined from further use.
Optimal storage, preparation and accountability of the BCG are the responsibility of the SATVI pharmacist, who is registered with the Pharmacy Council of South Africa. The SATVI pharmacy premises are registered per regulations with the relevant South African authorities.

8.2.2. Vaccine administration

Study vaccine MTBVAC and BCG will be administered by syringe and needle, by the Mantoux technique, in the adult participant's non-dominant arm and infant's left arm in the deltoid region. The needle and syringe are then disposed of in the biohazard sharps container.

8.3 Treatment allocation and randomization

Participants within each cohort will be randomly assigned to one of 2 groups (vaccine or control) according to the randomisation envelope. After expiry of BCG SSI on 30 June 2016 if enrolment is not yet complete, enrolment into infant cohort 3 will be unblinded with MTBVAC only until the cohort is complete (n=12)

The treatment allocation at the investigator site will be performed by the SATVI study pharmacist.

The SATVI pharmacist in charge of preparing the vaccination will have access the randomization program. Upon providing a participant number the randomization system will use the minimization algorithm to determine the treatment number to be used for the participant. Note that as soon as the target number of participants in a specific group has been reached, the enrolment will be frozen for this group.
(Refer to Appendix 7)

8.4 Product accountability

The SATVI pharmacist and clinical study team will maintain complete records for the ordering, receipt, accountability, preparation and administration of the study vaccine. Source documents and CRFs will note the lot number and expiry date of BCG used.

Section 9: Safety assessment

Vaccination with BCG at birth is routine practice in many countries of the world, including in South Africa. The vaccine is considered very safe, with very few complications being reported despite its wide coverage. Therefore, the risk to the safety of participants in this trial is considered minimal. Nevertheless, the study has been designed to identify adverse reactions early through intensive follow-up of participants and the appropriate management of side-effects by qualified staff.

Vaccination with MTBVAC has been shown to be safe and tolerable in a Phase 1a trial in healthy adults in Lausanne, Switzerland. The data in preclinical animal models also support that MTBVAC is safe and well tolerated. It presents a comparable biodistribution and clearance profile to BCG. We expect that MTBVAC will be at least as safe as BCG in newborns.

9.1 Means of safety assessment

Assessment of safety, reactogenicity and adverse reactions will be performed by qualified study staff at all study visits, in-between study visits through diary card completion, through telephonic contact with participant caregivers, and through active self-reporting by participants or participant caregivers. Study visits will be scheduled at critical time-points in the expected clinical course of reaction to BCG vaccination, and unscheduled visits and home visits will be arranged as necessary.

Safety parameters to be assessed
Solicited and unsolicited adverse reactions will be monitored carefully, documented, discussed with the participant and participant caregiver, and managed appropriately.

The following parameters will be assessed:

- Solicited systemic reactions: fever, irritability, myalgia, headache, disruptions in feeding patterns, lethargy, skin rash, loss of weight or failure to thrive
- Unsolicited systemic reactions: participants or participant caregivers will be encouraged to report any unusual symptoms or signs. Any unusual medical history or unusual physical findings will be reported to an Investigator.
- Local injection site reactions: redness, swelling, ulceration, induration, fluctuation, scarring, tenderness, axillary or cervical lymphadenopathy, abscess formation or drainage, or any other abnormal findings.

9.1.1. Assessment at vaccination

Following vaccination, each participant will be observed in the vaccination clinic for at least 60 minutes for any signs or symptoms of local or systemic intolerance.

9.1.2. Assessment at study visits

An Investigator, or a trained research nurse under the supervision of an Investigator, will assess participants at all study visits for solicited, unsolicited and local injection site reactions. A focused medical history and physical examination will be performed at the visit. Any adverse reactions will be documented. Abnormal or unexpected findings will be reported immediately to a study Investigator, and appropriately managed. All findings, as well as the need for continued self-monitoring and self-reporting, will be discussed with the participant or participant caregiver.

9.1.3. Continuous assessment in between study visits

Monitoring of safety parameters will continue in between scheduled visits through the completion of a diary card by adult participants or infant participants' mothers/caregivers. Use of the card will be carefully demonstrated by study staff to participants or participant's caregivers. Diary cards will be completed for a period of 2 weeks, with daily systemic temperature monitoring for 7 days' post vaccination. A telephone contact number and address will be clearly noted on the diary card and appointment card, and participants or participant caregivers will be carefully instructed to contact a study team member if adverse reactions occur. If necessary, an unscheduled visit will be arranged. In addition, surveillance of hospital records will be maintained by the SATVI surveillance team for the duration of the study, in order to identify admissions of study participants.

9.2 Management of adverse reactions

Adverse reactions will be recorded by the study research nurse and reported to the study Investigator for assessment. Appropriate medical management will be performed. If necessary, referral will be made to the state primary healthcare service, the Worcester secondary hospital facility, a medical specialist, or a participant's private doctor. The SATVI Investigators and study staff have wide experience with vaccination and BCG reactions and are trained in the management of adverse reactions.

9.3 Reporting and recording of adverse reactions

All safety parameters, adverse reactions and adverse events will be recorded in source documentation and captured in the study database. The Principal Investigator (PI) or a delegated Investigator will notify the local medical monitor, sponsor, UCT HREC, and MCC of any serious adverse events or trends in adverse reactions (see section on SAEs for expedited reporting measures). Regular listings and summaries of all adverse reactions will be compiled, examined by the PI, and discussed with the study team, sponsor, the local medical monitor (LMM) and the UCT HREC.

The reporting period for SAEs is from enrolment to the final study visit. The reporting period for systemic AEs that are not serious is 4 weeks after vaccination. Injection site AEs will be reported from enrolment to the final study visit. All AEs will be followed-up by the study team until resolution. If the AE is not fully resolved by the last study visit, it

will be designated ongoing, but the AE will continue to be followed up by the study team after the last visit until the AE is designated resolved, or resolved with sequelae. Scarring of the injection site lesion is considered a normal reaction, and will not be considered an ongoing AE if present at the final study visit.

9.4 Classification of adverse events

ICH E6: Good Clinical Practice defines an adverse event (AE) as "any untoward medical occurrence in a patient or clinical investigation participant administered a pharmaceutical product regardless of its causal relationship to the study treatment. An AE can be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of medicinal (investigational) product. The occurrence of an AE may come to the attention of study personnel during study visits and interviews or by a study recipient presenting for medical care."

All AEs will be reported, irrespective of whether they are deemed to be caused by MTBVAC or BCG vaccination or not. The study Investigator will classify all adverse events according to severity, seriousness, expectedness, status and relatedness.

Severity:
The investigator will use a protocol defined grading system to assess all AEs (see Appendix 2).
For grading Safety Laboratory Events, the investigator will use the "Table of Toxicity Reference Ranges" and the "Total Bilirubin Table for Term and Preterm Neonates" (see appendix 3 and 4)

For events not included in the protocol-defined grading system, the following guidelines will be used to quantify intensity:
• Mild: events require minimal or no treatment and do not interfere with the participant's daily activities.
• Moderate: events result in a low level of inconvenience or concern. Moderate events may cause some interference with functioning and may require minimal treatment.
• Severe: events interrupt a participant's usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually incapacitating.
• Life threatening: Any adverse drug experience that places the participant, in the view of the investigator, at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that had it occurred in a more severe form might have caused death.

Changes in the severity of an AE should be documented to allow an assessment of the duration of the event at each level of intensity. Adverse events characterized as intermittent require documentation of onset and duration of each separate episode. The highest level of severity of a continuous episode AE will be reported, even if the severity changes during the course of the AE duration.

Expectedness:

Unexpected AEs are any AEs of which the specificity or severity is not consistent with the risk information described in the package insert or other medical literature.

Relatedness

All AEs will be classified into 1 of 5 classes of relatedness to the study vaccine. In making the classification of relatedness the Investigator will consider the temporal association between the AE and vaccine administration, the biological plausibility of the AE being caused by MTBVAC or BCG administration, and the presence, if any, of other conditions that could cause the AE.

