

**A Randomized, Placebo Controlled, Partially Blinded Phase II Study to Evaluate Safety, Immunogenicity, and Prevention of Infection with *Mycobacterium tuberculosis* of AERAS-404 and BCG Revaccination in Healthy Adolescents**

**Investigational Product:** **AERAS-404**  
**Bacille Calmette Guerin (BCG) Vaccine SSI**

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Signature

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**TABLE OF CONTENTS**

**LIST OF ABBREVIATIONS ..... 5**

**STUDY ABSTRACT ..... 7**

**1 INTRODUCTION..... 11**

1.1 Background ..... 12

1.2 Description of AERAS-404 Vaccine ..... 13

1.3 Nonclinical Experience with AERAS-404 (HyVac4) Vaccine..... 14

1.4 Clinical Experience with AERAS-404..... 14

1.5 Clinical Experience with Vaccines Related to AERAS-404..... 17

1.6 Description of BCG..... 18

1.7 Rationale for Study..... 19

**2 STUDY OBJECTIVES AND DESIGN..... 20**

2.1 Objectives..... 20

2.2 Design and Endpoints..... 21

**3 STUDY PROCEDURES ..... 24**

3.1 Schedule of Subject Evaluations ..... 24

3.2 Subject Selection ..... 27

3.2.1 Recruitment and Informed Consent ..... 27

3.2.2 Screening..... 28

3.2.3 Inclusion Criteria ..... 28

3.2.4 Exclusion Criteria ..... 29

3.2.5 Screening Clinical Assessments and Laboratory Tests ..... 30

3.3 Study Randomization ..... 30

3.4 Blinding..... 31

3.4.1 Unblinding for Clinical Emergencies ..... 31

3.5 Vaccine Administration..... 32

3.6 Study Evaluations..... 33

3.6.1 Evaluation of *Mtb* infection ..... 33

3.6.2 Immunology Laboratory Evaluations ..... 34

3.6.3 Safety Evaluations ..... 36

3.6.3.1 Pre-vaccination and Post-Vaccination Monitoring of  
Subjects ..... 36

3.6.3.2 Clinical Assessments and Laboratory Tests..... 36

3.6.3.3 Adverse Events..... 37

3.6.3.4 Concomitant Medications ..... 37

3.6.4 Subject Follow-up and Contact..... 38

3.6.5 Loss to Follow-up ..... 38

**4 VACCINES..... 38**

4.1 Supplies ..... 38

4.2	Accountability .....	39
4.3	Receipt and Storage .....	39
4.4	Vaccine Preparation .....	40
4.5	Disposal of Unused Supplies.....	40
<b>5</b>	<b>SAFETY.....</b>	<b>40</b>
5.1	Responsibilities for Ensuring the Safety of Trial Subjects .....	40
5.1.1	Principal Investigator .....	40
5.1.2	Study Sponsor .....	41
5.1.3	Local Medical Monitor .....	41
5.1.4	Data Monitoring Committee.....	41
5.1.5	Institutional Review Boards and Ethics Committees.....	41
5.1.6	National Regulatory Authority .....	42
5.2	Safety Surveillance During the Study .....	42
5.3	Definition of Adverse Event .....	42
5.4	Assessing Severity.....	43
5.5	Assessing Causal Relationship (Relatedness) .....	44
5.6	Definition of Adverse Reaction.....	45
5.7	Solicited Adverse Events and Injection Site Reactions .....	45
5.8	Adverse Events of Special Interest (AESIs) .....	46
5.9	Assessing "Seriousness" and Serious Adverse Events.....	46
5.10	Assessing Expectedness .....	47
5.11	Definition of Suspected Unexpected Serious Adverse Reaction (SUSAR).....	47
5.12	Reporting of Serious Adverse Events .....	47
5.13	Other Events Requiring Immediate Reporting.....	48
5.14	Adverse Event Treatment, Follow-up, and Outcome.....	49
5.15	Follow-up of Subjects Who Become Pregnant .....	49
5.16	Subject Diary and Daily Temperature Monitoring.....	50
<b>6</b>	<b>PAUSING AND STOPPING RULES .....</b>	<b>51</b>
6.1	Rules for Discontinuing Study Injections in an Individual Participant.....	51
6.2	Rules for Suspension of the Entire Study.....	51
<b>7</b>	<b>STATISTICAL CONSIDERATIONS .....</b>	<b>52</b>
7.1	Subject Populations .....	52
7.2	Demographics and Protocol Compliance .....	52
7.3	Prevention of Infection (QFT-GIT Conversion) Analyses.....	53
7.4	Immunogenicity and Other Immunology Analyses .....	53
7.4.1	Immune Response Determined by Intracellular Cytokine Staining Assay .....	54
7.4.2	Additional Exploratory Analyses.....	54
7.4.2.1	RNA Transcriptomics and Whole Blood Absolute Blood Cell Counts.....	54
7.4.2.2	Quantification of Soluble Immune Mediators by Multiplex ELISA.....	55
7.4.2.3	Correlates of Risk/Correlates of Protection .....	55

7.4.2.4	Novel IGRAs.....	55
7.5	Safety Analyses .....	55
7.5.1	Adverse Events .....	55
7.5.2	Clinical Laboratory and Vital Sign Parameters .....	56
7.6	Sample Size Considerations .....	56
7.7	Plan for Statistical Summaries and Analyses .....	57
7.7.1	Primary Data Review .....	57
7.7.2	Final Study Report .....	57
7.8	Computer Methods .....	57
<b>8</b>	<b>DATA COLLECTION, MONITORING, AND RECORD RETENTION.....</b>	<b>58</b>
<b>9</b>	<b>HUMAN SUBJECTS.....</b>	<b>58</b>
9.1	Ethics and Regulatory Considerations .....	58
9.2	Institutional Review Board or Independent Ethics Committee.....	60
9.3	Informed Consent and Assent .....	61
<b>10</b>	<b>STUDY COMPLETION .....</b>	<b>62</b>
<b>11</b>	<b>PUBLICATIONS .....</b>	<b>62</b>
<b>12</b>	<b>CHANGES IN THE PROTOCOL .....</b>	<b>62</b>
12.1	Changes from Version 1.0 to Version 2.0 (Amendment 1).....	62
12.2	Changes from Version 2.0 to Version 3.0 (Amendment 2).....	65
12.3	Changes from Version 3.0 to Version 4.0 (Amendment 3).....	67
12.4	Changes from Version 4.0 to Version 5.0 (Amendment 4).....	68
<b>13</b>	<b>REFERENCES.....</b>	<b>70</b>
<b>APPENDIX A</b>	<b>Detailed Description of Study Visits.....</b>	<b>74</b>
	<b>SAE and SUSAR Reporting Schemes .....</b>	<b>81</b>
<b>APPENDIX B</b>	<b>Toxicity Table (Modified from Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events).....</b>	<b>83</b>
<b>APPENDIX C</b>	<b>Adverse Events of Special Interest .....</b>	<b>90</b>

## LIST OF ABBREVIATIONS

βHCG	beta human chorionic gonadotropin
µg	microgram(s)
Ab	antibody
AE	adverse event(s)
AESI	adverse event of special interest
ALP	alkaline phosphatase
ALT	alanine aminotransferase
AST	aspartate aminotransferase
BCG	Bacillus Calmette-Guérin
BMI	body mass index
BUN	blood urea nitrogen
CBC	complete blood count
CFR	Code of Federal Regulations (US)
CRF	case report form(s)
DMC	Data Monitoring Committee
DOH	Department of Health
EDC	electronic data capture
ELISA	enzyme-linked immunosorbent assay
ELISPOT	enzyme-linked immunospot
GCP	Good Clinical Practices
HIV	human immunodeficiency virus
IB	Investigator’s Brochure
ICH	International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICS	intracellular cytokine staining
ID	intra-dermal
IEC	Independent Ethics Committee
IFN-γ	interferon gamma
IGRA	interferon gamma release assay
IL-2	interleukin-2
IM	intramuscular
INH	isoniazid
IPT	isoniazid preventive therapy
IRB	Institutional Review Board
IUD	intrauterine device
LLN	lower limit of normal
MedDRA	Medical Dictionary for Regulatory Activities
mL	milliliter(s)
<i>Mtb</i>	<i>Mycobacterium tuberculosis</i>
NTP	National TB Programme
NTM	non-tuberculous mycobacteria
PBMC	peripheral blood mononuclear cell(s)

QFN	QuantiFERON
QFT-GIT	QuantiFERON®-TB Gold in-Tube
RNA	ribonucleic acid
SAE	serious adverse event(s)
SAER	supplemental serious adverse event report
SATVI	South African Tuberculosis Vaccine Initiative
SOC	system organ class
SUSAR	suspected unexpected serious adverse reaction(s)
TB	tuberculosis
TNF	tumor necrosis factor
TST	tuberculin skin test
ULN	upper limit of normal
US	United States
WBC	white blood cell
WHO	World Health Organization

## STUDY ABSTRACT

### TITLE:

A Randomized, Placebo Controlled, Partially Blinded Phase II Study to Evaluate Safety, Immunogenicity, and Prevention of Infection with *Mycobacterium tuberculosis* of AERAS-404 and BCG Revaccination in Healthy Adolescents

### RATIONALE:

This clinical trial will evaluate safety, immunogenicity, and prevention of *Mtb* infection (QFT-GIT conversion), of AERAS-404 and BCG revaccination in previously BCG vaccinated adolescents. QFT-GIT negative adolescents in the area where this study will be conducted have previously been shown to have very high rates of TST and QFT-GIT conversion, associated with increased risk of active TB disease in subsequent years. A TB vaccination strategy incorporating BCG revaccination or AERAS-404 vaccination in adolescents or young adults, if found to prevent *Mtb* infection, would likely have a major impact on TB disease, TB transmission, and control of the epidemic. If revaccination with BCG or vaccination with AERAS-404 is shown to prevent infection with *Mtb* in this proof of concept study in adolescents, additional larger scale studies examining the impact on TB disease in more diverse populations would be warranted.

### OBJECTIVES:

#### Primary Objectives:

- To evaluate the safety profile in HIV-uninfected, remotely BCG vaccinated adolescents of
  - AERAS-404
  - BCG revaccination
- To evaluate prevention of *Mtb* infection, as measured by rates of conversion using a QFT-GIT assay, by
  - AERAS-404 compared to placebo
  - BCG revaccination compared to placebo

#### Secondary Objectives

- To evaluate prevention of *Mtb* infection, as measured by rates of sustained conversion using a QFT-GIT assay, by
  - AERAS-404 compared to placebo
  - BCG revaccination compared to placebo
- To investigate the immunogenicity in HIV-uninfected, remotely BCG vaccinated adolescents of
  - AERAS-404
  - BCG revaccination

### DESIGN:

This Phase II, randomized, 3-arm, placebo controlled, partially blinded, clinical trial will be conducted in 990 healthy, HIV-uninfected, QFT-GIT negative, previously BCG vaccinated adolescents. The trial will be conducted at the South African Tuberculosis Vaccine Initiative (SATVI) site in the Worcester region of the Western Cape region of South Africa, where

epidemiological studies involving thousands of adolescents have been conducted over the last decade to characterize rates of *Mtb* infection and active TB disease in this age group. Additional sites with similar TB epidemiology may also be used.

Subjects will be enrolled in two sequential cohorts and within each cohort subjects will be randomized in a 1:1:1 ratio to receive either AERAS-404 or saline placebo on Days 0 and 56, or BCG Vaccine SSI on Day 0. The first 90 subjects (30 from each arm) will form the Safety & Immunogenicity Cohort and will be subject to more intensive collection of safety data, with data reviewed by the Data Monitoring Committee (DMC), principal investigator and local medical monitor. Selected immunogenicity assays, including whole blood intracellular cytokine staining (ICS), will also be performed in this cohort. The remaining 900 subjects will be enrolled into the Correlates Cohort. All 990 subjects in the study will be evaluated for safety and biomarker outcomes, and for prevention of *Mtb* infection.

The primary *Mtb* infection endpoint will be QFT-GIT conversion from a negative to positive test, using the manufacturer's recommended threshold of 0.35 IU/mL, at any time-point after Day 84 and through end of follow-up for the primary endpoint (see Figure 2-1). The 84-day 'wash-out' period is stipulated in order to exclude subjects who may have already been *Mtb* infected, but not yet converted their QFT-GIT test at screening, thus subjects who convert their QFT-GIT at Day 84 will not be included in the analyses of prevention of *Mtb* infection.

The duration of the trial will be endpoint-driven, using a design targeting accrual of 64 primary endpoints. The primary analysis will be triggered when at least 64 endpoints are accrued in the study (both cohorts) AND the median follow up time is at least 15 months, with a maximum individual follow-up time to detect initial QFT-GIT conversion of 24 months. If an initial reduction in infection rate in the BCG or AERAS-404 groups is seen in the primary analysis of QFT-GIT conversion, follow up for the entire study may be extended to examine the duration of prevention of *Mtb* infection.

The trial sample size of 990 subjects is determined by the primary prevention of *Mtb* infection objective (measured by prevention of conversion to a positive QFT-GIT test). A potential mechanism of action of vaccination might be to allow initial *Mtb* infection (and QFT-GIT conversion), but to prevent establishment of sustained latent *Mtb* infection (and thus persistent QFT-GIT conversion), resulting in QFT-GIT reversion to negative. Thus the study will also examine the persistence of QFT-GIT conversion.

All subjects with primary QFT-GIT conversion will be followed for an additional 6 months post-conversion to ascertain the sustained QFT-GIT conversion and QFT-GIT reversion endpoints. The secondary *Mtb* infection endpoint will be the combination of QFT-GIT conversion from a negative to positive test, using the manufacturer's recommended threshold of 0.35 IU/mL, at any time-point after Day 84 and through end of follow-up for the primary endpoint, AND persisting without QFT-GIT reversion from a positive to a negative test through 6 months after QFT-GIT conversion (see Figure 2-1).

Immunological outcomes will include intracellular cytokine staining (ICS) on PBMCs and whole blood, transcriptomics on RNA from whole blood, absolute blood cell subset counting by flow cytometry (for deconvolution of transcriptomics), quantification of soluble immune mediators by multiplex ELISA (all in the Safety & Immunogenicity Cohort only), and exploratory assays using PBMCs, plasma, and RNA samples for correlates of risk/protection analyses in all subjects.

### **ANALYSIS OF PREVENTION OF INFECTION**

The primary *Mtb* infection endpoint will be QFT-GIT conversion from a negative to positive test, using the manufacturer's recommended threshold of 0.35 IU/mL, at any time-point after Day 84 and through end of follow-up for the primary endpoint. The frequency of QFT-GIT negativity/positivity will be summarized for each treatment group at screening, on Day 84, on Month 6, and for all subsequent assessments. The log-rank statistic will be used to test the null hypothesis of no difference in the rates of *Mtb* infection over the follow-up period between each of the AERAS-404 and BCG groups compared to the placebo group. The log-rank test statistic compares estimates of the hazard functions of the vaccine groups at each observed event time. It is constructed by computing the observed and expected number of events in the groups at each observed event time and then summing these to obtain an overall summary across all time points where there is an event. As subjects convert their QFT-GIT tests over time (experience a primary endpoint) or are lost to follow up the number of subjects at risk over time will change. As this is a proof of concept study, these two log-rank tests (AERAS-404 compared to placebo and BCG revaccination compared to placebo) will be performed with a 1-sided Type 1 error of 0.1. There will be no adjustment for multiplicity to control the Type 1 error rate over the two tests. The rationale for not performing this adjustment is related to the fact that the tests pertain to efficacy assessments of two unrelated vaccines. If these two evaluations were performed in two separate trials then no adjustment for multiplicity would be made. Thus, one should not be compelled to adjust for this multiplicity simply because the two evaluations are performed administratively in a single trial.

The secondary *Mtb* infection endpoint will be the combination of QFT-GIT conversion from a negative to positive test, using the manufacturer's recommended threshold of 0.35 IU/mL, at any time-point after Day 84 and through end of follow-up for the primary endpoint, AND persisting without QFT-GIT reversion from a positive to a negative test through 6 months after QFT-GIT conversion. The methods used for the analysis of this endpoint will be the same as that for the primary *Mtb* infection endpoint, but the number of endpoints will be smaller as subjects who experience primary QFT-GIT conversion but then have a reversion to negative at one or both of the repeat tests will not be counted.

### **ANALYSIS OF IMMUNOLOGY**

The primary variables of interest for preliminary assessment of immune response to vaccine will be the percentage of CD4+ and CD8+ T cells that express IFN- $\gamma$ , TNF, IL-2, IL-17, IL-22, CD107a, and/or CD154 alone or in combination in response to stimulation with peptide pools representing the entire amino acid sequence of the mycobacterial antigens Ag85B and TB10.4, and BCG antigens. Due to high backgrounds associated with the CD107a response, CD107a

expression in the absence of any other functional response will be ignored. Response will be measured by flow cytometry in the intracellular cytokine staining (ICS) assay.

### **ANALYSIS OF SAFETY**

The safety profile of BCG revaccination, AERAS-404 and saline placebo will be described by treatment group. The primary variable for evaluation of the safety profile will be the number and percentage of unsolicited and solicited adverse events recorded at all available post-vaccination time points.

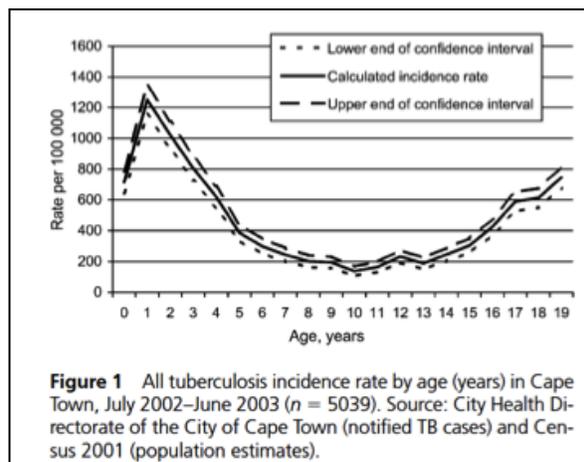
## 1 INTRODUCTION

There were an estimated 8.7 million new cases of tuberculosis (TB) worldwide in 2011, (13% co-infected with HIV) and 1.4 million people died from TB, including almost one million deaths among HIV-negative individuals (WHO, 2012). Although new cases of TB have been falling worldwide for several years and the TB mortality rate has decreased 41% since 1990, Africa is not on track to reach TB mortality targets by 2015. South Africa ranks 3<sup>rd</sup> among the top twenty high TB burden countries worldwide, with 500,000 new TB cases each year, and the highest for TB incidence per population (993 per 100,000). Improved control of TB among young adults would have significant impact on the South African TB epidemic (Dye and Williams, 2008; Abu-Raddad et al, 2009). The spread of multi-drug and extensively drug resistant TB also highlights the need for an effective vaccine against this ancient disease.

Clinical development of TB vaccines is hampered by the lack of biologic correlates of protection or validated preclinical models, which provide evidence of likely efficacy for advancement into large scale trials. As an example, despite protection in some animal models and immunogenicity in target populations, the novel vaccine MVA85A did not offer additional protection against TB disease or *Mtb* infection in infants beyond that offered by BCG vaccination at birth (Tameris et al, 2013). Several additional candidate vaccines are advancing in clinical development, but the resources to test all of these vaccines in costly and lengthy clinical trials are likely to fall short. Infection with *Mtb* is a much more frequent event than TB disease, so the size and duration of a clinical trial to test for prevention of infection with *Mtb* is much less than that of a study testing for prevention against TB disease. While a TB vaccine would not need to prevent infection with *Mtb* to prevent TB disease, prevention of infection with *Mtb* would be an important marker of biologic impact, and likely efficacy, since lasting prevention of *Mtb* infection would interrupt the cycle of disease and transmission.

A vaccine targeting adolescents or young adults would have maximal impact on TB control (Dye and Williams, 2008; Abu-Raddad et al, 2009; Dye, 2000), since TB incidence after adolescence is high (see Figure 1) and it is adolescents and adults who are responsible for the vast majority of transmission (Mahomed et al, 2011a). Thus adolescents and young adults are an important target population for TB vaccines. Boosting of BCG-induced immunity with novel TB vaccines, or with BCG revaccination, might prevent progression to active pulmonary TB disease in young adulthood and the cycle of transmission.

Bacille Calmette Guerin (BCG) Vaccine SSI is registered in South Africa for prevention of TB in unvaccinated infants, children, and adults. Protection against TB is highly variable, and likely to be related to regional differences in environmental mycobacterial exposure. (Fine, 1995). Some evidence supports the hypothesis that BCG vaccination offers protection against *Mtb* infection in young children (Soysal et al, 2005; Eisenhut et al 2009; Basu et al, 2012).



There is also some evidence that BCG revaccination of adolescents offers modest protection in temperate geographical areas (Barret et al, 2011; Pereira et al 2012; Rodrigues et al, 2005). It is also possible that BCG revaccination of adolescents might protect against primary *Mtb* infection, but this hypothesis has yet to be tested and is one of the objectives of this study.

AERAS-404 is a novel TB vaccine originally developed by SSI (Copenhagen, Denmark) and currently being jointly developed by SSI, Sanofi Pasteur and Aeras. AERAS-404 is intended to be deployed as a booster vaccine following BCG. Initial safety and immunogenicity have been demonstrated in adults in several clinical trials as described below and in the Investigator's Brochure (IB). While a closely related vaccine (see Section 1.5) is currently being evaluated in adolescents, this will be the first use of AERAS-404 in adolescents. A dose-escalating safety and immunogenicity trial in South African infants using AERAS-404 (IMPAACT P1113/C-015-404) has been approved by the Medicines Control Council and is expected to begin enrollment in 2013. Demonstration of a biologic impact against infection with *Mtb* in adolescents would provide additional rationale for a Phase 2b study of protection against TB disease of AERAS-404 in infants or an adolescent/young adult population.

## 1.1 Background

The high incidence of *Mtb* infection and active TB disease among adolescents in high TB burden countries suggests that trials assessing vaccine-induced protection are critical in this age group. Approximately 50% of high school students in the Worcester region, near Cape Town, South Africa, test QuantiFERON®-TB Gold in-tube (QFT-GIT) positive (Mahomed et al, 2011a). The incidence of active TB disease in this same adolescent study population is similarly high (450 per 100,000 person years) (Mahomed et al, 2013). Further, the increase in active TB disease incidence that begins in adolescence occurs in parallel with a very high incidence of QFT-GIT conversion, with QFT-GIT conversion rates (the denominator being QFT-GIT negative individuals) increasing by 14% to 17% per year between 13-17 years of age (*unpublished SATVI data*).

Adolescents who are QFT-GIT positive have an almost three-fold higher incidence of active TB disease in subsequent years, compared to QFT-GIT negative adolescents (640 vs 220 per 100,000 person years) (Mahomed et al, 2011b). However, the highest risk of TB disease occurs in adolescents with recent QFT-GIT conversion. Adolescents who were known to have converted from QFT-GIT negative to QFT-GIT positive over a preceding two-year period had eight-fold higher TB disease incidence in subsequent years, compared to adolescents who had remained QFT-GIT negative (1,460 vs 170 per 100,000 person years) (Machingaidze et al, 2012). Therefore, QFT-GIT conversion has clear biological importance as a risk factor for active TB disease in this age group. It follows that demonstration of prevention of QFT-GIT conversion would be a critical step towards control of TB disease incidence among young adults. This clinical trial will test whether vaccination with AERAS-404 or BCG revaccination prevents QFT-GIT conversion among healthy, HIV-uninfected, QFT-GIT negative, previously BCG vaccinated adolescents.

The QFT-GIT assay has limitations as a test for *Mtb* infection, due to intra-subject and dynamic variability around the diagnostic threshold recommended by the manufacturer and by the US Centers For Disease Control And Prevention (CDC) (0.35 IU/mL) (Pai, 2012; Mazurek et al, 2010). In a study of short-term serial QFT-GIT testing in South Africa, QFT-GIT assays were associated with a 4% false positive conversion rate, supporting the recommendation that an “uncertainty zone” of 0.2 – 0.7 IU/mL (i.e., increasing the positive threshold value) be applied to interpretation of QFT-GIT results (van Zyl-Smit et al, 2009). QFT-GIT assay variability appears maximal in low burden settings with low rates of TB infection. Repeat testing of individual QFT-GIT samples in a low burden setting showed that the normal expected range of within-subject variability is  $\pm 0.24$  IU/mL (coefficient of variation 27%) for individuals with a QFT-GIT response within this “uncertainty zone” (Metcalf et al, 2013). Serial QFT-GIT testing of Canadian health care workers, with expected TST conversion rates of less than 1%, has also shown unexpectedly high QFT-GIT conversion rates (5%) unrelated to occupational TB exposure risk (Zwerling et al, 2013). These unexpected QFT-GIT conversions were associated with a high rate of spontaneous reversion (62%).

Findings from high TB burden settings with high risk of *Mtb* infection, such as South Africa, where the rate of QFT-GIT conversion is similar to the expected rate of TST conversion, are in contrast to the pattern observed in serial testing of low risk study populations, where the rate of QFT-GIT conversion has been unexpectedly high compared to TST (Pai, 2012; Zwerling et al, 2013). For example, in South African adolescents, the QFT-GIT assay performs equally as well as the TST for prediction of subsequent active TB disease (Mahomed et al, 2011b). In a high risk South African community, the QFT-GIT conversion rate among QFT-GIT baseline negative adolescents was less than the rate of TST conversion (5mm threshold) over the same period (17% vs. 24%) (*unpublished SATVI data*). These preliminary data indicate that QFT-GIT conversion is an appropriate endpoint to define *Mtb* infection, and vaccine-induced prevention of *Mtb* infection, for clinical trials among South African adolescents.

