A Phase 2a Randomised Controlled Dose-Defining Trial of the Safety and Immunogenicity of MTBVAC in Healthy, BCG Naïve, HIV Unexposed, South African Newborns

Protocol Number: 202

Study Phase: 2a

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Trial Acronym: MTBVAC-Newborns

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CONFIDENTIALITY STATEMENT

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PROTOCOL SIGNATURE SHEET

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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 South African Health Products Regulatory Authority (SAHPRA), 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.

1 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

AE	adverse event
ALP	alkaline phosphatase
ALT	alanine aminotransferase
AST	aspartate aminotransferase
BCG	Bacille Calmette-Guérin
BUN	blood urea nitrogen
CFR	Code of Federal Regulations
CFU	colony forming unit
DAFF	Department of Agriculture, Forestry and Fisheries
DSMB	Data Safety Monitoring Board
eCRF	electronic case report form
EDC	Electronic Data Capture
EDCTP	European and Developing Countries Clinical Trials Partnership
ELISA	enzyme linked immunosorbent assay
GCP	Good Clinical Practice
GGT	gamma glutamyl transferase
HIV	human immunodeficiency virus
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
ICMJE	International Committee of Medical Journal Editors
ICS	intracellular cytokine staining
IEC	Independent Ethics Committee
IFN	interferon
Ig	immunoglobulin
IGRA	interferon gamma release assay
IRB	Institutional Review Board
IRE	Immediately Reportable Event
LFT	liver function test
LMM	local medical monitor
MedDRA	Medical Dictionary for Regulatory Activities
Mtb	*Mycobacterium tuberculosis*
PBMC	peripheral blood mononuclear cell(s)
PCR	polymerase chain reaction
PHA	phytohemagglutinin
PID	participant identification number
POI	prevention of infection
PT	prothrombin time
PTT	partial thromboplastin time
QFT	QuantiFERON®-TB
SAE	serious adverse event
SAER	Supplemental SAE Report
SAHPRA	South African Health Products Regulatory Authority
SATVI	South African Tuberculosis Vaccine Initiative

SOP	Standard Operating Procedure
SUSAR	suspected unexpected serious adverse reaction
SSAR	Standard Safety Analysis Report
TB	tuberculosis
TBVI	TuBerculosis Vaccine Initiative
TCD	Triclinium Clinical Development (Pty) Ltd
TCD RA	Triclinium Regulatory Department
TNF	tumor necrosis factor
TSC	Trial Steering Committee
ULN	upper limit of normal
US	United States
WB	whole blood
WBC	white blood cell
WHO	World Health Organization

PROTOCOL SUMMARY

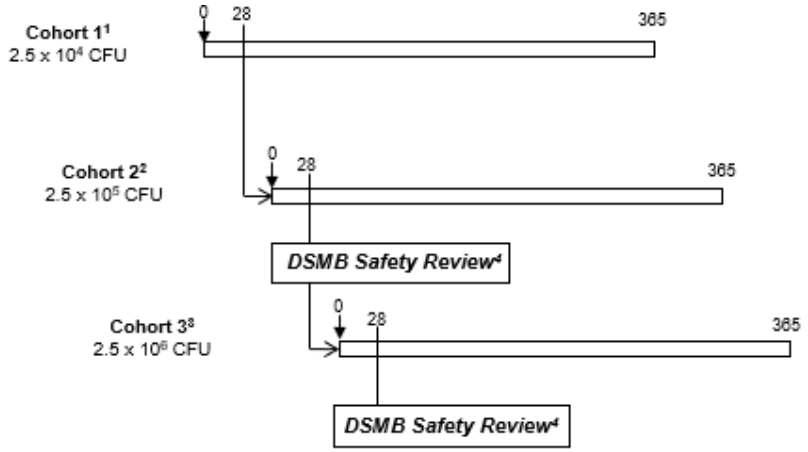
Title:	A Phase 2a Randomised Controlled Dose-defining Trial of the Safety and Immunogenicity of MTBVAC in healthy, BCG naïve, HIV unexposed, South African newborns
Phase:	2a
Population:	Ninety-nine HIV unexposed, BCG naïve, newborns without known household exposure to *M. tuberculosis*
Number of Sites:	One site in South Africa: South African Tuberculosis Vaccine Initiative (SATVI) Brewelskloof Hospital Worcester South Africa
Study Duration:	The estimated study duration (first participant vaccinated to completion of data collection) is approximately 36 months.
Study Participation Duration:	365 days
Description of Agent or Intervention:	MTBVAC at three dose levels: 2.5 x 10^4 CFU, 2.5 x 10^5 CFU and 2.5 x 10^6 CFU. The active control is BCG vaccine. Participants will receive a single dose of MTBVAC or BCG administered intradermally on Study Day 0.
Objectives:	Primary: • To evaluate safety and reactogenicity of MTBVAC at escalating dose levels compared to BCG vaccine in healthy, BCG naïve, HIV unexposed, South African newborns • To evaluate the immunogenicity of MTBVAC at escalating dose levels compared to BCG vaccine in healthy, BCG naïve, HIV unexposed, South African newborns Secondary: • To evaluate QFT conversion rates in neonates receiving escalating dose levels of MTBVAC Future exploratory: Samples will be kept for future analyses: • To evaluate exploratory immune responses to escalating dose levels of MTBVAC and to BCG (including, but not necessarily

limited to, analysis of innate response cytokines, specific IgG antibody responses, donor-unrestricted T-cell responses).

- To differentiate vaccine-induced immune responses from natural Mtb infection.

Design:	Double-blind, randomised, active BCG control, MTBVAC dose-finding trial with 3 cohorts of healthy, BCG naïve, HIV unexposed, South African newborns (24 BCG; 75 MTBVAC)
Estimated Time to Complete Enrollment:	The estimated time to complete enrollment is approximately 9 months.

Schematic of Study Design:



↓ = vaccination

[1] Cohort 1: n=25 MTBVAC (2.5 x 10^4 CFU) and n=8 BCG
[2] Cohort 2: n=25 MTBVAC (2.5 x 10^5 CFU) and n=8 BCG
[3] Cohort 3: n=25 MTBVAC (2.5 x 10^6 CFU OR 2.5 x 10^5 CFU (n=13) plus 2.5 x 10^4 CFU (n=12)) and n=8 BCG
[4] At each DSMB, all available safety data from previous cohorts enrolled will be reviewed. If any of the safety criteria is not met for the 2.5 x 10^6 CFU dose for Cohort 3, Cohorts 1 and 2 will be expanded so that 33 additional neonates will be randomly allocated to receive the 2.5 x 10^5 CFU dose (n=13), the 2.5 x 10^4 CFU dose (n=12) or BCG (n=8) by default.

2 KEY ROLES

Study Monitor	TCD, Triclinium Clinical Development (Pty) Ltd. Design House 121 Amkor Road Pretoria 0157 South Africa
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Professor Mark Hatherill, University of Cape Town (UCT), South Africa

Clinical and Regulatory Affairs Monitor	TCD, Triclinium Clinical Development (Pty) Ltd. Design House 121 Amkor Road Pretoria 0157 South Africa

3 BACKGROUND INFORMATION AND SCIENTIFIC RATIONALE

3.1 Background Information

A new effective tuberculosis (TB) vaccine is essential to achieve World Health Organization (WHO) End TB goals and ultimately to eliminate TB by 2050 (1). Bacille Calmette Guerin (BCG), currently the only licensed vaccine against TB, protects against disseminated TB (miliary and meningitic disease) in children, but provides inconsistent protection against the predominant form of pulmonary TB in all age groups. A new improved TB vaccine is needed that is effective against all forms of TB disease in infants, children, and adults; and safe in immune-compromised persons, including those with HIV infection. One potential vaccination approach targets adults, the drivers of the TB epidemic, to prevent transmission within the community including to susceptible children. Modeling shows that a moderately effective adult TB vaccine would be the single best tool to achieve TB elimination by 2050 (2). However, the optimal strategy would be to combine serial mass campaigns in adults, and universal newborn vaccination to provide direct protection to susceptible children who experience a high incidence rate of TB infection. Implementation of this strategy will require demonstration of safety, immunogenicity, and efficacy of a new TB vaccine in both newborns and adults.

The most robust evidence for vaccine-induced protection against TB disease in humans comes from children who received a live attenuated mycobacterial vaccine – BCG, a live-attenuated *Mycobacterium bovis* originally isolated from cattle. Systematic review suggests that BCG confers variable protection against TB disease in infants and school-age children, provided that they have not already been infected with *Mycobacterium tuberculosis* (or potentially exposed to other non-tuberculous mycobacteria) (3). However, the majority of adults in countries with high TB endemicity are latently infected (4). Therefore, although a new effective adult vaccine would be highly desirable to prevent active TB disease and halt TB transmission, adults might be the most difficult population to protect. Newborn infants are the only human population without prior mycobacterial exposure in TB endemic countries. Therefore, parallel clinical developments of MTBVAC are crucial to provide safety, immunogenicity and efficacy data in *M. tuberculosis* naïve and *M. tuberculosis* exposed populations, in order to deliver an effective, long-term TB vaccine strategy for both adults and newborns.

3.2 Rationale

BCG lacks >100 genes when compared to the human TB pathogen *M. tuberculosis* (5), that are thought to be pathogen-beneficial for the successful interaction of *M. tuberculosis* with the human immune system (6,7). Therefore, it is hypothesized that a successful vaccine against TB should incorporate several of these *M. tuberculosis* genes. Within European TB vaccine projects and TBVI, Prof. Carlos Martin (Unizar) and Prof. Brigitte Gicquel (Pasteur Institute, France) genetically engineered a uniquely attenuated strain, MTBVAC, based on a *M. tuberculosis* clinical isolate of the Euro-American lineage (8), the most widespread and

commonly transmitted lineage between humans by the aerosol route (9). The attenuation of MTBVAC is based on two independent, stable genetic deletions of two major virulence factors in *M. tuberculosis*: *phoP* coding for the transcription factor PhoP and *fadD26* coding for the synthesis of PDIM (8,10,11). MTBVAC contains most of the genes deleted from BCG and presents a wider collection of mycobacterial antigens to the host immune system. Live-attenuated strategies are also considered the best approach for conferring durable T-cell immune responses (12). It is our hypothesis that, as a live attenuated *M. tuberculosis*, MTBVAC provides a more durable T-cell response to a wider collection of mycobacterial antigens and confers improved protection compared to BCG. The Spanish vaccine manufacturer Biofabri has produced and characterized live-attenuated MTBVAC as a freeze-dried preparation in compliance with GMP, WHO, EMA and FDA recommendations to assure the quality, safety and efficacy of BCG vaccines. The final freeze-dried MTBVAC product has shown a robust safety, biodistribution and persistence profile comparable to licensed BCG; fulfills regulatory requirements; and has shown promising immunogenicity and protective efficacy in relevant animal models (8). Recent studies in newborn mice show that MTBVAC is safe, does not affect growth and organ development and, notably, confers superior immunogenicity and efficacy against pulmonary forms of TB, compared to BCG (13).

MTBVAC entered a first-in-human Phase 1a trial in 2012. Safety and immunogenicity of three escalating dose levels of MTBVAC (5×10^3, 5×10^4, 5×10^5 CFU) was evaluated in comparison with BCG SSI in healthy Swiss adults without history of BCG vaccination or exposure to TB or HIV (14). Vaccination with MTBVAC demonstrated an excellent safety profile, comparable to BCG. MTBVAC was at least as immunogenic as BCG; and at the same dose level as BCG, the MTBVAC group showed greater frequency of polyfunctional CD4+ central memory T-cells. A transient increase in CFP-10-specific ELISPOT positivity was observed at day 28 in three adult participants who received MTBVAC (14).

Based on these promising first-in-human data, a Phase 1b evaluation of MTBVAC in healthy newborns started in 2015 at the SATVI site in South Africa (ClinicalTrials.gov NCT02729571). This trial evaluated safety and immunogenicity of three escalating dose levels of MTBVAC (2.5×10^3, 2.5×10^4, 2.5×10^5 CFU) for the first time in BCG naïve newborns in a TB endemic region. Twenty-eight infants received MTBVAC (9 in each of the first two dose groups, and 10 in the third), preceded by a pilot safety group of adults, previously BCG-vaccinated at birth, who received MTBVAC (2.5×10^5 CFU). Vaccination of both adults and newborns was completed without any significant safety signal. Immunogenicity results indicate that one dose of MTBVAC 2.5×10^4 or 2.5×10^5 CFU given within 96 hours of birth induced substantial cytokine-expressing (IFN-γ, IL-2, or TNF-α) antigen-specific CD4 T-cell responses that persisted for six months until the final study day. MTBVAC 2.5×10^5 CFU dose evoked the greatest magnitude response at day 70 post vaccination, whereas the lowest MTBVAC dose of 2.5×10^3 CFU was less immunogenic and did not induce a durable response at day 180. Vaccination with MTBVAC at 2.5×10^4 or 2.5×10^5 CFU is immunogenic in neonates from a TB endemic setting.

