

A Phase I/IIa Double-Blind, Randomized, Placebo-Controlled, Dose-Finding Study to Evaluate the Safety and Immunogenicity of AERAS-456 in HIV-negative Adults with and without Latent Tuberculosis Infection

Investigational Product: AERAS-456

Aeras Protocol Number: C-035-456

Sponsor: Aeras
1405 Research Boulevard
Rockville, Maryland 20850 USA
Phone: 001-301-547-2900
Fax: 001-301-547-2901

Sponsor's Authorized Representative: Ann Ginsberg, MD, PhD
Vice President, Scientific Affairs, and
Acting Chief Medical Officer

Principal Investigator Agreement:

I, the undersigned, have reviewed this protocol and agree to conduct this protocol in accordance with Good Clinical Practices (ICH-GCP), the ethical principles set forth in the Declaration of Helsinki, and with local regulatory requirements.

Signature

Date

Printed Name

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LIST OF ABBREVIATIONS

βhCG	beta human chorionic gonadotropin
μmol	micromole(s)
Ag	antigen
AE	adverse event(s)
ALP	alkaline phosphatase
ALT	alanine aminotransferase
AST	aspartate aminotransferase
BCG	Bacillus Calmette-Guérin
BUN	blood urea nitrogen
CBC	complete blood count
CFR	Code of Federal Regulations (US)
CIOMS	Council for International Organizations of Medical Sciences
CRF	case report form(s)
CTA	Clinical Trials Application
dL	deciliter
EDC	electronic data capture
ELISA	enzyme-linked immunosorbent assay
ELISpot	enzyme-linked immunospot
FDA	United States Food and Drug Administration
g	gram
GCP	good clinical practices
GMP	good manufacturing practices
HIV	human immunodeficiency virus
ICH	International Conference on Harmonization of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICS	intracellular cytokine staining
ID	identification
IEC	Independent Ethics Committee
IFN-γ	interferon gamma
IM	intramuscular
IND	Investigational New Drug Application
INR	international normalized ratio
IRB	Institutional Review Board
IUD	intrauterine device
IV	intravenous
IWRS	Interactive web response system
kDa	kilodalton
L	liter
LDH	lactate dehydrogenase
LFT	liver function test
LLN	lower limit of normal
LTBI	latent tuberculosis infection

MAF	medical assessment form
MedDRA	Medical Dictionary for Regulatory Activities
mEq	milliequivalent
mg	milligram
mL	milliliter(s)
mm	millimeter(s)
mmol	millimole(s)
nmol	nanomole(s)
<i>Mtb</i>	<i>Mycobacterium tuberculosis</i>
PBMC	peripheral blood mononuclear cell(s)
PI	principal investigator
PPD	purified protein derivative
PRBC	packed red blood cells
PT	preferred term
QFT	QuantiFERON®-TB test
RNA	ribonucleic acid
SAE	serious adverse event(s)
SAER	serious adverse event report
SFU	spot-forming unit
SMC	Safety Monitoring Committee
SOC	system organ class
SOP	standard operating procedure
SSI	Statens Serum Institut
SUSAR	suspected unexpected serious adverse reaction(s)
TB	tuberculosis
TST	tuberculin skin test
UK	United Kingdom
ULN	upper limit of normal
US	United States
WBC	white blood cell
WHO	World Health Organization

STUDY ABSTRACT

TITLE:

A Phase I/IIa Double-Blind, Randomized, Placebo-Controlled, Dose-Finding Study to Evaluate the Safety and Immunogenicity of AERAS-456 in HIV-negative Adults with and without Latent Tuberculosis Infection

RATIONALE:

Bacillus Calmette-Guerin (BCG) is currently the only licensed vaccine for the prevention of tuberculosis (TB). There is currently no licensed vaccine for the prevention of TB disease in individuals with latent *Mycobacterium tuberculosis* (*Mtb*) infection (LTBI). AERAS-456 is being developed to prevent TB disease in individuals with and without LTBI. AERAS-456 has two components: H56, a recombinant fusion protein of the *Mtb* antigens Ag85B, ESAT-6, and Rv2660c; and IC31[®], an oligonucleotide-based adjuvant designed to elicit a cell-mediated immune response. AERAS-456 is proposed to protect against TB disease by inducing or boosting existing CD4 responses against *Mtb* antigens expressed during both active disease (Ag85B and ESAT-6) and latency (Rv2660c). AERAS-456 has been demonstrated to be immunogenic and protective before and after TB exposure in mouse and non-human primate models.

Study C-035-456 is the second study in humans and will evaluate safety and immunogenicity of AERAS-456 in adults with and without LTBI. The first in humans study of AERAS-456 (C-032-456) is a Phase I open-label study in 25 adults (17 with LTBI, 8 without LTBI) conducted at 1 site in South Africa. Eight LTBI(-) subjects have received 3 doses of intramuscular (IM) injections of 15/500 (15 µg H56/500 nmol IC31 KLK) AERAS-456 on Study Days 0, 56, and 112, and have completed 6 months follow up after last vaccination. Eight and 9 LTBI(+) subjects have completed 3 vaccinations with 15/500 and 50/500, respectively, of AERAS-456 on Study Days 0, 56, and 112. All 17 LTBI(+) subjects have been followed up for at least 28 days after the last vaccination. No safety issues judged to be vaccine-related have been identified in this study. The last subject visit of Study C-032-456 is scheduled for December 2012.