Optimum use of QFT-GIT conversion as an infection end-point requires further exploration of the effect of alternative definitions of positive and negative test results on rates of QFT-GIT conversion, reversion, and protection in multiple populations and in high and low burden countries. These alternative conversion thresholds will be evaluated as exploratory objectives in this clinical trial.

## 1.2 Description of AERAS-404 Vaccine

AERAS-404 has two components: the H4 antigen and the IC31<sup>®</sup> adjuvant. The term HyVac4 refers to the H4 antigen and IC31 adjuvant in non-clinical development by SSI. The term AERAS-404 refers to the H4 antigen and IC31 adjuvant in clinical development sponsored by Aeras (Rockville, Maryland USA). The current formulation of AERAS-404 is a field-reconstituted vaccine with H4 antigen and IC31 adjuvant supplied in different vials. The components are dissolved in a sterile aqueous buffer containing tris-hydroxymethylaminomethane (Tris) and sodium chloride (NaCl).

## H4 Antigen

The H4 antigen is a fusion protein created from two *Mtb* antigens: antigen 85B (Ag85B) and TB10.4. Ag85B is also referred to as  $\alpha$ -antigen and is a 30-kDa mycolyl transferase protein (Horwitz et al, 2000; Belisle et al, 1997). TB10.4 is one of three members of the very similar ESAT-6 group of proteins found in *Mtb* culture supernatants. TB10.4 induces broad immune responses in T cells isolated from TB subjects compared to BCG-vaccinated donors and unvaccinated donors (Skjot et al, 2000; Skjot et al, 2002).

## IC31 Adjuvant

The IC31 adjuvant is a proprietary adjuvant of Intercell AG (Intercell, Vienna, Austria). IC31 is a combination of a leucine-rich peptide, named KLK, and a synthetic oligonucleotide, named ODN1a. KLK enhances the uptake of antigens into the antigen-presenting cell and increases the immune response to peptide antigens. ODN1a is a synthetic bacterial deoxyribonucleic acid (DNA) analogue that resembles a CpG pattern that will direct the adaptive immune response toward a T helper cell type-1 (Th-1) pattern with production of interferon- $\gamma$  (IFN- $\gamma$ ) and interleukin-12 (IL-12). The amount of adjuvant given may affect the immune response. The optimal molar ratio of KLK to ODN1a in mice is 25:1 (Lingnau et al, 2002). This same molar ratio is used in all formulations of AERAS-404 tested in clinical studies. For simplicity and clarity hereafter, amounts and concentrations of IC31 adjuvant will be expressed as molar equivalents of KLK.

### 1.3 Nonclinical Experience with AERAS-404 (HyVac4) Vaccine

Multiple toxicology studies have been conducted in mice and rabbits, in both BCG-primed and non-primed animals. Administration of HyVac4 was not associated with overt signs of toxicity and did not demonstrate mortality, adverse clinical signs, effects on body weight or food consumption, body temperature, ophthalmology, or clinical pathology.

The H4 antigen used in AERAS-404 has been evaluated in mice at 0.5, 5.0 and 15 mcg, in guinea pigs at 20 mcg, and in rabbits at 150 mcg in combination with IC31 adjuvant. HyVac4 is immunogenic in mice and induced a significant additive protective efficacy against subsequent aerosol challenge with *Mtb* compared to BCG alone (Billeskov et al, 2012; Skeiky et al, 2010; Dietrich et al, 2005). Administration of 20 mcg of H4 antigen after BCG prime has been shown to protect guinea pigs from aerosol challenge with *Mtb* compared to BCG alone (Skeiky et al, 2010). Ag85B has been previously demonstrated in the guinea pig model to induce substantial protective immunity against aerosol challenge with the highly virulent Erdman strain of *Mtb* (Horwitz et al, 2000).

Additional details regarding the nonclinical experience and results of toxicology studies with HyVac4 can be found in the IB.

### 1.4 Clinical Experience with AERAS-404

AERAS-404 has been studied in 198 adults who were enrolled in 4 Phase I studies as shown in Table 1-1; an additional 36 subjects received placebo. Subjects received 2 or 3 doses of the vaccine or placebo at the doses indicated. Three studies were conducted in European adults (a

TB non-endemic population) and one was conducted in a TB endemic population (South Africa). Subjects were previously BCG-vaccinated, either in childhood or as part of a prime-boost regimen. All were QuantiFERON (QFN) negative (non-latently infected) prior to enrollment.

**Table 1-1 Clinical Studies of AERAS-404 Performed to Date**

Study Number	Population/Location	Treatment regimen H4(µg)/IC31(nmol)	Number of doses/ Dosing schedule	Number receiving vaccine
C-005-404	18-45y / Sweden BCG pos, QFN neg, HIV neg	50,150 / 0,100,500	1 or 2 / SD 0, 56	56 (24 received H4 alone)
C-006-404	18-45y / Finland BCG pos, QFN neg, HIV neg	5,15,50,150 / 100,500	2 / SD 0, 56	50
C-011-404	18-45y / South Africa BCG pos, QFN neg, HIV neg	5,15,50,150 / 500	2 / SD 0, 56	32
C-013-404	18-45y / Switzerland BCG neg, QFN neg, HIV neg	(BCG prime at SD -42) 50 / 500	3 / SD 0, 56, 231 or 2 / SD 56, 231	60

No vaccine-related serious adverse events have occurred and the vaccine has generally been well tolerated at all doses and regimens evaluated. Injection site reactions (pain, swelling, and erythema) have been mild or moderate. Reactions have occurred at the site of tuberculin skin tests (TST) and BCG vaccination sites as described below. Systemic adverse reactions include mild to moderate fatigue, myalgia, headache, arthralgia, and mild to severe pyrexia. Asymptomatic transient isolated proteinuria was seen in subjects receiving the vaccine and placebo in all four trials and was more frequent in South African adults, including at baseline (prior to vaccination). Proteinuria did not recur with revaccination and this finding is not considered to be clinically significant. One subject had a reactivation of Graves’ disease; and one subject was diagnosed with celiac disease at the end of the study; neither event was considered related to study vaccine.

In study C-005-404 a TST result less than 10 mm was an eligibility requirement. AERAS-404 was shown to induce post-vaccination hypersensitivity reactions (HSR) at the sites of recently administered TSTs, with 14/21 (66.7%) subjects who received AERAS-404 after TST experiencing a post-vaccination TST site HSR, characterized by warmth, erythema and induration, with onset from several hours to two days after Study Day 0 vaccination, and duration from one to 28 days, typically resolving without treatment. Two of the 14 subjects also experienced pruritus at the TST site. For this reason, the TST was removed as a study procedure from C-005-404 and subsequent protocols.

Local BCG injection site reactions are well documented adverse events in subjects who receive BCG. Induration appears at the site of the BCG injection which is followed by a local lesion (redness and swelling) that may ulcerate some weeks later. In study C-013-404 all study participants received BCG at Study Day -42, before receiving AERAS-404 or placebo at Study Days 0, 56, and 231. Redness was the most common local BCG injection site reaction with all

subjects (100%) experiencing redness after BCG vaccination. Redness persisted in the majority of the subjects throughout the study, with at least 60% of subjects in each group having redness at the final study assessment. Swelling was the second most common local BCG injection site reaction. None of the redness or swelling at the BCG injection site met protocol-specified toxicity grading scale criteria for a severe (Grade 3 or higher) adverse event. BCG injection site ulceration occurred after BCG vaccination in both placebo and AERAS-404 recipients. There was no dose-related increase in frequency of BCG site ulceration after receiving AERAS-404. Overall, there was similar distribution of BCG local reactions in subjects who received AERAS-404 and placebo, with no dose response of BCG site reactions noted in subjects who received 2 doses or 3 doses of AERAS-404 and no evidence of acute worsening of BCG site reactions after AERAS-404 administration.

Table 1-2 presents the number and percentage of subjects in all four Phase 1 studies of AERAS-404 experiencing adverse events related to AERAS-404 for events occurring in  $\geq 5\%$  of subjects.

**Table 1-2 Adverse Events in the AERAS-404 Treatment Groups in Adults with Event Rate of  $\geq 5\%$  and Number of Subjects for Each Event**

Related Adverse Event <sup>a</sup> MedDRA Preferred Term <sup>b</sup>	AERAS-404 (N=198) <sup>c</sup> n <sup>d</sup> (%)
Injection site pain	109 ( 55.1)
Fatigue	95 ( 48.0)
Headache	82 ( 41.4)
Myalgia	76 ( 38.4)
Injection site swelling	39 ( 19.7)
Injection site erythema	37 ( 18.7)
Arthralgia	28 ( 14.1)
Pyrexia	25 ( 12.6)
Neutrophil count decreased	18 ( 9.1)
Application site hypersensitivity <sup>e</sup>	14 ( 7.1)
Chills	13 ( 6.6)
Diarrhoea	12 ( 6.1)
Protein urine	12 ( 6.1)
Red blood cells urine	12 ( 6.1)
Haemoglobin decreased	11 ( 5.6)
Nausea	10 ( 5.1)

- Adverse event data through the end of the study for C-005-404, C-006-404, C-011-404, and C-013-404; includes adverse events that occurred on or after the first dose of AERAS-404
- For the purpose of summarizing cumulative data across multiple studies, adverse events were coded using the same version of MedDRA, which may differ from the version of MedDRA that was used to code adverse events in an individual study.
- N is number of subjects who received at least 1 dose of AERAS-404 (HyVac4)
- n is number of subjects with at least 1 related adverse event for the preferred term in question
- Application site hypersensitivity refers to reactions at the tuberculin skin test application site

A more detailed description of post-vaccination adverse events on all AERAS-404 clinical trials in adults can be found in the IB. As noted, while an infant study is expected to begin enrolling in

2013, and while a related vaccine is currently being evaluated in adolescents, this will be the first use of AERAS-404 in adolescents.

Immune responses in the previous adult trials have been used to determine the dose regimen to be used in this study. Among participants in study C-006-404, increases in ELISpot responses were seen among all groups receiving AERAS-404, and peaked at Day 84. Responses were strongest among participants receiving IC31 at 500 nmol, with comparable responses between groups receiving 5, 15, and 50 mcg H4. ICS responses showed Ag85B-specific cluster of differentiation 4 (CD4+) T-cells among all treatment groups, peaking at 2 or 4 weeks after the second dose of AERAS-404. The highest magnitude of responses was noted among the 5, 15, and 50 mcg H4 dose groups. CD4+ responses were predominantly of bifunctional or polyfunctional profile. By comparison, TB10.4-specific CD4+ responses were lower in frequency and of lower magnitude compared to Ag85B-induced CD4 responses.

Samples from participants in C-011-404 were evaluated following stimulation with Ag85B. Responses were noted in each dose group by 7-color Aeras ICS, with the highest responses being noted in the 15/500 H4:IC31 group predominantly against the Ag85B peptide pool. No new T-cell responses were noted among participants receiving the highest level of antigen, 150 mcg H4. *Mtb*-specific CD4 T-cell responses did not significantly change in the 5/500 and 50/500 H4:IC31 groups following the second dose of study vaccine, whereas a significant increase of *Mtb*-specific CD4 T-cell responses was observed in the 15/500 H4:IC31 group (mean 0.237%) at D84/182 as compared to both baseline ( $P < 0.0001$ ) and D14/28 ( $P = 0.0364$ ). Responses were more highly polyfunctional in the 5/500 and 15/500 H4:IC31 groups.

In Study C-013-404, the dosage studied, 50/500 H4:IC31, was selected before 15/500 H4:IC31 was found to induce a more favorable immune profile in adults. Two or three doses of AERAS-404 boosted recent BCG priming and induced Ag85B antigen-specific CD4 T cells above that seen with placebo control. These responses were primarily polyfunctional (IFN- $\gamma$ , IL-2 and TNF- $\alpha$ ) and bi-functional (IL-2 and TNF- $\alpha$ ). In subjects receiving 3 doses of AERAS -404, the magnitude of Ag85B-specific CD4 T cell responses on Study Day 259 (28 days after the third dose) was comparable to that seen on Study Day 84 (28 days after the second dose). The three dose regimen was seen to only slightly outperform the two dose regimen as measured by the magnitude of Ag85B-specific CD4 T-cell responses.

In summary, CD4 responses have consistently been induced by vaccination with AERAS-404. Responses were higher after two vaccinations of the 15/500 dose compared to the higher and lower doses, and did not significantly increase with a third vaccination. Based on these data, a dose of 15/500 H4:IC31 administered on a 0, 56 Day schedule has been selected as most appropriate for this trial in adolescents.

## 1.5 Clinical Experience with Vaccines Related to AERAS-404

Two fusion protein vaccines using similar antigens and the same adjuvant used in AERAS-404 are also in clinical development.

**AERAS-456 (H56:IC31)**

AERAS-456 is a fusion protein of the Ag85B, ESAT-6, RV2660 antigens formulated with IC31 adjuvant, and is being jointly developed by Aeras and SSI. One previous Phase 1 trial (C-032-456) has been conducted in South African adults. Twenty-five BCG-vaccinated adults were enrolled in 3 groups stratified by baseline QuantiFERON TB Gold status (latently infected) and H56/IC31 dosage as follows: 8 healthy subjects not infected with *Mtb* (dose 50/500 H56:IC31), 8 *Mtb* infected subjects (dose 15/500 H56:IC31), and 9 *Mtb* infected subjects (dose 50/500 H56:IC31). Three doses were administered in all three groups at intervals of 56 days. Subjects were followed up for 210 days.

No vaccine related SAEs and no severe AEs were reported. The most common systemic adverse events were headaches, proteinuria and upper respiratory tract infections with most judged not to be vaccine related. All local AEs were mild to moderate and transient without medical interventions. AERAS-456 was immunogenic in BCG-vaccinated adults with and without latent infection. The vaccine induced a dominant CD4+ T cell responses dominated by polyfunctional CD4+ T cells. A greater boosting effect was observed in LTBI (+) subjects than in LTBI (-) subjects.

**Hybrid-1:IC**

The Hybrid-1:IC vaccine is being developed by SSI, and consists of the Hybrid 1 fusion protein of the *Mtb* antigens ESAT-6 and Ag85B in combination with IC31. Three Phase I clinical trials in healthy adults have been completed. These studies enrolled a total of 95 healthy, HIV (-) adults with and without latent TB infection, 73 of whom received up to 2 vaccinations with 50 mcg antigen and 100 or 500 nmol of IC31 (van Dissel et al, 2010; van Dissel et al, 2011). A Phase II trial evaluated two vaccinations of 40 HIV infected adults with CD4 counts >350, and was completed in 2013. Another Phase II trial in South African adolescents with and without latent TB infection is currently enrolling; a total of 240 subjects will receive 1 or 2 doses of 15 or 50 mcg antigen and 500 nmol of IC31.

Two possible vaccine related SAEs were observed (elevated CPK and AST/ALT values, not associated with clinical abnormalities) but the Hybrid-1:IC vaccine has generally been well tolerated, with most adverse events mild or moderate. No safety concerns have been identified, including in populations with HIV infection and latent TB infection, and in adolescents.

**1.6 Description of BCG**

BCG Vaccine SSI is manufactured by Statens Serum Institut (SSI), Copenhagen, Denmark and imported and distributed in South Africa for the national immunization program by Biovac, Johannesburg, South Africa. BCG Vaccine SSI is registered in South Africa for prevention of TB in children and adults. BCG, an attenuated, live culture of the *Bacillus Calmette-Guérin*, was originally attenuated between 1906 and 1919 by serial passage of an *M. bovis* strain. The Statens Serum Institut in Copenhagen, Denmark derives this vaccine from the Danish BCG strain 1331. SSI BCG is supplied by the manufacturer in amber 10-dose vials containing 0.75 mg lyophilized SSI BCG. The vaccine will be reconstituted with Sauton SSI diluent supplied by the manufacturer, according to the manufacturer's instructions. After reconstitution, 1 adult dose (0.1 mL) contains 2 to 8 x 10<sup>5</sup> CFU. Sauton SSI Diluent is composed of magnesium sulphate,

dipotassium phosphate, citric acid, monohydrate L-asparagine monohydrate, ferric ammonium citrate and glycerol at 85%, which is reconstituted with sterile water for injection.

There is considerable experience with BCG vaccine, which is licensed in South Africa for prevention of TB in children and adults. Local and systemic reactogenicity in various populations is well described (FitzGerald 2000; Hoft et al 1999; Lotte et al 1984; Nicol et al 2002). Injection site and regional complications, such as extensive local ulceration, local subcutaneous abscesses, and suppurative lymphadenitis, occur in less than 1 per 5 million vaccinations. Induration and redness of the skin at the site of intradermal BCG vaccination typically develops within several days and represents a normal response to BCG vaccination. The induration gradually resolves over several days and is followed by a small local superficial ulcer. The ulcer opens and drains for 4 weeks on average, and spontaneously heals within 2-3 months, usually leaving a small scar. A brief period of minor, asymptomatic enlargement of the regional cervical and axillary lymph nodes (< 1 cm) is common. Systemic adverse events due to BCG occur very infrequently. Fever, headache and non-injection site cutaneous manifestations occur in less than 1% of vaccinees. Severe systemic adverse events, such as osteitis and disseminated BCG disease, are rare (about 1 per 5 million vaccinations) and usually occur in immune-compromised infants. Severe local or systemic complications of BCG would not be expected in the healthy, HIV-uninfected, *Mtb* uninfected adolescents to be recruited in this trial.

Adolescents in this trial will have received prior BCG vaccination at birth. BCG revaccination appears to be safe and well tolerated in adolescents and young adults (Pereira et al 2012; Rodrigues et al, 2005; Bottiger et al 1983). In a large trial of BCG revaccination in more than 7,000 Brazilian adolescents, only 25 of the participants had adverse reactions and no deaths or cases of disseminated BCG disease were reported (Pereira et al 2012; Rodrigues et al, 2005). Other published trials of BCG revaccination have concluded that BCG revaccination is not associated with significantly more AEs than primary BCG vaccination. In a trial of BCG revaccination in 2,997 fourteen to fifteen year old Swedish adolescents, open vaccination lesions were reported in 4% (mean diameter of open lesions 4mm) of adolescents receiving the Danish BCG vaccine that will be used in this trial (Bottiger et al 1983). South African Tuberculosis Vaccine Initiative (SATVI) data from an ongoing trial of BCG revaccination and isoniazid preventive therapy (South African National Clinical Trials Register DOH-27-0212-3995) indicate that BCG revaccination is safe and well tolerated in young adults. Eighty-two subjects in this ongoing trial were enrolled with a TST  $\geq 15$ mm. Data from these *Mtb*-infected young adults show that injection site reactions are mostly of mild intensity, with ulceration of the injection site resolving within three months. No BCG-related SAEs or major safety concerns have arisen.

## 1.7 Rationale for Study

This clinical trial will evaluate safety, immunogenicity, and prevention of *Mtb* infection (QFT-GIT conversion), of AERAS-404 and BCG revaccination in healthy, HIV-uninfected, QFT-GIT negative, previously BCG vaccinated adolescents. QFT-GIT negative adolescents in the area where this study will be conducted have previously been shown to have very high rates of TST and QFT-GIT conversion, associated with increased risk of active TB disease in subsequent years (Mahomed et al, 2011b; Machingaidze et al, 2012). A TB vaccination strategy incorporating BCG revaccination or AERAS-404 vaccination in adolescents or young adults, if

found to prevent *Mtb* infection, would likely have a major impact on TB disease, TB transmission, and control of the epidemic (Dye and Williams, 2008; Abu-Raddad et al, 2009; Dye, 2000). If revaccination with BCG or vaccination with AERAS-404 is shown to prevent infection with *Mtb* in this proof of concept study in adolescents, additional larger scale studies examining the impact on TB disease in more diverse populations would be warranted.

## 2 STUDY OBJECTIVES AND DESIGN

### 2.1 Objectives

#### Primary Objectives:

- To evaluate the safety profile in HIV-uninfected, remotely BCG vaccinated adolescents of
  - AERAS-404
  - BCG revaccination
- To evaluate prevention of *Mtb* infection, as measured by rates of conversion using a QFT-GIT assay, by
  - AERAS-404 compared to placebo
  - BCG revaccination compared to placebo

#### Secondary Objectives:

- To evaluate prevention of *Mtb* infection, as measured by rates of sustained conversion using a QFT-GIT assay, by
  - AERAS-404 compared to placebo
  - BCG revaccination compared to placebo
- To investigate the immunogenicity in HIV-uninfected, remotely BCG vaccinated adolescents of
  - AERAS-404
  - BCG revaccination

As this is a novel study design, generation of data and hypotheses to be tested in future vaccine trials is an important overarching goal. Thus the exploratory objectives below will also be investigated:

- To evaluate prevention of *Mtb* infection, as measured by rates of reversion to a negative from a positive QFT-GIT assay, by
  - AERAS-404 compared to placebo
  - BCG revaccination compared to placebo
- To explore the effect of alternative QFT-GIT test threshold values on rates of QFT-GIT conversion, QFT-GIT reversion, and prevention of *Mtb* infection
- To identify immune correlates of risk for *Mtb* infection
- To identify immune correlates of vaccine-induced protection against *Mtb* infection induced by AERAS-404 or BCG revaccination
- To evaluate alternative IGRAs or immune markers for diagnosis of *Mtb* infection or as markers of exposure to non-tuberculous mycobacteria (NTM)
- To explore trends in TB disease incidence after AERAS-404 vaccination or BCG revaccination

- To explore trends in QFT-GIT prolonged/sustained conversions and late reversions (i.e., more than 6 months post initial conversion) in early QFT-GIT converters (i.e., among those who converted at Month 6 or Month 12 of follow-up)

## 2.2 Design and Endpoints

This Phase II, randomized, 3-arm, placebo controlled, partially blinded, clinical trial will be conducted in 990 healthy, HIV-uninfected, QFT-GIT negative, previously BCG vaccinated adolescents. The trial will be conducted at the SATVI site in the Worcester region of the Western Cape region of South Africa, where epidemiological studies involving thousands of adolescents have been conducted over the last decade to characterize rates of *Mtb* infection and active TB disease in this age group (Mahomed et al, 2011b; Mahomed et al, 2013). Additional sites with similar TB epidemiology may also be used.

Subjects will be recruited from high schools or directly from the community. The all-TB disease incidence in the Worcester region is greater than 1% per year (2011 DOH data). TB disease incidence in 12-18 year old adolescents attending high school is 450 per 100,000; approximately 40% of adolescents are *Mtb* infected at entry to high school; and HIV prevalence among adolescents is less than 1% (Mahomed et al, 2011b; Mahomed et al, 2013).

Subjects will be randomized in a 1:1:1 ratio to receive either two doses of AERAS-404 (15 mcg H4/500 nmol IC31), or two doses of placebo at equivalent volume, administered by intramuscular injection on Day 0 and Day 56; or one dose of BCG Vaccine SSI (2-8 x 10<sup>5</sup> CFU) administered by intradermal injection on Day 0. Allocation to the AERAS-404 and saline placebo trial arms will be double-blinded. Since BCG causes a recognizable local injection site reaction, the BCG revaccination trial arm will be unblinded. Laboratory staff performing QFT-GIT, immunogenicity, and correlates assays will be blinded to all three interventions.

Subjects will be enrolled in two sequential cohorts and within each cohort subjects will be randomized in a 1:1:1 ratio to receive either AERAS-404, saline placebo, or BCG Vaccine SSI. The first 90 subjects (30 from each arm) will form the Safety & Immunogenicity Cohort and will be subject to more intensive collection of safety data, with data reviewed by the Data Monitoring Committee (DMC), principal investigator and local medical monitor. Selected immunogenicity assays, including whole blood intracellular cytokine staining (ICS), will also be performed in this cohort. The remaining 900 subjects will be enrolled into the Correlates Cohort. All 990 subjects in the study will be evaluated for safety and biomarker outcomes, and for prevention of *Mtb* infection.

Allocation of subjects by treatment group and study cohort is shown in Table 2-1.

**Table 2-1 Distribution of Subjects by Treatment Group and Study Cohort**

Study Cohort	Planned Number of Subjects <sup>a</sup>			Total
	Treatment Group			
	Placebo	BCG	AERAS-404	
Safety & Immunogenicity	30	30	30	<b>90</b>
Correlates	300	300	300	<b>900</b>
<b>Total</b>	<b>330</b>	<b>330</b>	<b>330</b>	<b>990</b>

a. Subject numbers are approximate due to block randomization.

Safety outcomes will include solicited adverse events (AEs) reported through 7 days after each vaccination and unsolicited adverse events reported through 28 days after each vaccination, injection site assessments performed through Day 84, and adverse events of special interest (AESIs), serious adverse events (SAEs), and suspected unexpected serious adverse reactions (SUSARs) reported until end of study. The Data Monitoring Committee (DMC) will review unblinded safety data from the Safety & Immunogenicity Cohort twice: data through Day 7 and data through Day 84. The DMC may request additional information, or a pause in recruitment and vaccination, while safety data are being evaluated. The DMC will make a formal recommendation on the continued conduct of the trial after each safety review; if pausing rules have not been met enrollment will continue while the reviews are being conducted. Each subject will be followed for safety for a minimum of 6 months after the last vaccination.