Data from this Phase 1b infant trial indicates that MTBVAC vaccination may cause interferon gamma release assay (IGRA) conversion in infants, unrelated to natural *M. tuberculosis* infection. This phenomenon is analogous to the known effect of BCG vaccination on tuberculin

skin test (TST) conversion. Three of 8 (37,5%), six of 8 (75%) and 7 of 9 (77,8%) infants who received MTBVAC at 2.5 x 10^3, 2.5 x 10^4 and 2.5 x 10^5 dose respectively, demonstrated a positive QuantiFERON (QFT) test 180 days after MTBVAC administration, in the absence of known exposure to TB, compared to none of seven BCG recipients. At one year of age none of 8 (0%), two of 8 (25%) and 4 of 9 (44%) infants who received MTBVAC at 2.5 x 10^3, 2.5 x 10^4 and 2.5 x 10^5 dose respectively, demonstrated a positive QFT result. This new data offers a strong indication of the immunogenicity of MTBVAC in newborns, which requires confirmation in a larger study. Further, since QFT conversion is used as a tool to diagnose *M. tuberculosis* infection in adults and older children, and as an endpoint in exploratory prevention of *M. tuberculosis* infection (POI) vaccine trials, it is critical that the mechanism of MTBVAC-induced QFT conversion and reversion is fully explored. If MTBVAC is shown to be efficacious against TB disease, these data will be important for development of alternative interferon-gamma release assays (IGRA) for *M. tuberculosis* infection that are not cross-reactive with MTBVAC.

Biofabri, industrial manufacturer and exclusive licensee of MTBVAC, together with intellectual property owner Unizar, has set two independent clinical development pathways for MTBVAC. One pathway focuses on development of MTBVAC for prevention of TB disease in previously BCG vaccinated adults. This clinical trial is based on the clinical development pathway of MTBVAC as a preventive pediatric vaccine that could potentially replace newborn BCG vaccination. A Phase 2a dose-defining study is a pre-requisite for selecting optimal dose for future efficacy trials of MTBVAC in newborns in TB endemic countries.

Reflecting the clinical strategy to move straight from the Phase IIa to Phase III study with a single selected dose, the design of the proposed Phase III incorporates three case-driven assessments for futility and efficacy. Such a pathway for the MTBVAC clinical development would allow for the neonatal vaccine to be made available sooner (estimated 5 years) with the potential to offer a greater magnitude of protection compared to current BCG vaccine. Such a saving in time would have a major public health benefit when considering that the BCG vaccine has been reported to have variable protective effect depending on the form of TB and the geographical location. The impact of TB in children is significant: in 2019, over one million (1 190 000) children globally became ill with TB and 230 000 died from the illness and therefore it is prudent to develop a more effective vaccine as soon as possible, within safety and regulatory requirements.

3.3 Potential Risks and Benefits

3.3.1 Potential Risks

The safety profile of MTBVAC at the highest dose to be tested (2.5 x 10^6 CFU) is not yet known in infants. Therefore, vaccination of newborns will be staggered to allow gradual evaluation of safety and reactogenicity at each escalation of MTBVAC dose. Progression from the 2.5 x10^5 to the 2.5 x10^6 CFU dose level, for which safety in infants has not yet been demonstrated, will require that all of the following criteria have been met: (1) no pre-defined pausing rules have been observed at lower MTBVAC dose levels in this trial; (2) favourable review of day 28

safety data of all lower MTBVAC dose groups by the Data Safety Monitoring Board (DSMB); (3) favourable review of safety data from the current pilot Phase 1b infant trial by the DSMB; and (4) favourable review of day 28 safety data for the 2.5 x 10^6 CFU dose level in QFT negative adults in the parallel adult trial by the DSMB. In the event that any one of these safety criteria are not met, Cohorts 1 and 2 will be expanded so that 33 additional neonates will randomly allocated to receive the 2.5 x 10^4 CFU dose (n=12), the 2.5 x 10^5 CFU dose (n=13) or BCG (n=8) by default. If there is a delay in adult data, dose escalation in infants will either be delayed, or Cohorts 1 and 2 be expanded as mentioned above.

MTBVAC Data Safety Monitoring Board Charter has been created as a guidance.

It is not yet known whether MTBVAC offers equivalent, better, or worse protection against TB compared to BCG. Therefore, measures to minimize risk of TB will be put in place throughout the study and, after completion of all Study D365 visits in each cohort and unblinding of the clinical team, 'catch up' BCG vaccination will be given to all MTBVAC vaccinated infants once the last participant of each cohort has performed the D365 visit, provided that the individual recipients have a negative screen for active TB disease and new household contacts, and do not have a new positive QFT result at D365. Risk of incident TB disease will be minimized by exclusion of infants with known TB exposure, including prior maternal or family history. Newborns born to HIV infected mothers, with increased risk of TB, will not be enrolled. All infants will be screened actively for TB symptoms at each study visit to diagnose TB disease early and pre-empt severe morbidity. Infants diagnosed with TB disease will remain in safety follow-up, but will be referred immediately for TB treatment, provided free of charge by health services. In the previous South African study we saw no confirmed TB cases in either adults or infants who received MTBVAC. One adult MTBVAC recipient with recent HIV seroconversion was diagnosed with aseptic meningitis and received 6 months treatment for unconfirmed TB meningitis. Two infant MTBVAC recipients (one QFT+; one QFT-) received TB treatment in the public health system for unconfirmed TB; a rate (2/28; 7%) that is similar to the rate of unconfirmed TB in a previous infant trial in this community (13% over 2 years)[29]

The QFT assay is widely used in older children and adults as a tool to aid in the diagnosis of *M. tuberculosis* infection. Given the preliminary evidence of MTBVAC vaccine induced QFT conversion in the current Phase 1b infant study, it is postulated that infants who receive MTBVAC will demonstrate QFT conversion events two months after vaccination (D56), followed by a high rate of reversion, leading to declining rates of QFT positivity at D182 and D365. The same response kinetic was observed for BCG-specific T cell responses in a prior longitudinal study in newborns (15). The observed effect of MTBVAC on QFT conversion is analogous to the known effect of BCG on false positive TST conversion; MTBVAC might be expected to have a similar effect on TST conversion.

Based on observed rates of QFT positivity 1 year after MTBVAC vaccination in infants, half or more of infants who receive MTBVAC may remain QFT positive at end of study and longer. However, it will not be possible to differentiate MTBVAC-induced QFT conversion from QFT conversion as a consequence of natural *M. tuberculosis* infection. Therefore, risk of natural *M. tuberculosis* infection will be minimized by exclusion of infants with known TB exposure, including prior maternal or family history. Infants who demonstrate QFT conversion soon after

MTBVAC administration are more likely to have converted due to vaccine effect than infants who demonstrate QFT conversion later during follow-up, who are more likely to have converted due to natural *M. tuberculosis* infection. Early (D56) post-vaccination QFT conversion in infants with low risk of natural *M. tuberculosis* exposure is assumed to be vaccine-induced and will not be treated with isoniazid preventive therapy (IPT). Infants who demonstrate early QFT conversion at D56 will undergo enhanced screening for potential new TB exposure and TB symptoms during all scheduled study visits, and in addition, during interval telephonic contacts at D120 and D270. QFT samples will be collected at D182 and D365 from infants who tested QFT positive at D56, and stored for later analysis, but since a positive QFT result would not be interpretable, these data will not be used for clinical decision-making. All infants who received MTBVAC and tested QFT positive will receive a card at the end of study indicating that they have received an experimental vaccine that may interfere with interpretation of future IGRA and TST tests for TB infection.

QFT assays will be repeated at D182 and D365 in infants who tested QFT negative at D56. These results will be made available for clinical decision-making. Infants with new QFT conversion events at D182 or D365, and any infant with a reported new household TB contact regardless of QFT status, will be referred to the public health services for IPT since these infants would potentially benefit from TB prophylaxis against *M. tuberculosis* infection as per SA national guidelines, provided that signs and symptoms of active TB disease are absent.

Known Potential Benefits

All infant participants will benefit from enhanced TB screening and facilitated referral and improved access to healthcare providers.

4 OBJECTIVES

4.1 Study Objectives

Primary Objectives

The primary objectives of this study are:

- To evaluate safety and reactogenicity of MTBVAC at escalating dose levels compared to BCG vaccine in healthy, BCG naïve, HIV unexposed, South African newborns.
- To evaluate the immunogenicity of MTBVAC at escalating dose levels compared to BCG vaccine in healthy, BCG naïve, HIV unexposed, South African newborns.

Secondary Objective

The secondary objective of this study is:

- To evaluate QFT conversion rates in neonates receiving escalating dose levels of MTBVAC.

Future Exploratory Objective

Samples will be kept for future analyses:

- To evaluate exploratory immune responses to escalating dose levels of MTBVAC and to BCG (including, but not necessarily limited to, analysis of innate response cytokines, specific IgG antibody responses, donor-unrestricted T-cell responses).
- To differentiate vaccine-induced immune responses from natural Mtb infection

4.2 Study Outcome Measures

4.2.1 Primary Outcome Measures

The primary outcome measures are:

Safety and reactogenicity endpoints:

- Solicited systemic adverse events: fever, irritability, vomiting, diarrhea, drowsiness, poor feeding, skin rash.
- Solicited injection site reaction adverse events: pain, redness, swelling, ulceration, drainage, and regional lymphadenopathy
- Unsolicited adverse events and serious adverse events

Immunogenicity endpoints:

- Frequencies of antigen-specific IFN-γ, TNF-α, IL-2, IL-17 and/or IL-22-expressing CD4 and CD8 T cells measured by 12-hour whole blood (WB) intracellular cytokine staining (ICS) assay at Study Days 28, 56, 182, and 365. Stimulating agents at Study Days 28 and 56 will include MTBVAC, ESAT-6, CFP-10, EspC and a “megapool” of peptides containing immunodominant *M. tuberculosis* epitopes (16). Stimulating agents at Study Days 182 and 365 will include MTBVAC, ESAT-6, CFP-10, EspC and a “megapool” of peptides containing immunodominant *M. tuberculosis* epitopes (16) as well as a modified version of the “megapool” but containing only those epitopes shared by both BCG and MTBVAC.

4.2.2 Secondary Outcome Measures

The secondary outcome measures are:

- Quantitative and qualitative results of the QFT Gold Plus assay at Study Day 56, 182 and 365.

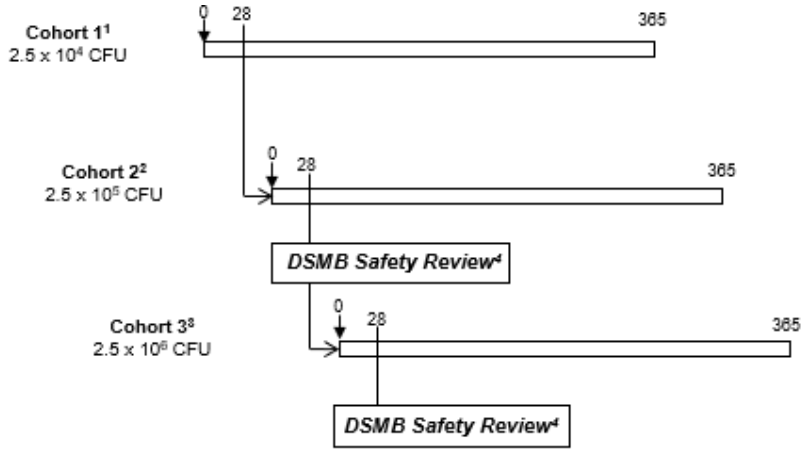
5 STUDY DESIGN

This double-blind, randomized, active controlled, MTBVAC dose-finding trial will evaluate the safety, reactogenicity, immunogenicity, and potential for QFT conversion and reversion of the candidate TB vaccine MTBVAC in HIV uninfected BCG naïve newborns, administered in 3 cohorts at a single intradermal dose of 2.5 x 10^4 CFU (Cohort 1, n=25), 2.5 x 10^5 CFU (Cohort 2, n=25), and either 2.5 x 10^6 CFU (n=25) or 2.5 x 10^5 CFU (n=13) plus 2.5 x 10^4 CFU (n=12) (Cohort 3), compared to BCG vaccine (24 BCG; 75 MTBVAC).