AEs will be classified in 1 of 5 classes of relatedness:

- Definite: there is no doubt that the AE is caused by MTBVAC or BCG or the administration of the vaccines. All injection site reactions will be designated definitely related.
- Probable: the AE is likely to be caused by the study vaccine, but the Investigator cannot completely rule out other causes. On the balance of probabilities, the AE is more likely to have been caused by study vaccine administration than by another cause.
- Possible: it is possible, but not certain, that the AE is caused by the vaccines. There may be other possible causes of the AE, or the AE occurs too long after the MTBVAC or BCG administration or lacks biological plausibility to be designated as probably or definitely related. On the balance of probabilities, the AE is more likely to have been caused by another condition than by MTBVAC or BCG administration.
- Unlikely: an alternative cause for the AE is present and the Investigator deems that it unlikely that MTBVAC or BCG could have caused the AE, but definitive evidence for an alternative cause is not present e.g. clinical diagnosis of community acquired pneumonia but without culture of an organism
- Not related: the Investigator has no doubt that the AE was not caused by MTBVAC or BCG vaccination e.g. physical trauma

For analysis purposes, all AEs classified as definite, probable or possible will be deemed related to study vaccine administration.

Status

AEs will be reported as “Ongoing”, “Resolved without sequelae”, “Resolved with sequelae” or “Death”. The status of each AE will be updated as new information becomes available.

Seriousness

Seriousness refers to the outcome of an adverse event. Seriousness is determined by both the principal investigator and the local medical monitor. If either principal investigator or local medical monitor determines an event to be serious, it will be classified as such. If any of the following outcomes are present then the adverse event is serious:

- It results in **death** i.e., the AE caused or led to the fatality. Serious does not describe an event which hypothetically might have caused death if it were more severe.
- It was immediately **life-threatening** i.e., the AE placed the participant at immediate risk of dying. It does not refer to an event which hypothetically may have led to death if it were more severe.
- It required inpatient **hospitalization** or prolonged hospitalization beyond the expected length of stay. Hospitalizations for scheduled treatments and elective medical/surgical procedures related to a pre-existing condition that did not increase in severity or frequency following receipt of study vaccine, are **not** serious by this criterion. Hospitalization is defined as a hospital admission or an emergency room visit for a period greater than 24 hours.
- It resulted in a persistent or significant **disability/incapacity** i.e., substantial reduction of the participant's ability to carry out activities of daily living.
- It resulted in a **congenital anomaly or birth defect** i.e., an adverse finding in a child or fetus of a participant exposed to the study vaccine prior to conception or during pregnancy.
- Other **medically important conditions** that may not result in death, threaten life or require hospitalization i.e., the AE does not meet any of the above serious criteria, may be considered a serious adverse event when, based on appropriate medical judgment, they may jeopardize the participant and require medical or surgical intervention to prevent one of the serious outcomes listed in these criteria (e.g., allergic bronchospasm requiring intensive treatment in an emergency room or at home; blood dyscrasias or convulsions that do not result in hospitalization).

A serious adverse event is an adverse event meeting the outcome criteria for seriousness regardless of relationship to an administered medicinal product. When an adverse event is judged to be serious, unexpected, and related to the investigational product, it is a Suspected Unexpected Serious Adverse Reaction (SUSAR) and is subject to expedited reporting.

9.5 Reporting of serious adverse events (SAE) and unanticipated problems

Serious adverse events, which include SUSARs, will be reported by the PI to the sponsor, local medical monitor, UCT HREC and the MCC in an expedited fashion. SUSARs are reported even after the trial is over, if the principal investigator or local medical monitor becomes aware of them.

Serious adverse events will be assessed by the investigator and the local medical monitor according to their roles for severity, causal relationship to the study vaccine, and expectedness. The onset and resolution dates of the event and the action taken in response to the event will be documented. If the event has not resolved by the final study visit, it will be documented as "ongoing" on the CRF. However, follow-up of the SAE must continue until resolved. Information recorded on the CRF must be substantiated in the source documents.

SAEs are to be reported to the sponsor within 24 hours of the investigator site becoming aware of the event, even if all information concerning the event is not yet known at that time. The MCC stipulates that a SUSAR is to be reported to the MCC

within 7 calendar days if it is fatal or life threatening, and within 15 calendar days if the SUSAR is not fatal or not life threatening. Other SAEs should be submitted as part of 6 monthly reports to the MCC in a line-listing format.
The UCT HREC are to be notified as soon as possible but not later than 7 days of all SAE's, SSAR's and SUSAR's. New information which may impact the conduct of a study should be reported to HREC within 3 days.
Follow-up reports for the SAE are to be provided by the site as new information becomes available or when the status of the SAE changes.
Fatal or life-threatening serious adverse events that the investigator suspects are related to the vaccination should be telephoned to the local medical monitor immediately upon the investigator's awareness of the event. If the local medical monitor or PI is required by the protocol or chooses to suspend enrollment s/he shall immediately create a written memorandum for record to the study file and immediately notify the sponsor, UCT HREC and MCC of this act.
Contact information for all safety personnel are contained in the Team Contact List which will be stored on site in the Site Regulatory Binder and maintained by the PI.

9.6 Roles and responsibilities

The PI and the study team, the local medical monitor, and the sponsor have joint responsibilities for participant safety.
The ***principal investigator*** has a personal responsibility to closely monitor trial participants and an inherent authority to take whatever measures necessary to ensure their safety. The principal investigator has the authority to terminate, suspend or require changes to a clinical trial for safety concerns and may delay an individual's study vaccine administration or pause study vaccine administration in the whole trial if the investigator has some suspicion that the study vaccine or experimental device might place a participant at significant risk. The principal investigator determines severity and causality with respect to the investigational vaccine for each adverse event. The PI is blinded to study arm allocation. The PI has a responsibility to report to the sponsor and LMM on study progress issues, participant safety issues, or any other issues that may affect the conduct of the study.
The ***local medical monitor (LMM)*** is a licensed physician, not affiliated to the investigator team, who has an objective responsibility for participant safety. The LMM is the sponsor's representative for matters pertaining to participant safety, The LMM will be based in the Cape Town/Worcester area. The local medical monitor will oversee the safety of study participants through regular reviews of the progress of the study and AEs encountered. The LMM may make an assessment of severity and causality for adverse events that may upgrade the degree of severity and causality determined by the principal investigator, and must be consulted on all SAEs and protocol pausing rules. In cases where the PI and LMM disagree on the classification of AEs, the most conservative classification will be upheld e.g. the most severe classification with the highest degree of relatedness. The PI will provide the LMM with a copy of the vaccine package insert and experimental device investigator's brochure (IB) and promptly provide any new safety information received by the PI. The LMM will receive all protocol revisions and may receive other documents related to the study.

The LMM will be in communication with the PI at the enrollment site and the sponsor for any event that needs further evaluation. The LMM will not be directly involved with the trial, will not be under the PI's supervision, and will preferably be in a different department and will have no financial, intellectual, proprietary or professional interest in outcome of the study. The local medical monitor, like the principal investigator, is blinded to study arm allocation. He/she may, in circumstances where it is required for him/her to assess participant safety, be unblinded to study arm allocation. In cases where the LMM has been unblinded, care will be taken not to unblind the PI and research team. All communications between the LMM and sponsor and PI will be documented in the site file, and important safety determinations made by the LMM will be contained within a file-note in the site file.

The **Data Safety Monitoring Board (DSMB)** will be responsible for oversight of participant safety and data integrity on behalf of the Sponsor throughout the trial. Membership of the DSMB will include at least three independent members, including the Chair, and an independent statistician. The DSMB may, at its discretion, consult or co-opt content experts as part of its deliberations. The DSMB will establish its own constitution. Minutes will be kept of all meetings of the DSMB, and written summary reports of the DSMB's decisions will be produced and kept in the regulatory site file.