The primary *Mtb* infection endpoint will be QFT-GIT conversion from a negative to positive test, using the manufacturer's recommended threshold of 0.35 IU/mL, at any time-point after Day 84 and through end of follow-up for the primary endpoint (see Figure 2-1) (Mazurek et al, 2010). The 84-day 'wash-out' period is stipulated in order to exclude subjects who may have already been *Mtb* infected but not yet converted their QFT-GIT test at screening, thus subjects who convert their QFT-GIT at Day 84 will not be included in the analyses of prevention of *Mtb* infection.

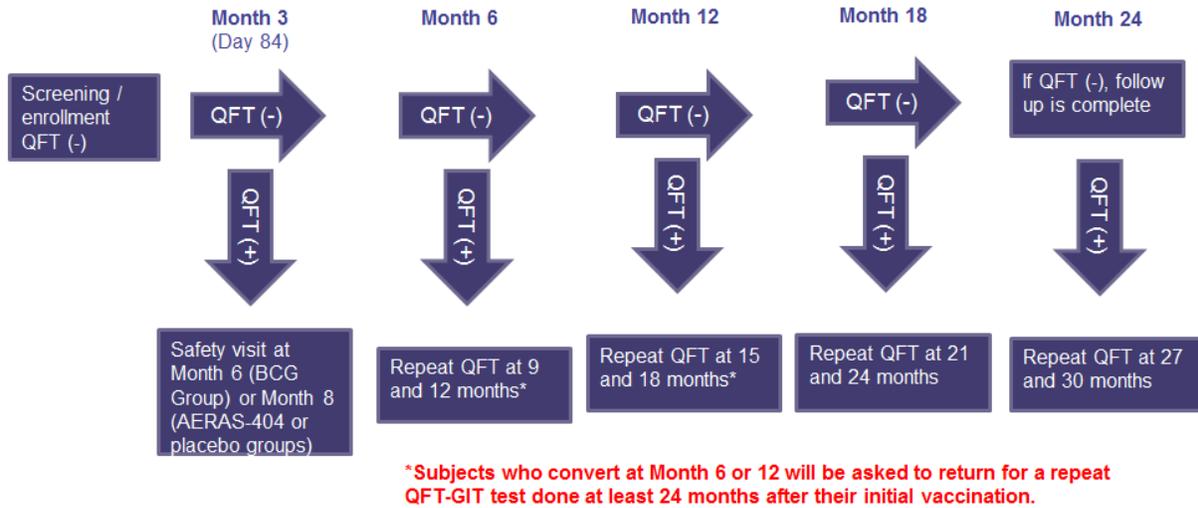
The duration of the trial will be endpoint-driven, using a design targeting accrual of 64 primary endpoints. The primary analysis will be triggered when at least 64 endpoints are accrued in the study (both cohorts) AND the median follow up time is at least 15 months, with a maximum individual follow-up time to detect initial QFT-GIT conversion of 24 months. Enrollment is expected to take approximately 12 months and the primary analysis is expected to occur approximately 21 months from the first subject enrolled. Therefore, duration of individual subject follow-up for the primary analysis is expected to range from 9 – 21 months (maximum 24 months), with 6 months of additional follow up for those who convert to QFT-GIT positive after Day 84 as shown in Figure 2-1. If an initial reduction in infection rate in the BCG or AERAS-404 groups is seen in the primary analysis of QFT-GIT conversion, follow up for the entire study may be extended to examine the duration of prevention of *Mtb* infection.

The trial sample size of 990 subjects is determined by the primary prevention of *Mtb* infection objective (measured by prevention of conversion to a positive QFT-GIT test). A potential mechanism of action of vaccination might be to allow initial *Mtb* infection (and QFT-GIT conversion), but to prevent establishment of sustained latent *Mtb* infection (and thus persistent

QFT-GIT conversion), resulting in QFT-GIT reversion to negative. Thus the study will also examine the persistence of QFT-GIT conversion.

All subjects with primary QFT-GIT conversion will be followed for an additional 6 months post-conversion to ascertain the sustained QFT-GIT conversion and QFT-GIT reversion endpoints. Subjects with an initial QFT-GIT conversion at Month 6 or 12 will be asked to return for a final QFT-GIT evaluation and assessment for TB signs and symptoms at least 24 months after their initial vaccination. The secondary *Mtb* infection endpoint will be the combination of QFT-GIT conversion from a negative to positive test, using the manufacturer's recommended threshold of 0.35 IU/mL, at any time-point after Day 84 and through end of follow-up for the primary endpoint, *AND* persisting without QFT-GIT reversion from a positive to a negative test through 6 months after QFT-GIT conversion. The exploratory *Mtb* infection endpoints will be defined by alternative threshold values for QFT-GIT conversion and reversion (Pai, 2012; van Zyl-Smit et al, 2009; Metcalfe et al, 2013), alternative time periods for definition of persistent QFT-GIT conversion, and the evaluation of novel IGRAs which are currently being developed; details on these endpoint cut-off values will be included in the statistical analysis plan.

The secondary analysis will be triggered after 6 months of additional post-conversion follow-up for individuals who convert, regardless of the rate of QFT-GIT reversion. Thus, total duration of the trial is expected to be 27-30 months (21-24 months, plus 6 months additional follow up for converters), with a maximum total duration of 42 months (12 months enrollment, 24 month maximum individual follow up, plus 6 months additional follow up for converters). The schedule of follow up for each individual subject is contingent on results of the QFT-GIT tests conducted at Month 3 (Day 84), and Months 6, 12, 18, and 24. For example, a subject who has negative QFT-GIT tests at screening, Month 3, and Month 6, but who has a positive QFT-GIT at Month 12, will then have two additional visits with QFT-GIT testing at Months 15 and 18, at which time follow up will be complete for that subject. A schematic of follow up and QFT-GIT testing is shown in Figure 2-1.



**Figure 2-1 Schematic of Follow Up and QFT-GIT Testing**

Based on previous data from baseline QFT-GIT negative adolescents at the SATVI site, we expect the incidence of active TB disease in the study population to approximate 0.2% (estimate 2 cases per year of follow-up) (Mahomed et al, 2011b). While the total number of active TB disease cases is expected to be very low and the study is not powered to detect a difference in rates of active TB disease among the vaccine groups, data on the number and type of cases of active TB disease (as defined in Section 3.6.1) will be captured as part of the assessment of safety.

Immunological outcomes will include intracellular cytokine staining (ICS) on PBMCs and whole blood, transcriptomics on RNA from whole blood, absolute blood cell subset counting by flow cytometry (for deconvolution of transcriptomics), quantification of soluble immune mediators by multiplex ELISA (all in the Safety & Immunogenicity Cohort only), and exploratory assays using PBMCs, plasma, RNA, and absolute blood cell subset counting samples for correlates of risk/protection analyses in all subjects.

### 3 STUDY PROCEDURES

#### 3.1 Schedule of Subject Evaluations

A Summary Schedule of Evaluations depicting visit-specific procedures is provided in Table 3-1 for subjects receiving BCG and Table 3-2 for subjects receiving AERAS-404 or placebo. Written informed consent and assent must be obtained before any screening procedures are performed. See Appendix A for a more detailed description of the evaluations.

**Table 3-1 Schedule of Subject Evaluations (Subjects receiving BCG, N=330)**

Study Visit Day (D) or Month (M) →	Screen	0	D7	D28	D70	D84 <sup>c</sup>	M6 (D168) <sup>d,e</sup> M12 (D336) M18 (D504) M24 (D672)
Eligibility criteria verification	X	X					
Medical history	X						
Physical examination	X						
Urine βHCG (all females)	X	X					
QuantiFERON®-TB Gold in-tube (mL)	3					3	3
HIV-1 (mL) with HIV counselling	3						
Urinalysis	X		X <sup>b</sup>				
Serum chemistry (mL) <sup>g</sup>	5		5 <sup>b</sup>				
CBC, differential, platelets (mL)	5		5 <sup>b</sup>				
Vital signs	X	X					
Interval history		X	X	X	X	X	X
Focused physical examination <sup>a</sup>		X	X	X	X	X	X
Distribute/review diary cards		X <sup>b</sup>	X <sup>b</sup>				
Solicited adverse events (incl. con. meds.)		X	X				
Unsolicited adverse events (incl. con. meds.)		X	X	X			
SAEs, AESIs, and SUSARs (incl. con. meds.)		X	X	X	X	X	X
Solicited and unsolicited injection site reaction adverse events (incl. con. meds.)		X	X	X	X	X	
Site of injection examination		X	X	X	X	X	
TB symptom screen	X			X	X	X	X
BCG administration		X					
Whole blood assay (mL)		6 <sup>b</sup>			6 <sup>b</sup>		
Absolute blood count (mL)		0.5	0.5				0.5
RNA (mL)		2.5	2.5				2.5
PBMC for ICS (mL)		8 <sup>b</sup>			8 <sup>b</sup>		
Serum for multiplex ELISA (mL)		3 <sup>b</sup>	3 <sup>b</sup>				
PBMC, plasma, and serum for correlates of risk/protection (mL)		16			16		40 <sup>i</sup>
Whole blood for evaluation of novel IGRA (mL) <sup>f</sup>	7	7				7	7
PBMC for evaluation of NTM exposure (mL)		10					

Study Visit Day (D) or Month (M) →	Screen	0	D7	D28	D70	D84 <sup>c</sup>	M6 (D168) <sup>d,e</sup> M12 (D336) M18 (D504) M24 (D672)
Per visit phlebotomy volume (mL) <sup>h</sup>	23	53	16	0	32.5	10	53 (M6,M12)/ 13 (M18,M24)
Cumulative phlebotomy volume (mL) <sup>h</sup>	23	76	92	92	124.5	134.5	266.5

- If indicated by interval history
- Subjects in the Safety & Immunogenicity Cohort only
- Subjects who convert from QFT-GIT(-) to (+) at Day 84 will have a final visit at 168 days ±14 days after vaccination for assessment of SAEs, AESIs, SUSARs, and TB symptom screen
- Month 6, 12, 18, and 24 study visits will be completed by subjects who did not convert from QFT-GIT (-) to (+) at the previous visit; the Month 24 visit will be the final visit for subjects who remain QFT-GIT(-) throughout the study.
- Subjects who convert from QFT-GIT (-) to (+) at Month 6-24 visits will have two additional visits with repeat QFT-GIT testing, assessment of SAEs, AESIs, SUSARs, and TB symptom screen 84 days ±14 days and 168 days ±14 days after conversion. Up to 10 mL of blood will be collected at each of those visits, including blood for absolute blood count and RNA. Subjects who initially convert to QFT-GIT(+) at Month 6 or 12 will be asked to have a final QFT-GIT test, blood collection for absolute blood count and RNA, and TB symptom screen done at least 24 months after their initial vaccination.
- May not be collected at all time points shown.
- Serum chemistry includes AST, ALT, alkaline phosphatase, total bilirubin, creatinine, and BUN.
- Blood volumes are approximate.
- Study Months 6 and 12 only.

**Table 3-2 Schedule of Subject Evaluations (Subjects receiving AERAS-404 [N=330] or placebo [N=330])**

Study Visit Day (D) or Month (M) →	Screen	0	D3	D7	D28	D56	D63	D70	D84 <sup>c</sup>	M6 (D168) <sup>d,e</sup> M12 (D336) M18 (D504) M24 (D672)
Eligibility criteria verification	X	X				X				
Medical history	X									
Physical examination	X									
Urine βHCG (all females)	X	X				X				
QuantiFERON®-TB Gold in-tube (mL)	3								3	3
HIV-1 (mL) with HIV counselling	3									
Urinalysis	X			X <sup>b</sup>			X <sup>b</sup>			
Serum chemistry (mL) <sup>g</sup>	5			5 <sup>b</sup>			5 <sup>b</sup>			
CBC, differential, platelets (mL)	5			5 <sup>b</sup>			5 <sup>b</sup>			
Vital signs	X	X				X				
Interval history		X	X	X	X	X	X	X	X	X
Focused physical examination <sup>a</sup>		X	X	X	X	X	X	X	X	X
Distribute/review diary cards		X <sup>b</sup>		X <sup>b</sup>		X <sup>b</sup>	X <sup>b</sup>			
Solicited adverse events (incl. con. meds.)		X	X	X		X	X			
Unsolicited adverse events (incl. con. meds.)		X	X	X	X	X	X	X	X	
SAEs, AESIs, and SUSARs		X	X	X	X	X	X	X	X	X

Study Visit Day (D) or Month (M) →	Screen	0	D3	D7	D28	D56	D63	D70	D84 <sup>c</sup>	M6 (D168) <sup>d,e</sup> M12 (D336) M18 (D504) M24 (D672)
(incl. con. meds.)										
Solicited injection site reaction adverse events (incl. con. meds.)		X	X	X	X	X	X	X	X	
Site of injection examination		X	X	X	X	X	X	X	X	
TB symptom screen	X				X	X			X	X
Study vaccine administration		X				X				
Whole blood assay (mL)		6 <sup>b</sup>						6 <sup>b</sup>		
Absolute blood count (mL)		0.5	0.5							0.5
RNA (mL)		2.5	2.5							2.5
PBMC for ICS (mL)		8 <sup>b</sup>						8 <sup>b</sup>		
Serum for multiplex ELISA (mL)		3 <sup>b</sup>	3 <sup>b</sup>							
PBMC, plasma, and serum for correlates of risk/protection (mL)		16						16		40 <sup>i</sup>
Whole blood for evaluation of novel IGRA (mL) <sup>f</sup>	7	7							7	7
PBMC for evaluation of NTM exposure (mL)		10								
Per visit phlebotomy volume (mL) <sup>h</sup>	23	53	6	10	0	0	10	32.5	10	53 (M6,M12)/ 13 (M18,M24)
Cumulative phlebotomy volume (mL) <sup>h</sup>	23	76	82	92	92	92	102	134.5	144.5	276.5

a. If indicated by interval history

b. Subjects in the Safety & Immunogenicity Cohort only

c. Subjects who convert from QFT-GIT(-) to (+) at Day 84 will have a final visit at 168 days ±14 days after second vaccination (Month 8, Study Day 224) for evaluation of SAEs, AESIs, SUSARs, and TB symptom screen

d. The Month 6, 12, 18, and 24 study visits will be completed by subjects who did not convert from QFT-GIT (-) to (+) at the previous visit; the Month 24 visit will be the final visit for subjects who remain QFT-GIT(-) throughout the study

e. Subjects who convert from QFT-GIT (-) to (+) at Month 6-24 visits will have two additional visits with repeat QFT-GIT testing, assessment of SAEs, AESIs, SUSARs, and TB symptom screen 84 days ±14 days and 168 days ±14 days after conversion. Up to 10 mL of blood will be collected at each of those visits, including blood for absolute blood count and RNA. Subjects who initially convert to QFT-GIT(+) at Month 6 or 12 will be asked to have a final QFT-GIT test, blood collection for absolute blood count and RNA, and TB symptom screen done at least 24 months after initial vaccination.

f. May not be collected at all time points shown.

g. Serum chemistry includes AST, ALT, alkaline phosphatase, total bilirubin, creatinine, and BUN.

h. Blood volumes are approximate.

i. Study Months 6 and 12 only.

## 3.2 Subject Selection

### 3.2.1 Recruitment and Informed Consent

Subjects will be recruited from high schools or communities in the Worcester region, and potentially elsewhere with similar TB epidemiology, through liaison with Education Department officials, teachers, parent organizations, and community organizations. Various methods of

recruitment may be used, such as classroom information sessions, advertising, referrals, word-of-mouth, or solicitation through subjects previously known to the clinical site. All recruitment materials will be approved by the Institutional Review Board (IRB) or Independent Ethics Committee (IEC). Interested subjects will be invited to participate in the informed consent and assent process. Informed consent will be obtained by the use of a written consent form approved by the IRB or IEC and signed and dated by the parent/legal guardian at the time of consent. Similarly, informed assent will be obtained by the use of a written assent form approved by the IRB or IEC and signed and dated by the subject at the time of assent. Potential subjects will be interviewed to ensure that they meet all entry criteria relating to history. The clinical investigator or designee will conduct the consent and assent discussions on an individual basis with each subject and parent/legal guardian and will allow adequate time for all questions to be addressed. Written informed consent and assent will be obtained prior to conducting any study-related procedures. A copy of the signed consent and assent forms shall be given to the subject and parent/legal guardian prior to conducting any study-related procedures.

Subjects who converted to a positive QFT-GIT at Month 6 or 12 will be approached for an additional QFT-GIT test, blood collection for absolute blood count and RNA, and evaluation for signs/symptoms of TB to be conducted at least 24 months after their initial vaccination. Informed consent will be obtained by the use of a written supplemental consent form approved by the IRB or IEC, and signed and dated by subject or by the subject's parent/legal guardian if subject is less than 18 years of age. Similarly, informed assent will be obtained by the use of a written assent form approved by the IRB or IEC, and signed and dated by the subject if the subject is less than 18 years of age.

### 3.2.2 Screening

After informed consent is obtained, subjects will be screened to assess eligibility for the study. For identification purposes each subject will be assigned a unique 12-digit subject number by an interactive voice/web response system (IVRS/IWRS) that consists of the last two digits of the Aeras product number (04), the last two digits of the protocol number (40), a 2-digit site number assigned by Aeras, followed by a 5-digit number sequentially assigned by the system. (For example, if the clinical site number is 99, the first subject to be screened for protocol C-040-404 would receive the number 044099-00001, where all except "-00001" were pre-assigned by Aeras.) This subject number will be used throughout the study.

Abnormal results and findings resulting in ineligibility will be discussed with the subject, who will be referred for follow-up care with their healthcare provider if necessary.

Eligibility for randomization will be based on the inclusion and exclusion criteria described below. The investigator must document confirmation of eligibility prior to randomization.

### 3.2.3 Inclusion Criteria

**Subjects must meet all of the following criteria at the time of randomization:**

1. Has completed the written informed consent and assent process
2. Is age  $\geq 12$  years and  $\leq 17$  years on Study Day 0

3. Agrees to stay in contact with the study site for the duration of the study, provide updated contact information as necessary, and has no current plans to move from the study area for the duration of the study
4. For female subjects: agrees to avoid pregnancy from 28 days prior to Study Day 0 and for the full duration of the study. Women physically capable of pregnancy (not sterilized and still menstruating or within 1 year of the last menses) in sexual relationships with men must use an acceptable method of avoiding pregnancy during this period. Acceptable methods of avoiding pregnancy include a sterile sexual partner, sexual abstinence (not engaging in sexual intercourse), hormonal contraceptives (oral, injection, transdermal patch, or implant), vaginal ring, intrauterine device (IUD), or the combination of a condom or diaphragm with spermicide
5. Has general good health, confirmed by medical history and physical examination
6. Had BCG vaccination at least 5 years ago documented through medical history or by presence of healed BCG scar
7. Tests QFT-GIT negative at screening, using the manufacturer's recommended threshold of 0.35 IU/mL

### 3.2.4 Exclusion Criteria

**Subjects must have none of the following at the time of randomization:**

1. Acute illness on Study Day 0
2. Axillary temperature  $\geq 37.5^{\circ}\text{C}$  on Study Day 0
3. Abnormal laboratory values from the most recent blood collected prior to randomization as follows:
  - Laboratory evidence of hematologic disease (white blood cell count  $< 3000/\text{mm}^3$  or  $> 11,500/\text{mm}^3$ ; hemoglobin  $< 0.9$  times the lower limit of normal of the testing laboratory, by age and gender; absolute neutrophil count  $< 1300/\text{mm}^3$ ; absolute lymphocyte count  $< 1000/\text{mm}^3$ )
  - ALT, AST, alkaline phosphatase, total bilirubin, creatinine, blood urea nitrogen (BUN)  $> 1.25$  times the upper limit of normal of the testing laboratory
4. Greater than Grade 1 on the Toxicity Scale urinalysis result (with the exception of hematuria in a menstruating female), or urinalysis abnormality judged clinically significant by the investigator
5. History or evidence of any clinically significant systemic disease, or any acute or chronic illness that might affect the safety, immunogenicity, or efficacy of study vaccine in the opinion of the investigator
6. History of treatment for active TB disease or latent *Mtb* infection
7. History or evidence, including chest X-ray, of active TB disease
8. Shared residence with an individual receiving anti-TB treatment, or known to have incompletely treated culture or smear positive TB, at screening
9. History of autoimmune disease or immunosuppression
10. Used immunosuppressive medication within 42 days before Study Day 0 (inhaled and topical corticosteroids are permitted)
11. Received immunoglobulin or blood products within 42 days before Study Day 0

12. Received any investigational drug therapy or investigational vaccine within 182 days before Study Day 0, or planned participation in any other clinical trial during the study period
13. Received investigational TB vaccine, other than BCG, at any time prior to Study Day 0
14. Planned administration/administration of a licensed vaccine in the period starting 28 days before and ending 28 days after each dose of study vaccine
15. History or laboratory evidence of any past or present possible immunodeficiency state including, but not limited to, any laboratory indication of HIV-1 infection
16. History of allergic disease or reactions, including eczema, likely to be exacerbated by any component of the study vaccine
17. History of alcohol or drug abuse
18. All female subjects: currently pregnant or lactating/nursing; or positive urine pregnancy test during screening
19. Received a tuberculin skin test (TST) within 3 months (90 days) prior to Study Day 0.
20. Any current medical, psychiatric, occupational, or substance abuse problems that, in the opinion of the investigator, will make it unlikely that the subject will comply with the protocol

### 3.2.5 Screening Clinical Assessments and Laboratory Tests

Unless noted otherwise, the window period within which all screening evaluations must be completed, and the results reviewed by the investigator to confirm eligibility of subjects, is 21 days prior to and including Study Day 0.

Subjects will provide a detailed medical history and undergo a physical examination. Demographic characteristics (date of birth, gender, and race/ethnicity) will also be collected. A dipstick urinalysis, urine pregnancy test (all females), QFT-GIT test, and an HIV rapid test with repeat confirmation of all positive tests, will be performed. Any new abnormal findings will be discussed with the subject and referral will be made for follow-up care if necessary.

Screening laboratory tests (including serum chemistry, complete blood count, differential blood count, platelets, and hepatic enzymes) will be performed during the screening process. Results from these laboratory tests will serve as study-entry baseline values. Abnormal results and findings that make the subject ineligible will be discussed with the subject and the subject will be referred for follow-up care with their healthcare provider if necessary. All screening laboratory specimens will be processed according to laboratory SOPs available from the clinical laboratory(ies) designated for the study. Information about the laboratory(ies), including any instructions for performing and interpreting specific tests, will be maintained in the investigator's study files.

### 3.3 Study Randomization

Subjects will be randomized to the study based on a randomly-generated sequence of subject identification numbers (randomization schedule) managed by a validated IVRS/IWRS. The randomization schedule will be prepared by a statistician who will not be involved in the analysis of the study in order to maintain the blind of the study team. The day of randomization for each subject will be Study Day 0. Randomization will be in blocks for each school or community, as

the epidemiology of TB transmission may vary from one school or community to another. Randomization will also be blocked such that 30 subjects from each vaccine group will be enrolled in the Safety & Immunogenicity Cohort. Enrollment for the Safety & Immunogenicity Cohort will be from a subset of schools or communities selected to be representative of the study population as a whole in terms of socio-economic status and TB risk. Subjects who discontinue participation will not be replaced.

### **3.4 Blinding**

Assignment of subjects to the BCG Vaccine SSI arm will be unblinded (i.e. subjects and clinic staff will be aware of study product allocation), with the exception that laboratory staff performing the QFT-GIT and other immunological endpoint assays will remain unaware of study product allocation.

Assignment of subjects to the AERAS-404 and saline placebo arms will be blinded, such that subjects, site study staff, and laboratory staff will remain unaware of study product allocation. Since AERAS-404 and the saline placebo have different appearances, the syringes containing AERAS-404 or comparator will be masked so that the study injection administrator (the study team member in the clinic who will be administering the injections) can also remain blinded.

The Aeras investigational product manager (and/or designee) will be unblinded in order to manage study vaccine inventory. The other unblinded persons on the study are the study vaccine manager (and designee, if appointed) and the study monitor(s) responsible for monitoring study vaccines. All unblinded persons must take care to not reveal individual subject treatment regimen assignments to any other member of the study team.

The study vaccine manager (and designee) must be a designated study team member, such as the study pharmacist, who is not an employee of Aeras and who will have no other clinical or regulatory responsibilities associated with the conduct of the study during the entire study period. Unblinded study personnel must not participate in the evaluation of adverse events. A Delegation of Authority Log will be maintained by the site and will identify the individual(s) authorized to function as the study vaccine manager, i.e., individuals with access to study blinding information.

Labels accompanying the syringes of prepared AERAS-404 and placebo will not indicate which study vaccine is in the syringe. Identical syringes and needles will be used for preparation and administration of AERAS-404 and placebo.

#### **3.4.1 Unblinding for Clinical Emergencies**

If there is an urgent clinical requirement to know a subject's treatment assignment, the principal investigator will request the urgent unblinding of a subject's treatment by completing the Subject Unblinding by Site form in the IWRS. The designated study management team will be notified of the unblinding immediately via the IWRS system alert.