Three dose levels of MTBVAC (2.5 x 10^3, 2.5 x 10^4, 2.5 x 10^5 CFU) have been tested in the current Phase 1b trial in healthy, BCG-naïve, South African newborns; and four dose levels (5 x 10^3, 5 x 10^4, 5 x10^5, and 5 x 10^6 CFU) are being tested in a parallel trial among *M. tuberculosis* infected and uninfected South African adults. It is our hypothesis that the optimal safe and immunogenic MTBVAC dose for infants lies in the range of 2.5 x 10^4 (10-fold lower than BCG) to 2.5 x 10^6 CFU (10-fold higher than BCG). Therefore, this proposed infant trial aims to define the MTBVAC dose-response curve above and below the optimal MTBVAC dose, straddling a 3-log dose range. Final selection of the MTBVAC dose for use in the 3rd cohort will be data driven, based upon the most recent safety and immunogenicity data from all ongoing and completed trials of MTBVAC in infants and adults at that time. Infant safety data are not yet available for the highest (2.5 x 10^6 CFU) MTBVAC dose. Therefore, selection of the 2.5 x 10^6 CFU MTBVAC dose would be contingent upon demonstration of an acceptable safety profile (as defined in Section 3.3.1), both for all lower MTBVAC doses in infants, and for the 5 x 10^6 CFU MTBVAC dose in QFT negative adults (data expected end 2018/beginning 2019). If these safety criteria for the highest MTBVAC dose are not fulfilled, the 2.5 x 10^5 CFU and 2.5 x 10^4 CFU MTBVAC doses would be selected for the last cohort, as described in Section 3.3.1. This approach will build upon data from the current infant pilot study and should allow detailed comparison of safety and immunogenicity of the postulated optimal MTBVAC dose within a 3-log dose range, in a larger infant study population. Refer to Figure 5-1.

In addition, given the crucial importance of understanding the immunological basis for MTBVAC vaccine-induced QFT conversion, and defining the time course and magnitude (IFN-g concentration) of MTBVAC vaccine-induced QFT conversion and reversion events, serial QFT assays will be performed in parallel with whole blood T cell assays.

Figure 5-1 Study Flow Diagram



↓ = vaccination

[1] Cohort 1: n=25 MTBVAC (2.5 x 10^4 CFU) and n=8 BCG
[2] Cohort 2: n=25 MTBVAC (2.5 x 10^5 CFU) and n=8 BCG
[3] Cohort 3: n=25 MTBVAC (2.5 x 10^6 CFU OR 2.5 x 10^5 CFU (n=13) plus 2.5 x 10^4 CFU (n=12)) and n=8 BCG
[4] At each DSMB, all available safety data from previous cohorts enrolled will be reviewed. If any of the safety criteria is not met for the 2.5 x 10^6 CFU dose for Cohort 3, Cohorts 1 and 2 will be expanded so that 33 additional neonates will be randomly allocated to receive the 2.5 x 10^5 CFU dose (n=13), the 2.5 x 10^4 CFU dose (n=12) or BCG (n=8) by default.

6 STUDY ENROLLMENT AND WITHDRAWAL

6.1 Participant Inclusion Criteria

Participants must meet all of the following criteria at the time of randomization:

1. Male or female neonates within 96 hours of birth.
2. Written informed maternal consent, including permission to access maternal antenatal, postnatal and infant medical records.
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 ≥ 2450 grams.
6. Apgar score at 5 minutes ≥ 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 ≥ 37 weeks.
9. Mother has not participated in a clinical trial within 3 months prior to the infant's birth, and if breast-feeding, mother should not participate in a clinical trial during the 12 months of study follow up.
10. Mother has never participated in a TB vaccine trial before.
11. Infant may not participate in any other clinical trial from birth, and for the 12 months of follow up.

6.2 Participant Exclusion Criteria

Participants must have none of the following at the time of randomization:

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. History of close contact with a TB patient, antenatal or postnatal, whether maternal, other family member or another household member who has not yet completed TB treatment.
6. Clinically suspected neonatal sepsis.
7. Any severe congenital malformation.
8. History or evidence of any systemic disease on examination, or any illness that in the opinion of the investigator may interfere with the evaluation of the safety and immunogenicity of the vaccine. Neonatal jaundice not considered clinically significant is not an exclusion.

6.3 Treatment Assignment Procedures

6.3.1 Randomization Procedures

Participants will be randomized using a pre-prepared block randomization schedule, linked to study enrolment number.

Ninety-nine (99) infant participants will be recruited, randomized and allocated into 3 groups: BCG (single dose level 2.5 x 10^5 CFU/0.05 mL, n=24); or MTBVAC at three different dose levels as a single intradermal dose of 2.5 x 10^4 CFU (Cohort 1, n=25), 2.5 x 10^5 CFU (Cohort 2, n=25), and either 2.5 x 10^6 CFU (n=25) or 2.5 x 10^5 CFU (n=13) plus 2.5 x 10^4 CFU (n=12) (Cohort 3) (24 BCG; 75 MTBVAC).

Vaccination of newborns will be staggered to allow gradual evaluation of safety and reactogenicity at each escalation of MTBVAC dose. Progression from the 2.5 x 10^5 to the 2.5 x 10^6 CFU dose level, for which safety has not yet been demonstrated, will require that all of the following criteria have been met: (1) no pre-defined pausing rules have been observed at lower MTBVAC dose levels in this trial; (2) favourable review of day 28 safety data of all lower MTBVAC dose groups by the Data Safety Monitoring Board (DSMB); (3) favourable review of safety data from the current pilot Phase 1b infant trial by the DSMB; and (4) favourable review of day 28 safety data for the 2.5 x 10^6 CFU dose level in QFT negative adults in the parallel adult trial by the DSMB. In the event that any one of these safety criteria are not met, Cohorts 1 and 2 will be expanded so that 33 additional neonates will randomly allocated to receive the 2.5 x 10^4 CFU dose (n=12), the 2.5 x 10^5 CFU dose (n=13) or BCG (n=8) by default. If there is a delay in adult data, dose escalation in infants will either be delayed, or Cohorts 1 and 2 be expanded as mentioned above.

6.3.2 Masking Procedures

MTBVAC and BCG vaccine will be prepared, and allocation concealed by the site pharmacist. All other site staff will be blinded to vaccine allocation, throughout the follow-up period. Individually-sealed code break envelopes will be held by the pharmacist in case emergency unblinding is necessary.

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. It is not yet known whether MTBVAC offers equivalent or better protection against TB compared to BCG. Therefore, measures to minimize risk of TB will be put in place

throughout the study and, after completion of all Study D365 visits in each cohort and unblinding of the clinical team, 'catch up' BCG vaccination will be given to all MTBVAC vaccinated infants once the last participant of each cohort performed the Post D365 visit.

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's 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 Section 10.9).

6.3.3 Reasons for Withdrawal

Participants are free to withdraw from participating in the study at any time.

Rules governing the pausing and stopping of investigational product administration between individuals within a single cohort and between cohorts are presented in Section 10.5.

6.3.4 Handling of Withdrawals

Participants who withdraw from participation in the study will be asked to complete a final visit (see Schedule of Events in Appendix A for procedures to be conducted at next visit, which depend on timing of discontinuation from the study). Participants who discontinue the study will not be replaced.

6.3.5 Termination of Study

Rules for pausing or stopping the study are presented in Section 10.5.

7 STUDY INTERVENTION/INVESTIGATIONAL PRODUCT

7.1 Study Product Description

7.1.1 Acquisition

MTBVAC (live attenuated derivative of *Mtb*) was developed at University of Zaragoza, Spain, by Prof. Carlos Martin, and licensed to Biofabri, SL. MTBVAC and excipient solution for dilution will be supplied by Biofabri.

BCG vaccine (BCG SSI, a WHO pre-qualified BCG vaccine)

Formulation, Packaging, and Labeling

The composition of MTBVAC is shown in Table 7-1. Refer to the most recent version of the Vaccine Management Manual for further details on formulation and packaging for MTBVAC.

Table 7-1 Composition of MTBVAC Study Vaccine

Vaccine Dose[a]	Formulation (approximately per standard dose)	Presentation	Volume
MTBVAC 2.5 x 10^5	1.5 – 8.5 x 10^5 CFU Sucrose Sodium glutamate	Lyophilized pellet in vials (20 doses)	0.05 mL/dose[b]
MTBVAC 2.5 x 10^6	1.5 – 8.5 x 10^6 CFU Sucrose Sodium glutamate	Lyophilized pellet in vials (20 doses)	0.05 mL/dose[b]

a MTBVAC 2.5 x 10^4 dose to be prepared by dilution with excipient solution of 2.5 x 10^5 formulation.
b After reconstitution with 1 mL/vial sterilized water for injection

Refer to the package insert for formulation details of BCG.

7.1.2 Product Storage and Stability

Upon receipt of investigational product, the study vaccine manager must immediately inspect all vials for damage. Investigational product will be shipped with a continuous temperature-monitoring device. Any damage or discrepancies from the packing list must be documented and promptly discussed with Biofabri and the study monitor to determine the appropriate action.

MTBVAC must be stored at -15 to -30°C in a secured location with no access for unauthorized personnel. MTBVAC excipient solution must be stored at 2-8°C.

BCG must be stored at the manufacturer's recommended temperature range of 2-8°C.

Refer to the most recent version of the Vaccine Management Manual for detailed instructions regarding investigational product storage.

7.2 Dosage, Preparation and Administration of Study Intervention/Investigational Product

The administered volume is 0.05 mL/dose for all doses of investigational product and BCG. Refer to the most recent version of the Vaccine Management Manual for detailed instructions regarding investigational product preparation. Refer to Section 6.3.2 for masking procedures.

Participants will be administered one dose of the MTBVAC vaccine or BCG active control on Study Day 0. The vaccine dose will be administered intradermally in the deltoid area. Opsite plaster (breathable) will be used to cover the site of injection immediately after vaccination; the plaster will be removed for the 60 minutes post-vaccination injection site examination and then replaced. The participant's caregiver will be provided with additional plasters and will be instructed to change the plaster as needed. Participants will be instructed to protect the plaster and site of vaccination from water. Participants will be provided with a plastic sample bag and clear instructions on how to replace the plaster and will be asked to retain used plasters 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.

7.3 Accountability Procedures for the Study Intervention/Investigational Product(s)

MTBVAC will be shipped from Biofabri to the clinical trial site. The frequency of product distribution and amount of product distributed will depend on the rate of enrollment of study participants. A WHO pre-qualified BCG vaccine will be supplied by the Sponsor from a registered local supplier or imported under the applicable South African Medicines Control Council regulations.

The study vaccine manager is required to maintain accurate investigational product accountability records. Instructions and forms to be completed and kept for accountability will be provided to the study vaccine manager. If the study vaccine manager wishes to use clinical trial site-specific accountability forms, these must be reviewed and approved in advance by Biofabri. Upon completion of the study, all investigational product management records will be copied, and the copies returned to Biofabri or its designee. The originals must be maintained at the clinical trial site with the rest of the study records.

Biofabri must provide authorization for the disposition of any unused investigational product or BCG. Unused investigational product will be destroyed according to the facility's SOPs. Any disposal of investigational product conducted at the clinical trial site must be documented in the study file.

7.4 Assessment of Participant Compliance with Study Intervention/Investigational Product

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

7.5 Concomitant Medications/Treatments

The collection of information on concomitant medications used by participants following vaccination will coincide with the collection period of adverse events (see Section 10.1). In addition, information on all concomitant medications associated with the treatment of adverse events and serious adverse events will be collected throughout the study.

Concomitant medication includes prescription and non-prescription drugs or other treatments, and any vaccines other than the investigational product. The name of the medication, treatment start and stop dates (or 'ongoing'), and indication must be recorded on the Concomitant Medications electronic case report form (eCRF). The indication recorded on the Concomitant Medications eCRF must correspond to a medical term/diagnosis recorded on the Adverse Event (AE) eCRF, or to a pre-existing condition noted in the participant's medical history, or be noted as prophylaxis, e.g., dietary supplement or routine vaccinations.

8 STUDY SCHEDULE

A Schedule of Events depicting all visit-specific procedures is provided in Appendix A, including the acceptable time windows. Detailed descriptions of procedures to be carried out at each visit are presented in Sections 0 through 8.6.

All participants who are assigned a participant identification number and receive investigational product will be followed according to the protocol unless consent is withdrawn.

Participants will be instructed to contact a study team member to report new signs and symptoms or new or worsening adverse events and will be referred for medical attention as applicable.

During each clinic visit, participants will be reminded to notify a study team member of the following:

- The occurrence of AEs and SAEs during the respective reporting periods.
- Receipt of any concomitant medications during the applicable reporting period.
- Plans to move or if contact information changes.
- If participant has decided to withdraw from the study.
- Change in general health status.
- Any other change in status that may affect the participant's participation (e.g. plan to participate in another investigational study).