The DSMB will convene after the day 28 post-vaccination reactogenicity and safety data from adults are available. Enrolment of neonates may not commence until a favourable safety review by the DSMB. The DSMB will convene ad hoc when certain protocol-defined safety conditions or pausing rules have been met, or to consider interim safety or other issues. The DSMB will convene after the day 28 post-vaccination reactogenicity and safety data from each infant cohort are available. Vaccination of neonates of the next cohort may not commence until a favourable safety review by the DSMB of the previous cohort. The safety review of cohort 2 will occur after Day 28 for the first 9 participants in order to allow maximum time for enrolling cohort 3 blinded until expiry of BCG SSI. The Day 28 safety data of the remaining 3 infants in cohort 2 will be made available to the DSMB once the visits have been performed.

The DSMB will consider the comparative frequencies and comparative relative risk of Serious Adverse Events (SAEs), grade 3 or 4 adverse events for injection site reactions and for systemic side-effects. The DSMB will also consider trends in the incidence of all adverse events within and between the study groups. After convening the DSMB will provide a recommendation to the TSC regarding continuation or discontinuation of enrolment in the trial, as well as any other recommendations or stipulations it deems necessary.
The DSMB will be provided with reports of related SAEs and SUSARs and 6-monthly line-listings of unrelated SAEs.

The DSMB will have the authority to request additional information and results of investigations from the Investigators; to request partial or complete unblinding; to pause enrolment and vaccination while considering safety issues; to request

amendments to the trial protocol, standard operating procedures, or informed consent forms; or to halt the trial if significant risks to participant safety are identified

The **Institutional Review Board or Ethics Committee** (UCT HREC) has institutional responsibility for the safety of research participants. The Institutional Review Board or Ethics Committee has the authority to terminate, suspend or require changes to a clinical trial.
Since the ***national regulatory authority*** (the MCC in South Africa) receives all expedited reports it also has the authority to terminate, suspend or require changes to a clinical trial.

9.7 Pausing rules

Enrolment to study will be paused if certain pre-defined conditions are met. Follow-up of study participants and pre-enrolment recruitment may continue while a study pause is in effect. The trial can be paused independently by either the PI, or the local medical monitor. Written notification via fax or e-mail of the study pause must be made to the PI, local medical monitor, UCT HREC, sponsor, DSMB and MCC within 24 hours of its effect and a memorandum recorded in the study site file.
If the principal investigator pauses study vaccine administration in a trial under the rules in this section and additional clinical information becomes available that reduces the principal investigator's assessment of causality, severity or toxicity grade such that the adverse event's causality, severity or toxicity grade no longer requires pausing then the principal investigator, with the agreement of the local medical monitor, may resume study vaccine administration with a memorandum to the study regulatory binder. In such a case a formal study pause need not be affected.
In all cases where the study has been paused, the DSMB will be convened, except in the case where the PI revises his assessment of a study pause condition before the meeting is convened, as additional information becomes available to him, to review safety reports and any other pertinent data following the study pause. Additional information, results of investigations, or unblinded data may show that potential risks to participants are lower, or less generalized, than suspected. If, after meeting and deliberating on all relevant information, the DSMB determines that it is safe for the study to continue, the study pause may be lifted and enrolment and vaccination may continue. Restrictions may be placed, if appropriate, on further study conduct, additional safety or monitoring measures may be put in place, or recruitment and vaccination may be stopped completely if participant safety cannot be guaranteed. The sponsor, PI, LMM, UCT HREC and MCC will be notified in writing via fax or email of the decisions and recommendations, and a signed memorandum of the deliberations will be provided to the sponsor, UCT HREC, MCC, and will also be kept in the site regulatory binder. Any changes to the protocol required as a condition of study enrolment resumption must be approved by, the Institutional Review Board/Ethics Committee (UCT HREC) and the MCC.
Pausing rules:

- SUSAR deemed to be definitely, probably or possibly related to MTBVAC/BCG administration

- SAE deemed to be definitely, probably, or possibly related to MTBVAC / BCG administration
- Grade 3 or 4 adverse event deemed to be definitely, probably or possibly related to MTBVAC /BCG administration (excluding injection site reactions) in 3 (16%) of adult participants in stage 1 of the study, or 6 (16%) of infant participants in stage 2

9.8 Discontinuation criteria and lost-to-follow up

All participants enrolled and vaccinated will remain in follow-up per the visit schedule for safety monitoring purposes. It is possible that in some cases misbleeds or missed visits may occur, but this will not influence follow-up or safety monitoring per protocol. Participants who are unavailable for some or all scheduled visits will not be designated lost to follow-up until their final visit date has passed. Repeated attempts will be made by study staff to locate such a participant and gather relevant data, unless the participant or participant's guardian has withdrawn consent.

9.9 Emergency unblinding

If the PI is of the opinion that unblinding of a particular participant's randomization result is essential to treat an adverse event and that the participant's safety would be compromised without this information, the PI may unblind the randomization result for a specific participant. In such cases, the PI should if at all possible consult the LMM first for concurrence. If emergency unblinding has occurred, a memorandum will be filed in the site regulatory binder detailing the occurrence.
The DSMB must, if possible, immediately be informed of any unblinding.

Section 10: Data analysis and sample size

10.1 Sample size considerations

The sample size for the adult stage of the study (stage 1) was selected as adequate for an initial review of the safety profile, rather than for statistical reasons. This is accepted practice for early phase trials designed to detect only relatively common safety events, major safety signals, or important trends.

10.2 Analysis of data

Statistical analysis will be performed on data from all participants who received study vaccine at enrolment. Listings of participants who did not complete all trial procedures, such as missed visits or misbleeds, and other protocol deviations that may affect the analysis, will be provided for the overall sample and by study arm. No imputation for missing data will be performed. Data will be transformed as appropriate prior to analysis but results calculated from transformed values will be reported in original units for clarity. This trial is not designed for treatment efficacy analysis, but immunogenicity analyses and analysis of clinical reactogenicity will be reported.

10.2.1. Description and analysis of baseline characteristics of the study sample

Baseline demographic and clinical characteristics will be summarized using means and standard deviations, or proportions, for the overall sample and by study group. Between-group comparisons will be performed using the most appropriate statistical tests.

10.2.2. Description and analysis of immunologic outcomes

For each of the outputs from the relevant immunology parameters, descriptive statistics including means, standard deviations, medians, and intra-quartile range, will be determined. Between-group comparisons of means or medians at each time point, as well as changes between time points post revaccination, will be performed using Student's the most appropriate statistical tests.

The primary variables of interest for preliminary assessment of the immune response to the study vaccine will be the percentage of BCG-specific CD4 and CD8 T cells that produce any or a combination of relevant cytokines. Response will be measured by flow cytometry in the intracellular cytokine staining (ICS) assay. The difference in T cell response between treatment regimens across all post-immunization time points as measured by percentage CD4 and CD8 response will be compared using a linear mixed effects model or other regression models as appropriate.

10.2.3. Description and analysis of reactogenicity and safety endpoints

The safety profile will be described by study arm and for all enrolled participants. The primary variable for evaluation of the safety profile will be the number and percentage of unsolicited and solicited adverse events and injection site reactions recorded at all available post-vaccination time points. These will be reported and analyzed by study arm. Summary statistics for continuous and categorical parameters will be provided.

Serious adverse events will be recorded through enrolment to the final study visit for all participants. Listings will be provided for all participants with serious adverse events and by study arm.

The number (percentage) of participants with adverse events will be summarized by MedDRA system organ class (SOC) and preferred term (PT). Additional summaries will present the number and percentage of participants with adverse events by severity and by relationship to the BCG vaccination; each participant will be counted once per preferred term at the greatest severity or most related state recorded for that term. Listings will be provided for all participants who have discontinued study participation prematurely due to an adverse event.

Between-group comparisons will be performed using the Student's t-test or Wilcoxon Rank Sum test for continuous variables and Chi-square, or Fisher Exact test for categorical variables.

Section 11: Ethics/Protection of Human participants

11.1 Ethical Standard

The PI will ensure that the study is conducted in full conformity with the 2013 version of the Declaration of Helsinki, with the South African GCP guidelines and the International Conference for Harmonization of Good Clinical Practice (ICH-GCP) regulations and guidelines, whichever affords the greatest protection to the participant.