If the IWRS system has been closed or is otherwise unavailable, the investigator will make a written request to the vaccine manager for urgent unblinding of a participant's treatment. The request must include the subject identification number, the date, a brief justification of the clinical requirement for unblinding, and the investigator's signature. The request will be kept in the study file. Upon receipt of proper written request, the vaccine manager or designee will disclose the treatment group to the investigator. Aeras must be notified immediately of any clinically required break of the study blind on an Immediately Reportable Event Form.

It is recommended that the principal investigator consults with the local medical monitor and study medical team prior to unblinding of a subject. However, in urgent circumstances at the principal investigator's discretion, the site can proceed with the unblinding of a subject without prior consultation with the study team.

### **3.5 Vaccine Administration**

On Study Day 0, subjects will receive their study injection as soon as possible after randomization, and after their baseline immunology blood collection and other required assessments.

#### **BCG**

For subjects randomized to the BCG group, a single 0.1mL dose of BCG Vaccine SSI will be administered intradermally in the left upper arm (deltoid region) on Study Day 0, using the standard Mantoux technique. BCG Vaccine SSI will be administered in an unblinded fashion.

#### **AERAS-404 and Placebo**

For subjects randomized to the blinded AERAS-404 and saline placebo groups, one injection will be administered intramuscularly (IM) in the left upper arm (deltoid) on Study Day 0. On Study Day 56, subjects who have not met any of the criteria for discontinuation of study injections (see Section 6.1) will receive a second injection in the right upper arm. In cases of short term, reversible conditions, such as acute febrile or respiratory illness or evidence of significant active infection, the second study injection should be deferred until the subject has recovered; the allowable time period for deferral of the second dose is 16 days (i.e., Study Day 56-2/56+14 days).

The study vaccine manager will send the AERAS-404 and placebo to the clinic as a unit-dose syringe, which will be identified with the subject identification number, date and time of dose preparation, and the volume prepared. A medically qualified study team member must be present in the clinic at the time of all study injection administrations.

Before administering the injection, the study injection administrator must inspect the syringe and vaccine volume, checking that the syringe is identified with the correct subject identification number and checking the date and time the dose was prepared.

The syringe will contain 0.5 mL of AERAS-404 or placebo. The study injection will be administered IM by the study injection administrator into the deltoid area using standard aseptic technique.

The study vaccine manager will refer to the Vaccine Management Manual (provided under separate cover) for detailed instructions for AERAS-404 and placebo storage and preparation.

### 3.6 Study Evaluations

The 90 subjects enrolled in the Safety & Immunogenicity Cohort will have more intensive evaluation of safety and immunologic data. The remaining 900 subjects will be enrolled into the Correlates Cohort. All 990 subjects in the study will be evaluated for safety and biomarker outcomes, and for prevention of *Mtb* infection.

#### 3.6.1 Evaluation of *Mtb* infection

QFT-GIT assays will be performed at screening, on Day 84 (end of the wash-out period), on Month 6, and periodically thereafter until end of study, as described in Section 2.2. QFT-GIT assays will be performed according to site SOPs to reduce variability due to effects such as blood draw technique, duration of incubation, amount of blood incubated, temperature of sample storage, etc. The primary evaluation of *Mtb* infection will be QFT-GIT conversion from a negative to positive test, using the manufacturer's recommended threshold of 0.35 IU/mL, at any time-point after Day 84 and through end of follow-up for the primary endpoint.

The exploratory evaluation of *Mtb* infection will be defined by alternative threshold values for QFT-GIT conversion and reversion (Pai, 2012; van Zyl-Smit et al, 2009; Metcalfe et al, 2013); alternative time periods for definition of persistent QFT-GIT conversion; and development and validation of novel IGRAs.

The South African National TB Programme (NTP) does not recommend a course of isoniazid preventive therapy (IPT) for HIV-uninfected adults and children older than 5 years of age with asymptomatic (latent) *Mtb* infection, due to the high risk of reinfection after completion of IPT in this high TB prevalence region. Subjects in this trial will be managed according to current NTP guidelines. However, since QFT-GIT conversion is known to be associated with increased risk of active TB disease, symptom screening for early diagnosis and treatment of incident TB disease will be a safety priority, and all subjects who are found to be QFT-GIT positive (including at screening) will be educated on the symptoms of TB and the benefits of early diagnosis and treatment. Screening for symptoms compatible with incident TB disease, including unexplained cough, fever, night sweats, or loss of weight for longer than two weeks duration will continue throughout the study. Subjects with one or more compatible symptoms will be asked to provide two sputum samples for smear microscopy, mycobacterial culture, and GeneXpert. Subjects who are sputum smear negative or unproductive may also undergo chest radiography or additional investigations, including repeat HIV testing, as clinically indicated. Subjects with a microbiological, radiological, and/or clinical diagnosis of TB disease will be referred to the NTP for TB treatment (directly observed, short course), which is provided free of charge by the NTP. For the purposes of the exploratory TB disease objective, a bacteriologically confirmed case of active TB disease will be defined as clinical evidence of TB with positive *Mtb* MGIT culture, or GeneXpert MTB/RIF (Cepheid, USA), from sputum or an extra-pulmonary site. A clinical case

of active TB disease will be defined as clinical and/or radiographic evidence of active TB disease that is not bacteriologically confirmed (as defined above).

### **3.6.2 Immunology Laboratory Evaluations**

A summary of immunologic assays to be performed on blood specimens is shown in Table 3-3. Staff at the clinical research site will refer to the most current version of the Specimen Management Manual (provided under separate cover) for further instructions and additional information on specimen collection and processing. All blood volumes are approximate.

**Table 3-3 Summary of Immunology Laboratory Evaluations**

Sample type	Assay	Blood volume	To be conducted by	Study Days (D)/ Study Months (M)
<b>Immunogenicity (Safety &amp; Immunogenicity Cohort only, n = 90)</b>				
Immunogenicity (primary) PBMC	Intracellular cytokine staining (ICS)	8mL (CPT)	Aeras or South Africa Endpoint Laboratory, Cape Town	D0, D70
Immunogenicity (secondary) Whole blood in Sodium Heparin	Intracellular cytokine staining (ICS)	6mL (Na Hep)	Site laboratory	D0, D70
<b>Early innate signature (Safety &amp; Immunogenicity Cohort only, n = 90)</b>				
Serum from clotted blood	Quantification of soluble immune mediators by multiplex ELISA	3mL (clotted tube)	TBD	D0, D3 for AERAS-404/Placebo D0, D7 for BCG
<b>Correlates of Protection (Safety &amp; Immunogenicity and Correlates Cohorts, n=990)</b>				
PBMC, plasma, and serum	TBD	Up to 16mL (2 CPT tubes, 1 clotted tube) (2mL plasma; 2mL serum)	TBD	D0, D70
		Up to 40mL (4 CPT tubes, 1 clotted tube) (2mL plasma; 2mL serum)	TBD	M6, M12
Whole blood in PAXgene <sup>a</sup>	Transcriptomics on RNA	2.5mL (Paxgene)	TBD	D0, D3 for AERAS-404/Placebo D0, D7 for BCG M6, M12, M18, M24, and 3 and 6 months after QFT conversion for all groups
Whole blood in Lithium Heparin microtainer <sup>a</sup>	Absolute blood cell subset counting by flow cytometry (deconvolution of transcriptomics)	0.5mL	Site laboratory	D0, D3 for AERAS-404/Placebo D0, D7 for BCG M6, M12, M18, M24, and 3 and 6 months after QFT conversion for all groups

- a. Subjects who initially convert to QFT-GIT(+) at Month 6 or 12 will be asked to have a final blood collection for absolute blood count and RNA done at least 24 months after their initial vaccination.

### **3.6.3 Safety Evaluations**

#### **3.6.3.1 Pre-vaccination and Post-Vaccination Monitoring of Subjects**

Subjects will have vital signs taken prior to each study vaccination. Subjects will remain in the clinic under close observation for at least 30 minutes after receiving study vaccine or BCG. Vital signs will be repeated before subjects leave the clinic. Allergic reactions to vaccination are possible, therefore, appropriate drugs and medical equipment to treat acute 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.

#### **3.6.3.2 Clinical Assessments and Laboratory Tests**

Clinical assessments and laboratory tests to be performed at each visit are summarized in Tables 3-1 and 3-2.

##### **Abnormal Clinical Assessments and Laboratory Tests**

Results from clinical laboratory tests obtained on the study must be promptly reviewed by the investigator (or a designee who is a medically qualified study team member) as per site policy to determine if abnormalities exist. If the laboratory value is abnormal and has increased in toxicity grade (see Appendix C for toxicity grading scales) from pre-vaccination values, it must be reported as an adverse event and repeated promptly at the investigator's discretion to demonstrate resolution. 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 adverse events of clinical significance.

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

### 3.6.3.3 Adverse Events

The collection periods for adverse events are shown in Table 3-4.

**Table 3-4 Adverse Event Collection Periods**

Type of Event	Collection Period
Unsolicited adverse events	28 days post each vaccination <sup>a</sup>
Solicited adverse events	7 days post each vaccination (with diary cards to be used for 7 days after each vaccination for Safety & Immunogenicity Cohort only)
Solicited and unsolicited injection site reaction adverse events	BCG Group: 84 days post vaccination AERAS-404/Placebo Groups: 28 days post each vaccination <sup>a</sup>
Serious adverse events, adverse events of special interest, and SUSARs	Entire study period
a. Adverse events will be collected through the end of the study visit window for each adverse event collection period (i.e., 28 days after each vaccination + 5-day window = 33 days)	

For this study, solicited adverse events to be collected include the following: pain, redness and swelling at the site of injection; fever; myalgia; arthralgia; fatigue; headache; anorexia; hives; and chills. Solicited injection site reaction adverse events include (in addition to pain, redness, and swelling) ulceration, drainage, and axillary lymphadenopathy. Solicited adverse events of local injection site reactions (i.e., pain at injection site, redness at injection site, swelling at injection site, ulceration at the injection site, or drainage at the injection site) will be considered causally related to study injection (adverse reaction). Adverse events of special interest (see Appendix D) will be collected passively during participant safety evaluations.

### 3.6.3.4 Concomitant Medications

The collection of information on concomitant medications used by subjects following vaccination will coincide with the collection period of adverse events (solicited and unsolicited adverse events; and solicited and unsolicited injection site reaction adverse events; see Table 3-4). In addition, information on all concomitant medications associated with the treatment of serious adverse events and adverse events of special interest will be collected throughout study follow up. Information on any medications being taken to treat TB disease will also be collected throughout the study.

Concomitant medication includes prescription and non-prescription drugs or other treatments, and any vaccines other than the study vaccines. The name of the medication, treatment start and stop dates (or ‘ongoing’), route of administration, and indication must be recorded on the Concomitant Medications case report form (CRF). The indication recorded on the Concomitant Medications CRF must correspond to a medical term/diagnosis recorded on the adverse event (AE) CRF, or to a pre-existing condition noted in the subject’s medical history, or be noted as prophylaxis, e.g., dietary supplement.

### 3.6.4 Subject Follow-up and Contact

All subjects who are assigned a subject identification number and receive study vaccine will be followed according to the protocol unless consent is withdrawn.

Subjects will be instructed to contact a study team member to report new diagnoses or new or worsening adverse events and to come to the study clinic if medical attention is needed, provided the urgency of the situation permits. 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.

Study visits will be scheduled out of school hours (if applicable) whenever possible. During each clinic visit, subjects 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 the subject has decided to withdraw from the study
- Change in general health status
- Any other change in status that may affect the subject’s participation (e.g., plan to participate in another clinical trial)

All deviations from protocol procedures, evaluations, and/or visits will be documented according to the Case Report Form Guidelines provided by Aeras Data Management or their designee. When possible, missed visits and procedures must be rescheduled and performed at the nearest possible time point to the original schedule.

### 3.6.5 Loss to Follow-up

If the site’s study team members are unable to establish contact with a subject who misses a scheduled study visit, the clinical site must make every possible effort to re-establish contact and document such efforts. If contact is re-established, then the subject will resume participation in the study.

If contact with the subject cannot be re-established by the subject’s calculated final study visit date, then a determination of “lost to follow-up” can be made.

## 4 VACCINES

### 4.1 Supplies

#### AERAS-404

AERAS-404 is an investigational vaccine manufactured by Sanofi Pasteur (Toronto, Canada) and Statens Serum Institut (SSI; Copenhagen, Denmark). AERAS-404 has two components: the H4 antigen and the IC31 adjuvant. The reconstitution of the vaccine components, H4 antigen (manufactured at Sanofi Pasteur) and IC31 adjuvant (supplied by SSI), will take place at the study clinic.

*H4 antigen* is composed of a purified recombinant fusion protein. The H4 antigen is presented as a sterile, clear, colorless, or slightly yellow solution in single dose vials. Each 0.5 mL of H4 antigen solution contains 75 mcg of H4 antigen at a concentration of 150 mcg/mL. H4 antigen is formulated in a buffer consisting of 10 mmol/L Tris-HCl, pH 8.3. The presentation is 0.20 mL/vial (label claim) with an actual fill volume of 0.50 mL ± 0.05 mL. The AERAS-404 antigen will be mixed further with adjuvant prior to injection (reconstitution).

*IC31 adjuvant* is composed of ODN1a and KLK peptide. When thawed, the single-dose IC31 adjuvant vials contain 0.8 mL of translucent adjuvant solution. Each 0.8 mL of IC31 solution contains the following components: IC31 adjuvant at 1000 nmol KLK + 40 nmol ODN1a solubilized in 10 mmol/L Tris-HCl, pH 7.4, 168.75 mmol/L sodium chloride.

### **Control Product (Placebo)**

The normal saline placebo (control) will be sourced by the study vaccine manager in sterile ampoules or vials. A separate ampoule or vial will be used for each participant in order to ensure sterility.

### **BCG**

BCG (BCG Vaccine SSI, powder and solvent for suspension for injection) will be supplied by the study site. After reconstitution, 1 dose (0.1 mL) for adults and children aged 12 months and over contains *Mycobacterium bovis* BCG (Bacillus Calmette-Guerin), Danish strain 1331, live attenuated, 2-8 x 10<sup>5</sup> cfu.

## **4.2 Accountability**

The study vaccine manager is required to maintain accurate study vaccine 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 site-specific accountability forms, these must be reviewed and approved in advance by Aeras. Upon completion of the study, all study vaccine management records will be copied and the copies returned to Aeras or its designee. The originals must be maintained at the clinical site with the rest of the study records.

## **4.3 Receipt and Storage**

Upon receipt of study vaccine supplies, the study vaccine manager must immediately inspect all vials for damage. AERAS-404 (H4 antigen and IC31 adjuvant) will be shipped with a continuous temperature-monitoring device. Any damage or discrepancies from the packing list must be documented and promptly discussed with Aeras and the study monitor to determine the appropriate action.

AERAS-404 (H4 antigen and IC31 adjuvant) must be stored at less than or equal to -15°C in a secured location with no access for unauthorized personnel. BCG must be stored at 2-8°C in a secured location with no access for unauthorized personnel. The normal saline placebo will be stored at room temperature in the study pharmacy.

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

#### **4.4 Vaccine Preparation**

##### **AERAS-404 and Control (Placebo)**

Refer to the most recent version of the Vaccine Management Manual (VMM) for detailed instructions regarding AERAS-404 and placebo preparation.

##### **BCG**

BCG will be prepared and administered as per the manufacturer's recommendations. BCG Vaccine SSI will be prepared by the study pharmacist from multi-dose vials dispensed according to the package insert using aseptic technique. Each vial of BCG Vaccine SSI will be reconstituted with diluted Sauton SSI as specified in the package insert. Reconstituted vaccine may be kept at 4-8°C for up to 4 hours. Exposure to light should be kept to a minimum. Any reconstituted vaccine not used within 4 hours must be discarded.

#### **4.5 Disposal of Unused Supplies**

Aeras must provide authorization for any unused study vaccine and supplies to be destroyed. Unused supplies will be destroyed according to the facility's SOPs. Any disposal of study vaccine conducted at the clinical site must be documented in the study file.

## **5 SAFETY**

### **5.1 Responsibilities for Ensuring the Safety of Trial Subjects**

The national regulatory authority, the study sponsor (Aeras), 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.

#### **5.1.1 Principal Investigator**

The principal investigator has a personal responsibility to closely monitor trial subjects and an inherent authority to take whatever measures necessary to ensure their safety. The principal investigator has the authority to terminate, suspend or require changes to a clinical trial for safety concerns and may delay an individual's study vaccine administration or pause study vaccine administration in the whole trial if the investigator has some suspicion that the study vaccine might place a subject at significant risk. Where specified, the responsibilities of the principal investigator may be delegated to a medically qualified team member (designee). The principal investigator or designee determines severity and causality with respect to the study vaccine for each adverse event. For blinded studies the principal investigator is blinded, in which case the study vaccine may consist of a placebo, an active control, or the investigational product.

### 5.1.2 Study Sponsor

The sponsor (Aeras) also has an institutional responsibility to ensure subject safety. This responsibility is vested in a local medical monitor and a data monitoring committee (DMC).

### 5.1.3 Local Medical Monitor

The local medical monitor is the sponsor's representative and is a physician or surgeon in their country of residence. 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 the adverse event. The local medical monitor, in consultation with the sponsor, may assess the severity and causality for adverse events and may upgrade the degree of severity and causality determined by the principal investigator or designee. The local medical monitor, like the principal investigator, will be blinded for the subjects in the AERAS-404 and placebo study groups.

### 5.1.4 Data Monitoring Committee

The DMC will operate according to its charter. If study vaccine administration is paused by the local medical monitor or the principal investigator, the DMC will be convened. Based on its review and the protocol stopping rules (Section 6.2) the DMC will make recommendations in the DMC minutes to Aeras regarding further conduct of the study and further administration of study vaccine. The DMC 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 DMC 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 DMC minutes. The DMC may recommend suspension or resumption of enrollment and study vaccine administration after review of safety data. However, the sponsor will make the final decision to suspend or resume study activities. The recommendations of the DMC, along with the sponsor's decision, will be communicated to the investigators and the IRB/Ethical Committees and the national regulatory authorities. The sponsor or its designee agrees to abide by any directives issued by the national regulatory authority or the Institutional Review Board.

The DMC will review safety data for the Safety & Immunogenicity cohort as described in Section 2.2. The DMC will also review unblinded data after sufficient QFT-GIT conversion endpoints have accumulated for the primary analysis of prevention of infection with *Mtb*, and if an impact on infection (positive or negative) is seen will advise the Sponsor accordingly. The Sponsor may then elect to extend follow up of all subjects to explore the duration of prevention of infection after vaccination, or to terminate the study.

### 5.1.5 Institutional Review Boards and Ethics Committees

The Institutional Review Board or Ethics Committee has institutional responsibility for the safety of research subjects. The Institutional Review Board or Ethics Committee has the authority to terminate, suspend or require changes to a clinical trial.

### 5.1.6 National Regulatory Authority

Since the national regulatory authority (such as the Medicines Control Council for South Africa) receives all expedited safety reports it also has the authority to terminate, suspend or require changes to a clinical trial.

## 5.2 Safety Surveillance During the Study

Subjects will be monitored and safety data collected by way of clinical interviews and examinations, evaluations of daily diaries conducted by study team members, and through reports of laboratory evaluations. Time points and the specific data collected for each of these evaluations are described in Section 3 and the protocol appendices.

The principal investigator and local medical monitor will review safety data on an ongoing basis to ensure that study pausing rules are not met.

## 5.3 Definition of Adverse Event

An adverse event (AE) is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An adverse event can therefore be any unfavorable and unintended sign (including abnormal laboratory findings), symptom or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product.

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

Any adverse change from the subject's baseline condition (determined from screening evaluations conducted to confirm study eligibility) that occurs following the administration of the study vaccine 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 study vaccine. Intermittent conditions such as headaches in adults or irritability in infants 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 study vaccine. Adverse events include but are not limited to: adverse 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 subject, signs and symptoms noted by the subject, concomitant disease with onset or increased severity after study vaccine administration, and changes in laboratory safety parameters occurring after study vaccine administration.

**The reporting period for all adverse events is specified in Section 3.** Adverse events will be reported on the Adverse Event CRF using a recognized medical term or diagnosis that accurately reflects the event. Adverse event evaluations will be reviewed by the principal investigator or by a designated medically qualified practitioner. Adverse event CRF pages are 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. All adverse events must be followed until resolution is demonstrated. The resolution date will be recorded on the CRF as the last date on which the subject experienced the adverse event. If an adverse event resolution date is uncertain the principal investigator or designee should estimate the completion date based on medical judgment and interview of the subject. 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 intervisit 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 CRF 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 Adverse Event CRF and a Supplemental SAE Report (SAER) form will be completed.

#### 5.4 Assessing Severity

The safety concepts of “severity” and “seriousness” are distinct concepts (see Section 5.8). 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 (or designee, who is a healthcare professional; is someone the investigator deems qualified to review adverse event information, to provide a medical evaluation of the event, and to classify the event based upon medical judgment and the severity categories described below) is responsible for assessing the severity of the event and the causal relationship of the event to the study vaccine.

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**

A Toxicity Table is 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 CRF, 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. In the Toxicity Table certain local reactions such as erythema (redness) and swelling are graded according to size. Laboratory values are graded according to level of deviation from the normal range.

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 subject.
- “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 subject.
- “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 CRF with the onset and resolution dates encompassing the entire duration of the event.

## 5.5 Assessing Causal Relationship (Relatedness)

For all adverse events, the investigator and the sponsor (the local medical monitor) will determine a **causal relationship**, to the study vaccine without knowledge, for the blinded portion of the study, of whether AERAS-404 or placebo 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 study vaccine 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 subject’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 favors 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 subject’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 favors 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).

The principal investigator and the local medical monitor both determine causality. It is expected that communication and consultation may occur in the assessment of the causality of adverse events. The greatest degree of causal relationship (definite > probable > possible > unlikely related > not related) determined by either the investigator or local medical monitor after their discussions will determine the ultimate classification of the adverse event. Definite, probable and possible are considered to be related. Not related and unlikely related are considered to be unrelated.

Every effort should be made by the investigator to determine the existence of any pre-existing conditions (e.g., headache in adults or rashes in infants 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 CRF as baseline history and substantiated by appropriate source documentation. Intermittent conditions such as headaches in adults or irritability in infants 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 study vaccine.

## 5.6 Definition of Adverse Reaction

An adverse reaction is an adverse event judged to be related to study vaccine (see Section 5.3 for adverse event definition).

**Related adverse events (adverse reactions) are defined as those judged by the investigator or local medical monitor to be possibly, probably, or definitely related to study vaccine.**

## 5.7 Solicited Adverse Events and Injection Site Reactions

Solicited adverse events are events the subject 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: pain, redness and swelling at the site of injection; fever; myalgia; arthralgia; fatigue; headache; anorexia; hives; and chills. Solicited injection site reaction adverse events include (in addition to pain, redness, and swelling) ulceration, drainage, and axillary lymphadenopathy. Solicited adverse events of local injection site reactions (i.e., pain at injection site, redness at injection site, swelling at injection site, ulceration at injection site, or drainage at injection site) will be considered causally related to study vaccine (adverse reaction).

**The reporting period during which *solicited* adverse events will be evaluated is specified in Section 3.** The solicited adverse event reporting period begins with the day of vaccination. All subjects in the Safety & Immunogenicity cohort will be provided a diary card to record temperature and information regarding occurrences of these specific events for the 7 days post-vaccination (see further details in Section 5.16).

Adverse events and solicited adverse events including assessment of local injection site reactions will be assessed by the investigator for severity, causal relationship to the study vaccine, possible etiologies, and whether the event meets criteria as a serious adverse event (and therefore requires immediate notification to the medical monitor).

Presence of ulceration and/or scarring at the site of injection and axillary lymphadenopathy of the injection arm(s) are considered to be adverse events that are causally related to the study vaccine and are of special interest. Site of injection ulceration (including presence of drainage) and axillary lymphadenopathy will be actively evaluated during through Day 84. These events will be recorded on the Adverse Event CRF as applicable.

Visible injection-site abnormalities other than expected site reactions should be photographed. The types of events to be photographed should be findings that are grossly abnormal or beyond the expected injection site reactions. These types of reactions may be reactions that appear rapidly or are larger or more severe than usual. The purpose of the photograph is for information sharing purposes with the sponsor. The types of events that should be photographed include, but are not limited to, allergic type reactions, such as prompt injection site redness, angioedema, and anaphylactic reaction.

In the event that the clinical presentation meets the definition of a serious adverse event, an SAER form must be completed and the event reported per protocol instructions.

## 5.8 Adverse Events of Special Interest (AESIs)

Adverse events of special interest (AESIs) are clinical events which are potentially immune mediated, and are listed in Appendix D.

## 5.9 Assessing "Seriousness" and Serious Adverse Events

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

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

considered a serious adverse event when, based on appropriate medical judgment, they may jeopardize the subject 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, or the development of drug dependency or drug abuse).

A **serious adverse event** is an adverse event meeting the outcome criteria for seriousness regardless of relationship to an administered medicinal product.

### 5.10 Assessing Expectedness

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 the person of the local medical monitor, determines expectedness. If the assessment is that the adverse event is **expected** no further action is required. If the local medical monitor's assessment is that the adverse event is **unexpected**, then the event may represent a SUSAR or expedited SAE (see Sections 5.10 and 5.11).