If the clinical trial site's study team members are unable to establish contact with a participant who misses a scheduled study visit, the clinical trial site must make every possible effort to re-establish contact and document such efforts. If contact is re-established, then the participant will resume participation in the study. If contact with the participant cannot be re-established by the participant's calculated Study Day 365 visit date, then a determination of "lost to follow-up" should be made on the date of the last attended study visit.

Upon completion of the study, participants will be provided a letter and a card that briefly describe their participation in the study.

8.1 Screening

Written informed consent must be obtained before any screening procedures are performed.

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 women who have consented 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 newborn child 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. The mother will be given a date when she should attend the clinic for the study day 0 visit. If the mother's discharge from the birthing unit is delayed beyond 96 hours the infant will not be eligible for the study.

Screening procedures

- Maternal:
  Written informed consent
  Assign study participant identification (PID) number
  Medical history
  Confirm HIV status (mother to be tested if result is not available or if the test was not done within 30 days prior to delivery)
  History of active TB or household TB contact

- Infant:
  On Road to Health Card: confirm no BCG given at birthing unit, confirm birth weight, gestational age and Apgar score meet eligibility criteria.

8.2 Enrollment/Baseline

Study Day 0 (within 96 hours of birth)

Pre-vaccination:

1. Verbally reconfirm maternal consent.
2. Confirm absence of acute illness in infant.
3. Medical history and update of concomitant medication.
4. Screening for potential new TB exposure and TB symptoms.
5. Vital signs:

- Heart rate, respiratory rate, weight, recumbent length.
- Axillary temperature: must be <38°C.

6. History and physical examination by investigator.
7. Confirm eligibility for study entry and vaccination.
8. Complete Vaccine Request Form, send to study vaccine manager (study pharmacist) who will randomise participant into a study arm according to the next available treatment number in the final randomisation schedule, and then prepare study vaccine.

Vaccination (by Vaccination Nurse):

1. Inspect syringe and vaccine volume, confirm PID number, date and time of dose preparation.
2. Administer study vaccine intradermally into left deltoid area of the infant's arm. Record date and time of vaccination, presence of a bleb. Apply Opsite plaster.

Post-vaccination:

1. Monitor participant for acute adverse events for at least 60 minutes after vaccination.
2. Obtain 60±10 minutes post-immunization vital signs (pulse, axillary temperature).
3. Examine site of injection 60±10 minutes post-immunization (includes measurement of redness, swelling, and ulceration); replace Opsite plaster.
4. Record any adverse events, including systemic solicited adverse events, unsolicited adverse events, solicited and unsolicited injection site reactions, and serious adverse events (including concomitant medications).
5. Distribute and review diary card and diary card instructions with participant; review injection site care instructions and distribute Opsite plasters and plastic Ziploc bags.

8.3 Follow-up

Study Day 7

Allowable window for clinic visit is 7±3 days from Study Day 0 visit date.

1. Verbally reconfirm consent.
2. Update medical history including use of medications.
3. Screening for potential new TB exposure and TB symptoms.
4. Record feeding practice.
5. Vital signs (heart rate, respiratory rate, axillary temperature, weight and recumbent length).
6. Focused physical examination.
7. Collect and review solicited AE and medication diary card entries for completeness and accuracy. Redistribute AE and medication diary card.
8. Record any adverse events, including systemic solicited adverse events, unsolicited adverse events, solicited and unsolicited injection site reactions, and serious adverse events (including concomitant medications).
9. Examine site of injection (includes measurement of redness, swelling, and ulceration) and take a pus swab if discharge present.

10. Blood collection for:
    - Serum chemistry: includes AST, ALT, GGT, total bilirubin, creatinine.
    - Hematology: includes full blood count with differential, and platelet count.

Study Day 28

Allowable window for clinic visit is 28±3 days from Study Day 0 visit date.

1. Verbally reconfirm consent.
2. Update medical history including use of medications.
3. Screening for potential new TB exposure and TB symptoms.
4. Record feeding practice.
5. Vital signs (heart rate, respiratory rate, axillary temperature, weight and recumbent length).
6. Focused physical examination.
7. Collect and review solicited AE and medication diary card entries for completeness and accuracy.
8. Record any adverse events, including systemic solicited adverse events, unsolicited adverse events, solicited and unsolicited injection site reactions, and serious adverse events (including concomitant medications).
9. Examine site of injection (includes measurement of redness, swelling, and ulceration) and take a pus swab if discharge present.
10. Blood collection for:
    - Immunogenicity WB-ICS

Study Day 56

Allowable window for clinic visit is 56±7 days from Study Day 0 visit date.

1. Verbally reconfirm consent.
2. Update medical history including use of medications.
3. Screening for potential new TB exposure and TB symptoms.
4. Record feeding practice.
5. Vital signs (heart rate, respiratory rate, axillary temperature, weight and recumbent length).
6. Focused physical examination.
7. Record any adverse events, including unsolicited adverse events, solicited and unsolicited injection site reactions, and serious adverse events (including concomitant medications).
8. Examine site of injection (includes measurement of redness, swelling, and ulceration) and take a pus swab if discharge present.
9. Blood collection for:
    - Immunogenicity WB-ICS
    - QuantiFERON®-TB Gold Plus test

Study Day 120 ±7 days and Study Day 270 ±7 days

Home / telephonic visit if infant tested QFT positive at D56 to enquire about TB signs and symptoms and general well-being

Study Day 182

Allowable window for clinic visit is 182 ±14 days from Study Day 0 visit date.

1. Verbally reconfirm consent.
2. Update medical history including use of medications.
3. Screening for potential new TB exposure and TB symptoms.
4. Record feeding practice.
5. Vital signs (heart rate, respiratory rate, axillary temperature, weight and recumbent length).
6. Focused physical examination.
7. Record any adverse events, including unsolicited adverse events and serious adverse events (including concomitant medications).
8. Examine site of injection (includes measurement of redness, swelling, and ulceration) and take a pus swab if discharge present.
9. Blood collection for:
   - Immunogenicity WB-ICS
   - QuantiFERON®-TB Gold Plus

Partial Cohort Unblinding of Data Post Study Day 182

Once the last Cohort 3 Study D182 visit has been completed, an independent biostatistician be unblinded to available study data. At this point Cohort 1 and Cohort 2 participants will have completed all study visits and these participants and clinical team will be unblinded post Study D365 visit. All available safety data for all cohorts up to the last Cohort 3 Study D182 visit completion; and immunogenicity data for all cohorts up to each cohort's Study D182 visit, will be analysed by the independent, unblinded biostatistician and provided to DSMB for review to determine MTBVAC dose selection for the Phase III study. The Sponsor will review blinded data only in order to keep the blind until post Study D365 visit. The DSMB will be provided with the same blinded data along with unblinding vaccine recipient lists. The DSMB review will allow for a blinded recommendation and input into the dose selection for the Phase III study. For all cohorts, unblinding of the clinical team and participants will take place post Study D365 visit.

Note: The immunology team will remain blinded until immunology database lock.

8.4 Final Study Visit

Study Day 365

Allowable window for clinic visit is 365 ±14 days from Study Day 0 visit date.

1. Verbally reconfirm consent.
2. Update medical history including use of medications.
3. Screening for potential new TB exposure and TB symptoms.
4. Record feeding practice.
5. Vital signs (heart rate, respiratory rate, axillary temperature, weight and recumbent length).
6. Focused physical examination.
7. Record any serious adverse events (including concomitant medications) and unsolicited adverse events.
8. Examine site of injection (includes measurement of redness, swelling, and ulceration) and take a pus swab if discharge present.
9. Blood collection for:
   - Immunogenicity WB-ICS
   - QuantiFERON®-TB Gold Plus

Post Cohort Unblinding of Participants Post Study Day 365

Participant, and subsequent clinical team unblinding will occur when all infants in a given cohort have attained their Study Day 365 visit.

1. MTBVAC recipients who have tested QFT positive will be given a card stating that they have received an investigational vaccine that may interfere with interpretation of future IGRA and TST tests.
2. BCG rescue dose will be offered to all MTBVAC recipients in this cohort, given that the individual recipients have a negative screen for active TB disease and new household contacts, and do not have a new positive QFT result at D365. Any MTBVAC recipient receiving IPT will be offered the BCG rescue dose after completion of IPT.
3. The site will contact the participant's caregiver telephonically (or perform a home visit if no telephone available) 14 days post vaccination with the BCG rescue dose to enquire about the infant's wellbeing and BCG injection site reaction, and respond appropriately to any adverse reaction.

Note: The immunology team will remain blinded until immunology database lock.

8.5 Early Termination Visit

Visit to be conducted if participant discontinues from study prior to Study Day 365.

1. Update medical history including use of medications.
2. Screening for potential new TB exposure and TB symptoms.
3. Record feeding practice.

4. Vital signs (heart rate, respiratory rate, axillary temperature, weight and recumbent length).
5. Focused physical examination.
6. Record any adverse events, including unsolicited adverse events, and serious adverse events (including concomitant medications).
7. Examine site of injection (includes measurement of redness, swelling, and ulceration) and take a pus swab if discharge present.
8. Blood collection for:
   - QuantiFERON-PLUS TB
   - Confirm contact details for possible BCG rescue dose once all infants in cohort have completed D365 visit.

8.6 Unscheduled Visit

For emergencies and other unscheduled visits to a medical facility other than the study clinic, medical records will, to the extent possible, be obtained by the investigator.

Should visits to the clinical trial site occur outside of the scheduled study visits, these will be documented in the source notes as an unscheduled visit and captured in the Electronic Data Capture system as an unscheduled visit. Data will be reviewed as per all scheduled visits.

9 STUDY PROCEDURES/EVALUATIONS

9.1 Clinical Evaluations

Participants will have vital signs taken prior to study vaccination. Participants will remain in the clinic under close observation for at least 60 minutes after receiving investigational product. Vital signs will be repeated at 60±10 minutes post-vaccination before participants leave the clinic. Allergic reactions to vaccination are possible; therefore, appropriate paediatric drugs and medical equipment to treat acute paediatric anaphylactic reactions must be immediately available, and a medically qualified study team member trained to recognize and treat anaphylaxis must be present in the clinic during the entire vaccination procedure and post-vaccination monitoring period.

Clinical assessments to be performed at each visit are summarized in Appendix A and listed by visit in Section 8.

Participants' caregivers will receive, and be instructed in, the operation of a daily adverse event diary, ruler (for measurement of redness, swelling, and ulceration), and a digital thermometer to measure axillary temperature, to be used during the 7 days post-vaccination diary period after investigational product administration. The daily adverse event diary is a tool to help aid the principal investigator or designee to engage in a conversation with the participant's caregiver about any AEs that may have occurred between visits. During scheduled visits through the specified diary period and after investigational product administration, the daily diary will be collected and reviewed by the principal investigator or designee at which time any clinical details required for complete understanding of the information recorded will be obtained. If possible, diaries not brought to the scheduled visit should be obtained before adverse event assessment can be performed and events discussed with the principal investigator or designee. If a diary is lost, the principal investigator or designee will discuss the occurrence of any solicited adverse events with the participant's caregiver and document the discussion. Information on the diary card will not be directly recorded onto eCRFs. The diary card will be considered source documentation and adverse event information obtained from the diary card will be recorded and completely assessed on the adverse event eCRF. Axillary temperatures below 38°C will not be considered fevers.

Any change to an observation or event recorded by the participant's caregiver on the diary card (e.g., the severity level of an event is changed after interviewing the participant's caregiver), based on the investigator's evaluation of the event, must be explained by notation in source documentation by the principal investigator or designee.

9.1.1 Screening for TB and referral for Isoniazid Preventive Therapy

All infants will undergo screening for potential new TB exposure and TB symptoms at each visit and caregivers will be encouraged to report such symptoms or any new household TB contact if they occur in between visits. If TB symptoms or potential new TB exposure are

detected these infants will be investigated for active TB disease and may be referred to the public health clinic for initiation of TB treatment if indicated or IPT if active TB is ruled out.

Early (D56) post-vaccination QFT conversion in infants with low risk of natural *M. tuberculosis* exposure is assumed to be vaccine-induced and will not be treated with isoniazid preventive therapy (IPT). Infants who demonstrate early QFT conversion at D56 will undergo enhanced screening for potential new TB exposure and TB symptoms during all scheduled study visits, and in addition, during interval telephonic contacts at D120 and D270. QFT samples will be collected at D182 and D365 from infants who tested QFT positive at D56, and stored for later analysis, but since a positive QFT result would not be interpretable, these data will not be used for clinical decision-making. All infants who received MTBVAC and tested QFT positive will receive a card at the end of study indicating that they have received an experimental vaccine that may interfere with interpretation of future IGRA and TST tests for TB infection..