11.2 Institutional Review Board (IRB)

This protocol and information and consent documents will be submitted to the Human Research Ethics Committee (HREC) of the University of Cape Town (UCT) for prior approval before starting the study. This HREC is responsible for regulatory and ethical oversight of the activities of the study staff at the sites and institutions described in this protocol. Any amendments to the protocol or information and consent documents will be submitted to the HREC, for prior approval before bringing into operation the contents of such an amendment.

11.3 Informed Consent Process

Informed consent is a process that is initiated prior to the individual agreeing to participate in the study and continues throughout the individual's participation in the study. For the infant participants, consent will be obtained from the participant's legal guardian. In most cases, this will be the participant's mother who would be asked to give consent before the birth of her baby. In exceptional cases where re-consent need to signed after the infant's birth e.g. amended Informed Consent Form (ICF), death of mother, parent no longer available, a legal guardian may sign consent on behalf of the participant. In cases where the mother is herself a minor (younger than 18 years of age), her legal guardian is required to sign informed consent on her behalf.

Potential participants will be identified through approaching members of the community or at their mother's routine antenatal clinic visits in liaison with the local state healthcare services. General information about the study will be given through individual contacts. Individual face-to-face informed consent sessions will then be held between potential participants and the study team member prior to written consent and signing of the informed consent document. This discussion session will take place in the privacy of a dedicated room at the clinic or at the SATVI field site, or at the participant's home. Adequate time will be allowed for discussion and questions, and all attempts will be made to create an unrushed, confidential, and safe atmosphere that is conducive to open discussion.

The final approved English language informed consent documents will be translated by a reputable translation agency contracted to SATVI, into isiXhosa and Afrikaans. The isiXhosa and Afrikaans versions will then be submitted to the UCT HREC for acknowledgment. The information and informed consent document will be given to the potential participant to read (or to be read to them) before the informed consent

process begins. If possible, the document will be given to the participant at least the day before the informed consent process.

Written informed consent will be obtained from all potential participants or their legal guardians in their own language for study participation and for all study interventions, including access to medical records and the results of HIV testing, prior to enrolment in the study. If a participant or his/her legal guardian is illiterate an independent witness may be used. The informed consent sessions will be conducted by study team members who have undergone training in the protocol and consent documents, who have been judged proficient in the informed consent process, and who have been delegated this task by the PI. The consenter may be a research nurse, field worker, or medical officer. Members of the research team will be drawn from similar communities as the participants and it is expected that within the study team there will be staff that are fluent in each of the local home languages (Afrikaans, English, and isiXhosa). A medically qualified member of the study team will be available to answer any questions that the consenter is unable to answer. The consenter will discuss with the potential participant the study information and consent document that has been approved by the UCT HREC, and detail the reason for the study, the study procedures, risks, benefits, and the rights of participants, including the right not to take part, or to withdraw without prejudice. The aspects of the study that are research-specific and those that are standard of care will be explained.

Any questions the potential participant may have will be answered. Participants or participant mother's understanding of the information and consent documents will be assessed by the study staff member taking consent using four key questions on: (1) the reason for the study; (2) the study procedures to be performed; (3) the risks and benefits; and (4) the right to withdraw without prejudice. The study information will be reviewed by the study staff member until the person who is giving consent has demonstrated adequate understanding of all four key areas. If all questions have been answered satisfactorily, the participant has demonstrated adequate understanding, and the participant indicates their wish to participate, they will be asked to give their informed consent by personally signing and dating the consent form in the presence of the staff member. The informed consent process, as well as specific questions or uncertainties that the participant has expressed during the informed consent session, will be documented in the source documentation. A copy of the signed informed consent document will be given to the participant for their records.

Illiterate persons will indicate their consent using a thumbprint, in the presence of an impartial witness. The witness will not date the document for illiterate participants on behalf of the participant. The date of illiterate participants' informed consent will be determined by the date of the witness signature. In cases where the mother-to-be of the potential participant is under the age of 18 years, and is therefore considered a minor, she must give her informed assent by co-signing the informed consent document, but written consent must be given by her guardian. The University of Cape Town REC and the national Department of Health require that informed consent must be obtained from a parent for participation in research by any minor under the age of

18 years. Parental consent will be obtained using the standard information and consent document.
All participants will have the right to withdraw their consent at any time throughout the course of the study.

In addition to the main Inform Consent Form, parental / caregiver consent may be requested for TB investigations and for photographing the baby, with emphasis on the injection site, at vaccination or during follow up. Photographs will be used for safety review and for study-related presentation or publication purposes.

11.4 Exclusion of Women, Minorities, and Children (Special Populations)

11.4.1. Women and minorities

For the infant stage of the study (stage 2) only women will be asked to sign informed consent in this study due the nature of the intended study population. Male and female infant participants will be enrolled. For the adult stage of the study (stage 1) male and female participants will be approached for recruitment.

Estimates of racial/ethnic group distribution of study participants are based upon demographic data from the regional health report and from the experience with enrolment in ongoing and completed SATVI studies. The expected racial/ethnic distribution of the population throughout the study region is: 99% Black (24% African and 75% so-called Colored ethnic groups); 0.1% Asian; and 1% White. As would be expected in an African population, the Hispanic and Latino groups are not represented. It is expected that the racial/ethnic distribution of participants in this study will follow a similar pattern to study population in previous SATVI studies. However, the population of the local district from which participants will be enrolled is increasing due to in-migration. It is thought that the majority of new residents are Black African and the representation of this group may increase. No participants will be excluded from the study on the basis of race or ethnicity.

11.4.2. Age of pregnant women recruited

Pregnant women who are approached for recruitment into the study will not be excluded on the basis of age alone. Under-age mothers-to-be who are not married are considered minors by law, and will require written consent from their legal guardian. In such a case the mother-to-be would also provide written assent.

11.5 Participant Confidentiality

The utmost effort will be made to avoid breach of participant confidentiality, in all discussions with study staff, handling of documentation, and management of the study data, as far as possible within the law. This confidentiality is extended to results of laboratory tests. No information concerning study participants will be released to any third party, for example the participant's general practitioner, without prior written approval from the participant. If the participant gives their written informed consent to

do so, participant contact details may be divulged to researchers at the University of Cape Town who might wish to contact the participant to take part in future research.
Study data will be entered into a dedicated custom-designed electronic database that is secure and password restricted. Access to records will be restricted to study staff only, participant folders will be stored on site in lockable, fire proof cabinets, and access to computer records will be password restricted. Participant and laboratory data with potential identifiers will be recorded on study data capture forms and the database using a unique and coded participant identification number only. Access to data with participant identifiers in study files, or elsewhere, will be restricted to the Principal Investigator, Co-Investigators, the clinical trial staff, and the SATVI Data Manager. The study monitor will have access to participant records only as necessary for performance of their study-specific functions.
No participant data will be reported in such a way that participants might be recognised from any presentation or publication of the study findings.

11.6 Study Discontinuation

The study may be paused or terminated prematurely by the PI, DSMB, UCT HREC, or local medical monitor, in the event that a serious adverse event, or a pattern of non-serious adverse events, is judged causally related to study vaccination and jeopardizes the safety of future study participants. The investigators will abide by the decision of the HREC, LMM, DSMB or MCC, if a decision to temporarily halt or prematurely terminate the study is made. Study pause rules and other safety measures are described in section 9.7.

11.7 Future Use of Stored Specimens

Documentation will be kept per laboratory SOPs to reflect storage location of samples for later retrieval. Participant identifiers will not be displayed on samples or in laboratory documentation. Laboratory staff will remain blinded to study arm allocation and participant identity. Blood collected for storage (e.g. for RNA analysis and PBMC, see section 7) may be stored for future use for additional TB-related tests after the completion of this study. These samples will remain anonymous. Storage of blood samples will be discussed in the informed consent form (ICF) and the participant will be given the option to have the infant participant's blood samples destroyed after study completion. A decision not to allow storage of blood samples will not influence participation in the main study and will not prejudice the participant in any way. See section 6.3.2 for a detailed discussion of measures for long term sample storage.

11.8 Participant Reimbursement

It is expected that participants and their caregivers will experience some inconvenience and possible additional transport costs in attending the study visits. Participants will be compensated for their inconvenience, time, and possible financial loss, in the amount of ZAR 150 (approximately $10 USD) per study-specific visit. This amount is consistent with the external recommendations of the South African Medicines Control Council and with SATVI internal recommendations for participant compensation in clinical studies. Where necessary, transport will be provided to participants by the study team.