### 5.11 Definition of Suspected Unexpected Serious Adverse Reaction (SUSAR)

When an adverse event is judged to be related to an investigational product, such as AERAS-404, and also is judged to be serious and unexpected, it is a SUSAR (suspected unexpected serious adverse reaction) and is subject to expedited reporting.

### 5.12 Reporting of Serious Adverse Events

All serious adverse events, which include SUSARs, are reported to the sponsor and to the World Wide Safety Center for the entire study period (see protocol appendices). SUSARs are reported even after the trial is over, if the sponsor, local medical monitor or principal investigator becomes aware of them. The site will be provided with specific reporting procedures including the SAE eCRF and any supplemental reporting forms to be used. Serious adverse events will be reported on the SAE eCRF using a recognized medical term or diagnosis that accurately reflects the event.

Serious adverse events will be assessed for severity, causal relationship to the study vaccine, and expectedness by the investigator and the local medical monitor according to their roles (as described in Sections 5.1.1 and 5.1.3). The onset and resolution dates of the event and medical care 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 or the condition has stabilized. Information recorded on the eCRF must be substantiated in the source documents.

The SAE eCRF for that event must be completed by the principal investigator or his/her designee, within one business day of the clinical site becoming aware of the event. The SAE eCRF should be completed with all information known at the time. In case the eCRF cannot be completed, the Supplemental SAE Report (paper form) should be completed by the principal

investigator or his/her designee, and scanned and emailed, or faxed to the World Wide Safety Center and to the local medical monitor.

Fatal or life-threatening serious adverse events that the investigator suspects are related to the study vaccine should be telephoned to the local medical monitor immediately upon the investigator's awareness of the event. If the local medical monitor 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 telephonically notify the sponsor 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 study sponsor.

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

- Protocol number and name and contact number of the investigator
- Subject ID number (and initials and date of birth, if available)
- Date subject received study vaccine
- Serious adverse event(s) and date of event onset
- Current status of subject

Aeras has authorized the World Wide Safety Center to execute its responsibilities for expedited safety report submission to the appropriate regulatory authorities within specific time periods of being notified of the event (within 7 or 15 calendar days depending the character of the SUSAR); therefore, it is important that the investigator submit additional information requested as soon as it becomes available.

Aeras will notify the DMC and the manufacturer of AERAS-404 of all SUSARs within 3 working days of becoming aware of an event and will provide all follow-up information in a timely manner.

### 5.13 Other Events Requiring Immediate Reporting

The investigator must report the following events by scanning and emailing, or faxing the appropriate form to the local medical monitor within 24 hours of becoming aware of the event:

- Withdrawal of consent during the study for medical reasons (Immediately Reportable Event Form)
- Emergency unblinding (Immediately Reportable Event Form)
- Protocol violation affecting the safety of a subject or involving the vaccination process (Immediately Reportable Event Form)
- Adverse event thought to be an allergic reaction to the study vaccine (Immediately Reportable Event Form, unless event meets SAE criteria)
- Any event that, in the opinion of the investigator, precludes further administration of the study vaccine (Immediately Reportable Event Form, unless meets SAE criteria)
- Pregnancy (Immediately Reportable Event Form, and Pregnancy Notification Form)

#### **5.14 Adverse Event Treatment, Follow-up, and Outcome**

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 CRF.

Adverse events will be considered resolved when the condition returns to normal or returns to the subject'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 subject completes the study.

Follow-up for serious adverse events must continue until resolution and the outcome reported to Aeras, 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 study vaccine, the event must be reported to Aeras.

#### **5.15 Follow-up of Subjects Who Become Pregnant**

If a subject becomes pregnant during the study, she should be encouraged to continue in the study for safety follow-up. Follow-up should continue for pregnancy outcome including premature terminations, and data are to be included in the safety reports.

The investigator must notify the local medical monitor of the pregnancy immediately (even if already known to have resulted in spontaneous or elective abortion) by emailing the scanned copy or faxing the Pregnancy Notification Form to the medical monitor. At a minimum, the estimated date of conception, the estimated due date, and the date the subject received the study vaccine should be provided.

If a subject becomes pregnant, she will not have any interventions done as normally mandated by the protocol. The subject will undergo all other evaluations according to the Summary Schedule(s) of Evaluations.

The health status of the mother and child, the date of delivery, and the child's sex, birth weight and multiparity should be reported to the safety monitor after delivery, using a Pregnancy Notification Form. If delivery occurs before the final study visit, the subject should continue to be followed for SAEs through the final study visit unless withdrawal of consent has occurred. If delivery occurs after the final study visit, the investigator should attempt to maintain contact with the subject to obtain information after delivery.

Pregnancy will not be recorded as an adverse event. However, pregnancy outcomes will be recorded in the World Wide Safety Database. If the pregnancy results in a miscarriage or a planned termination, the event (spontaneous abortion or elective abortion) will be reported as an adverse event or serious adverse event per the investigator's judgment (e.g., if it was a medically important or life-threatening event that meets the definition of a serious adverse event).

A congenital anomaly or birth defect (i.e., an adverse finding in a child or fetus of a subject exposed to the study vaccine before conception or during pregnancy) must be reported as a serious adverse event.

If it is determined after completion of the study that a subject became pregnant during the study, the subject should notify the investigator. The pregnancy must be reported to the local medical monitor and the status of the mother and child after delivery will be obtained and reported, when possible.

#### **5.16 Subject Diary and Daily Temperature Monitoring**

Subjects in the Safety & Immunogenicity Cohort will receive, and be instructed in, the operation of a daily adverse event diary and a digital thermometer to be used during the specified post-vaccination diary period after vaccine administration. The daily adverse event diary is a tool to help aid the principal investigator and/or designee to engage in a conversation with the subject about any AEs that may have occurred between visits. During scheduled visits through the specified diary period after study vaccine 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 is performed and events discussed with the principal investigator and/or designee. Adverse events obtained from the diary, as determined by the principal investigator or designee, will not be directly recorded onto case report forms.

Any entry recorded by the subject on the diary card that differs from the opinion of the investigator's evaluation of the event (e.g., the severity level of an event is changed after interviewing the subject) must be explained by notation in source documentation.

## 6 PAUSING AND STOPPING RULES

These rules govern the pausing and stopping of study vaccine administration at any time during the study such as between doses (for multiple dose studies) for an individual, between individuals within a single dose group, and between dose groups.

### 6.1 Rules for Discontinuing Study Injections in an Individual Participant

Administration of additional study injections will be discontinued for an individual participant if he/she has any of the following:

- An objective clinical or laboratory parameter change which meets Grade 3 or Grade 4 severity, as defined in the protocol toxicity table, AND is judged to be possibly, probably, or definitely related to study injection\*
- Adverse event thought to be an allergic reaction to the study injection, including anaphylaxis or bronchospasm
- Any extensive rash (>40% body surface) on the thorax, abdomen, or limbs, including but not limited to urticaria, generalized petechiae, or erythema multiforme judged to be possibly, probably, or definitely related to study injection
- Development of active TB
- Development of autoimmune disease or immunosuppression, or an adverse event of special interest
- Receipt of investigational drug therapy or investigational vaccine (other than study injections received as part of this study)
- Any event that in the opinion of the principal investigator precludes administration of any further study injections
- Pregnancy

\*Excluding local injection site redness, induration, or ulceration that follow the normal expected course of BCG vaccination.

Subjects who are withdrawn from vaccination will be followed for a minimum of 6 months after the last study vaccination for safety.

### 6.2 Rules for Suspension of the Entire Study

The following rules will trigger pausing by the principal investigator, local medical monitor, or the sponsor of further enrollment and study vaccine administration, and DMC review of unblinded safety data:

1. One or more SAE(s) judged POSSIBLY, PROBABLY or DEFINITELY related to vaccination occurs  
*OR*
2. Anaphylaxis or bronchospasm within 4 hours of injection, indicative of an immediate hypersensitivity reaction to the study injection  
*OR*

3. It is determined that a SUSAR occurred  
*OR*
4. > 15% of subjects in the safety cohort experience a Grade 3 or higher event judged POSSIBLY, PROBABLY or DEFINITELY related to study vaccine\*, excluding local injection site reactions that decrease to < Grade 3 within 24 hours  
*OR*
5. an adverse event pattern of concern occurred

\*Excluding local injection site redness, induration, or ulceration that follow the normal expected course of BCG vaccination.

If the principal investigator, local medical monitor, or sponsor pauses administration of study vaccine in the study, the decision will be recorded in a memorandum to the study file and will trigger DMC review. If a recommendation to resume study enrollment and study vaccine administration is made, the DMC will record their judgment in a memorandum to the study file and notify Aeras. The DMC memorandum will be forwarded to the medical monitors and principal investigators. The DMC may recommend resumption of enrollment with changes to the protocol if it judges that such changes will eliminate or greatly reduce the safety risks specified in the stopping rules. However, the final decision to resume study activities or amend the protocol will be made by Aeras. The clinical site will be allowed to resume activities upon receipt of written notification from Aeras.

## 7 STATISTICAL CONSIDERATIONS

The planned statistical analyses for this study are outlined below. A detailed statistical analysis plan will be created and finalized prior to database lock and preparation of any unblinded preliminary data review and for preparation of the final study report (see Section 7.7).

### 7.1 Subject Populations

The safety population will consist of all randomized subjects who received at least one dose of BCG or study vaccine (AERAS-404 or placebo).

The per-protocol population for efficacy analyses will consist of all randomized subjects who received all scheduled doses of BCG, AERAS-404, or placebo and had no major protocol deviations (to be described in the statistical analysis plan). The intent-to-treat population for efficacy and immunogenicity analyses will consist of all randomized subjects.

### 7.2 Demographics and Protocol Compliance

Demographic parameters (age, gender, and race/ethnicity) and other baseline characteristics will be summarized by treatment group for all subjects in the safety population.

Listings of randomized subjects who missed any dose of BCG, AERAS-404, or placebo and number (percentage) of subjects with protocol deviations (to be defined in the statistical analysis plan) will be presented by treatment group.

### 7.3 Prevention of Infection (QFT-GIT Conversion) Analyses

The primary *Mtb* infection endpoint will be QFT-GIT conversion from a negative to positive test, using the manufacturer's recommended threshold of 0.35 IU/mL, at any time-point after Day 84 and through end of follow-up for the primary endpoint (see below). The frequency of QFT-GIT negativity/positivity will be summarized for each treatment group at screening, on Day 84, on Month 6, and for all subsequent assessments. The log-rank statistic will be used to test the null hypothesis of no difference in the rates of *Mtb* infection over the follow-up period between each of the AERAS-404 and BCG groups compared to the placebo group. The log-rank test statistic compares estimates of the hazard functions of the vaccine groups at each observed event time. It is constructed by computing the observed and expected number of events in the groups at each observed event time and then summing these to obtain an overall summary across all time points where there is an event. As subjects convert their QFT-GIT tests over time (experience a primary endpoint) or are lost to follow up the number of subjects at risk over time will change. As this is a proof of concept study, these two log-rank tests (AERAS-404 compared to placebo and BCG revaccination compared to placebo) will be performed with a 1-sided Type 1 error of 0.1. There will be no adjustment for multiplicity to control the Type 1 error rate over the two tests. The rationale for not performing this adjustment is related to the fact that the tests pertain to efficacy assessments of two unrelated vaccines. If these two evaluations were performed in two separate trials then no adjustment for multiplicity would be made. Thus, one should not be compelled to adjust for this multiplicity simply because the two evaluations are performed administratively in a single trial.

The secondary *Mtb* infection endpoint will be the combination of QFT-GIT conversion from a negative to positive test, using the manufacturer's recommended threshold of 0.35 IU/mL, at any time-point after Day 84 and through end of follow-up for the primary endpoint, AND persisting without QFT-GIT reversion from a positive to a negative test through 6 months after QFT-GIT conversion. The methods used for the analysis of this endpoint will be the same as that for the primary *Mtb* infection endpoint, but the number of endpoints will be smaller as subjects who experience primary QFT-GIT conversion but then have a reversion to negative at one or both of the repeat tests will not be counted.

As one possible mechanism of action of vaccination is to allow initial infection (QFT-GIT conversion) with subsequent clearance (and QFT-GIT reversion from positive to negative), rates of reversion (overall and as a proportion of those who initially converted) will be presented by treatment group as an exploratory analysis. Rates of conversion and reversion using alternative QFT-GIT threshold values will also be explored by treatment group.

### 7.4 Immunogenicity and Other Immunology Analyses

All immunogenicity analyses will be based on subjects who received at least one dose of study vaccine (AERAS-404, BCG, or placebo). Immunogenicity will be summarized for all time points as collected and as available for the Safety & Immunogenicity Cohort, or for all subjects. No imputation for missing data will be performed. Data will be transformed as appropriate prior to analysis.

Additional analyses may be performed based on subjects who complete all scheduled vaccinations with study vaccine (per-protocol population).

#### **7.4.1 Immune Response Determined by Intracellular Cytokine Staining Assay**

The primary variables of interest for preliminary assessment of immune response to vaccine will be the percentage of CD4+ and CD8+ T cells that express IFN- $\gamma$ , TNF, IL-2, IL-17, IL-22, CD107a, and/or CD154 alone or in combination in response to stimulation with peptide pools representing the entire amino acid sequence of the mycobacterial antigens Ag85B and TB10.4, and BCG antigens. Due to the high background observed with CD107a, cells expressing CD107a in the absence of any other functional response will be ignored. Response will be measured by flow cytometry in the intracellular cytokine staining (ICS) assay.

Median DMSO-subtracted cytokine responses and associated 95% confidence intervals (CI) or other descriptive statistics as appropriate will be used to summarize percentage T cell responses. Summaries of T cell response will be presented by T cell type (CD4 and CD8) and by stimulation antigen. Summaries will include immune response at all available pre- and post-vaccination immunology time points, and change from pre-vaccination to post-vaccination time points.

Whole blood ICS will be conducted as a secondary immunologic endpoint, and will measure the frequencies and patterns of CD4+ and CD8+ T cells expressing Th1 and Th17 cytokines following stimulation of whole blood with peptide pools representing the entire amino acid sequence of the mycobacterial antigens Ag85B and TB10.4, as well as viable BCG from the vaccine vial. Stained cells will be acquired on a LSR Fortessa or LSRII flow cytometer. Analysis of these results will be performed as outlined above.

#### **7.4.2 Additional Exploratory Analyses**

Analytic approaches for the exploratory endpoints below will be informed by best practice and the most recent advances in biomarker discovery and systems biology.

##### **7.4.2.1 RNA Transcriptomics and Whole Blood Absolute Blood Cell Counts**

RNA samples will be analyzed by microarray or sequencing to measure the acute inflammatory response induced by vaccination and thereby determine if immunogenicity can be predicted by a single, early measurement, and to understand how this acute response influences the character and function of the adaptive response. Samples collected after QFT conversion will be used to explore a correlate of risk for developing TB disease which has been identified by SATVI, or to explore other correlates as informed by recent data. Because analysis of such data is sensitive to changes in blood leukocyte subsets, it is important to include a measure of blood cell subsets by multiparameter flow cytometry, thus absolute blood cell counts will be used to deconvolute RNA transcriptomic results.

#### 7.4.2.2 Quantification of Soluble Immune Mediators by Multiplex ELISA

Serum will be collected and analyzed for soluble immune mediators, informed by results of the transcriptomic analyses.

#### 7.4.2.3 Correlates of Risk/Correlates of Protection

PBMCs, plasma, and RNA samples will be collected and stored at time points likely to show peak responses for cellular, humoral, and transcriptomic responses respectively, and at 6 and 12 months (when *Mtb* infection will be assessed), for all subjects. If an effect of vaccination is seen on infection with *Mtb*, immunologic assays will be conducted to search for correlates of risk or protection. Functional assays such as mycobacterial growth inhibition may also be conducted.

#### 7.4.2.4 Novel IGRAs

Samples obtained during this study may be used to evaluate IGRA tests using antigens other than those in the QFT-GIT. Such assays would be useful to test vaccines containing ESAT-6 (one of the stimulating antigens in QFT-GIT) in the prevention of *Mtb* infection model. IGRAs using antigens from non-tuberculous mycobacteria (NTMs) may also be evaluated, as NTMs may be a cause of reduced specificity of the QFT-GIT, and may also interfere with demonstration of BCG efficacy (Wilson et al, 1995) through increased protection in control groups.

### 7.5 Safety Analyses

Safety analyses will be performed using the safety population as defined in Section 7.1. Count (percentage) summaries will be presented by treatment group for all subjects in the safety population.

#### 7.5.1 Adverse Events

The safety profile of BCG revaccination, AERAS-404 and saline placebo will be described by treatment group. The primary variable for evaluation of the safety profile will be the number and percentage of unsolicited and solicited adverse events recorded at all available post-vaccination time points. For all presentations of adverse events, additional summaries based on reporting period of adverse events following each study vaccination may also be presented.

The number (percentage) of subjects with adverse events will be summarized by MedDRA system organ class (SOC) and preferred term (PT). Additional summaries will present the number (percentage) of subjects with adverse events by severity and by relationship to study vaccine; each subject will be counted once per preferred term at the greatest severity or most related state recorded for that term.

Separate summaries of the number (percentage) of subjects with solicited adverse events will also be presented. Solicited adverse events will also be summarized by severity and relationship to study vaccine; each subject will be counted once per preferred term at the greatest severity or most related state recorded for that term.

Listings will be provided for subjects with serious adverse events and adverse events of special interest.

Listings will be provided for subjects who have discontinued prematurely due to an adverse event.

The number (percentage) of subjects with post-vaccination clinical laboratory values or vital sign values recorded as newly abnormal following study vaccination and meeting toxicity mild criteria (Grade 1) or above as specified in the Toxicity Table (Appendix C) will be tabulated at each post-vaccination time point and overall. Clinical laboratory and vital sign abnormalities will also be reported as adverse events and will be included in the summary of adverse events.

### 7.5.2 Clinical Laboratory and Vital Sign Parameters

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

## 7.6 Sample Size Considerations

Sample size for the trial is based on the power to detect a reduction in the rate of infection with *Mtb*, as determined by QFT-GIT conversion. As a proof of concept study looking for evidence of biologic impact, the trial is designed to distinguish a QFT-GIT conversion rate reduction of 50% compared to placebo for each vaccine (AERAS-404 and BCG) with power of 80% and a type 1 error rate of 10% (1-sided).

Trial design calculations are performed by computer simulation. The simulations estimate the statistical operating characteristic of a conditional binomial test which, due to relative rarity of the endpoint, provides an excellent approximation to the operating characteristic of the log-rank tests which will be used in the analyses of trial data. The following assumptions, based on preliminary data from SATVI (Mahomed et al, 2011b) were used in these simulations:

- Primary *Mtb* infection endpoint rate = 10% per year
- Rate of loss to follow up = 7% per year
- Average rate of enrollment = 20 subjects per week
- Maximum duration of follow up to primary conversion = 24 months with 6-monthly study visits

Under these assumptions, a total sample size of 990 is expected to provide 64 primary QFT-GIT conversion endpoints approximately 21 months after the first subject is enrolled. Follow up will continue until 64 endpoints are accrued and a median follow up duration of 15 months for individual subjects have both occurred. A 10% rate of QFT-GIT conversions per year is conservative (SATVI data have shown an average 17% conversion rate in adolescents); however, continuing to follow subjects until the required number of endpoints is accrued ensures adequate power for the primary endpoint.

Sustained *Mtb* infection, as measured by persistence of QFT-GIT conversion, is an important secondary endpoint, as clearance of initial infection could be a mechanism of action of a vaccine. In addition, a persistently positive result is less likely to be the result of assay technical errors. Preliminary data from SATVI has shown an annual reversion rate of 20% over one year (Mahomed et al, 2011b). Based on this rate, the power to distinguish a 50% reduction in the rate of persistent QFT-GIT conversion is estimated to be 70% (the power is lower with the smaller number of endpoints expected). The difference in the rate of reversion among those who initially convert will also be examined. If the rate of reversion in the placebo group is 20% and if the 50% reduction in the rate of persistent QFT-GIT conversion in the vaccine group is due solely to an increase in the rate of reversions, then the power to detect such a vaccine effect on reversion rate is > 90%. If some of the vaccine effect is in the prevention of initial conversion and some is in an increase in reversions then the secondary endpoint of persistent QFT-GIT conversion is more sensitive to detect impact of vaccine.

If no SAEs are observed among 330 subjects in each vaccine group (AERAS-404, BCG, and placebo), the exact upper 95% confidence bound on the rate of SAE occurrence would be 1%. In the safety cohort, a sample size of 30 in each vaccine group provides 90% power to detect at least one AE occurring at a frequency of 7% in each group.

## 7.7 Plan for Statistical Summaries and Analyses

### 7.7.1 Primary Data Review

After 64 primary QFT-GIT conversion endpoints and a median of 15 months follow up have both occurred a primary unblinded analysis of QFT-GIT conversion data will be conducted by an independent statistical group and results will be reviewed by the DMC. If an effect on QFT-GIT conversion is seen, follow up for the entire study cohort may be extended. Study investigators will remain blinded to treatment assignments for the duration of study follow up.

### 7.7.2 Final Study Report

The final study report will include all available safety data, immunogenicity data, clinical assessments, and concomitant medications through the final study visit. The database will be locked prior to unblinding and preparation of the final study report when all of the above data have been entered, reviewed, and all queries related to the data have been addressed.

Modifications or additions to the analyses described above will be included in the relevant statistical analysis plan(s). Any decisions to deviate from the planned analyses described in the protocol and in the statistical analysis plan will be described in detail in the final study report.

## 7.8 Computer Methods

Statistical analyses will be performed using SAS® version 9.1 or later under a Windows operating system.

## **8 DATA COLLECTION, MONITORING, AND RECORD RETENTION**

For the purpose of monitoring and auditing the study, source documentation will consist of existing medical records and/or study records developed and maintained by the investigator.

Data recorded on source documents will be transcribed onto case report forms (CRFs) provided by Aeras or entered using electronic case report forms (eCRFs) using an Electronic Data Capture (EDC) system provided and approved by Aeras.

The study will be monitored regularly by Aeras or its designee throughout the study period. For studies of unapproved investigational products, all study records (source documents, signed informed consent forms, copies of CRFs, IRB/IEC correspondence and approval letters, study vaccine management records) will be kept secured for a minimum of 2 years following the marketing of the investigational product or for 2 years after the discontinuation of the IND (or CTA, etc.). Records will be kept for longer if required by local regulations. The investigator will ensure that study records are not disposed of or removed from the clinical site without prior notification and approval from Aeras or its designee.

## **9 HUMAN SUBJECTS**

### **9.1 Ethics and Regulatory Considerations**

The study will be conducted according to the ethical principles set forth in the Declaration of Helsinki, ICH-GCP, Protection of Human Subjects (21 CFR 50), Institutional Review Boards (21 CFR 56), Obligations of Clinical Investigators (21 CFR 312), South African Good Clinical Practice Guidelines, and other local regulatory requirements.

Adolescents younger than 18 years of age require the consent of a parent or legal guardian, in addition to their own assent, for participation in clinical research in South Africa. Therefore, written informed assent will be obtained from each subject, and written informed consent will be obtained from the parent or legal guardian of each subject, prior to any protocol-specified procedures being conducted. Subjects who turn 18 during the course of the study will be re-consented as adults at the next scheduled study visit.

The protocol and informed assent and consent forms will be reviewed and approved by the IRB or IEC of the clinical site prior to any protocol-specified procedures being conducted. The investigator will inform the IRB/IEC as to the progress of the study on a regular basis, or at minimum, once a year.

There are potential known and unknown risks associated with both BCG revaccination and AERAS-404.

No AERAS-404 vaccine-related serious adverse events have occurred and AERAS-404 has generally been well tolerated at all doses and regimens evaluated. Injection site reactions (pain, swelling, and erythema) have been mild or moderate. Reactions have occurred at the site of

tuberculin skin tests (TST) and BCG vaccination sites as described in the IB. Systemic adverse reactions include mild to moderate fatigue, myalgia, headache, arthralgia, and mild to severe pyrexia. Asymptomatic transient isolated proteinuria was seen in subjects receiving the vaccine and placebo in all four trials but was more frequent in South African adults, including at baseline (prior to vaccination). Proteinuria did not recur with revaccination and this finding is not considered to be clinically significant. One subject had a reactivation of Graves' disease; and one subject was diagnosed with celiac disease at the end of the study; neither event was considered related to study vaccine.

There is considerable experience with BCG vaccine, which is licensed in South Africa for prevention of TB in children and adults. BCG revaccination has also been extensively studied in adolescents and young adults, as discussed in Section 1.6. Injection site reactions are common and typically include redness, induration, and a superficial ulcer which may last 2-3 months. Regional lymphadenopathy is also common. Complications such as extensive local ulceration, local subcutaneous abscesses, suppurative lymphadenitis, or severe systemic complications are very rare and are not expected in the healthy, HIV-uninfected, *Mtb* uninfected adolescents to be recruited in this trial.