QFT assays will be repeated at D182 and D365 in infants who tested QFT negative at D56. These results will be made available for clinical decision-making. Infants with new QFT conversion events at D182 or D365, and any infant with a reported new household TB contact regardless of QFT status, will be referred to the public health services for IPT since these infants would potentially benefit from TB prophylaxis against *M. tuberculosis* infection as per SA national guidelines, provided that signs and symptoms of active TB disease are absent.

9.2 Laboratory Evaluations

9.2.1 Clinical Laboratory Evaluations

Laboratory tests to be performed at each visit are summarized in Appendix A (including approximate phlebotomy volumes) and listed by visit in Section 8.

9.2.1.1.1. Special Assays or Procedures

A summary of immunologic assays to be performed on blood specimens is shown in Table 9-1.

Table 9-1 Summary of Immunology Laboratory Evaluations

Sample Type	Assay	Immunology Endpoint	Approximate Blood Volume (per Visit)	Study Days	Name and Location of Analysis Laboratory
Whole blood	12-hour WB ICS[a] for analysis of T cell responses	Primary	2 to 4 mL[b]	28, 56, 182, 365	SATVI, South Africa
QFT Gold Plus	QFT conversion by MTBVAC	Secondary,[c]	4 mL	56, 182, 365	SATVI, South Africa
Supernatants from whole blood ICS assay	ELISA/ Multiplex bead array for analysis of innate response cytokines	Future Exploratory	Not applicable	28, 56, 182, 365	SATVI, South Africa

a. Stimulation conditions for days 28, 56, 182 and 365 include MTBVAC, CFP-10, ESAT-6, EspC and a "megapool" of peptides containing immunodominant *M. tuberculosis* epitopes, Nil and PHA. The higher blood volume at days 182 and 365 allows for an additional stimulation condition of a modified version of the "megapool" but containing only those epitopes shared by both BCG and MTBVAC to be added.
b. Depending on age of infant as explained in footnote to Schedule of Events (Appendix A)
c. If QFT positive at D56, D182 and D365 QFT samples will be stored for later analysis

9.2.2 Specimen Preparation, Handling, and Shipping

9.2.2.1 Instructions for Specimen Preparation, Handling, and Storage

Staff at the clinical trial site will refer to the most current version of the Laboratory Manual (provided under separate cover) for further instructions and additional information on specimen collection and processing.

9.2.2.2 Specimen Shipment

Staff at the clinical trial site will refer to the most current version of the Laboratory Manual (provided under separate cover) for further instructions and additional information on specimen shipping.

10 ASSESSMENT OF SAFETY

Responsibilities for Ensuring the Safety of Trial Participants

The national regulatory authority, the study sponsor (Biofabri), the institution through which the research is performed, and all members of the principal investigator's clinical team, share responsibility for ensuring that participants in this trial are exposed to the least possible risk of adverse events that may result from participation in this protocol.

Principal Investigator

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 a participant's investigational product administration or pause investigational product administration in the whole trial if the investigator has some suspicion that the investigational product might place a participant at significant risk. The principal investigator determines severity and causality with respect to the investigational product for each adverse event.

Responsibilities of the principal investigator may be assigned to a designee who is a medically qualified team member (who must be a sub-investigator in the case of assessment of adverse events); however, the accountability for the specific task remains with the principal investigator.

Study Sponsor

The sponsor (Biofabri) also has an institutional responsibility to ensure participant safety. This responsibility is vested in the local medical monitor and a DSMB (see Section 10.7).

Local Medical Monitor

The local medical monitor is a credentialed physician or surgeon in their country of residence with the necessary expertise to act in such capacity. The local medical monitor reviews the safety of the product for protocols in a specific region and, in conjunction with the sponsor, determines expectedness of related serious adverse events. The local medical monitor, in consultation with the sponsor, may assess the causality for adverse events and may upgrade or downgrade the causality determined by the principal investigator. The local medical monitor, like the principal investigator, is blinded.

Institutional Review Boards and Ethics Committees

The IRB/IEC has institutional responsibility for the rights, safety, and welfare of participants in clinical trials. The IRB/IEC has the authority to terminate, suspend or require changes to a clinical trial.

National Regulatory Authority

Since the national regulatory authority receives all expedited safety reports, it also has the authority to terminate, suspend or require changes to a clinical trial.

10.1 Specification of Safety Parameters

Safety parameters that constitute the primary outcome measures of the study are described in Section 4.2.1. The time periods for collection of adverse events are as follows.

Unsolicited Adverse Events

Unsolicited adverse events will be collected from informed consent until trial completion.

Solicited Adverse Events

Solicited systemic adverse events will be collected post-vaccination through the Study Day 28 visit (with diary cards to be used for 7 days after vaccination).

Solicited injection site reactions will be collected post-vaccination through the Study Day 56 visit (with diary cards to be used for 28 days after vaccination).

Solicited adverse events are events the participant is specifically asked about. These adverse events are commonly observed soon after receipt of vaccines. For this study, solicited adverse events to be collected include:

- Solicited systemic adverse events:

  fever, irritability, vomiting, diarrhea, drowsiness, poor feeding, skin rash.

- Solicited injection site reaction adverse events:

  pain, redness, swelling, ulceration, drainage, and regional lymphadenopathy.

Solicited adverse events of injection site reactions will be considered causally related to investigational product.

All participants' caregivers will be provided a diary card to record axillary temperature and information regarding occurrences of these specific events for the first 7 days (for systemic adverse events) or 28 days (for injection site reaction adverse events) of the solicited adverse event reporting period.

Serious Adverse Events

Serious adverse events will be collected throughout the entire study period (i.e., 365 days post-vaccination).

Site of Injection Examination

Site of injection examinations will be performed at the visits noted in the schedule of events in Appendix A. The following will be assessed at each examination:

- Tenderness/pain, redness, swelling, warmth, ulceration, presence of infection/abscess, crusting, drainage, scarring, and regional lymphadenopathy.

Any of the above-mentioned injection site reactions that meet adverse event criteria according to the Toxicity Table in Appendix C will be recorded as adverse events.

10.2 Methods and Timing for Assessing, Recording, and Analyzing Safety Parameters

10.2.1 Adverse Events

Definition of an Adverse Event

An adverse event (AE) means any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. An adverse event (also referred to as an adverse experience) can be any unfavorable and unintended sign (e.g., an abnormal laboratory finding), symptom, or disease temporally associated with the use of a drug and does not imply any judgment about causality. An adverse event can arise with any use of the drug (e.g., off-label use, use in combination with another drug) and with any route of administration, formulation, or dose, including an overdose.

Medical conditions that exist prior to administration of the investigational product (pre-existing conditions) will be recorded in the participant's medical history to establish baseline. Day-to-day fluctuations in pre-existing conditions that do not represent a clinically significant change in the participant's status will not necessarily be reported as adverse events.

Any adverse change from the participant's baseline condition (determined from screening evaluations conducted to confirm study eligibility) that occurs following the administration of the investigational product will be considered an adverse event. This includes the occurrence of a new adverse event or the worsening of a baseline condition, whether or not considered related to the investigational product. Intermittent conditions may be present on Study Day 0 but may represent an adverse event if the intensity or duration of the event is worse than usual following receipt of investigational product. Adverse events include but are not limited to: adverse changes (including clinically significant laboratory and vital sign changes) from baseline that represent increases in toxicity grade according to the Toxicity Table (see protocol appendices), adverse changes in the general condition of the participant, signs and symptoms noted by the caregiver of the participant, concomitant disease with onset or increased severity after investigational product administration, and clinically significant abnormalities in laboratory safety parameters occurring after investigational product administration.

The reporting period for all adverse events is specified in Section 10.1. Adverse events will be reported using a recognized medical term or diagnosis that accurately reflects the event. Adverse event evaluations will be reviewed by the principal investigator or sub-investigator. AE information is to be completed by members of the study team designated in writing by the principal investigator. The onset and resolution dates of the event and action taken in response to the event will be documented.

Assessing Severity

The safety concepts of "severity" and "seriousness" are distinct concepts (see Section 10.2.3). "Severity" refers to a degree of clinical manifestation. "Seriousness" refers to defined outcomes from an adverse event. A severe adverse event is not always serious, and a serious adverse event is not always severe.

For all adverse events, the investigator is responsible for assessing the severity of the event and the causal relationship of the event to the investigational product.

The severity of all adverse events, including clinical findings and abnormal laboratory values, will be classified as one of the following grades:

1. **Mild**
2. **Moderate**
3. **Severe**

Toxicity Tables are provided in the protocol appendices for the assessment of severity of specified adverse events. The Toxicity Table Adverse Event Grades do not correlate directly with the classical severity grades of mild, moderate and severe. FOR THE PURPOSES OF RECORDING EVENTS ON THE eCRF, Toxicity Table Grade 1 events will be considered mild in severity, Toxicity Table Grade 2 events will be considered moderate in severity, and both Toxicity Table Grade 3 and 4 events will be considered as severe.

For adverse events not listed in the Toxicity Table determination of severity requires some level of interpretation as outlined below. The degree of incapacity caused by the adverse event and the level of medical intervention required for treatment may be helpful in assessing the overall severity of the adverse event.

For example:

- "Mild" events are generally regarded as noticeable but have no impact on normal activities; they may or may not require over-the-counter treatment managed by the participant's caregiver.
- "Moderate" events generally have some impact on an individual's normal activities and may require general symptomatic medical intervention by a healthcare professional or by the participant's caregiver.
- "Severe" adverse events may be incapacitating, leading to suspension of normal daily activities, and would generally require more immediate medical evaluation and intervention by a healthcare professional.

A change in severity of an adverse event will not be recorded as a new adverse event. Only the highest severity level that occurs during the entire period of the adverse event will be recorded on the eCRF with the onset and resolution dates encompassing the entire duration of the event.

Assessing Causal Relationship (Relatedness)

For all adverse events, the investigator and, for SAEs, the sponsor and local medical monitor, will determine a **causal relationship** to the investigational product without knowledge, for blinded studies, of whether active vaccine or active control was administered. A number of factors will be considered in making this assessment, including: 1) the temporal relationship of the event to the administration of the investigational product 2) whether an alternative etiology has been identified, and 3) biological plausibility.

The investigator will use the following guidelines to assess the causal relationship of an adverse event to study vaccine:

- **Not Related** to study vaccine (i.e., there is no evidence of a causal relationship; another etiology is known to have caused the adverse event. The alternative etiology should be documented in the participant's study record).
- **Unlikely Related** to study vaccine (i.e., there is less than a reasonable possibility that the adverse event was caused by study vaccine).
- **Possible** relationship to study vaccine (i.e., there is a reasonable possibility that the adverse event was caused by study vaccine. There must be a plausible mechanism for the event to be related to study vaccine. The evidence is inadequate to accept or reject, or favours rejection of, a causal relationship; an association exists between the event and the study vaccine but there may also be an alternative etiology, such as characteristics of the participant's clinical status or underlying condition).
- **Probable** relationship to study vaccine (i.e., it is likely that the adverse event was caused by administration of the study vaccine. The evidence favours acceptance of a causal relationship; an association exists between the event and receipt of the study vaccine and there is a plausible mechanism for the event to be related to the study vaccine, and an alternative etiology is not apparent).
- **Definite** relationship to study vaccine (i.e., the study vaccine is known to be the cause of the adverse event. The evidence establishes a causal relationship; an association exists between the event and receipt of the study vaccine and there is a plausible mechanism for the event to be related to the study vaccine and causes other than the study vaccine have been ruled out).

It is expected that communication and consultation with the sponsor and local medical monitor/research monitor will occur in the assessment of the causality of serious adverse events.

Every effort should be made by the investigator to determine the existence of any pre-existing conditions (e.g., medical conditions on Study Day 0 with onset prior to study vaccination) that

must be taken into consideration when assessing causal relationship of an adverse event. Pre-existing conditions should be recorded in the eCRF as baseline history and substantiated by appropriate source documentation. Intermittent conditions may not be present on Study Day 0 but may represent an adverse event if the intensity or duration of the event is worse than usual following investigational product.

Definition of an Adverse Reaction

An adverse reaction is an adverse event judged to be related to investigational product.

Assessing Expectedness of Adverse Events

Expected adverse events are adverse events consistent with the applicable product information provided by the sponsor (the investigator's brochure for an investigational product). The sponsor, in conjunction with the local medical monitor/research monitor, determines expectedness of related serious adverse events.

10.2.2 Reactogenicity (for Vaccine Studies and Some Therapeutic Trials)

Reactogenicity events are referred to as solicited adverse events. Refer to Section 10.1 for a list of solicited adverse events (systemic and injection site reactions) and timing of collection. Diary cards will be provided to participants for recording of solicited systemic adverse events (for 7 days) and solicited injection site reactions (for 28 days; see Section 9.1 for further details regarding diary cards).