Section 12: Clinical Monitoring structure

Clinical monitoring will be conducted to ensure that the human participant protection, study procedures, laboratory assessments, study intervention administration, and data collection processes are of high quality and meet GCP, ethical and regulatory guidelines and that the study is conducted in accordance with the protocol and SOPs.

12.1. Site monitoring

The investigator will permit authorized representatives of the sponsor, and of the respective local and national health and regulatory authorities to inspect facilities and records relevant to this study. Regular site visits will be conducted by an independent monitor and may include a review of, but not limited to, the study data, participants' medical records, source documents, CRFs, regulatory files, accountability records, informed consent forms, medical and laboratory reports. Study monitors may meet with investigators to discuss any problems and actions to be taken and document visit findings and discussions.

12.2 Local medical monitor

As described in section 9 of the protocol, a local medical monitor (LMM) will be appointed to oversee the safety of participants.

Section 13: Data handling and record keeping

13.1 Types of study documentation and study records

13.1.1. Source documentation

A folder for source documentation will be maintained by the site for each study participant. This folder will contain notes and records made by the study staff, including visits and procedures performed, results of observations, history taking and examinations, or any other notes relevant to the conduct of the study, protection and safety of the participant, or study data collected. Copies of relevant results or reports will be included in the source documentation folder. If copies of original documents are made, the staff member who makes the copy will certify that the copy is a true reflection of the original by following the SATVI site SOP for “certification of copies”. The source documentation participant folder will contain participant identifiers and will therefore only be accessible to the SATVI clinical team and monitors.

13.1.2. Case Report Forms (CRFs)

Relevant data will be transcribed by the clinical study team from source documentation to study CRFs. CRFs will not contain participant identifiers, and will identified only by the study number (enrolment number). CRFs will not be completed for recruitment or failed screening procedures.
The database will be password restricted and regular back-ups will be made on secure data-storage media. Electronic records on the database will contain relevant data to be used in the analysis of endpoints for the trial. The results of laboratory analysis may be kept on a separate secure database within the SATVI laboratory until laboratory analysis is complete, but may be reconciled with the main study database before the database is locked. All data recorded on the study CRFs. will be captured onto the database.

13.2 Numbering and coding of participant folders

All CRFs and electronic records will be identified by the study number only. The study number will be the same as the assigned enrolment number at the time of enrolment and vaccination. No participant identifiers or initials will be used on CRFs or on database records. The use of some identifiers on source documentation may be unavoidable, but access to these records will be restricted to the core clinical study team only and will not be available to data management staff, laboratory staff or any other person.

13.3 Study logs

The following logs will be maintained by the study team:

- Informed consent (IC) log: containing particulars of adults in study stage 1 and pregnant women in study stage 2 who were consented. This log will contain participant identifiers. Two separate IC logs will be maintained for each of the study stages.

- Screening log: containing results of screening at the time of birth and enrolment number (study stage 2) or at the time of screening of adult participants (stage 1). This log may contain participant identifiers. Two separate screening logs will be maintained for each of the study stages.
- Enrolment and vaccination log: containing the assigned enrolment number and details of BCG vaccination. This log will not contain participant identifiers and will not contain blinded data i.e. the outcome of randomization in neonates. Two separate enrolment and vaccination logs will be maintained for each of the study stages.
- Randomization log: the results of randomization by enrolment number in the case of study stage 2. This log will be accessible to unblinded team members only.

13.4 Data Management Responsibilities

All participant CRFs will be reviewed for completeness and accuracy in relation to source documents. All adverse events will be reviewed and graded for severity, relatedness, and seriousness by a study investigator. The data manager will be responsible for development and maintenance of the study database, periodic evaluation of missing data, range and logic checks, data access and security, and data back-up

13.5 Data Capture Methods

Data quality (QC) measures will be instituted in accordance with generic data QC procedures at the study site. All completed CRFs will be checked for completeness, accuracy and legibility. Data reported on the CRF that are derived from source documents or chart review, should be consistent with the source documents or the discrepancies should be explained. The study participant will not be contacted for CRF data validation. Required changes to data captured in the database will be made through a documented data query process that involves an audit trail. Details of the data query process, and other data management procedures, will be included in a data management plan.

13.6. Timing of reports

Line lists of all adverse events will be reported to the sponsor, UCT HREC, DSMB, MCC and local medical monitor, on a 6-monthly basis or per the guidelines or SOPs of each of these bodies. Serious adverse events will be reported to the sponsor within 24 hours of detection, to UCT HREC and MCC as per their respective guidelines.

13.7. Study records retention

All study documentation related to the study will be retained by SATVI until after the final study report or published manuscript is finalized. Documentation will be archived,

on the funder's expense, for a period of 15 years after study completion or 2 years after licensure of the investigational vaccine, whichever period is the shortest.

13.8. Protocol deviations

A protocol deviation will be defined as any action or inaction that is not in compliance with the protocol, study-specific SOPs, or Good Clinical Practice (GCP). The noncompliance may be either on the part of the participant, the investigator, laboratory, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly. These practices are consistent with Good Clinical Practice.

It is the responsibility of the site PI and study staff to use continuous vigilance to identify and report deviations within 5 working days of identification of the protocol deviation, or within 5 working days of the scheduled protocol-required activity. All deviations that have affected or may affect participant safety must be promptly reported to the UCT HREC, and regulatory authorities. Protocol deviations will be detected by ongoing vigilance of the study staff, review of participant CRFs prior to data capture, review of adverse events by investigators, internal QC procedures, and external monitoring visits.

All protocol deviations, as defined above, must be addressed in study participant source documents. A completed copy of the Protocol Deviation (PD) Form must be maintained in the Regulatory File, as well as in the participant's source document. Protocol deviations must be sent to the UCT HREC per their guidelines. The site PI/study staff is responsible for knowing and adhering to their HREC requirements.

Exemptions for inclusion and exclusion criteria stipulated in the protocol will not be allowed. The investigators will undertake prompt action to correct any identifiable factors underlying protocol deviations, which may include re-training of study staff where deemed necessary. All protocol deviations, possible sequelae, and corrective actions, will be recorded in the participant's source documentation. Any protocol deviations associated with adverse events or affecting participant safety will be reported to the UCT HREC and regulatory authorities at the time of reporting of such adverse events. All protocol deviations will be reviewed by the PI or designated investigator to determine whether the data from that participant should be included in the analysis of the study endpoints.

Section 15: Trial insurance

The sponsor will make provision for trial insurance to ensure compensation for study participants who experience injury or loss directly due to participation in the study.

Section 16: publication policy

Following completion of the study, the investigator may publish the results of this research in a scientific journal, or present the data at a scientific meeting, in consultation with the sponsor and funding collaborator as stated in the Trial agreement.

The International Committee of Medical Journal Editors (ICMJE) member journals have adopted a trials-registration policy as a condition for publication. This policy requires that all clinical trials be registered in a public trials registry such as ClinicalTrials.gov, which is sponsored by the National Library of Medicine.

Appendices

Appendix 1: phlebotomy blood volumes

This appendix is concerned with guidelines for infant phlebotomy volumes. Some guidelines on blood sample volume limits for pediatric research suggest that phlebotomy should not exceed 5% of total blood volume (TBV) and ideally should be less than 3% of the total blood volume. A maximum volume of 10% is recommended over a 4 week period. Some guidelines recommend a maximum allowable single blood draw of 3ml/kg.[26]

Table 3 below shows the expected weight and allowable blood volumes according to expected weight at each of the blood sampling visits. The weight estimations are for girls, since weight estimations in girls tends to be more conservative than for boys, and are based on the 10th percentile of expected weight for age – this combination therefore represents the strictest limitation. TBV determination is based on weight. The estimated maximum volume according to 3% of TBV represents the most conservative estimation of allowable blood volume.