Based on previous data from baseline QFT-GIT negative adolescents at SATVI, we expect the overall incidence of active TB disease in the study population to approximate 0.2% (estimate 2 cases per year of follow-up) (Mahomed et al, 2011b). Those adolescents who convert to a positive QFT-GIT during follow-up have increased risk of incident TB disease (approximately 1.4% per year), compared to adolescents who remain QFT-GIT negative (Machingaidze et al, 2012). Screening for symptoms of incident TB disease will be a safety priority, in order to ensure early diagnosis and treatment. In countries with very high TB prevalence, such as South Africa, a course of isoniazid (INH) preventive therapy (IPT) for HIV-uninfected persons with *Mtb* infection is not recommended by the South African NTP, due to the high risk of multiple exposures and reinfection. A randomized controlled trial of community-wide INH prophylaxis conducted in South Africa among gold miners at high risk of TB disease showed that short-term TB incidence was reduced by 63% compared to the control group. However, the effect of IPT wore off rapidly and no benefit was observed beyond 9 months (Aurum Institute, 2012). Even among HIV infected persons with latent TB, a course of IPT provides only short-term protection that is lost within the first year of treatment (Johnson et al, 2001).

In order to minimize potential risk to subjects related to study vaccination, the DMC will review safety data from the initial Safety & Immunogenicity cohort and may make recommendations on additional measures to ensure the safety of human subjects if any safety concerns arise.

Other potential risks include the risks associated with phlebotomy. The blood volume that will be collected at each visit, and the total blood volume that will be collected over the course of the study, are not deemed physiologically significant in adolescents aged 12 years and older and are unlikely to be associated with adverse effects in the healthy adolescents to be recruited.

HIV testing will be performed with appropriate pre- and post-test counseling. Adolescents in this community are sensitized to HIV and TB as public health problems. Ongoing epidemiological

studies and clinical trials of TB vaccines at the SATVI site have not found HIV testing to be a barrier to participation in this community.

Results of investigations, including HIV test and pregnancy test results, will only be divulged to the parent or legal/guardian with the subject's permission. Clinical information will not be released to other parties without written permission from the subject, except as necessary for monitoring or auditing of the study by Aeras, or its designee or applicable regulatory authorities. To maintain confidentiality, subject identification numbers will be used to identify the subject's laboratory specimens, source documents, CRF, study reports, etc. All study records will be maintained in a secured location.

Subjects will be compensated for their inconvenience and additional costs due to attendance at study visits in the amount of ZAR 150 per study visit.

After the study has been unblinded, the subject should be informed which study intervention the subject received. If AERAS-404 or BCG revaccination is found to be effective in prevention of *Mtb* infection, subjects allocated to the placebo arm may be offered AERAS-404 or BCG revaccination if QFT-GIT is negative at the end of the study. The decision of whether or not to offer AERAS-404 or BCG revaccination will be made by the study sponsor in conjunction with the IEC.

## 9.2 Institutional Review Board or Independent Ethics Committee

All the documents the IRB/IEC may need to fulfill its responsibilities, such as the protocol, protocol amendments, information concerning subject 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 study protocol and the informed consent form will be in the possession of the investigator/clinical 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 subjects 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 assent and consent forms or any other documents previously submitted for review/approval, of any new information that may adversely affect the safety of the subjects 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 documents or forms to be provided to the subject or parent/legal guardian (e.g., information cards, form letters from the investigator) and all forms of study advertising (flyers, brochures, print advertisements, radio or television scripts, etc.) must be approved by Aeras or its designee

prior to the clinical site submitting them to the IRB/IEC. Approval from the IRB/IEC must be obtained prior to providing the documents or forms to the subject or parent/legal guardian.

### **9.3 Informed Consent and Assent**

The principles of informed consent in the current edition of the Declaration of Helsinki and ICH-GCP/21 CFR 50.25 should be implemented prior to any protocol-specified procedures being conducted. Informed consent by the parent/legal guardian will be documented in writing on a consent form approved by the IRB/IEC. Similarly, subject informed assent will be documented in writing on an assent form approved by the IRB/IEC.

The informed consent and assent processes will be conducted in a private space, whether at the study clinic, at home, or at school, to maintain confidentiality. Consent and assent processes will be conducted in the subject's language of choice. All relevant information should be provided in both oral and written form in a way that is understandable to the subject and parent/legal guardian. Ample time and opportunity must be given for the subject and parent/legal guardian to inquire about details of the study. The written consent and assent documents will embody the elements of informed consent as described in the Declaration of Helsinki and will also comply with local regulations (e.g., South African GCP).

The investigator or the investigator's qualified designee will explain the nature of the study and inform the subject and parent/legal guardian that participation is voluntary and that the subject can leave the study at any time, without penalty or loss of benefits to which they are otherwise entitled. The subject and parent/legal guardian must be informed about the study's purpose including why the subject was selected to participate, study goals, expected benefits and risks, potential risks, and that some potential risks are unforeseeable. The subject and parent/legal guardian must be provided with a description of the procedures and the estimated duration of time required for participation in the study, as well as alternative interventions or courses of treatment, if applicable.

The subject and parent/legal guardian must receive an explanation as to whether any compensation and any medical treatments are available if injury occurs and, if so, what they are, where further information may be obtained, and who to contact in the event of a study-related injury. Subject and parent/legal guardian must be told who to contact for answers to any questions related to the study. The extent of the confidentiality of subject records must be defined and the subject must be informed that applicable data protection legislation applies.

The subject and parent/legal guardian must be informed that the monitor(s), auditor(s), IRB/IEC members, and the applicable regulatory authorities will be granted direct access to the subject's original study medical records for verification of protocol-specified procedures and/or data, without violating the confidentiality of the subject to the extent permitted by the applicable laws and regulations. The subject and parent/legal guardian must be informed that his/her signature on the informed assent or consent form indicates that he/she has decided to participate in the study, having read and discussed the information presented.

Modifications made by the investigator to an informed assent or consent form template provided to the investigator by Aeras or its designee will be reviewed and approved by Aeras or its designee prior to being submitted to the IRB/IEC.

The original, signed informed assent and consent forms for each subject and parent/legal guardian will be maintained by the investigator as part of the subject’s study records. A copy of the signed informed assent and consent forms will be provided to each subject and parent/legal guardian.

**10 STUDY COMPLETION**

At the discretion of Aeras, all materials and supplies provided to the investigator will be returned or disposed of in compliance with local regulatory requirements upon authorization from Aeras, upon study completion. The investigator or designated clinical site staff will notify the IRB/IEC when the study has been completed.

**11 PUBLICATIONS**

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 Aeras for review prior to their submission. Aeras reserves the right to delete any part or parts of such materials deemed to be confidential or proprietary.

**12 CHANGES IN THE PROTOCOL**

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

**12.1 Changes from Version 1.0 to Version 2.0 (Amendment 1)**

Section Number (Title)	Change/Rationale
<p><b>Section 3.1 (Schedule of Subject Evaluations)</b></p>	<p>Tables 3-1 and 3-2 were revised as follows:</p> <ul style="list-style-type: none"> <li>• For the Correlates Cohort, collection of solicited AEs (through Study Day 7) and unsolicited AEs (through Study Day 28) were added to the applicable visits. This change was made to collect more safety information since this study represents the first use of AERAS-404 in adolescents.</li> <li>• Blood collection (0.5 mL) for absolute blood count was added as follows: for the Correlates Cohort at Study Days 0 and 3 (AERAS-404/placebo) and Study Days 0 and 7 (BCG); and for the Safety &amp; Immunogenicity Cohort and Correlates Cohort at Months 6, 12, 18, and 24. These changes were made in order to have samples collected for absolute blood counts at the same time points as for RNA analysis, to deconvolute the RNA results at all time points.</li> <li>• Collection of serum for correlates of risk/protection was added at Study Days 0 and 70, and Months 6, 12, 18, and 24. This was done at the request of MCC to obtain antigen specific antibodies.</li> </ul>

Section Number (Title)	Change/Rationale
	<ul style="list-style-type: none"> <li>A footnote was added to indicate that all blood volumes are approximate.</li> <li>A typographical error (Table 3-2 only) that indicated that the Day 3 visit is for subjects in the Safety &amp; Immunogenicity Cohort only was corrected. In fact, all subjects who receive AERAS-404 or placebo will have a visit at Day 3.</li> </ul>
<b>Section 3.2.4 (Exclusion Criteria)</b>	<p>Exclusion criterion #2 was revised to refer to axillary temperature instead of oral temperature, to maintain consistency with the protocol toxicity table in Appendix C.</p> <p><b>Tracked changes:</b>            2. <del>Oral</del>Axillary temperature <math>\geq 37.5^{\circ}\text{C}</math> on Study Day 0</p>
<b>Section 3.4.1 (Unblinding for Clinical Emergencies)</b>	<p><b>Change/rationale:</b> The description for requesting unblinding of a subject’s treatment assignment in the event of urgent clinical requirement was corrected to reflect the process used with the IWRS.</p> <p><b>Tracked changes:</b>            ‘If there is an urgent clinical requirement to know a subject’s treatment assignment, the principal investigator will request the urgent unblinding of a subject’s treatment by following the “unblinding by site form call flow” process completing the Subject Unblinding by Site form in the IWRS.’</p>
<b>Section 3.6.2 (Immunology Laboratory Evaluations)</b>	<p>Table 3-3 was revised as follows:</p> <ul style="list-style-type: none"> <li>To reflect the changes made to the schedule of evaluation Tables 3-1 and 3-2 (see Changes for Section 3.1, above)</li> <li>To indicate that the volume of blood to be collected for ‘PBMC, plasma, and serum’ at Study Days 0 and 70 will be ‘up to 16mL’ (previously noted as ‘16mL’).</li> <li>To indicate in the ‘PBMC, plasma, and serum’ row that 2mL (previously 1mL) of plasma will be collected at each time point, and that 1 clotted tube will be used (in addition to the CPT tubes previously noted).</li> <li>To correct the volume of blood to be collected for ‘PBMC, plasma, and serum’ at Months 6, 12, 18, and 24 to read ‘up to 40mL’ (previously was noted as ‘16mL’).</li> <li>To delete the row for ‘Whole blood in PAXgene’ under ‘Early innate signature (Safety &amp; Immunogenicity Cohort only, n=90).’ This row was previously included in error, and represented a duplicate of information included under ‘Correlates of Protection (Safety &amp; Immunogenicity and Correlates cohorts, n=990).’</li> </ul>
<b>Section 3.6.3.3 (Adverse Events)</b>	<p>Table 3-4 was revised to reflect that unsolicited and solicited adverse events will now be collected for the Correlates Cohort, and that the diary card for solicited AEs will only be used for the Safety and Immunogenicity Cohort.</p>
<b>Section 4.1 (Supplies)</b>	<p>This section was revised to indicate that the normal saline placebo will be sourced as either ampoules or vials (previously only vials were indicated).</p>
<b>Section 4.3 (Receipt and Storage)</b>	<p>This section was revised to indicate that AERAS-404 must be stored at ‘less than or equal to <math>-15^{\circ}\text{C}</math>’ (previously ‘<math>-15^{\circ}\text{C}</math>’ was indicated).</p>
<b>Section 5.12 (Reporting of Serious Adverse Events)</b>	<p>This section was revised to reflect the method of reporting serious adverse events, and for clarity.</p> <p><b>Tracked changes:</b>  <del>All serious</del> Serious adverse events, which include SUSARs, are reported to the sponsor and to the World Wide Safety Center (administered by PPD, Inc.) for the entire study period (see protocol appendices). SUSARs are reported even after the trial is over, if the sponsor, local medical monitor or principal investigator becomes aware of them. The site will be provided with specific reporting procedures including the SAE eCRF Adverse Event CRF and any supplemental reporting forms to be used. Serious adverse events will be reported on the SAE eCRF Adverse Event CRF using a recognized medical term</p>

Section Number (Title)	Change/Rationale
	<p>or diagnosis that accurately reflects the event.</p> <p>Serious adverse events will be assessed <u>for severity, causal relationship to the study vaccine, and expectedness</u> by the investigator and the local medical monitor according to their roles (as described in Sections 5.1.1 and 5.1.3) <del>for severity, causal relationship to the study vaccine, and expectedness</del>. The onset and resolution dates of the event and <u>medical care</u> <del>the action</del> 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 <u>or the condition has stabilized</u>. Information recorded on the eCRF must be substantiated in the source documents.</p> <p>The SAE <del>eCRF Report form</del> <u>completed</u> for that event must be <u>completed, scanned and emailed, or faxed</u> by the principal investigator or his/her designee, <u>within 24 hours (one business calendar day)</u> of the clinical site becoming aware of the event, <del>to the local medical monitor and to the World Wide Safety Center at PPD</del>. The SAE eCRF should be completed with all information known at the time; <u>In case the eCRF cannot be completed</u>, the Supplemental SAE Report (paper form) should be completed <u>by the principal investigator or his/her designee</u>, and <del>both forms</del> scanned and emailed, or faxed <u>to (even if all information concerning the World Wide Safety Center and to the local medical monitor event is not yet known) within the first 24 hours of awareness of the event</u>.</p> <p>....</p> <p>Aeras has authorized the <del>PPD</del>-World Wide Safety Center to execute its responsibilities for <u>expedited</u> safety report submission to the appropriate regulatory authorities within specific time periods of being notified of the event (within 7 or 15 calendar days depending the character of the SUSAR); therefore, it is important that the investigator submit additional information requested as soon as it becomes available.</p> <p>Aeras will notify the DMC <u>and the manufacturer of AERAS-404</u> of all SUSARs within 3 working days of becoming aware of an event and will provide all follow-up information in a timely manner.</p>
<p><b>Section 7.3 (Prevention of Infection (QFT-GIT Conversion) Analyses)</b></p>	<p>A typographical error regarding reversion of QFT-GIT conversion was corrected.</p> <p><b>Tracked changes:</b> The methods used for the analysis of this endpoint will be the same as that for the primary <i>Mtb</i> infection endpoint, but the number of endpoints will be smaller as subjects who experience primary QFT-GIT conversion but then have a reversion to <u>negative</u> <del>positive</del> at one or both of the repeat tests will not be counted.</p>
<p><b>Appendix A (Detailed Description of Study Visits)</b></p>	<p>The visit descriptions were revised as follows:</p> <ul style="list-style-type: none"> <li>• To reflect the changes made to the schedule of evaluation Tables 3-1 and 3-2 (see Changes for Section 3.1, above).</li> <li>• To change oral temperature to axillary temperature, to maintain consistency with the protocol toxicity table in Appendix C.</li> <li>• To remove urine collection from the Study Day 3 visit for subjects receiving AERAS-404 or placebo in the Safety and Immunogenicity Cohort, for consistency with Table 3-2</li> <li>• To correct the window for the Month 6, 12, 18, and 24 visits for subjects receiving AERAS-404 or placebo to read ‘±14 days from Day 0’ (previously read ‘±14 days from Day 56’).</li> </ul>

Section Number (Title)	Change/Rationale
<b>Appendix C (Toxicity Table (Modified from Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events))</b>	The following changes were made to the definitions in the toxicity table: <ul style="list-style-type: none"> <li>One of the definitions for Grade 3 hemoglobin (any decrease) was corrected to read '≥2.79 mmol/L' (previously read '&gt;2.79 mmol/L').</li> <li>The definitions for Grade 1 and Grade 2 for hematuria (microscopic) in the toxicity table were changed to reflect the definitions used at the site.</li> </ul> <b>Tracked changes:</b> Grade 1: $65 - 10 \text{ cells/mm}^3 \text{RBC/HPF}$ Grade 2: $> 10 \text{ cells/mm}^3 \text{RBC/HPF}$
<b>Appendix D (Adverse Events of Special Interest)</b>	Autoimmune thrombotic/thromboembolic conditions and Tolosa-Hunt syndrome were added to the list of adverse events of special interest. This was done at the request of the DMC to fully assess the safety profile of the vaccine.
<b>Throughout the protocol</b>	Minor editorial changes were made for clarity.

## 12.2 Changes from Version 2.0 to Version 3.0 (Amendment 2)

Section Number (Title)	Change/Rationale
<b>Study Abstract</b>	The study abstract was revised to reflect the changes below.
<b>Section 2.2 (Design and Endpoints)</b>	The first 2 paragraphs of this section were revised to reflect that additional sites to SATVI may be used, and that subjects will be recruited directly from the community (in addition to high schools).
<b>Section 3.1 (Schedule of Subject Evaluations)</b>	Tables 3-1 and 3-2 were revised as follows: <ul style="list-style-type: none"> <li>Written informed consent and assent were removed from the screening procedures (text was added above the tables to indicate that written informed consent and assent must be obtained before any screening procedures are performed).</li> <li>Whole blood collection for evaluation of novel IGRA was added at screening.</li> <li>A window of ±14 days was added for each of the post QFT-GIT(-) to QFT-GIT(+) conversion visits.</li> </ul>
<b>Section 3.2.1 (Recruitment and Informed Consent)</b>	The first paragraph of this section was revised to reflect that subjects will be recruited directly from the community (in addition to high schools), and to add community organizations to the list of groups used to recruit subjects.
<b>Section 3.2.3 (Inclusion Criteria)</b>	Inclusion criterion #6 was deleted ('Has body mass index [BMI] for age and sex between the 5 <sup>th</sup> and 95 <sup>th</sup> centiles by Centers for Disease Control nomogram'). This criterion has resulted in the exclusion of approximately 15% of the first 200 participants screened who are otherwise healthy, with exclusions occurring both above and below the 5 <sup>th</sup> /95 <sup>th</sup> centiles. SATVI data indicate that BMI in this population has a bimodal distribution, with peaks at the high and low ends of the curve. Thus the use of a BMI nomogram does not appear to be appropriate for this population and excludes otherwise eligible participants without increasing safety.
<b>Section 3.2.4 (Exclusion Criteria)</b>	A new exclusion criterion was added to prevent the possibility of immunological interference by use of licensed vaccines in close temporal proximity to study vaccine administration: '14 Planned administration/administration of a licensed vaccine in the period starting 28 days before and ending 28 days after each dose of study vaccine'.
<b>Section 3.3 (Study Randomization)</b>	This section was revised to reflect that additional sites to SATVI may be used.  <u>Tracked Changes:</u> ....The day of randomization for each subject will be Study Day 0. Randomization will be in blocks for each school <u>or community</u> , as the epidemiology of TB transmission may vary from one school <u>or community</u> to another. Randomization will also be blocked such

Section Number (Title)	Change/Rationale
	that 30 subjects from each vaccine group will be enrolled in the Safety & Immunogenicity Cohort. Enrollment for the Safety & Immunogenicity Cohort will be from a subset of schools or <u>communities</u> selected to be representative of the study population as a whole in terms of socio-economic status and TB risk. Subjects who discontinue participation will not be replaced.
<b>Section 3.6.1 (Evaluation of <i>Mtb</i> Infection)</b>	The text regarding performance of QFT-GIT assays was clarified to indicate that the assay will be performed according to site SOPs.
<b>Section 3.6.2 (Immunology Laboratory Evaluations)</b>	Table 3-3 was revised to indicate that the secondary immunogenicity ICS assay and absolute blood cell subset counting by flow cytometry will be conducted at the applicable site laboratory (previously the SATVI laboratory was listed as conducting both of these assays).
<b>Section 3.6.3.3 (Adverse Events)</b>	Table 3-4 was revised to include the following clarifications regarding the collection periods for adverse events: <ul style="list-style-type: none"> <li>• The 28-day post-vaccination collection period for unsolicited AEs and 7-day post-vaccination period for solicited AEs refer to time periods after each vaccination</li> <li>• Solicited injection site reaction AEs will be collected for 84 days post-vaccination in the BCG group and 28 days post each vaccination in the AERAS-404 and placebo groups</li> <li>• For both unsolicited AEs and solicited injection site AEs, events will be collected through the end of the study visit window for each adverse event collection period (i.e., 28 days after each vaccination + 5-day window = 33 days)</li> </ul>
<b>Section 5.1.1 (Principal Investigator)</b>	The following text was added to define designee for the principal investigator: ‘Where specified, the responsibilities of the principal investigator may be delegated to a medically qualified team member (designee).’ Subsequent text in this section was revised to indicate that a designee for the principal investigator can determine the severity and causality with respect to study vaccine of adverse events.
<b>Section 5.3 (Definition of Adverse Event)</b>	The text in this section was revised to indicate that a designee for the principal investigator may estimate the resolution date of an adverse event if the resolution date is uncertain.
<b>Section 5.7 (Solicited Adverse Events and Injection Site Reactions)</b>	The following text was added to this section to describe the circumstances under which injection site reactions should be photographed: ‘Visible injection-site abnormalities other than expected site reactions should be photographed. The types of events to be photographed should be findings that are grossly abnormal or beyond the expected injection site reactions. These types of reactions may be reactions that appear rapidly or are larger or more severe than usual. The purpose of the photograph is for information sharing purposes with the sponsor. The types of events that should be photographed include, but are not limited to, allergic type reactions, such as prompt injection site redness, angioedema, and anaphylactic reaction.’
<b>Appendix A (Detailed Description of Study Visits)</b>	The visit descriptions were revised for consistency with the changes described above, and to add timing for the injection site examinations on the day of each injection (30±5 minutes post-immunization).
<b>Appendix C (Toxicity Table (Modified from Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events))</b>	The definitions for pediatric ( $\leq 17$ years) hypertension were revised to add values required for systolic and diastolic blood pressure to meet the criteria for a moderate or severe AE.

### 12.3 Changes from Version 3.0 to Version 4.0 (Amendment 3)

Section Number (Title)	Change/Rationale
Section 2.2 (Design and Endpoints)	A footnote was added to Table 2-1 to indicate that subject numbers are approximate due to block randomization.
Section 3.1 (Schedule of Subject Evaluations)	<p>Tables 3-1 and 3-2 were revised as follows:</p> <ul style="list-style-type: none"> <li>• Study Days were defined for the Month 6, 12, 18, and 24 visits, and the numbers of days post conversion were added for the 3-month and 6-month post-QFT-GIT-conversion visits, for clarification of timing.</li> <li>• HIV-1 testing was revised to indicate that this includes HIV counselling.</li> <li>• Collection of injection site reaction adverse events was revised to include unsolicited events (previously was only solicited events).</li> <li>• PBMC, plasma, and serum at Months 18 and 24 for correlates of protection were removed. This was done as the most useful correlates at the later time points are likely to be those determined from the absolute blood count and RNA.</li> <li>• Blood for absolute blood count and RNA was added at the visits after QFT conversion, to explore correlates of risk for TB disease.</li> </ul>
Section 3.2.4 (Exclusion Criteria)	<p>The following changes were made to the exclusion criteria:</p> <ul style="list-style-type: none"> <li>• Exclusion criterion #3 was revised to clarify the timing of blood collection for qualifying laboratory tests, and to add more specific criteria for each laboratory parameter that would result in exclusion of a subject from the study.</li> </ul> <p><b>Tracked Changes:</b>  <del>Clinically significant (and no more than Grade 1 on the Toxicity Scale)</del> Abnormal laboratory values from <u>the most recent</u> blood collected <del>within 21 days</del> prior to randomization as follows:</p> <ul style="list-style-type: none"> <li>• <u>Laboratory evidence of hematologic disease (white blood cell count &lt;3000/mm<sup>3</sup> or &gt;11,500/mm<sup>3</sup>; hemoglobin &lt;0.9 times the lower limit of normal of the testing laboratory, by age and gender; absolute neutrophil count &lt;1300/mm<sup>3</sup>; absolute lymphocyte count &lt;1000/mm<sup>3</sup>)</u> <del>hemoglobin, hematocrit, absolute neutrophil count, or absolute lymphocyte count below lower limit of normal (LLN)</del></li> <li>• <del>white blood cell count above upper limit of normal (ULN) or below LLN (i.e., must be within normal limits)</del></li> <li>• ALT, AST, alkaline phosphatase, total bilirubin, creatinine, blood urea nitrogen (BUN) <u>&gt;1.25 times the upper limit of normal of the testing laboratory</u> <del>above ULN</del></li> </ul> <ul style="list-style-type: none"> <li>• Exclusion criterion #4 was revised to clarify exclusion based on urinalysis results.</li> </ul> <p><b>Tracked Changes:</b>  <del>Evidence of clinically significant (and no more Greater than Grade 1 on the Toxicity Scale) systemic or local disease on</del> urinalysis <u>result (with the exception of hematuria in a menstruating female), or urinalysis abnormality judged clinically significant by the investigator</u></p>
Section 3.6.2 (Immunology Evaluations)	Table 3-3 was revised to reflect the changes made to the schedule of evaluation Tables 3-1 and 3-2 (see Changes for Section 3.1, above). In addition, absolute blood count and RNA will be collected at visits after QFT conversion, to explore correlates of risk.
Section 3.6.3.3 Adverse Events	Table 3-4 was revised to indicate that collection of injection site reaction adverse events includes unsolicited and solicited adverse events (previously was only solicited adverse events).