10.2.3 Serious Adverse Events

Definition of Serious Adverse Event

Seriousness refers to the outcome of an adverse event. Seriousness is determined by both the investigator and the local medical monitor. If the 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 investigational product, 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).
- 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. Recording and follow up of serious adverse events is discussed in Section 10.4.

Definition of Suspected Unexpected Serious Adverse Reaction (SUSAR)

When an adverse event is judged to be related to an investigational product, is judged to be serious and unexpected, and is in a participant who received active vaccine, it is a SUSAR (suspected unexpected serious adverse reaction) and is subject to expedited reporting.

10.2.4 Procedures to be Followed in the Event of Abnormal Laboratory Test Values or Abnormal Clinical Findings

Results from clinical assessments and laboratory tests obtained on the study must be reviewed by the investigator, and the participant managed in accordance with the clinical trial site policies. The clinical significance of any change in a vital sign or laboratory parameter will be determined by the investigator and may be reported as an adverse event at the discretion of the investigator (using the toxicity tables in Appendix B, C and D to grade severity). Additional laboratory tests may be performed if the investigator deems them to be necessary to fully evaluate an adverse event. In the event that the investigator elects to order non-protocol-specified laboratory tests, the investigator must record the rationale for the tests and a determination of clinical significance of the result in the source documents. The investigator must keep the medical monitor informed of vaccine-related adverse events of clinical significance.

Abnormal results and findings will be discussed as applicable with the participant, and the participant will be referred for follow-up with their healthcare provider if necessary.

10.3 Reporting Procedures

The reporting period for all adverse events is specified in Section 10.1. Reporting procedures for adverse events are described in Section 10.2.1.

10.3.1 Serious Adverse Events

Serious adverse events, which include SUSARs, will be reported by the PI to the sponsor, local medical monitor, UCT HREC and TCD in an expedited fashion. TCD RA in turn will notify SAHPRA according to their required timelines . SUSARs are reported even after the trial is over, if the principal investigator/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 eCRF. However, follow-up of the SAE must continue until resolved. Information recorded on the eCRF 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. SAHPRA stipulates that a SUSAR is to be reported to SAHPRA 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 SAHPRA 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 SAHPRA 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.

The sponsor will notify the DSMB and the manufacturers of MTBVAC and BCG of all SUSARs within 3 working days of becoming aware of an event and will provide all follow-up information in a timely manner.

Investigators must not wait to collect additional information to fully document the event before notifying the local medical monitor/research monitor of a serious adverse event. The initial notification should include the following (at minimum):

- Protocol number and name and contact number of the investigator
- Participant ID number (and initials and date of birth, if available)

- Date participant received investigational product
- Serious adverse event(s) and date of event onset
- Current status of participant

10.3.2 Suspected and Confirmed Tuberculosis Events

Any adverse event suspected to be or confirmed as tuberculosis disease will be assessed, documented and reported according to the same procedures as for serious adverse events (refer to section 10.3.1), even if the event does not meet any of the serious adverse event criteria as per section 10.2.3. Such suspected or confirmed tuberculosis disease events will be classified as Immediately Reportable Events (IREs). These 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 eCRF. However, follow-up of the IRE must continue until resolved. Information recorded on the eCRF must be substantiated in the source documents.

10.3.3 Other Immediately Reportable Events

The investigator must report the following events to the sponsor within 24 hours of becoming aware of the event:

- Withdrawal of consent during the study for safety reasons
- Emergency unblinding (see Section 10.6)
- Protocol violation affecting the safety of a participant or involving the vaccination process
- Adverse event thought to be an allergic reaction to the investigational product
- Any event that, in the opinion of the investigator, precludes further administration of the investigational product

10.4 Type and Duration of Follow-up of Participants after Adverse Events

The duration of follow up for AEs is described in Section 10.1.

Treatment of any adverse events will be determined by the investigator using his/her best medical judgment and according to current clinical practice guidelines. All applied measures as well as follow-up will be recorded in the appropriate eCRF.

Adverse events will be considered resolved when the condition returns to normal or returns to the participant's baseline status as established on Study Day 0, or when the condition has stabilized with the expectation that it will remain chronic.

The investigator will continue follow-up on adverse events, including laboratory abnormalities and solicited adverse events, until the event has resolved, is otherwise satisfactorily explained, or the participant completes the study. The resolution date will be recorded on the eCRF as the last date on which the participant experienced the adverse event. If an adverse event resolution date is uncertain the investigator should estimate the completion date based on medical judgment and interview of the participant. Approximate dates of resolution from interviews may be taken as adverse event resolution dates. Some examples of estimation of adverse event resolution are: 1) an asymptomatic laboratory abnormality on one visit that has not been followed-up between visits but has resolved by the next visit may be assumed to have resolved by the midpoint of the inter-visit interval; 2) A resolved adverse event that was treated may be assumed to have been resolved by the end of treatment. Adverse events that are still present at the end of the trial should be recorded as ongoing. Information recorded on the eCRF must be substantiated in the source documents. If an adverse event evolves into a condition that becomes "serious," it will be designated as serious on the AE eCRF and a Supplemental SAE Report (SAER) form will be completed.

Follow-up for serious adverse events must continue until resolution and the outcome reported to the sponsor, even if this extends beyond the serious adverse event reporting period (i.e., after the final study visit). For analysis purposes, the outcome for serious adverse events will be determined on the final study visit.

Outcome of all adverse events will be classified as one of the following:

- Resolved
- Resolved with sequelae
- Ongoing
- Death

If at any time after completion of the serious adverse event reporting period (the final study visit) the investigator becomes aware of a serious adverse event that is suspected by the investigator to be related to the investigational product, the event must be reported to the sponsor.

10.5 Pausing Rules

These rules govern the pausing and stopping of investigational product administration at any time during the study such as between individuals within a single cohort, and between cohorts. 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 SAHPRA 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 SAHPRA 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, SAHPRA, 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 SAHPRA.

The following rules will trigger pausing by the principal investigator, local medical monitor, or the sponsor of further screening, enrollment, and investigational product administration, and DSMB review of safety data:

1. Severe (e.g., painful, draining, infected [abscess]) or large (e.g., >10.00 cm induration and erythema, and >3.0 cm ulceration) injection site reaction, excluding local injection site reactions that decrease to <Grade 3 within 24 hours.
   OR
2. Large (≥5 cm) local regional lymphadenopathy or suppurative lymphadenitis.
   OR
3. One or more SAE(s) judged related to investigational product occurs.
   OR
4. Anaphylaxis or bronchospasm within 4 hours of injection, indicative of an immediate hypersensitivity reaction to the study injection.
   OR
5. Two or more participants in any given cohort experience a Grade 3 or higher event, excluding local injection site reactions, judged related to investigational product.
   OR
6. An adverse event pattern of concern occurred.

If the principal investigator, local medical monitor or sponsor pauses the study, the decision will be recorded in a memorandum to the study file and will trigger DSMB review. If a recommendation to resume study enrollment and investigational product administration is made, the DSMB will record their judgment in a memorandum to the study file and notify the sponsor. The DSMB memorandum will be forwarded to the medical monitors and principal investigator. The DSMB may recommend resumption of enrollment with changes to the protocol if it judges that such changes will reduce safety risks. However, the final decision to resume study activities, amend the protocol, or terminate the study will be made by the sponsor. The clinical trial site will be allowed to resume activities only upon receipt of written notification from the sponsor. Decisions regarding pausing and resumption of the study will be communicated promptly to the IRB/IEC by the principal investigator and to the applicable national regulatory authority by the sponsor (or designee).

In addition, the study may be discontinued at any time by the applicable national regulatory authority, the IRB/IEC, the sponsor, or other governmental agencies as part of their duties to ensure that research participants are protected.

10.6 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 local medical monitor 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.

10.7 Safety Oversight (DSMB)

The DSMB, composed of a panel of independent experts, will operate according to its charter. If investigational product administration is paused for safety reasons by the local medical monitor or the principal investigator, the DSMB will be convened. Based on its review and the protocol pause rules (Section 10.5) the DSMB will make recommendations to the sponsor regarding further conduct of the study and further administration of investigational product. The DSMB may review an individual SAE or it may choose to review adverse events, serious adverse events, solicited adverse events, and laboratory and vital signs data. The DSMB may unblind any amount of safety information needed to conduct their assessment. All procedures associated with this review, including objectives, data handling, and elements to be included for review will be documented in DSMB minutes. The DSMB may recommend suspension or resumption of enrollment and investigational product administration after review of safety data. However, the sponsor will make the final decision to suspend or resume study activities. The recommendations of the DSMB, along with the sponsor's decision, will be communicated to the investigators, IRB/IEC, and the national regulatory authorities. The sponsor or its designee agrees to abide by any directives issued by the national regulatory authorities or the IRB/IEC.

The DSMB will conduct three planned safety data reviews during dose-escalation, as described in Section 12.3.1. Two planned safety data reviews will solely focus on safety, with a third safety data review including the review of immunogenicity data together with safety data to confirm the safest and most immunogenic dose of MTBVAC, as described in Section 12.3.1 and 12.3.2.

10.8 Trial Steering Committee (TSC)

The Sponsor will establish a Trial Steering Committee (TSC) before the start of the clinical trial. The TSC will, based on a TSC charter, supervise clinical trial conduct as well as consider the advice from the DSMB. An independent expert member will chair the TSC that will furthermore be composed of 2 additional independent expert members. A representative of EDCTP will participate as an observer. The TSC will monitor trial progress and conduct and provide advice on scientific credibility. The TSC will consider the recommendations of the DSMB and will provide additional advice to the Sponsor as to whether a trial needs to be stopped, suspended, or resumed on scientific grounds and/or efficacy. However, the sponsor will make the final decision to stop, suspend or resume study activities. The TSC will meet annually in person and via Skype on a regular basis, or ad hoc when required. The TSC will reach advice by consensus and will minute meetings.

Any resolution leading to a protocol amendment, pausing rules or as per safety consideration, will be communicated to the regulatory authorities and Ethics Committee. This is in addition to the regular 6-monthly Study progress reports.

A European Ethics Committee, “**Comité Autonómico de Ética da Investigación de Galicia” (Sergas),** will be involved in the study as ethical advisor. This Committee will be informed from the beginning and throughout the study on Study Progress Reports, any Protocol amendment as well as any Safety Event that may occur.

11 CLINICAL MONITORING

11.1 Site Monitoring Plan

The study will be monitored regularly, according to the protocol-specific monitoring plan, by the sponsor or its designee throughout the study period. Monitoring will be a combination of on-site monitoring and central review of data in the electronic database. One hundred percent (100%) of data will be reviewed centrally and in-house review of data will be done on an ongoing basis with formal review meetings approximately monthly. On-site monitoring will occur approximately monthly during recruitment and vaccination periods and approximately every 2 months during follow up. On-site monitoring will include source data verification of approximately 80% of study data.

12 STATISTICAL CONSIDERATIONS

12.1 Study Hypotheses

No formal hypothesis testing will be performed, since the trial is not powered to do any formal testing (see sample size section below).

12.2 Sample Size Considerations

The sample size for this study was selected as being adequate for a preliminary assessment of the safety profile of MTBVAC in neonates, rather than for statistical reasons. The sample sizes of Phase 1 and 2a clinical trials are conventionally not powered to detect rare or infrequent events, but rather common safety events, major safety signals, or important trends. The sample size of 99 infants was selected as adequate for such initial evaluation of the safety profile of MTBVAC, rather than for statistical reasons (17-25).

12.3 Planned Interim Analyses

12.3.1 Safety Review

Pausing rules are described in Section 10.5. The DSMB (see Section 10.7) will conduct two safety data reviews during dose-escalation, at the following time points:

- Participants in Cohort 2 have completed the Study Day 28 visit.
- Participants in Cohort 3 have completed the Study Day 28 visit.

Dose-escalation will proceed as described in Section 5, as long as no pausing rules are triggered.

In addition to dose-escalation DSMB safety data reviews, an additional safety data review will take place at the following time point:

- Participants in Cohort 3 have completed the Study Day 182 visit.

Dose selection of MTBVAC for the Phase III study will proceed based on the results of the analysis of all of the safety data available for all cohorts up to the time that Cohort 3 Study D182 visit has been completed.