Table 3. Maximum blood volumes allowable at each sampling point

Age (weeks)	**10th percentile by body weight in kg (girls)**	**Estimated total blood volume (ml)**	**Maximum allowable blood volume/ 24 hours (milliliters)**			**Max. volume in 4 wk period (ml)**
			3 ml/kg	**3% of TBV**	**5% TBV**	**10% of TBV**
10	4.4	360	13.2	10.8	18	36
14	4.8	380	14.4	11.4	19	38

Weight estimates at the phlebotomy visits are determined from nomogram for standard weight growth charts for girls. Maximum allowable blood volumes are shown according to 3 different recommendations.

Appendix 2. Table of toxicity reference ranges for grading of adverse events

Local Site of Injection Symptoms	**Grade 1**	**Grade 2**	**Grade 3**	**Grade 4**
Tenderness (pain when area is touched)	Minimal or no limitation of use of limb	Limitation of use of limb OR greater than minimal interference with usual activities	Inability to perform usual activities	N/A
Erythema (Redness)*	>2 - ≤20 mm maximum diameter	>20 - ≤50 mm maximum diameter	>50 mm maximum	Local or extensive exfoliative dermatitis
Induration *	>2 - ≤20 mm maximum diameter	>20 - ≤50 mm maximum diameter	>50 mm maximum	Local or extensive exfoliative dermatitis
Ulceration *	>2- ≤15 mm maximum diameter	>15- ≤30 mm maximum diameter	>30 mm maximum diameter	Requires hospitalization or non-routine treatment
Scar at Injection Site*	>2- ≤15 mm maximum diameter	>15- ≤30 mm maximum diameter	>30 mm maximum diameter	Keloid or extensive scarring
Regional lymphadenopathy	Non-tender, isolated, <3cm, mobile, non-fluctuating	Tender, multiple matted nodes, fixated, non-fluctuating	Supparative lymphadenitis	Supparative lymphadenitis with fistula formation
* In addition to grading, record maximum diameter in mm				
General symptoms	**Grade 1**	**Grade 2**	**Grade 3**	**Grade 4**
Decreased oral intake	Minimal decrease in oral intake	Below 50% of normal oral intake in 24 hr	No oral intake in 24hr	N/A
Vomiting	1 episode in 24hr; no interference with activity	2-3 episodes in 24 hr OR some interference with activity	> 3 episodes in 24 hours OR prevents daily activity	N/A
Diarrhea	Unformed stool OR 1-3 more stools than baseline in 24 hr	Partially liquid stools OR 4-6 more stools than baseline in 24hr	Completely liquid stools OR >6 more stools than baseline in 24 hr	N/A
Irritability	Easily consolable; minimal or no interference with activity	Difficult to console; some interference with activity	Inconsolable; prevents daily activity	N/A
Lethargy	Minimal decrease in alertness; minimal interference with activity	Some interference with activity	Unable to achieve normal level of alertness; prevents daily activity	N/A

Illness or clinical adverse event	Minimal or no interference with activity	Some interference with activity not requiring medical intervention	Prevents daily activity and requires medical intervention	N/A
Vital Signs	**Grade 1**	**Grade 2**	**Grade 3**	**Grade 4**
Fever (Axillary)	38.0 – 38.4°C 100.4 – 101.1°F	38.5 - 40°C 101.2 - 104°F	>40°C >104°F	N/A
Tachycardia – beats per minute (infants)	181-200	201-220	>220	N/A
(adults)	101 – 115	116 – 130	>130	ER visit or hospitalization for arrhythmia
Bradycardia – beats per minute(infants)(adults)	96-105	91-95	<91	N/A
	50 – 54	45 – 49	<45	ER visit or hospitalization for arrhythmia
Tachypnea – breaths per minute (infants)	61-65	66-69	>69	N/A
(adults)	17 – 20	21 – 25	>25	Intubation
Hypertension (systolic) – mm Hg (adults only)	141 – 150	151 – 155	>155	ER visit or hospitalization for malignant hypertension
Hypertension (diastolic) – mm Hg (adults only)	91 – 95	96 – 100	>100	ER visit or hospitalization for malignant hypertension
Hypotension (systolic) – mm Hg (adults only)	85 - 89	80 – 84	<80	ER visit or hospitalization for hypotensive shock
Respiratory Distress/ Hypoxia	Wheezing, nasal flaring or retractions; minimal or no interference with activity	Some interference with activity or pulse oximetry <95%	Prevents normal activity or pulse oximetry <90%	N/A
All other AEs	No interference with normal daily activity, minor severity. No treatment required.	Minimal interference with normal daily activity, moderate severity, treatment may be required	Significant interference with normal daily activity, treatment required	Severe incapacitation, urgent treatment or hospital admission required

Appendix 3. Table of toxicity reference ranges for grading SAFETY LABORATORY EVENTS

Serum/Plasma Chemistry	Grade 1	Grade 2	Grade 3	Grade 4
Sodium – hyponatremia mEq/L or mmol/L:	132 – 134	130 – 131	<130	N/A
Sodium – hypernatremia mEq/L or mmol/L:	145- 146	147-148	>148	N/A
Potassium – hyperkalemia mEq/L or mmol/L ≤ 1 year: 1 - 2years:	 5.3 – 5.4 5.1 – 5.2	 5.5 – 5.6 5.3 – 5.4	 >5.6 >5.4	N/A
Potassium – hypokalemia mEq/L or mmol/L:	3.5 – 3.6	3.3 – 3.4	3.1 – 3.2	N/A
Glucose – hypoglycemia mg/dL: mmol/L:	55-59 3.0 – 3.2	50-54 2.8 – 2.9	<50 <2.8	N/A
Glucose – hyperglycemia Fasting - mg/dL: mmol/L: Random – mg/dL: mmol/L:	101 – 110 5.7 – 6.0 110 - 125 6.1 – 6.8	111 – 125 6.1 – 6.8 126 - 200 6.9 – 11.0	>125 >6.8 >200 >11.0	N/A
Blood urea nitrogen (BUN) – increased mg/dL: mmol/L:	21 – 24 7.5 – 8.9	25 – 28 9.0 – 10.0	>28 >10.0	N/A
Creatinine – increased mg/dL: umol/L:	0.8 – 0.9 66 - 82	1.0 – 1.2 83 – 100	>1.2 >100	N/A
Calcium – hypocalcemia mg/dL: mmol/L:	8.0 – 8.4 2.00 – 2.10	7.5 – 7.9 1.87 – 1.99	<7.5 <1.87	N/A
Calcium – hypercalcemia mg/dL: mmol/L:	11.1-11.3 2.78 – 2.84	11.4 – 11.6 2.85 – 2.92	>11.6 >2.92	N/A
Magnesium – hypomagnesemia mg/dL: mmol/L:	1.3 – 1.5 0.52 – 0.62	1.1 – 1.2 0.43 – 0.51	<1.1 <0.43	N/A
Phosphorus – hypophosphatemia mg/dL: mmol/L:	2.3 – 2.5 0.73 – 0.80	2.0 – 2.2 0.63 – 0.72	<2.0 <0.63	N/A
Albumin – hypoalbuminemia g/dL: g/L:	2.5 – 2.7 25 – 27	2.2 – 2.4 22 – 24	<2.2 <22	N/A
Total protein – hypoproteinemia g/dL: g/L:	4.4 – 4.6 44 – 46	4.1 – 4.3 41 – 43	<4.1 <41	N/A
Alkaline phosphatase (ALP) – increased	1.1 – 2.0 x ULN**	2.1 – 3.0 x ULN	3.1 – 10 x ULN	>10 x ULN
Liver Function Tests (LFT) AST, ALT, GGT – increased	1.1 – 2.5 x ULN	2.6 – 5.0 x ULN	5.1 – 10 x ULN	>10 x ULN

Serum/Plasma Chemistry	Grade 1	Grade 2	Grade 3	Grade 4
Bilirubin (with any increase in LFT) - increased	1.1 – 1.25 x ULN	1.26 – 1.5 x ULN	1.51 – 1.75 x ULN	>1.75 x ULN
Bilirubin (with normal LFT) - increased	1.1 – 1.5 x ULN	1.6 – 2.0 x ULN	2.1 – 3.0 – x ULN	>3.0 x ULN
Cholesterol – increased mg/dL: mmol/L:	 171 - 185 5.1 – 5.5	 186 – 199 5.6 – 6.0	 >199 >6.0	N/A
Pancreatic enzymes amylase, lipase – increased	1.1 – 1.5 x ULN	1.6 – 2.0 x ULN	2.1 – 5.0 x ULN	>5.0 x ULN