Section Number (Title)	Change/Rationale
<b>Section 3.6.3.4</b> Concomitant Medications	This section was revised to clarify the collection periods for concomitant medications, and to indicate that TB medications will be collected throughout the study.
<b>Section 5.1.4</b> (Data Monitoring Committee)	This section was revised as follows in relation to DMC review of unblinded QFT-GIT conversion endpoints when sufficient endpoints have accumulated for preliminary analysis: <ul style="list-style-type: none"> <li>To clarify that when the DMC advises the Sponsor after this review, both positive and negative impact on infection will be taken into account.</li> <li>To indicate that as an alternative to electing to extend follow-up based on this review, the Sponsor may elect to terminate the study.</li> </ul>
<b>Section 7.4.2.1</b> (RNA Transcriptomics and Whole Blood Absolute Blood Cell Counts)	The following text was added to this section: 'Samples collected after QFT conversion will be used to explore a correlate of risk for developing TB disease which has been identified by SATVI, or to explore other correlates as informed by recent data.'
<b>Section 7.4.2.3</b> Correlates of Risk/Correlates of Protection	This section was revised to reflect the changes made to the schedule of evaluation Tables 3-1 and 3-2 (see Changes for Section 3.1, above).
<b>Appendix A</b> (Detailed Description of Study Visits)	The visit descriptions were revised for consistency with the changes described above and for consistency with Schedule of Subject Evaluation Tables 3-1 and 3-2.

## 12.4 Changes from Version 4.0 to Version 5.0 (Amendment 4)

Section Number (Title)	Change/Rationale
<b>Section 2.1</b> (Objectives)	A new exploratory objective was added to evaluate early QFT converters (i.e., those that converted at Month 6 or 12) for sustained QFT conversions or late reversions so as to inform the design of subsequent POI studies. <b>Added:</b> To explore trends in QFT-GIT prolonged/sustained conversions and late reversions (i.e., more than 6 months post initial conversion) in early QFT-GIT converters (i.e., among those who converted at Month 6 or Month 12 of follow-up)
<b>Section 2.2</b> (Design and Endpoints), <b>Section 3.1</b> (Schedule of Subject Evaluations), <b>Section 3.6.2</b> (Immunology Laboratory Evaluations), and <b>Appendix A</b> (Detailed Description of Subject Visits)	These sections were updated to include the optional, additional visit for participants who converted to QFT-GIT+ at Month 6 or Month 12.

Section Number (Title)	Change/Rationale
<b>Section 3.2.1</b> (Recruitment and Informed Consent)	A requirement was added to obtain consent/assent for early QFT converters to participate in the optional, additional follow-up visit described above. <b>Added:</b> Subjects who converted to a positive QFT-GIT at Month 6 or 12 will be approached for an additional QFT-GIT test, blood collection for absolute blood count and RNA, and evaluation for signs/symptoms of TB to be conducted at least 24 months after their initial vaccination. Informed consent will be obtained by the use of a written supplemental consent form approved by the IRB or IEC, and signed and dated by subject or by the subject's parent/legal guardian if subject is less than 18 years of age. Similarly, informed assent will be obtained by the use of a written assent form approved by the IRB or IEC, and signed and dated by the subject if the subject is less than 18 years of age.

## 13 REFERENCES

Abu-Raddad LJ, Sabatelli L, Achterberg JT, Sugimoto JD, Longini IM, Jr., et al. (2009) Epidemiological benefits of more-effective tuberculosis vaccines, drugs, and diagnostics. *Proc Natl Acad Sci U S A* 106: 13980-13985.

Aurum Institute (2012). Thibela TB Fact Sheet.

Barreto ML, Pereira SM, Pilger D, Cruz AA, Cunha SS, et al. (2011) Evidence of an effect of BCG revaccination on incidence of tuberculosis in school-aged children in Brazil: second report of the BCG-REVAC cluster-randomised trial. *Vaccine* 29: 4875-4877.

Basu Roy R, Sotgiu G, Altet-Gomez N, Tsolia M, Ruga E, et al. (2012) Identifying predictors of interferon-gamma release assay results in pediatric latent tuberculosis: a protective role of bacillus Calmette-Guerin?: a pTB-NET collaborative study. *Am J Respir Crit Care Med* 186: 378-384.

Belisle JT, Vissa VD, Sievert T, Takayama K, Brennan PJ, Besra GS. (1997) Role of the major antigen of *Mycobacterium tuberculosis* in cell wall biogenesis. *Science* 276: 1420-1422.

Billeskov R, Elvang TT, Andersen PL, Dietrich J. (2012) The HyVac4 Subunit Vaccine Efficiently Boosts BCG-Primed Anti-Mycobacterial Protective Immunity. *PLoS ONE* 7: e39909.

Bottiger M, de Verdier C, Lind A, Beskow R, Linden G, Granath B. (1983) A comparative study of Danish (Statens Seruminstitut), Glaxo and Behringwerke vaccines--revaccination of schoolchildren. *Journal of biological standardization* 11:1-12.

Dietrich J, Aagaard C, Leah R, Olsen AW, Stryhn A, Doherty TM et al. (2005) Exchanging ESAT6 with TB10.4 in an Ag85B fusion molecule-based tuberculosis subunit vaccine: efficient protection and ESAT6-based sensitive monitoring of vaccine efficacy. *J Immunol* 174: 6332-6339.

Dye C (2000) Tuberculosis 2000-2010: control, but not elimination. *Int J Tuberc Lung Dis* 4: S146-152.

Dye C, Williams BG. (2008) Eliminating human tuberculosis in the twenty-first century. *J R Soc Interface* 5: 653-662.

Eisenhut M, Paranjothy S, Abubakar I, Bracebridge S, Lilley M, et al. (2009) BCG vaccination reduces risk of infection with *Mycobacterium tuberculosis* as detected by gamma interferon release assay. *Vaccine* 27: 6116-6120.

Fine PEM. (1995) Variation in protection by BCG: implications of and for heterologous immunity. *Lancet* 346:1339-45

FitzGerald JM. (2000) Management of adverse reactions to bacille Calmette-Guerin vaccine. *Clin Infect Dis*;31 Suppl 3:S75-6.

Hoft DF, Leonardi C, Milligan T, et al. (1999) Clinical reactogenicity of intradermal bacille Calmette-Guerin vaccination. *Clin Infect Dis*;28:785-90.

Horwitz MA, Harth G, Dillon BJ, Maslesa-Galic' S. (2000) Recombinant bacillus calmette-guerin (BCG) vaccines expressing the Mycobacterium tuberculosis 30-kDa major secretory protein induce greater protective immunity against tuberculosis than conventional BCG vaccines in a highly susceptible animal model. *Proc Natl Acad Sci USA* 97: 13853-13858.

Johnson J, et al. (2001) Duration of efficacy of treatment of latent tuberculosis infection in HIV-infected adults. *AIDS* 15:2137-47.

Lingnau K, Egyed A, Schellack C, Mattner F, Buschle M, Schmidt W. (2002) Poly-L-arginine synergizes with oligodeoxynucleotides containing CpG-motifs (CpG-ODN) for enhanced and prolonged immune responses and prevents the CpG-ODN-induced systemic release of pro-inflammatory cytokines. *Vaccine* 20: 3498-3508.

Lotte A, Wasz-Hockert O, Poisson N, Dumitrescu N, Verron M, Couvet E. (1984) BCG complications. Estimates of the risks among vaccinated subjects and statistical analysis of their main characteristics. *Advances in tuberculosis research Fortschritte der Tuberkuloseforschung Progres de l'exploration de la tuberculose* 21:107-93.

Machingaidze S, Verver S, Mulenga H, Abrahams DA, Hatherill M, et al. (2012) Predictive Value of Recent QuantiFERON Conversion for Tuberculosis Disease in Adolescents. *Am J Respir Crit Care Med* 186: 1051-1056.

Mahomed H, Hawkrigde T, Verver S, Geiter L, Hatherill M, et al. (2011a) Predictive factors for latent tuberculosis infection among adolescents in a high-burden area in South Africa. *Int J Tuberc Lung Dis* 15: 331-336.

Mahomed H, Hawkrigde T, Verver S, Abrahams D, Geiter L, et al. (2011b) The tuberculin skin test versus QuantiFERON TB Gold(R) in predicting tuberculosis disease in an adolescent cohort study in South Africa. *PLoS One* 6: e17984.

Mahomed H, Ehrlich R, Hawkrigde T, Hatherill M, Geiter L, et al. (2013) TB incidence in an adolescent cohort in South Africa. *PLoS One* 8: e59652.

Mazurek GH, Jereb J, Vernon A, LoBue P, Goldberg S, et al. (2010) Updated guidelines for using Interferon Gamma Release Assays to detect Mycobacterium tuberculosis infection - United States, 2010. *MMWR Recomm Rep* 59: 1-25.

Metcalf JZ, Cattamanchi A, McCulloch CE, Lew JD, Ha NP, et al. (2013) Test variability of the QuantiFERON-TB gold in-tube assay in clinical practice. *Am J Respir Crit Care Med* 187: 206-211.

Nicol M, Eley B, Kibel M, Hussey G. (2002) Intradermal BCG vaccination--adverse reactions and their management. *S Afr Med J* 92:39-42.

Pai M. (2012) Serial testing with TB interferon-gamma release assays: toward a nuanced understanding. *Chest* 142: 1366-1367.

Pereira SM, Barreto ML, Pilger D, Cruz AA, Sant'Anna C, et al. (2012) Effectiveness and cost-effectiveness of first BCG vaccination against tuberculosis in school-age children without previous tuberculin test (BCG-REVAC trial): a cluster-randomised trial. *Lancet Infect Dis* 12: 300-306.

Rodrigues LC, Pereira SM, Cunha SS, Genser B, Ichihara MY, et al. (2005) Effect of BCG revaccination on incidence of tuberculosis in school-aged children in Brazil: the BCG-REVAC cluster-randomised trial. *Lancet* 366: 1290-1295.

Skeiky YA, Dietrich J, Lasco TM, Stagliano K, Dheenadhayalan V, Goetz MA et al. (2010) Non-clinical efficacy and safety of HyVac4:IC31 vaccine administered in a BCG prime-boost regimen. *Vaccine* 28: 1084-1093.

Skjot RL, Oettinger T, Rosenkrands I, Ravn P, Brock I, Jacobsen S et al. (2000) Comparative evaluation of low-molecular-mass proteins from *Mycobacterium tuberculosis* identifies members of the ESAT-6 family as immunodominant T-cell antigens. *Infect Immun* 68: 214-220.

Skjot RL, Brock I, Arend SM, Munk ME, Theisen M, Ottenhoff TH et al. (2002) Epitope mapping of the immunodominant antigen TB10.4 and the two homologous proteins TB10.3 and TB12.9, which constitute a subfamily of the *esat-6* gene family. *Infect Immun* 70: 5446-5453.

Soysal A, Millington KA, Bakir M, Dosanjh D, Aslan Y, et al. (2005) Effect of BCG vaccination on risk of *Mycobacterium tuberculosis* infection in children with household tuberculosis contact: a prospective community-based study. *Lancet* 366: 1443-1451.

Tameris MD, Hatherill M, Landry BS, Scriba TJ, Snowden MA, et al. (2013) Safety and efficacy of MVA85A, a new tuberculosis vaccine, in infants previously vaccinated with BCG: a randomised, placebo-controlled phase 2b trial. *Lancet* [http://dx.doi.org/10.1016/S0140-6736\(13\)60177-4](http://dx.doi.org/10.1016/S0140-6736(13)60177-4).

van Dissel JT, Arend SM, Prins C, Bang P, Tingskov PN, et al. (2010) Ag85B-ESAT-6 adjuvanted with IC31 promotes strong and long-lived *Mycobacterium tuberculosis* specific T cell responses in naïve human volunteers. *Vaccine*. Apr 30;28(20):3571-81.

van Dissel JT, Soonawala D, Joosten SA, Prins C, Arend SM, et al. (2011) Ag85B-ESAT-6 adjuvanted with IC31® promotes strong and long-lived Mycobacterium tuberculosis specific T cell responses in volunteers with previous BCG vaccination or tuberculosis infection. *Vaccine*. Mar 3;29(11):2100-9.

van Zyl-Smit RN, Pai M, Peprah K, Meldau R, Kieck J, et al. (2009) Within-subject variability and boosting of T-cell interferon-gamma responses after tuberculin skin testing. *Am J Respir Crit Care Med* 180: 49-58.

WHO, Global Tuberculosis Report 2012. [http://www.who.int/tb/publications/global\\_report/en/](http://www.who.int/tb/publications/global_report/en/)

Wilson ME, Fineberg HV, Colditz GA. (1995) Geographic latitude and the efficacy of bacillus Calmette-Guerin vaccine. *Clin Infect Dis* 20:982–991.

Zwerling A, Benedetti A, Cojocariu M, McIntosh F, Pietrangelo F, et al. (2013) Repeat IGRA testing in Canadian health workers: conversions or unexplained variability? *PLoS One* 8: e54748.

## APPENDIX A Detailed Description of Study Visits

### **Screening Visit(s) (All Subjects)**

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

1. Verify study entry eligibility criteria are met
2. Medical history
3. Physical examination
4. Urine collection for urinalysis and (for females)  $\beta$ HCG
5. Vital Signs
6. TB Symptom Screen
7. Blood collection for
  - QuantiFERON<sup>®</sup>-TB Gold In-Tube
  - HIV-1 (with pre- and post-test counselling)
  - Serum chemistry (AST, ALT, alkaline phosphatase, total bilirubin, creatinine, BUN)
  - Hematology (hemoglobin, hematocrit, white cell count with differential and platelet count)
  - Whole blood for novel IGRA

### **Study Day 0 (All Subjects)**

#### **Pre- injection:**

1. Urine collection (females) for  $\beta$ HCG
2. Obtain pre-immunization vital signs (blood pressure, pulse, axillary temperature)
3. Interval medical history and, if indicated by history, focused physical examination.
4. Verify study entry eligibility criteria are met
5. Randomize
6. Blood collection for
  - PMBC and plasma for correlates of risk protection
  - Whole blood for RNA extraction/absolute blood count
  - Whole blood for novel IGRA
  - PBMC for evaluation of NTM exposure

#### **Safety & Immunogenicity Cohort Only:**

- Whole blood assay
- PMBC for ICS
- Serum for multiplex ELISA

#### **Injection:**

7. Administer either 1) BCG by intradermal injection or 2) AERAS-404 or placebo by intramuscular injection on the left upper arm (deltoid region). Record date and time of injection and which arm was injected.

#### **Post-injection:**

8. Monitor participant for solicited and unsolicited adverse events for at least 30 minutes after injection; record concomitant medications
9. Monitor participant for serious adverse events, adverse events of special interest, and SUSARs for at least 30 minutes after injection; record concomitant medications
10. Obtain 30 $\pm$ 5-minute post-immunization vital signs

11. Complete 30±5-minute post-immunization Study Day 0 study injection site examination (with photograph if there is a visible injection-site abnormality; see Section 5.7 for further details)

***Safety & Immunogenicity Cohort Only:***

12. Distribute diary cards

**Post Study Day 0 Visits (Subjects receiving BCG)**

**Study Day 7 (Subjects receiving BCG)**

*Allowable window for clinic visit is 7±1 days from Day 0*

1. Interval medical history and, if indicated by history, focused physical examination.
2. Monitor participant for solicited and unsolicited adverse events; record concomitant medications
3. Monitor participant for serious adverse events, adverse events of special interest, and SUSARs; record concomitant medications
4. Monitor participant for solicited and unsolicited injection site reaction adverse events; record concomitant medications
5. Complete Study Day 0 injection site examination (with photograph if there is a visible injection-site abnormality; see Section 5.7 for further details)
6. Whole blood collection for RNA extraction/absolute blood count

***Safety & Immunogenicity Cohort only***

7. Urine collection for urinalysis
8. Review diary cards.
9. Blood collection for
  - Serum chemistry (AST, ALT, alkaline phosphatase, total bilirubin, creatinine, BUN)
  - Hematology (hemoglobin, hematocrit, white cell count with differential and platelet count)
  - Serum for multiplex ELISA

**Study Day 28 (Subjects receiving BCG)**

*Allowable window for clinic visit is 28±5 days from Day 0*

1. Interval medical history and, if indicated by history, focused physical examination.
2. Monitor participant for unsolicited adverse events; record concomitant medications
3. Monitor participant for serious adverse events, adverse events of special interest, and SUSARs; record concomitant medications
4. Monitor participant for solicited and unsolicited injection site reaction adverse events; record concomitant medications
5. Complete Study Day 0 study injection site examination (with photograph if there is a visible injection-site abnormality; see Section 5.7 for further details)
6. TB Symptom Screen

**Study Day 70 (Subjects receiving BCG)**

*Allowable window for clinic visit is 70±5 days from Day 0*

1. Interval medical history and, if indicated by history, focused physical examination.
2. Monitor participant for serious adverse events, adverse events of special interest, and SUSARs; record concomitant medications
3. Monitor participant for solicited and unsolicited injection site reaction adverse events; record concomitant medications
4. Complete Study Day 0 study injection site examination (with photograph if there is a visible injection-site abnormality; see Section 5.7 for further details)
5. TB Symptom Screen
6. Blood collection for

- PBMC and plasma for correlates of risk/protection

***Safety & Immunogenicity Cohort only***

7. Blood collection for
  - Whole blood assay
  - PBMC for ICS

**Study Day 84 (Subjects receiving BCG)**

*Allowable window for clinic visit is 84±7 days from Day 0*

1. Interval medical history and, if indicated by history, focused physical examination.
2. Monitor participant for serious adverse events, adverse events of special interest, and SUSARs; record concomitant medications
3. Monitor participant for solicited and unsolicited injection site reaction adverse events; record concomitant medications
4. Complete Study Day 0 study injection site examination (with photograph if there is a visible injection-site abnormality; see Section 5.7 for further details)
5. TB Symptom Screen
6. Blood collection
  - QuantiFERON®-TB Gold In-Tube
  - Whole blood for novel IGRA

**Study Month 6, 12, 18 and 24 (Study Days 168, 336, 504, and 672) (Subjects receiving BCG; to be completed by subjects who did not convert from QFT-GIT(-) to QFT-GIT(+) at the previous visit)**

*Allowable window for clinic visit is 6, 12, 18, or 24 months (168, 336, 504, 672 days) ±14 days from Day 0*

1. Interval medical history and, if indicated by history, focused physical examination.
2. Monitor participant for serious adverse events, adverse events of special interest, and SUSARs; record concomitant medications
3. TB Symptom Screen
4. Blood collection
  - QuantiFERON®-TB Gold In-Tube
  - Whole blood for novel IGRA
  - [Study Months 6 and 12 only]PBMC and plasma for correlates of risk protection
  - Whole blood for RNA extraction/absolute blood count

**Study Month 6 (Study Day 168) (Subjects receiving BCG; to be completed by subjects who converted from QFT-GIT(-) to QFT-GIT(+) at Study Day 84)**

*Allowable window for clinic visit is 6 months (168 days) ±14 days from Day 0*

1. Monitor participant for serious adverse events, adverse events of special interest, and SUSARs; record concomitant medications
2. TB symptom screen

**84 and 168 Days after conversion from QFT-GIT(-) to QFT-GIT(+) at Months 6-24 (Subjects receiving BCG; to be completed by subjects who converted from QFT-GIT(-) to QFT-GIT(+) at Month 6-24 visits)**

*Allowable window for clinic visit is 84 or 168 days ±14 days after conversion*

1. Monitor participant for serious adverse events, adverse events of special interest, and SUSARs; record concomitant medications
2. TB symptom screen
3. Blood collection
  - QuantiFERON®-TB Gold In-Tube

- Whole blood collection for RNA extraction/absolute blood count

**Final visit for subjects who converted from QFT-GIT(-) to QFT-GIT(+) at Month 6 or 12 (Subjects receiving BCG)**

Written informed consent and assent must be obtained for additional follow-up before any extended follow-up procedures are performed.

*Allowable window for clinic visit is at least 24 months after initial vaccination but before the end of the study*

1. TB symptom screen
2. Record TB treatment, if applicable
3. Blood collection
  - QuantiFERON®-TB Gold In-Tube
  - Whole blood for RNA extraction/absolute blood count

**Post Study Day 0 Visits (Subjects receiving AERAS-404 or placebo)**

**Study Day 3 (Subjects receiving AERAS-404 or placebo)**

*Allowable window for clinic visit is 3±1 days from Day 0*

1. Interval medical history and, if indicated by history, focused physical examination.
2. Monitor participant for solicited and unsolicited adverse events; record concomitant medications
3. Monitor participant for serious adverse events, adverse events of special interest, and SUSARs; record concomitant medications
4. Monitor participant for solicited injection site reaction adverse events at Study Day 0 injection site; record concomitant medications
5. Complete Study Day 0 injection site examination (with photograph if there is a visible injection-site abnormality; see Section 5.7 for further details)
6. Whole blood collection for RNA extraction/absolute blood count

***Safety & Immunogenicity Cohort only***

7. Blood collection for
  - Serum for multiplex ELISA

**Study Day 7 (Subjects receiving AERAS-404 or placebo)**

*Allowable window for clinic visit is 7±1 days from Day 0*

1. Interval medical history and, if indicated by history, focused physical examination.
2. Monitor participant for solicited and unsolicited adverse events; record concomitant medications
3. Monitor participant for serious adverse events, adverse events of special interest, and SUSARs; record concomitant medications
4. Monitor participant for solicited injection site reaction adverse events at Study Day 0 injection site; record concomitant medications
5. Complete Study Day 0 injection site examination (with photograph if there is a visible injection-site abnormality; see Section 5.7 for further details)

***Safety & Immunogenicity Cohort only***

6. Urine Collection for urinalysis
7. Review diary cards.
8. Blood collection for
  - Serum chemistry (AST, ALT, alkaline phosphatase, total bilirubin, creatinine, BUN)
  - Hematology (hemoglobin, hematocrit, white cell count with differential and platelet count)

**Study Day 28 (Subjects receiving AERAS-404 or placebo)**

*Allowable window for clinic visit is 28±5 days from Day 0*

1. Interval medical history and, if indicated by history, focused physical examination.

2. Monitor participant for unsolicited adverse events; record concomitant medications
3. Monitor participant for serious adverse events, adverse events of special interest, and SUSARs; record concomitant medications
4. Monitor participant for solicited injection site reaction adverse events at Study Day 0 injection site; record concomitant medications
5. Complete Study Day 0 injection site examination (with photograph if there is a visible injection-site abnormality; see Section 5.7 for further details)
6. TB Symptom Screen

**Study Day 56 (Subjects receiving AERAS-404 or placebo)**

*Allowable window for clinic visit is 56-2/56+14 days from day 0*

**Pre- injection:**

1. Urine collection (females) for  $\beta$ HCG
2. Obtain pre-immunization vital signs (blood pressure, pulse, axillary temperature)
3. Interval medical history and, if indicated by history, focused physical examination.
4. Verify study entry eligibility criteria are met

**Injection:**

5. Administer AERAS-404 or placebo by intramuscular injection on the right upper arm (deltoid region). Record date and time of injection and which arm was injected.