12.3.2 Immunogenicity or Efficacy Review

An immunogenicity review will be completed by the DSMB to determine the most immunogenic (and safe) MTBVAC dose for dose selection for the Phase III study. This analysis is planned to take place once the last Study Day 182 visit of Cohort 3 has been completed and all available immunology samples across Cohort 1, 2, and 3 at Study D28, D56 and D182 visits have been processed. No unblinding of the clinical and immunology team to the

immunogenicity data analysis is expected for this review. An independent, unblinded biostatistician will complete the analysis on the raw immunogenicity data which will be provided by the immunology team. The independent, unblinded biostatistician will analyse MTBVAC dose selection based on magnitude of MTBVAC-specific CD4 T cell response; Comparison of CD4 T cells responses across increasing doses of MTBVAC and BCG; as well as evaluation of selected MTBVAC dose superiority over BCG vaccination. The results of the analysed immunogenicity data will be provided by the independent, unblinded biostatistician directly to the DSMB in a blinded manner along with the vaccine recipient listing. Sponsor will be provided with the same blinded data for review, excluding the vaccine recipient listing to maintain blinding of Cohort 3. The DSMB meeting will take place to assess the immunogenicity results, as described above, for all cohorts (Cohort 1, 2 and 3) up to Study D182 visit per cohort to determine the most relevant dose for the Phase III study. Unblinding of the clinical team will take place post Study Day 365 visit for all cohorts. In order to maintain data integrity and avoid compromising immunology analysis of samples collected at the last visit (Study Day 365 visit), the immunology team will remain blinded until immunology database lock.

Efficacy is not being assessed in this trial.

12.4 Final Analysis Plan

The planned statistical analyses for this study are outlined below. A detailed statistical analysis plan will be created and finalized prior to database lock. Any deviations from the planned analyses will be described in the final study report.

12.4.1 Participant Analysis Sets

The full analysis set will include all screened participants. The safety analysis set will include all participants who received investigational product. The immunogenicity analysis set will include all participants who received a complete study vaccination and has at least one evaluable immunogenicity result. Listings of participants who did not complete all trial procedures, such as missed visits or other protocol deviations that may affect the analyses, 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.

12.4.2 Demographics and Protocol Compliance

Baseline demographic (age, sex, and race) and other baseline characteristics will be summarized using descriptive statistics (at a minimum the following will be reported: means, medians, standard deviations, frequencies and proportions, as appropriate), for the overall sample (Safety Analysis Set) and by study group.

As participant enrollment to each cohort will be conducted based on timing of completion of study eligibility requirements, any imbalance in baseline characteristics between cohorts will also be examined.

Listings of randomized participants with protocol deviations will be provided.

12.4.3 Immunogenicity and Other Immunology Analyses

Immunogenicity will be summarized for all time points as collected and as available. No imputation for missing data will be performed. Data will be transformed as appropriate prior to analysis.

For each of the outputs from the relevant immunology parameters, descriptive statistics including means, standard deviations, medians, and interquartile 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 (informally) as outlined in the Statistical Analysis Plan.

12.4.3.1 Whole Blood ICS

The primary variables of interest for the assessment of the immune response to the study vaccine will be the frequencies of MTBVAC-specific CD4+ and CD8+ T cells that produce any or a combination of relevant cytokines. Response will be measured by flow cytometry using a qualified whole blood intracellular cytokine staining (ICS) assay (24).

T cell responses will be summarized by treatment group and time point. The difference in T cell response between treatment regimens across all post-immunization time points as measured by percentage CD4 and CD8 responses will also be modeled using linear mixed effects models or other regression models, as appropriate.

12.4.3.2 QFT Gold Plus

Qualitative and quantitative results for the QFT Gold Plus test at each available time point will be summarized using participant count (percentage) summaries and descriptive statistics. The proportions of QFT positive participants at various time points may also be compared using chi-square tests or Fisher's exact tests. Appropriate figures will be used to illustrate the observed responses over time.

12.4.4 Safety Analyses

12.4.4.1 Adverse Events

The primary endpoint is the frequency of adverse events (AEs) by MTBVAC dose compared to BCG. In order to assess the primary endpoint, analysis of the data will be preceded by a

blinded data review meeting in order to address population determination and miscellaneous data queries pertinent to analysis of the primary endpoint.

Safety and reactogenicity data will be evaluated by study arm and cohort in all participants in the safety analysis set. The primary variable for evaluation of safety will be the frequency of unsolicited, solicited adverse events and injection site reactions at all available post-vaccination time points. The number and percentage of participants with adverse events will be summarized by MedDRA system organ class (SOC) and preferred term (PT), as well as by severity and relationship to study vaccine. Informal between-group comparisons will be performed using the Chi-square or Fisher Exact test. The Cochran-Mantel-Haenzel test will be used to compare the effect of dose on reactogenicity (injection site adverse events).

Separate listings will be provided for participants with serious adverse events. SAEs may also be summarized by MedDRA system organ class and preferred term.

12.4.4.2 Clinical Laboratory and Vital Sign Parameters

For each clinical laboratory parameter and vital sign parameter pre-specified in the protocol, summary statistics for continuous parameters will be presented by treatment group for all pre- and post-vaccination assessments and for change from pre-vaccination to post-vaccination assessments.

The number and percentage of participants with post-vaccination Grade 3 or higher clinical laboratory values or vital sign values (Appendices B, C and D) will be tabulated at each post-vaccination time point and overall. Clinical laboratory and vital sign abnormalities will also be reported as adverse events as applicable and will be included in the summaries of adverse events

12.4.5 Computer Methods

Statistical analyses will be performed using SAS® (Version 9.4 or higher, SAS Institute Inc., Cary, NC, USA), under a Windows operating system.

13 SOURCE DOCUMENTS AND ACCESS TO SOURCE DATA/DOCUMENTS

For the purpose of monitoring and auditing the study, source documentation will consist of any existing medical records and study records developed and maintained by the investigator for this study. Monitor(s), auditor(s), IRB/IEC members, and the applicable regulatory authorities will be granted direct access to the participant's original study medical records for verification of protocol-specified procedures and/or data. The above-mentioned agencies will only have access to a participant's personal details if they come to the study site to monitor or audit the study. Any data sent to these agencies will be de-identified.

14 QUALITY CONTROL AND QUALITY ASSURANCE

The clinical trial site will be required to have a Quality Management Plan and an Investigator Oversight Plan in place for this trial. The Quality Management Plan will specify quality control procedures to be implemented by the trial site staff to ensure the protocol is being followed, data are entered accurately into the study database, investigational product is handled correctly, and all staff are appropriately trained. Additionally, the site will be required to have SOPs in place for management of the investigational product handling, adverse event and serious adverse events, and the informed consent process.

Independent trial monitors will provide an additional layer of quality control through regular monitoring visits. Monitoring will include review of site compliance with the quality management plan, investigator oversight plan, SOPs, and the protocol. Further details on the site monitoring plan can be found in Section 11.1.

An independent auditor will conduct a site audit during the trial to ensure that the trial is performed, and the data is generated, documented and reported in accordance with Good Clinical Practice and applicable regulatory requirements.

15 ETHICS/PROTECTION OF HUMAN PARTICIPANTS

15.1 Ethical Standards

The study will be carried out in accordance with this protocol, the guidelines and ethical principles set forth in the Declaration of Helsinki (last updated 2013), Good Clinical Practice (ICH E6 (R2)) and Guidelines for Good Practice in the Conduct of Clinical Trials with Human Participants in South Africa (SA GCP), Second Edition, 2006 or latest version.

Written maternal informed consent will be obtained from each participant prior to any protocol-specified procedures being conducted.

The protocol and informed consent form will be reviewed and approved by the IRB or IEC of the participating clinical trial site prior to any protocol-specified procedures being conducted. The investigator is responsible for ensuring that the protocol is reviewed by an IRB/IEC with the appropriate composition (per clinical trial site guidelines). The investigator will inform the IRB/IEC as to the progress of the study at least annually and as defined by IRB/IEC policy.

HIV testing will be performed with appropriate pre- and post-test counseling.

The protocol may not be modified without written approval from the sponsor. All changes to the protocol must be submitted to the IRB/IEC and must be approved by the IRB/IEC and applicable regulatory agencies prior to their implementation.

The investigator or designated clinical trial site staff will notify the IRB/IEC and applicable regulatory agencies when the study has been completed (i.e., the clinical database has been locked). After the study has been unblinded, the participant should be informed by the investigator which treatment (MTBVAC or BCG) the participant received.

15.2 Institutional Review Board

All the documents the IRB/IEC may need to fulfill its responsibilities, such as the protocol, protocol amendments, informed consent form, information concerning participant recruitment, payment or compensation procedures, etc., will be submitted to the IRB/IEC by the investigator. The IRB's/IEC's written, unconditional approval of the appropriate version of the study protocol and the informed consent form will be in the possession of the investigator/clinical trial site staff prior to the conduct of any protocol-specified procedures.

Modifications to the protocol may not be implemented without prior written IRB/IEC approval except when necessary to eliminate immediate hazards to the participants or when the modification involves only logistical or administrative aspects of the study. Such logistical or administrative modifications will be submitted to the IRB/IEC in writing by the investigator, and a copy of the correspondence to verify the submission will be maintained.

The investigator must inform the IRB/IEC of modifications to the informed consent form or any other documents previously submitted for review/approval and any new information that may adversely affect the safety of the participants or the conduct of the study, provide an annual update and/or request for re-approval, and advise the IRB/IEC when the study has been completed.

Any clinical trial site-generated documents or forms to be provided to the participant (e.g., information cards, letters from the investigator), and all forms of study advertising (flyers, brochures, print advertisements, radio or television scripts, etc.) must be approved by Biofabri or its designee prior to the clinical trial site submitting them to the IRB/IEC. Approval from the IRB/IEC must be obtained prior to providing the documents or forms to the participant.

15.3 Informed Consent Process

For 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 needs to be 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. Individual face-to-face informed consent sessions will then be held between potential participant's legal guardian 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. Where possible, paternal consent will also be sought.

The final approved English language informed consent documents will be translated and back translated by a reputable translation agency into required languages. The translated documents will then be submitted to the UCT HREC for acknowledgment. The information and informed consent document will be given to the potential participant's legal guardian to read (or to be read to them) before the informed consent process begins. If possible, the document will be given to the participant's legal guardian at least the day before the informed consent process.

Written informed consent will be obtained from all potential participants' 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's 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's legal guardian 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.

Since MTBVAC might interfere with interpretation of future IGRA and TST tests for *M. tuberculosis* infection after the end of the study, mothers will be informed of this phenomenon and the risk of receiving unnecessary IPT for a false positive test for *M. tuberculosis* infection. We will emphasise that all infants, especially those testing QFT positive at D56 will be carefully monitored for signs and symptoms of TB, with two extra telephonic contacts for infants QFT+ at D56; and that mothers of MTBVAC recipients who test QFT positive will receive a card at end of study stating that they have received an experimental vaccine that may interfere with future IGRA and TST tests for *M. tuberculosis* infection.

Any questions the potential participant's legal guardian may have will be answered. If all questions have been answered satisfactorily, the participant's legal guardian has demonstrated adequate understanding, and the participant's legal guardian 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's legal guardian 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's legal guardian for her 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's legal guardian. The date of an illiterate participant's legal guardian's 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.

Informed consent is an ongoing process. At every clinic visit it will be verbally reconfirmed that the participant is voluntarily consenting to the study and understands the significant aspects of the study (e.g., purpose, risks, duration).

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

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.

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. 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.

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.

15.5 Participant Confidentiality

To maintain confidentiality, participant identification numbers will be used to identify the participant's laboratory specimens, source documents, eCRF, study reports, etc. All study records will be maintained in a secured location. Clinical information will not be released without written permission from the participant except as necessary for monitoring or auditing of the study by Biofabri or its designee or applicable regulatory authorities. 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.

15.6 Study Discontinuation

Rules governing the pausing and stopping of investigational product administration between individuals within a single cohort and between cohorts are presented in Section 10.5. There are no procedures for participants to continue therapy, as this is a single-dose study.

15.7 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 may be stored for future use for additional TB-related tests after the completion of this study if consent was obtained for this. 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.

16 DATA HANDLING AND RECORD KEEPING

The investigator is responsible to ensure the accuracy, completeness, legibility, and timeliness of the data reported. All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data.

Data reported in the eCRF derived from source documents should be consistent with the source documents.

16.1 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 maternal consent. 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 and data entry staff only and will not be available to any other person.

16.2 Study logs

The following logs will be maintained by the study team:

- Informed consent (IC) log: will contain particulars of pregnant women who were consented. This log will contain participant identifiers.
- Screening and Enrolment log: will contain results of screening process This log will not contain participant identifiers and will not contain blinded data i.e. the outcome of randomization.
- 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.

16.3 Data Management Responsibilities

All source documents and laboratory reports must be reviewed by the clinical team and data entry staff, who will ensure that they are accurate and complete. Adverse events must be graded, assessed for severity and causality, and reviewed by the site investigator as described in Section 10.2.1.