Hematology	Grade 1	Grade 2	Grade 3	Grade 4
Hemoglobin g/dL ≤ 21 days 22 – 35 days 36 days – 56 days ≥ 57 days ≤ 6 months 6 mo - 2yr	 12.0 – 13.0 9.5 – 10.5 8.5 – 9.4 9.0 – 9.4 10.0 – 10.4	 10.0 – 11.9 8.0 – 9.4 7.0 – 8.4 8.5 – 8.9 9.5 – 9.9	 9.0 – 9.9 7.0 – 7.9 6.0 – 6.9 <8.5 <9.5	 <9.0 <7.0 <6.0 N/A N/A
WBC – increased cells/mm^3	18,700 – 22,000	22,100 – 25,000	>25,000	N/A
WBC – decreased cells/mm^3	4,500 – 5,500	3,500 – 4,400	<3,500	N/A
Lymphocytes - decreased cells/mm^3	2,000-2,700	1,500-1,900	<1,500	N/A
Neutrophils - decreased Age (> 7 days – 3 mo) cells/mm^3: (≥ 3 mo) cells/mm^3:	 1,000 – 1,300 750-990	 750 – 999 500-740	 < 750 <500	 N/A N/A
Eosinophils – increased cells/mm^3	850 – 1,500	1,501 – 5,000	>5,000	N/A
Platelets - decreased cells/mm^3	125,000 – 140,000	100,000 – 124,000	<100,000	N/A
Prothrombin Time (PT)/International normalized ratio (INR) – increased	1.1 – 1.2 x ULN**	1.3 – 1.4 x ULN	>1.4 x ULN	N/A
Partial thromboplastin time (PTT) – increased	1.1 – 1.2 x ULN	1.3 – 1.4 x ULN	>1.4 x ULN	N/A
Fibrinogen – increased mg/dL: g/L:	 400 – 500 4.00 – 5.00	 501 – 600 5.01 – 6.00	 >600 >6.00	N/A
Fibrinogen – decreased mg/dL: g/L:	 150 - 170 1.50 – 1.70	 125 – 149 1.25 – 1.49	 <125 <1.25	N/A

Appendix 4. Total Bilirubin Table for Term and Preterm Neonates

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Total Bilirubin[19], High *(mg/dL; µmol/L)[20]*				
Term Neonate[21] *< 24 hours of age*	*4 to < 7* *68.4 to < 119.7*	*7 to < 10* *119.7 to < 171*	*10 to < 17* *171 to < 290.7*	*≥ 17* *≥ 290.7*
24 to < 48 hours of age	*5 to < 8* *85.5 to < 136.8*	*8 to < 12* *136.8 to < 205.2*	*12 to < 19* *205.2 to < 324.9*	*≥ 19* *≥ 324.9*
48 to < 72 hours of age	*8.5 to < 13* *145.35 to < 222.3*	*13 to < 15* *222.3 to < 256.5*	*15 to < 22* *256.5 to < 376.2*	*≥ 22* *≥ 376.2*
72 hours to < 7 days of age	*11 to < 16* *188.1 to < 273.6*	*16 to < 18* *273.6 to < 307.8*	*18 to < 24* *307.8 to < 410.4*	*≥ 24* *≥ 410.4*
7 to 28 days of age (breast feeding)	*5 to < 10* *85.5 to < 171*	*10 to < 20* *171 to < 342*	*20 to < 25* *342 to < 427.5*	*≥ 25* *≥ 427.5*
7 to 28 days of age (not breast feeding)	*1.1 to < 1.6 x ULN*	*1.6 to < 2.6 x ULN*	*2.6 to < 5.0 x ULN*	*≥ 5.0 x ULN*
Preterm Neonate[20] *35 to < 37 weeks' gestational age*	*Same as for **Total Bilirubin, High, Term Neonate** (based on days of age).*	*Same as for **Total Bilirubin, High, Term Neonate** (based on days of age).*	*Same as for **Total Bilirubin, High, Term Neonate** (based on days of age).*	*Same as for **Total Bilirubin, High, Term Neonate** (based on days of age).*
32 to < 35 weeks' gestational age and < 7 days of age	*NA*	*NA*	*10 to < 14* *171 to < 239.4*	*≥ 14* *≥ 239.4*
28 to < 32 weeks' gestational age and < 7 days of age	*NA*	*NA*	*6 to < 10* *102.6 to < 171*	*≥ 10* *≥ 171*
< 28 weeks' gestational age and < 7 days of age	*NA*	*NA*	*5 to < 8* *85.5 to < 136.8*	*≥ 8* *≥ 136.8*
7 to 28 days of age (breast feeding)	*5 to < 10* *85.5 to < 171*	*10 to < 20* *171 to < 342*	*20 to < 25* *342 to < 427.5*	*≥ 25* *≥ 427.5*
7 to 28 days of age (not breast feeding)	*1.1 to < 1.6 x ULN*	*1.6 to < 2.6 x ULN*	*2.6 to < 5.0 x ULN*	*≥ 5.0 x ULN*

[19] Severity grading for total bilirubin in neonates is complex because of rapidly changing total bilirubin normal ranges in the first week of life followed by the benign phenomenon of breast milk jaundice after the first week of life. Severity grading in this appendix corresponds approximately to cut-offs for indications for phototherapy at grade 3 and for exchange transfusion at grade 4.

[20] A laboratory value of 1 mg/dL is equivalent to 17.1 µmol/L.

[21] Definitions: Term is defined as ≥ 37 weeks' gestational age; near-term, as ≥ 35 weeks' gestational age; preterm, as < 35 weeks' gestational age; and neonate, as 0 to 28 days of age.

Appendix 5 Vaccine supplies, packaging and accountability

1. Vaccine and/or other supplies

BIOFABRI will supply the following amounts of study vaccines and/or other supplies, sufficient to administer the corresponding doses to all subjects as described in the present protocol.

- 54 vials of MTBVAC, 20 standard doses per vial (0.05 mL).
- The corresponding MTBVAC excipient solution vials (4.5 mL) for making the dilutions.

2. Secondary packaging

The vaccines will be packed in labelled boxes. The box label will contain, as a minimum, the following information: study number, lot number, instructions for vaccine administration, the statement “contains GMO” and any other relevant regulatory requirements.

3. Vaccine shipment

From BIOFABRI to South African Tuberculosis Vaccine Initiative (SATVI).
Study vaccines will be sent from BIOFABRI TO SATVI after the GMP and GCP releases which are documented and archived.
Shipments are made in tamper evident parcels containing:

- a copy of Certificate of Analysis if required by local regulatory authorities
- a Delivery Note describing: study and centre number, investigational site name and address, recipient, products name, quantity supplied, lot numbers, expiry dates and storage requirements
- 2 temperature control devices

All shipments shall be received and acknowledged by the appropriate recipient at SATVI.

The following documents should be completed and returned to BIOFABRI, S.L. on reception of vaccine shipment:

- Delivery Note
- Temperature control devices.

These documents and devices should then be returned to:
Attention of Ingrid Murillo
Qualified Person
BIOFABRI, S.L
Fax : +34 986 345 201
E-mail: ingrid.murillo@biofabri.es
Temperature recording charts will be obtained at BIOFABRI to check that cold chain has not been broken. Study vaccines will not be used at SATVI until confirmation of

suitability is provided by BIOFABRI. In case of any temperature deviation, the official approval for the use of vaccine must be obtained from BIOFABRI.

4. Vaccine accountability

At all times the figures on supplied, used and remaining vaccine doses should match. At the end of the study, it must be possible to reconcile delivery records with those of used and unused stocks. An explanation must be given of any discrepancies.
After approval from BIOFABRI, used and unused study vaccine vials/syringes should be destroyed at the study site using locally approved biosafety procedures and documentation unless otherwise described in the protocol. If no adequate biosafety procedures are available at the study site, the used and unused vaccine vials/syringes are to be returned to an appropriate BIOFABRI site for destruction.