**Post-injection:**

6. Monitor participant for solicited and unsolicited adverse events for at least 30 minutes after injection; record concomitant medications
7. Monitor participant for serious adverse events, adverse events of special interest, and SUSARs for at least 30 minutes after injection; record concomitant medications
8. Monitor participant for solicited injection site reaction adverse events at Study Day 56 injection site; record concomitant medications
9. Obtain 30 $\pm$ 5-minute post-immunization vital signs
10. Complete 30 $\pm$ 5-minute post-immunization Study Day 56 study injection site examination (with photograph if there is a visible injection-site abnormality; see Section 5.7 for further details)
11. TB symptom screen

***Safety & Immunogenicity Cohort only***

12. Distribute diary cards

**Study Day 63 (Subjects receiving AERAS-404 or placebo)**

*Allowable window for clinic visit is 7 $\pm$ 1 days from Day 56*

1. Interval medical history and, if indicated by history, focused physical examination.
2. Monitor participant for solicited and unsolicited adverse events; record concomitant medications
3. Monitor participant for serious adverse events, adverse events of special interest, and SUSARs; record concomitant medications
4. Monitor participant for solicited injection site reaction adverse events at Study Day 56 injection site; record concomitant medications
5. Complete Study Day 56 injection site examination (with photograph if there is a visible injection-site abnormality; see Section 5.7 for further details)

***Safety & Immunogenicity Cohort only***

6. Urine Collection for urinalysis
7. Review diary cards.
8. Blood collection for

- Serum chemistry (AST, ALT, alkaline phosphatase, total bilirubin, creatinine, BUN)
- Hematology (hemoglobin, hematocrit, white cell count with differential and platelet count)

**Study Day 70 (Subjects receiving AERAS-404 or placebo)**

*Allowable window for clinic visit is 14±3 days from Day 56*

1. Interval medical history and, if indicated by history, focused physical examination.
2. Monitor participant for unsolicited adverse events; record concomitant medications
3. Monitor participant for serious adverse events, adverse events of special interest, and SUSARs; record concomitant medications
4. Monitor participant for solicited injection site reaction adverse events at Study Day 56 injection site; record concomitant medications
5. Complete Study Day 56 injection site examination (with photograph if there is a visible injection-site abnormality; see Section 5.7 for further details)
6. Blood collection for
  - PBMC and plasma for correlates of risk/protection

***Safety & Immunogenicity Cohort only***

7. Blood collection for
  - Whole blood assay
  - PMBC for ICS

**Study Day 84 (Subjects receiving AERAS-404 or placebo)**

*Allowable window for clinic visit is 28±5 days from Day 56*

1. Interval medical history and, if indicated by history, focused physical examination.
2. Monitor participant for unsolicited adverse events; record concomitant medications
3. Monitor participant for serious adverse events, adverse events of special interest, and SUSARs; record concomitant medications
4. Monitor participant for solicited injection site reaction adverse events at Study Day 56 injection site; record concomitant medications
5. Complete Study Day 56 injection site examination (with photograph if there is a visible injection-site abnormality; see Section 5.7 for further details)
6. TB symptom screen
7. Blood collection for
  - QuantiFERON®-TB Gold In-Tube
  - Whole blood for evaluation of IGRA

**Study Month 6, 12, 18 and 24 (Study Days 168, 336, 504, and 672) (Subjects receiving AERAS-404 or placebo; to be completed by subjects who did not convert from QFT-GIT(-) to QFT-GIT(+) at the previous visit)**

*Allowable window for clinic visit is 6, 12, 18, or 24 months (168, 336, 504, 672 days) ±14 days from Day 0*

1. Interval medical history and, if indicated by history, focused physical examination.
2. Monitor participant for serious adverse events, adverse events of special interest, and SUSARs; and record concomitant medications
3. TB Symptom Screen
4. Blood collection for
  - QuantiFERON®-TB Gold In-Tube
  - Whole blood for evaluation of IGRA
  - [Study Months 6 and 12 only]PBMC and plasma for correlates of risk protection
  - Whole blood for RNA extraction/absolute blood count

**Study Month 8 (Study Day 224) (Subjects receiving AERAS-404 or placebo; to be completed by subjects who converted from QFT-GIT(-) to QFT-GIT(+) at Study Day 84)**

*Allowable window for clinic visit is 6 months (168 days)  $\pm$ 14 days from Day 56*

1. Monitor participant for serious adverse events, adverse events of special interest, and SUSARs; record concomitant medications
2. TB symptom screen

**84 and 168 Days after conversion from QFT-GIT(-) to QFT-GIT(+) at Months 6-24 (Subjects receiving AERAS-404 or placebo; to be completed by subjects who converted from QFT-GIT(-) to QFT-GIT(+) at Month 6-24 visits)**

*Allowable window for clinic visit is 84 or 168 days  $\pm$ 14 days after conversion*

1. Monitor participant for serious adverse events, adverse events of special interest, and SUSARs; record concomitant medications
2. TB symptom screen
3. Blood collection
  - QuantiFERON<sup>®</sup>-TB Gold In-Tube
  - Whole blood collection for RNA extraction/absolute blood count

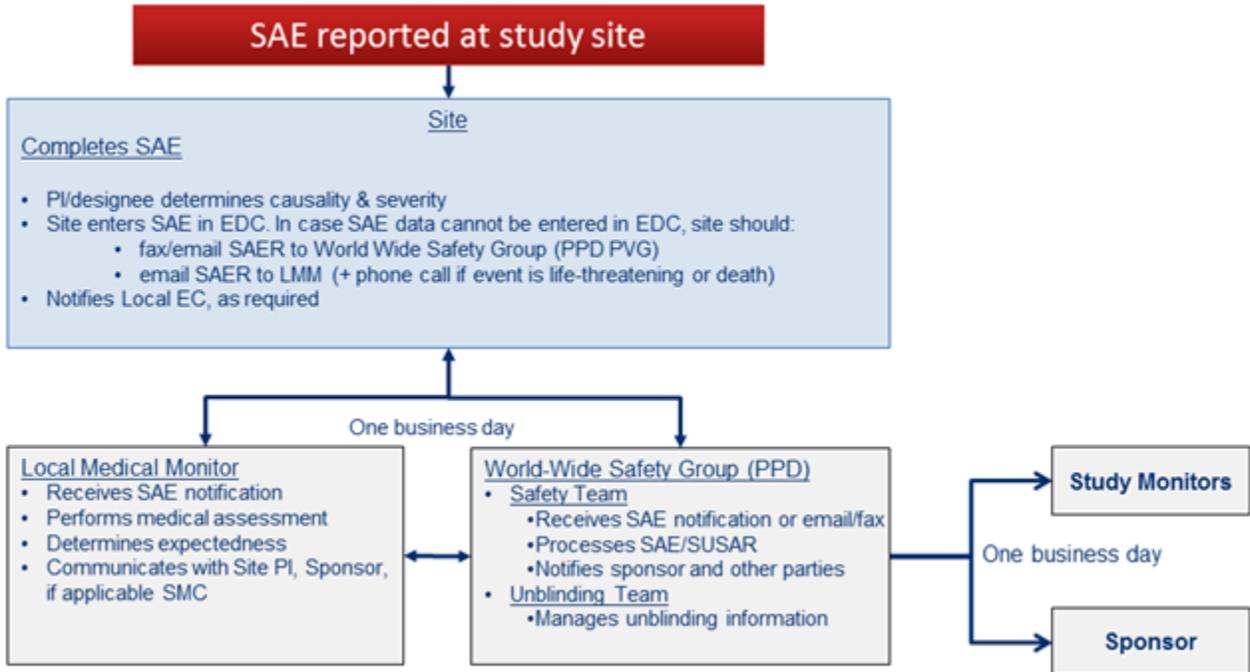
**Final visit for subjects who converted from QFT-GIT(-) to QFT-GIT(+) at Month 6 or 12 (Subjects receiving AERAS-404 or placebo)**

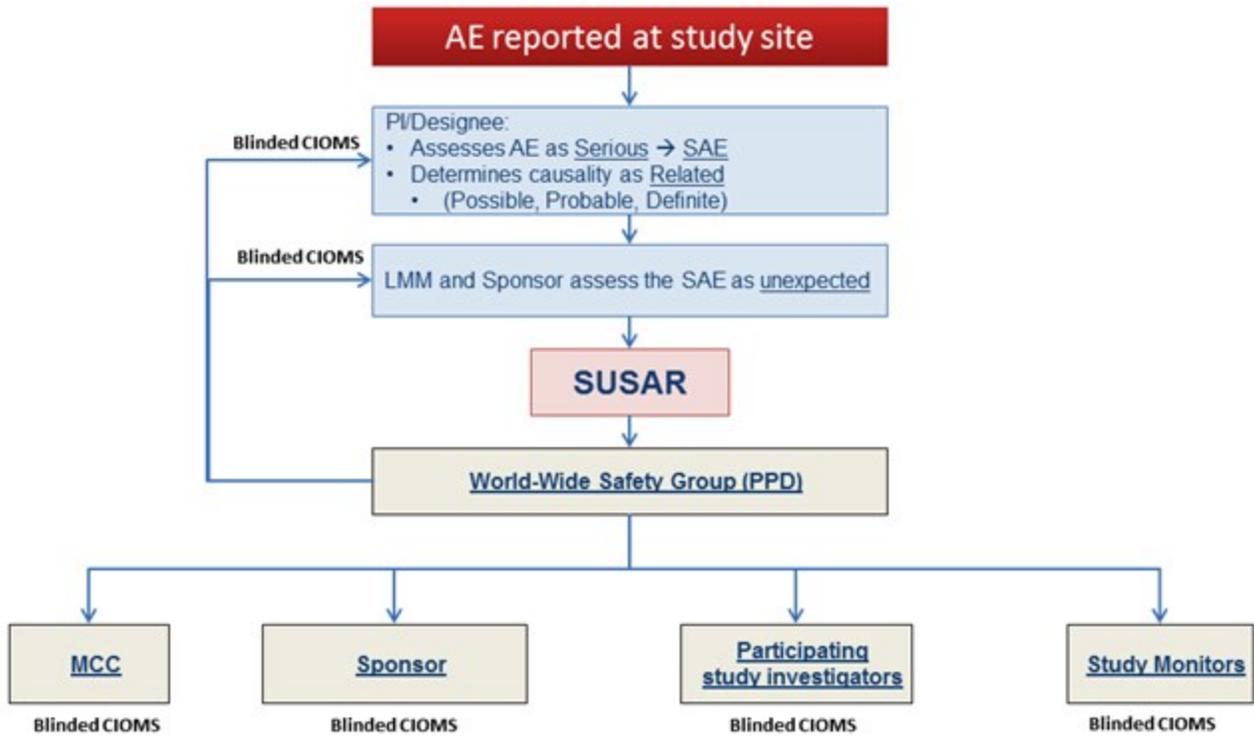
Written informed consent and assent must be obtained for additional follow-up before any extended follow-up procedures are performed.

*Allowable window for clinic visit is at least 24 months after initial vaccination but before the end of the study*

1. TB symptom screen
2. Record TB treatment, if applicable
3. Blood collection
  - QuantiFERON<sup>®</sup>-TB Gold In-Tube
  - Whole blood for RNA extraction/absolute blood count

## SAE and SUSAR Reporting Schemes





**APPENDIX B Toxicity Table (Modified from Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events)**

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
<b>ESTIMATING SEVERITY GRADE</b>				
Clinical adverse event NOT identified elsewhere in this AE Grading Table	Symptoms causing no or minimal interference with usual social & functional activities	Symptoms causing greater than minimal interference with usual social & functional activities	Symptoms causing inability to perform usual social & functional activities	Symptoms causing inability to perform basic self-care functions OR Medical or operative intervention indicated to prevent permanent impairment, persistent disability, or death
<b>SYSTEMIC</b>				
Acute systemic allergic reaction	Localized urticaria (wheals) with no medical intervention indicated	Localized urticaria with medical intervention indicated OR Mild angioedema with no medical intervention indicated	Generalized urticaria OR Angioedema with medical intervention indicated OR Symptomatic mild bronchospasm	Acute anaphylaxis OR Life-threatening bronchospasm OR laryngeal edema
Chills	Symptoms causing no or minimal interference with usual social & functional activities	Symptoms causing greater than minimal interference with usual social & functional activities	Symptoms causing inability to perform usual social & functional activities	NA
Fatigue Malaise	Symptoms causing no or minimal interference with usual social & functional activities	Symptoms causing greater than minimal interference with usual social & functional activities	Symptoms causing inability to perform usual social & functional activities	Incapacitating fatigue/ malaise symptoms causing inability to perform basic self-care functions
Fever (axillary)	38.0 – 38.4°C 100.4 – 101.1°F	38.5 - 40°C 101.2 - 104°F	>40°C >104°F	N/A
Pain (indicate body site)  DO NOT use for pain due to injection (See Injection Site Reactions: Injection site pain)  See also Headache, Arthralgia, and Myalgia	Pain causing no or minimal interference with usual social & functional activities	Pain causing greater than minimal interference with usual social & functional activities	Pain causing inability to perform usual social & functional activities	Disabling pain causing inability to perform basic self-care functions OR Hospitalization (other than emergency room visit) indicated

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Unintentional weight loss	NA	5 – 9% loss in body weight from baseline	10 – 19% loss in body weight from baseline	≥ 20% loss in body weight from baseline OR Aggressive intervention indicated [e.g., tube feeding or total parenteral nutrition (TPN)]
<b>INFECTION</b>				
Infection (any other than HIV infection)	Localized, no systemic antimicrobial treatment indicated AND Symptoms causing no or minimal interference with usual social & functional activities	Systemic antimicrobial treatment indicated OR Symptoms causing greater than minimal interference with usual social & functional activities	Systemic antimicrobial treatment indicated AND Symptoms causing inability to perform usual social & functional activities OR Operative intervention (other than simple incision and drainage) indicated	Life-threatening consequences (e.g., septic shock)
<b>INJECTION SITE REACTIONS</b>				
Injection site pain (pain without touching)  Or  Tenderness (pain when area is touched)	Pain/tenderness causing no or minimal limitation of use of limb	Pain/tenderness limiting use of limb OR Pain/tenderness causing greater than minimal interference with usual social & functional activities	Pain/tenderness causing inability to perform usual social & functional activities	Pain/tenderness causing inability to perform basic self-care function OR Hospitalization (other than emergency room visit) indicated for management of pain/tenderness
Injection site reaction (localized)				
<b>Adult &gt; 15 years</b>	Erythema OR Induration of 5x5 cm – 9x9 cm (or 25 cm <sup>2</sup> – 81cm <sup>2</sup> )	Erythema OR Induration OR Edema > 9 cm any diameter (or > 81 cm <sup>2</sup> )	Ulceration OR Secondary infection OR Phlebitis OR Sterile abscess OR Drainage	Necrosis (involving dermis and deeper tissue)
<b>Pediatric ≤ 15 years</b>	Erythema OR Induration OR Edema present but ≤ 2.5 cm diameter	Erythema OR Induration OR Edema > 2.5 cm diameter but < 50% surface area of the extremity segment (e.g., upper arm/thigh)	Erythema OR Induration OR Edema involving ≥ 50% surface area of the extremity segment (e.g., upper arm/thigh) OR Ulceration OR Secondary infection OR Phlebitis OR Sterile abscess OR Drainage	Necrosis (involving dermis and deeper tissue)

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Pruritus associated with injection  See also Skin: Pruritus (itching - no skin lesions)	Itching localized to injection site AND Relieved spontaneously or with < 48 hours treatment	Itching beyond the injection site but not generalized OR Itching localized to injection site requiring ≥ 48 hours treatment	Generalized itching causing inability to perform usual social & functional activities	NA
<b>SKIN – DERMATOLOGICAL</b>				
Cutaneous reaction – rash	Localized macular rash	Diffuse macular, maculopapular, or morbilliform rash OR Target lesions	Diffuse macular, maculopapular, or morbilliform rash with vesicles or limited number of bullae OR Superficial ulcerations of mucous membrane limited to one site	Extensive or generalized bullous lesions OR Stevens-Johnson syndrome OR Ulceration of mucous membrane involving two or more distinct mucosal sites OR Toxic epidermal necrolysis (TEN)
Pruritus (itching – no skin lesions)  (See also Injection Site Reactions: Pruritus associated with injection)	Itching causing no or minimal interference with usual social & functional activities	Itching causing greater than minimal interference with usual social & functional activities	Itching causing inability to perform usual social & functional activities	NA
<b>CARDIOVASCULAR</b>				
Hypertension				
<b>Adult &gt; 17 years</b> (with repeat testing at same visit)	140 – 159 mmHg systolic  OR 90 – 99 mmHg diastolic	160 – 179 mmHg systolic  OR 100 – 109 mmHg diastolic	≥ 180 mmHg systolic  OR ≥ 110 mmHg diastolic	Life-threatening consequences (e.g., malignant hypertension) OR Hospitalization indicated (other than emergency room visit)
<b>Pediatric ≤ 17 years</b> (with repeat testing at same visit)	NA	91 <sup>st</sup> – 94 <sup>th</sup> percentile adjusted for age, height, and gender (systolic and/or diastolic)  146 - 168 mmHg systolic  OR 91 - 102 mmHg diastolic	≥ 95 <sup>th</sup> percentile adjusted for age, height, and gender (systolic and/or diastolic)  ≥168 mmHg systolic  OR ≥102 mmHg diastolic	Life-threatening consequences (e.g., malignant hypertension) OR Hospitalization indicated (other than emergency room visit)

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Hypotension	NA	Symptomatic, corrected with oral fluid replacement	Symptomatic, IV fluids indicated	Shock requiring use of vasopressors or mechanical assistance to maintain blood pressure
<b>GASTROINTESTINAL</b>				
Anorexia	Loss of appetite without decreased oral intake	Loss of appetite associated with decreased oral intake without significant weight loss	Loss of appetite associated with significant weight loss	Life-threatening consequences OR Aggressive intervention indicated [e.g., tube feeding or total parenteral nutrition (TPN)]
<b>Comment:</b> Please note that, while the grading scale provided for Unintentional Weight Loss may be used as a <u>guideline</u> when grading anorexia, this is not a requirement and should not be used as a substitute for clinical judgment.				
Diarrhea	Transient or intermittent episodes of unformed stools OR Increase of $\leq 3$ stools over baseline per 24-hour period	Persistent episodes of unformed to watery stools OR Increase of 4 – 6 stools over baseline per 24-hour period	Bloody diarrhea OR Increase of $\geq 7$ stools per 24-hour period OR IV fluid replacement indicated	Life-threatening consequences (e.g., hypotensive shock)
Nausea	Transient (< 24 hours) or intermittent nausea with no or minimal interference with oral intake	Persistent nausea resulting in decreased oral intake for 24 – 48 hours	Persistent nausea resulting in minimal oral intake for > 48 hours OR Aggressive rehydration indicated (e.g., IV fluids)	Life-threatening consequences (e.g., hypotensive shock)
Vomiting	Transient or intermittent vomiting with no or minimal interference with oral intake	Frequent episodes of vomiting with no or mild dehydration	Persistent vomiting resulting in orthostatic hypotension OR Aggressive rehydration indicated (e.g., IV fluids)	Life-threatening consequences (e.g., hypotensive shock)
Headache	Symptoms causing no or minimal interference with usual social & functional activities	Symptoms causing greater than minimal interference with usual social & functional activities	Symptoms causing inability to perform usual social & functional activities	Symptoms causing inability to perform basic self-care functions OR Hospitalization indicated (other than emergency room visit) OR Headache with significant impairment of alertness or other neurologic function
Insomnia	NA	Difficulty sleeping causing greater than minimal interference with usual social & functional activities	Difficulty sleeping causing inability to perform usual social & functional activities	Disabling insomnia causing inability to perform basic self-care functions

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
<b>RESPIRATORY</b>				
Bronchospasm (acute)	FEV1 or peak flow reduced to 70 – 80%	FEV1 or peak flow 50 – 69%	FEV1 or peak flow 25 – 49%	Cyanosis OR FEV1 or peak flow < 25% OR Intubation
Dyspnea or respiratory distress				
<b>Adult ≥ 14 years</b>	Dyspnea on exertion with no or minimal interference with usual social & functional activities	Dyspnea on exertion causing greater than minimal interference with usual social & functional activities	Dyspnea at rest causing inability to perform usual social & functional activities	Respiratory failure with ventilatory support indicated
<b>Pediatric &lt; 14 years</b>	Wheezing OR minimal increase in respiratory rate for age	Nasal flaring OR Intercostal retractions OR Pulse oximetry 90 – 95%	Dyspnea at rest causing inability to perform usual social & functional activities OR Pulse oximetry < 90%	Respiratory failure with ventilatory support indicated
<b>MUSCULOSKELETAL</b>				
Arthralgia See also Arthritis	Joint pain causing no or minimal interference with usual social & functional activities	Joint pain causing greater than minimal interference with usual social & functional activities	Joint pain causing inability to perform usual social & functional activities	Disabling joint pain causing inability to perform basic self-care functions
Arthritis See also Arthralgia	Stiffness or joint swelling causing no or minimal interference with usual social & functional activities	Stiffness or joint swelling causing greater than minimal interference with usual social & functional activities	Stiffness or joint swelling causing inability to perform usual social & functional activities	Disabling joint stiffness or swelling causing inability to perform basic self-care functions
Myalgia (non-injection site)	Muscle pain causing no or minimal interference with usual social & functional activities	Muscle pain causing greater than minimal interference with usual social & functional activities	Muscle pain causing inability to perform usual social & functional activities	Disabling muscle pain causing inability to perform basic self-care functions

### LABORATORY

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
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Hemoglobin (Hgb)	10.0 – 10.9 g/dL 6.18 – 6.79 mmol/L OR Any decrease 2.5 – 3.4 g/dL 1.58 – 2.13 mmol/L	9.0 – 9.9 g/dL 5.55 - 6.17 mmol/L OR Any decrease 3.5 – 4.4 g/dL 2.14 – 2.78 mmol/L	7.0 – 8.9 g/dL 4.34 - 5.54 mmol/L OR Any decrease ≥ 4.5 g/dL ≥ 2.79 mmol/L	< 7.0 g/dL < 4.34 mmol/L
<b>Comment:</b> The decrease is a decrease from baseline				
Platelets, decreased	100,000 – 124,999/mm <sup>3</sup> <i>100.000 x 10<sup>9</sup> – 124.999 x 10<sup>9</sup>/L</i>	50,000 – 99,999/mm <sup>3</sup> <i>50.000 x 10<sup>9</sup> – 99.999 x 10<sup>9</sup>/L</i>	25,000 – 49,999/mm <sup>3</sup> <i>25.000 x 10<sup>9</sup> – 49.999 x 10<sup>9</sup>/L</i>	< 25,000/mm <sup>3</sup> < 25.000 x 10 <sup>9</sup> /L
WBC, decreased	2,000 – 2,500/mm <sup>3</sup> <i>2.000 x 10<sup>9</sup> – 2.500 x 10<sup>9</sup>/L</i>	1,500 – 1,999/mm <sup>3</sup> <i>1.500 x 10<sup>9</sup> – 1.999 x 10<sup>9</sup>/L</i>	1,000 – 1,499/mm <sup>3</sup> <i>1.000 x 10<sup>9</sup> – 1.499 x 10<sup>9</sup>/L</i>	< 1,000/mm <sup>3</sup> < 1.000 x 10 <sup>9</sup> /L
WBC, increased	10,800 – 15,000/mm <sup>3</sup> <i>10.8 – 15.0 x 10<sup>9</sup>/L</i>	15,001 – 20,000/mm <sup>3</sup> <i>15.1 – 20.0 x 10<sup>9</sup>/L</i>	20,001 – 25,000/mm <sup>3</sup> <i>20.1 – 25.0 x 10<sup>9</sup>/L</i>	>25,000/mm <sup>3</sup> >25.0 x 10 <sup>9</sup> /L

**LABORATORY**

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
<b>CHEMISTRIES</b> <i>Standard International Units are listed in italics</i>				
Alkaline Phosphatase	1.25 – 2.5 x ULN <sup>†</sup>	2.6 – 5.0 x ULN <sup>†</sup>	5.1 – 10.0 x ULN <sup>†</sup>	> 10.0 x ULN <sup>†</sup>
ALT (SGPT)	1.25 – 2.5 x ULN	2.6 – 5.0 x ULN	5.1 – 10.0 x ULN	> 10.0 x ULN
AST (SGOT)	1.25 – 2.5 x ULN	2.6 – 5.0 x ULN	5.1 – 10.0 x ULN	> 10.0 x ULN
Blood urea nitrogen (BUN)	23 – 26 mg/dL <i>8.3 – 9.5 mmol/L</i>	27 – 31 mg/dL <i>9.6 – 11.2 mmol/L</i>	>31 mg/dL <i>&gt;11.2 mmol/L</i>	Requires dialysis
Creatinine – elevated	1.5 – 1.7 mg/dL <i>121 - 145 umol/L</i>	1.8 – 2.0 mg/dL <i>146 – 170 umol/L</i>	2.1 – 2.5 mg/dL <i>171 – 208 umol/L</i>	>2.5 mg/dL or requires dialysis >208 umol/L or requires dialysis
Bilirubin (Total)	1.1 – 1.5 x ULN	1.6 – 2.5 x ULN	2.6 – 5.0 x ULN	> 5.0 x ULN
<b>URINALYSIS</b> <i>Standard International Units are listed in italics</i>				
Hematuria (microscopic)	5 – 10 cells/mm <sup>3</sup>	> 10 cells/mm <sup>3</sup>	Gross, with or without clots OR with RBC casts	Transfusion indicated
Proteinuria, random collection	1 +	2 – 3 +	4 +	NA
Proteinuria, 24 hour collection				

<b>Adult and Pediatric          ≥ 10 years</b>	200 – 999 mg/24 h <i>0.200 – 0.999 g/d</i>	1,000 – 1,999 mg/24 h <i>1.000 – 1.999 g/d</i>	2,000 – 3,500 mg/24 h <i>2.000 – 3.500 g/d</i>	> 3,500 mg/24 h <i>&gt; 3.500 g/d</i>
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## APPENDIX C Adverse Events of Special Interest

Adverse events of special interest (AESIs) are events which are potentially immune mediated and include:

- Acute disseminated encephalomyelitis (ADEM)
- Addison's Disease
- Anti-neutrophil Cytoplasmic Antibody (ANCA)-associated Vasculitis
- Ankylosing Spondylitis
- Anti-phospholipid Syndrome
- Autoimmune Bullous Skin Diseases
- Autoimmune Hemolytic Anemia
- Autoimmune Hepatitis
- Autoimmune Thrombotic/Thromboembolic Conditions
- Basedow's Disease
- Behcet's Syndrome
- Bell's Palsy
- Carditis
- Celiac Disease
- Crohn's Disease
- Cutaneous Lupus
- Demyelinating Disease
- Dermatomyositis
- Diabetes Mellitus, Insulin Dependent (IDDM)
- Erythema Nodosum
- Glomerulonephritis
- Guillain Barre Syndrome
- Grave's Disease
- Idiopathic Thrombocytopenic Purpura (ITP)
- Inflammatory Bowel Disease (non-specific)
- Juvenile Rheumatoid Arthritis
- Mixed Connective Tissue Disease
- Multiple Sclerosis
- Myasthenia Gravis
- Myelitis/Transverse Myelitis
- Myocarditis
- Nephritis
- Optic neuritis
- Pericarditis
- Polymyalgia Rheumatica
- Polymyositis
- Primary Biliary Cirrhosis
- Primary Sclerosing Cholangitis
- Psoriasis

Psoriatic Arthritis  
Raynaud's Phenomenon  
Rheumatoid Arthritis  
Sarcoidosis  
Scleroderma  
Sjogren's Syndrome  
Spondylo-arthropathy  
Stevens-Johnson Syndrome  
Systemic Lupus Erythematosus  
Temporal Arteritis  
Thyroiditis  
Tolosa-Hunt Syndrome  
Ulcerative Colitis  
Ulcerative Proctitis  
Uveitis  
Vasculitis  
Vitiligo  
Wegener's Granulomatosis