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site principal investigator. During the study, the investigator must maintain complete and accurate documentation for the study.

Biofabri or its designee will be responsible for data management, quality review, analysis, and reporting of the study data.

16.4 Data Capture Methods

Data quality control (QC) measures will be instituted in accordance with generic data QC procedures at the study site. All completed eCRFs will be checked for completeness, accuracy and legibility. Data reported on the eCRF that are derived from source documents or chart review, should be consistent with the source documents or the discrepancies should be explained.

Data for this study will include safety (systemic solicited adverse events, injection site reactions, unsolicited adverse events, and serious adverse events) and laboratory (immunogenicity and QFT Gold Plus) outcome measures.

16.4.1 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.

16.4.2 Case Report Forms (CRFs)

Relevant data will be transcribed by the clinical study team from source documentation to study eCRFs. eCRFs will not contain information that will identify the individual participating in the trial who will be identified only by the study number (screening number). eCRFs 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 will be reconciled with the main study database before the database is locked. All data recorded on the study eCRFs will be captured in the database.

16.5 Timing/Reports

Line lists of all adverse events will be reported to the sponsor, UCT HREC, DSMB, SAHPRA 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 SAHPRA as per their respective guidelines.

Data will be reviewed on a regular basis throughout the trial. DSMB reviews of safety data will be conducted at specified time points during dose-escalation, and on an ad hoc basis if the study is paused for safety reasons (see Section 10.7 for further details of DSMB reviews). A DSMB review on safety and immunogenicity data will be conducted after completion of the last Cohort 3 Study D182 visit. This safety and immunogenicity review will assist in dose selection for the Phase III study and will include all available safety data for all cohorts up to the last Cohort 3 Study D182 visit, as well as all immunogenicity data up to Study D182 visit per each cohort.

Coding (medical history, concomitant medications, and adverse events) will occur on an ongoing basis throughout the study, including prior to DSMB reviews. A final coding review and approval will be carried out prior to database lock.

The blind will be maintained throughout the trial, unless there is a need to unblind a participant for safety reasons (see Section 10.6) or unblinding is requested by the DSMB (see Section 10.7). Unblinding of an independent biostatistician only is planned once the last Cohort 3 Study D182 visit has been completed in preparation for a safety and immunogenicity review (see Sections 8.3 and 12.3.2). Unblinding of the data for preparation of the final study report is planned when the database has been locked, which will occur after all data (with the exception of data relating to the immunology and exploratory objectives) have been entered, reviewed, and all queries related to the data have been addressed.

16.6 Types of Data

Data for this study will include safety (systemic solicited adverse events, injection site reactions, unsolicited adverse events, and serious adverse events) and laboratory (immunogenicity and QFT Gold Plus) outcome measures.

16.7 Study Records Retention

All study records (source documents, signed informed consent forms, IRB/IEC correspondence and approval letters, investigational product management records, and other essential documents) will be kept secured for at least a minimum of 15 years or until, at least, 2 years after the last approval of a marketing application and until there are no pending or contemplated marketing applications or at least 15 years have elapsed since the formal discontinuation of clinical development of the investigational product. Additional storage will be according to the guidance of the applicable national regulatory authority or authorities. The investigator will ensure that study records are not disposed of or removed from the clinical trial site without prior notification and approval from Biofabri or its designee.

16.8 Protocol Deviations

All participant-level deviations from protocol procedures, evaluations, and/or visits must be documented. When possible, missed visits and procedures must be rescheduled and

performed at the nearest possible time point to the original schedule. Deviations will be reported to the IRB/IEC and applicable national regulatory authorities per their guidelines.

17 PUBLICATION POLICY

The final study report will be made available to the principal investigator for purposes of publications. The principal investigator and study staff must send all manuscripts, abstracts, and presentations using data from this study to Biofabri for review prior to their submission. Biofabri jointly and separately reserve the right to delete any part or parts of such materials deemed to be confidential or proprietary.

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. It is the responsibility of the sponsor to register this trial in an acceptable registry prior to the start of participant enrollment.

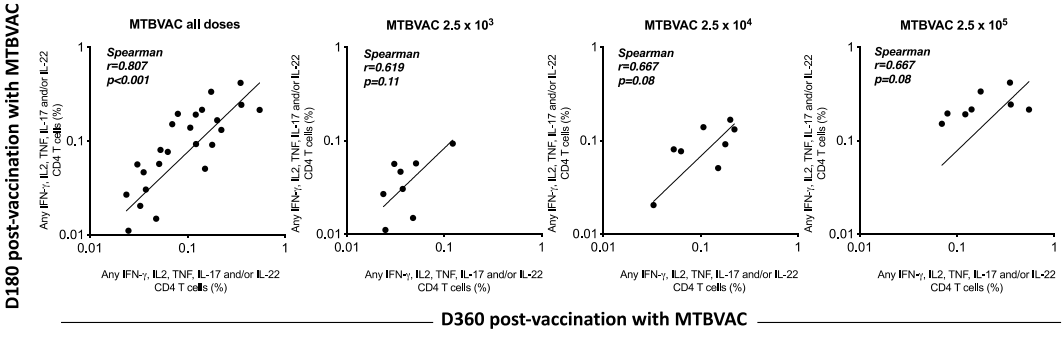
The ICMJE defines a clinical trial as any research project that prospectively assigns human subjects to intervention or comparison groups to study the cause-and-effect relationship between a medical intervention and a health outcome. Studies designed for other purposes, such as to study pharmacokinetics or major toxicity (e.g., Phase 1 trials), would be exempt from this policy.

18 CHANGES TO THE PROTOCOL

Changes between protocol versions are described below.

18.1 Changes from Version 3.0 to Version 4.0

Section Number (Title)	Change/Rationale
Title page	Protocol version and date updated.
Section 2 Key Roles	TCD address updated (Drive vs Road).
Section 3.2 Rationale	Grammar update. Rationale updated to include explanation for additional safety and immunogenicity analysis/review which will determine the optimal dose level for the Phase III study. Saving time to start Phase III will be a major public health benefit as indicated in the text. Included confirmation of when the clinical team would be unblinded, for clarity.
Section 6.3.2 Masking Procedures	Included confirmation of when the clinical team would be unblinded for clarity. Grammar update. Inclusion of “D” to confirm the Study D365, visit instead of just 365 visit, to clarify.
Section 8.3 Follow-up	Updated naming of study visit to avoid ambiguity and for clarity of the addition of 7 (days).

Section Number (Title)	Change/Rationale
Section 8.3 Follow-up (Study Day 182)	Unblinding of data for safety and immunogenicity to independent biostatistician in order to determine most optimal dose for Phase III study without compromising data integrity. Confirmation that the immune response to MTBVAC at D182 is predictive of the memory response at D365 is included below: The original study was designed to analyse the antigen-specific CD4 T cell response induced by escalating doses of MTBVAC at timepoints defined to represent an acceptable assessment of the immune response kinetics (at days 28, 56, 182 and 365 post vaccination). Based on previous studies (Soares et al, JID, 2013, Tameris et al, Lancet Resp. Med., 2019) vaccine-induced T-cell immune responses are expected to peak between 7 and 10 weeks post-vaccination (D28 and D56 in our trial), then to gradually wane over the subsequent 10 months of follow up. In the context of an interim analysis necessary for the vaccine dose selection to inform the MTBVAC phase 3 trial, we determined if assessment of immune responses at D182 post-MTBVAC vaccination is predictive of memory responses at D365. We reanalysed immunogenicity data from the phase IIa trial (Tameris et al, Lancet Resp. Med., 2019), and assessed the strength of correlation between MTBVAC-specific total CD4 T cell responses (IFN-g, IL-2, TNF, IL-17 and/or IL-22) at D180 and D360 following MTBVAC vaccination (Figure 1). At the vaccine dose analysed (2.5 x 10^3 CFU, 2,5 x 10^4 CFU and 2.5 x 10^5 CFU), the MTBVAC-specific CD4 T cell responses between D180 and D360 were strongly correlated, suggesting that D182 is predictive of vaccine induced memory response at 1 year post-vaccination. In addition, we will also determine if the cytokine co-expression patterns indicate evidence of excessive T cell differentiation, which can lead to poor long-term memory responses.  **Figure 1. Correlation between any IFN-γ, IL-2, TNF and/or IL-17 CD4 T-cell responses at D180 and D360 in response to MTBVAC stimulation following MTBVAC vaccination at the indicated doses in the phase IIa MTBVAC dose escalation clinical trial (**NCT02729571**).** Spearman coefficient and associated p-values are shown.
Section 8.4 Follow-up (Final Study Visit)	Included confirmation of when the clinical team would be unblinded for clarity. Updated grammar.

Section Number (Title)	Change/Rationale
Section 10.7 Safety Oversight (DSMB)	Included when DSMB to review overall available safety data to confirm if any safety issues for any cohort doses. DSMB to confirm safest dose for Phase III study.
Section 12.3.1 Planned Interim Analyses – Safety Review	Included when DSMB to review overall available safety data to confirm if any safety issues for any cohort doses. DSMB to confirm safest dose for Phase III study.
Section 12.3.2 Planned Interim Analyses – Immunogenicity or Efficacy Review	Included when DSMB to review overall immunogenicity data up to D182 for all cohorts to confirm which dose is most immunogenic and whether MTBVAC is superior to BCG immunogenically. This will assist in dose selection for the Phase III study.
Section 12.4.3 Immunogenicity and Other Immunology Analyses (Final Analysis)	Clarification of supporting document involved.
Section 16.4 Data Capture Methods	Grammar update. Inclusion of injection site reactions previously not included in previous versions of the protocol.
Section 16.5 Timing/Reports	To collate information mentioned in previous sections. Included when DSMB to review overall immunogenicity data up to D182 and all available safety data for all cohorts to confirm which dose is most safe and immunogenic. This will assist in dose selection for Phase III.
Section 16.6 Types of Data	Inclusion of injection site reactions previously not included in previous versions of the protocol.
Header	Protocol version and date updated.

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SUPPLEMENTS/APPENDICES

APPENDIX A. SCHEDULE OF EVENTS

Evaluation	Screen	Study day Visit Window (hrs/days)							
		D0	D7	D28	D56	D182	D365	Post D365	Early termination
	NA	+96 hrs	±3d	±3d	±7d	±14d	±14d	NA	NA
Informed consent process	M[a]								
Medical history	M								
TB symptoms or household TB contact	M	x	x	x	x	x	x	x	x
Maternal HIV result available	M								
Verbally reconfirm consent	M	M	M	M	M	M	M	M	
Verify eligibility criteria	x	x							
Vital signs, length, weight		x	x	x	x	x	x	x	x
Record feeding practice		x	x	x	x	x	x	x	x
Physical examination		x	x	x	x	x	x	x	x
Recording of concomitant medication		x	x	x	x	x	x	x	x
Randomization		x							
MTBVAC/BCG administration		x							
Safety blood sampling (mL)[b]			2						
QuantiFERON®-TB Gold Plus (mL)[b]					4[d]	4	4		4
Whole blood (mL)[b] (12-hour WB-ICS)				2	2	4	4		
Distribute/review diary cards		x	x						
Solicited systemic adverse events (incl. concomitant medications)		x	x	x					
Unsolicited adverse events (incl. concomitant medications)		x	x	x	x	x	x		x
Solicited and unsolicited injection site reaction adverse events (incl. concomitant medications)		x	x	x	x				
Serious adverse events (incl. concomitant medications)		x	x	x	x	x	x		x
Site of injection examination, swab if discharge		x	x	x	x	x	x		x
Rescue dose BCG post unblinding of cohort[c]								x	
Approximate per visit phlebotomy volume (mL)[a]			2	2	6	8	8		4
Cumulative phlebotomy volume (mL)[b]			2	4	10	18	26		NA

[a] Maternal - the mother signs once, antenatally , and thereafter reconfirms consent orally at each visit

[b] Acceptable infant blood sample volumes in clinical research are limited to <3% of the total blood volume (TBV), and no more than 3mL/kg over an 8 week period

[c] Telephonic contact/ home visit approximately 14 days post BCG to confirm infant's wellbeing incl. BCG injection site reaction

[d] If QFT positive at D56 a home/telephonic visit will be done at D120±7d and 270±7d to monitor infant's wellbeing, specifically enquiring about TB signs and symptoms

APPENDIX B. 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 [10]0.4 – 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

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APPENDIX C. 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
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

Serum/Plasma Chemistry	Grade 1	Grade 2	Grade 3	Grade 4
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 D. 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[a], High (mg/dL; µmol/L)[b] **Term Neonate**[c] < 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[c] 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

[a] 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.

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

[c] 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.