5. Transfers of clinical vaccines or products from country medical department or dispatch centre to study sites or between sites

Not applicable.

Appendix 6: Tabular summary of study visits

Schedule of events in study stage 1 (adults)

Phase		Screen	Post-vaccination safety follow-up						
Month		**-2 to -1**	**0**	**1**	**1**	**1**	**2**	**3**	**6**
Day		**-30 to -0**	**0**	**7**	**14**	**28**	**56**	**90**	**180**
Visit		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Dose			**1**						
Informed consent		•							
Confirmation of eligibility		•	•						
Medical history		•							
Physical examination and all other screening tests									
Pregnancy test: β-HCG-serum (3 ml)		•							
β-HCG –urine			•						
Safety blood samples (Biochemical and haematological analysis)		•		•		•			
HIV test		•							
Quantiferon (3 ml)		•							•
Focused Physical examination		•	•	•	•	[a]	[a]	[a]	[a]
Resting vital signs incl. temperature measurement		•	•	•	•	•	•	•	•
Recording of concomitant medication		•	•	•	•	•	•	•	•
Vaccination			•						
Injection site assessment		•	•	•	•	•	•	•	•
Diary cards	Distribution by study team		•	•					
	Return			•	•				
AE and medication form	Distribution by study team		•	•	•	•	•	•	
	Return				•	[a]	[a]		
Recording of solicited symptoms			•	•					
Non-serious AEs post vaccination			•	•	•	•	•	•	
Reporting of SAEs, SUSARS			•	•	•	•	•	•	

• is used to indicate a study procedure that requires documentation in the individual CRF

[a] Only examine if abnormalities.

Schedule of events in study stage 2 (infants)

Phase		Screen Mother	Post-vaccination follow-up								
Month		**-2 to -1**	**0**	**1**	**1**	**1**	**3**	**3**	**6**	**6.5**	**12**
Day		**-60 to -0**	**0**	**7**	**14**	**28**	**70**	**91**	**180**	**+/-194**	**360**
Visit		**-1**	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**
Dose			**1**								
Informed consent (mother and legal guardian)		•									
Confirmation of eligibility criteria		•	•								
Medical history		•									
HIV test		•									
Physical examination			•	•	•	•	[a]	[a]	[a]		•
Resting vital signs			•	•	•	•	•	•	•		•
Recording of concomitant medication pre-/post-vaccination			•	•	•	•	•	•	•		
Pre- and post-vaccination assessment incl. temperature measurement			•	•	•	•	•	•	•		
Vaccination			•								
Diary cards	Distribution		•	•							
	Return			•	•						
AE and medication form	Distribution		•	•	•	•	•	•	•		
	Return			•	•	•	[a]	[a]			
Recording of solicited symptoms			•	•	•	•	•	•	•		•
Recording of non-serious AEs			•	•	•	•	•	•	•		•
Reporting of SAEs and SUSARS			•	•	•	•	•	•	•		•
Microbiological analyses											
Swabs at injections site[b]				•	•	•	•	•	•		
Biochemical and haematological analysis											
Safety blood samples (2 ml)				•		•					
Primary Immunogenicity endpoints											
Whole Blood ICS (WB-ICS) Assay *(0.75 ml at D7 and 2.5 ml for the rest)*[c]				•		•	•		•		
QuantiFERON (QFT) (3 ml)									•		•
Blood volume per visit in ml:				2.75	0	4.5	2.5	0	5.5		
Cumulative blood volume (ml):				2.75	2.75	7.25	9.75	9.75	15.26		
BCG rescue dose given to all MTBVAC vaccinated infants[d]										•	

• is used to indicate a study procedure that requires documentation in the individual CRF

[a] Only examine if abnormalities.

[b] Swabs at injection site for microbiology analysis will be conducted in all infants that show site-opening event and will be repeated until 2 negative cultures are obtained.

[c] Stimulation of blood cells (whole blood) with live mycobacteria BCG, MTBVAC, or "mega pool" of mycobacteria peptides, or phytahemagglutinin (PHA), or left unstimulated. D7 stimulation conditions include half the blood volume [250 µL (0.25 mL)] and only Nil, MTBVAC and BCG. Evaluation of the frequency of specific CD4+ and CD8+ T cells that secrete cytokines in response to the stimulation.

[d] visit at 2 weeks post BCG for recording BCG receipt to all infants

Appendix 7: Randomisation procedures

1. Purpose

This clarification describes the final randomisation (and related) procedures to be followed in the execution of the applicable protocol.

2. Final randomisation procedures to be followed in this trial

2.1 Screening and treatment numbers

Screening number:

Screening numbers will be assigned in a sequential order to participants in the order in which they are screened for participation in the trial. Screening numbers will comprise three digits, with leading zeros, i.e. 001, 002, 003... Screening numbers for adult participants will be prefixed with the letter "A" to identify them as adults. Similarly, infant participants will be identified by the prefixed letter "B". Examples of adult and infant screening numbers are A001, A017, B005 and B020.

A participant's screening number will serve as their primary identifier during the trial.

Treatment number:

Treatment numbers will be allocated sequentially as participants are randomised. When a participant is randomised the next available treatment number for their group, i.e. adults or infants. As for screening numbers, treatment numbers will comprise three digits, with leading zeros, i.e. 001, 002, 003..., to identify the sequential order in which participants are assigned. This number will be prefixed with a binary code, "00" or "01", to indicate whether or not a participant is a replacement. Finally, the number will be completed by a prefixed "T01" or "T02", to identify the participant as adult or infant, respectively. Examples of treatment numbers are T01-00-001 (first randomised adult participant) and T02-01-002 (replacement participant for the second randomised infant participant).

Treatment numbers are used for the sole purpose of keeping track of the treatment assigned to a particular participant and is not to be used for any labelling purposes to identify a participant.

2.2 Randomisation lists

Triclinium, will generate four randomisation lists, two for the adult stage and two for the infant stage of the trial. Two lists are required for each stages, since participants may be replaced on this trial. Each list will indicate the treatment number, the treatment associated with that treatment number, room to add the participants primary identifier, and a space for the signature of the responsible pharmacist following treatment allocation.

Randomisation lists are to be used during randomisation / treatment assignment.

Following the expiry of BCG SSI and if the enrolment of infant cohort 3 is incomplete, enrolment will continue unblinded with MTBVAC only until there are 12 infants in the cohort.

2.3 Code break envelopes

Triclinium will also generate separate, individually-sealed code break envelopes for each trial participant that will be used in an emergency to break the blind for individual participants, if necessary. Code break envelopes will use treatment numbers to identify a particular participant, since the screening number is not known prior to randomisation.

Code break envelopes are to be used to only for breaking the blind, if required.

Appendix 8: Summary of protocol changes

Cover Page & Page 2: Protocol and date version updated

Page 2. Study Monitor: Contact detail changed

Page 2. Clinical and Regulatory Affairs Monitor: Contact detail changed

Page 16. Protocol Summary Study Endpoints. Table 1: Column D360 added. Detail of blood volume added

Page 16. Protocol Summary Study Endpoints. Table 1: D360 mention added at the legend

Page 19. Protocol Summary Study Procedures. Infant Stage: D360 added

Pages 38, 42, 57, 60, 63, 65, 66, 68, 74 and Infex (section 11.5): Term "subject" replaced by "participant"

Page 43. Exploratory immunology endpoints: D360 added

Page 52. Visit 8. Follow-up Visit (Day 360 ± 14 days): End of Study visit clarifications**.** Specific assessment added

Page 52. Infant face to face telephone or home visit (M9, M 15, M18, M21, M24): Wording text concerning face to face visits for all Infants with positive QFT test at D180

Page 58. Storage of MTBVAC: Appendix number corrected

Page 75. Future Use of Storage Specimens: Section number corrected at the end of the paragraph.

Page 90. Schedule of events in study Stage 2 (Infants): Column D360, specific safety assessments marked

Footer: Protocol version date updated.

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