For the purposes of study C-035-456, LTBI will be diagnosed based on a positive QuantiFERON TB Gold test (QFT) at screening. Subjects will not receive a screening tuberculin skin test (TST), due to post-vaccination reactions at TST sites observed in previous clinical trials of other vaccines similar to AERAS-456, as well as the potentially confounding effects of the TST on baseline measurements of immune response to *Mtb* antigens.

This study is designed to select an optimal dose regimen of AERAS-456 for further clinical development. The optimal dose regimen will be selected based on safety and immunogenicity in an initial cohort of LTBI(-) subjects and expanded to LTBI(+) subjects. Dose selection for the initial cohort is based on preclinical study results and preliminary safety data from the ongoing Phase I study C-032-456. The rationale for conducting initial dose finding in LTBI(-) subjects is based on clinical experience from prior H1 studies (H1 is a recombinant fusion protein of the *Mtb* antigens Ag85B and ESAT-6, formulated in IC31 adjuvant). In these studies, the variability

in baseline immunogenicity parameters was greater among LTBI(+) subjects compared to LTBI(-) subjects, making differences between vaccine-induced cellular responses easier to distinguish in the LTBI(-) population. Furthermore, cellular immune responses as measured by ELISpot were comparable between the LTBI(+) and LTBI(-) subjects for the same H1 dosage.

Previous BCG vaccination is a criterion for study entry for all subjects. AERAS-456 is being developed for use in countries with a high incidence of *Mtb*, many of which administer universal BCG at birth. Subjects with HIV co-infection or TB disease will be excluded from Study C-035-456; however, they may be included in future studies.

The lack of understanding of host defense mechanisms against *Mtb* infection and the lack of immune correlates of protection to TB vaccines are hampering the development of novel TB vaccines. There is an urgent need for clear immunological markers to predict and evaluate the immunogenicity and efficacy of these vaccines and to optimize vaccine regimens. In order to assess the immune responses to vaccination, Aeras will utilize two assays. The qualified ELISpot assay allows for very sensitive detection of the number of cells secreting the Th1 cytokine, IFN- γ , in response to vaccination. In addition, the ICS assay will be used to further assess the quality of the immune response. While the assay is not as sensitive as the ELISpot, it has the added benefit of examining multiple T cell functions (IFN- γ , IL-2, TNF- α , IL-17, CD107a, and CD154) and associating those functions with T cell phenotypes (CD3, CD4, CD8, and memory), resulting in a much more robust assessment of the immune responses generated by vaccination. These assays will allow for the assessment of the magnitude and quality of the immune responses to vaccination and also increase the likelihood that an immune correlate may be identified in future efficacy trials.

Due to the present lack of a biomarker for protection or surrogate marker for risk of TB disease, TB vaccine Phase IIb efficacy studies are tremendously costly and time consuming. For this reason there is a need to carefully select which vaccines are chosen to proceed to Phase IIb. Recent evidence suggests that the BCG vaccine is able to prevent *Mtb* infection as detected by the gamma interferon release assay (IGRA) (Eisenhut et al 2009). Thus the ability to further prevent infection with *Mtb* could be a positive selection criterion for a novel TB vaccine. Studies using prevention of TB infection as a biological outcome can be done with a much lower number of study participants and shorter study duration than prevention of TB disease efficacy studies. In line with this thinking, a prevention of *Mtb* infection study using QFT is currently in planning, and AERAS-456 is one of the vaccine candidates being considered for inclusion in the trial. One issue specific to the AERAS-456 vaccine is that it contains the *Mtb* antigen ESAT-6, which is an IFN- γ inducer in the QFT assay used for diagnosis of *Mtb* infection. This may potentially cause false positive QFT responses. Data from human TB vaccine studies so far show that QFT responses wane relatively quickly after vaccination with an ESAT-6 containing vaccine. Thus data suggests that this issue may very well be overcome (van Dissel et al 2011). However, more data are needed on the kinetics of QFT responses after AERAS-456 vaccination. QFT responses will therefore be measured at baseline, at 2 time points during the study, and at the end of the study in LTBI (-) subjects.

The reasons for conducting clinical trials of AERAS-456 in South Africa (and other sub-Saharan African countries) are as follows: 1) this vaccine is intended for licensing in South Africa and other sub-Saharan African countries. 2) South Africa has a very high rate of LTBI, particularly in the Western Cape Province, 50% overall, but up to 80% in the third decade of life (Wood et al, 2010). 3) Since the rate of LTBI and indeed active tuberculosis is so high, the utility of INH prophylaxis that has been found effective in low prevalence areas is not feasible in South Africa, and this has been so recognized in WHO recommendations (WHO, 1974). Thus, a vaccine that prevents reactivation from latency would have a significant positive impact on public health of South Africa. 4) The University of Cape Town and other organizations in the Republic are preeminent in the field of tuberculosis vaccinology. 5) The simultaneous presence of a high rate of LTBI, a high incidence of active TB, an unmet medical need, and a sophisticated research infrastructure make this clinical research study especially appropriate for South Africa.

OBJECTIVES:**Primary Objective**

The primary objective of this study is to evaluate the safety profile of multiple dosage levels and dosing regimens of AERAS-456 administered to HIV-negative, BCG-vaccinated adults with and without LTBI and with no history or evidence of TB disease.

Secondary Objectives

The secondary objectives of this study are:

- To evaluate the immunogenicity of multiple dosage levels and dosing regimens of AERAS-456 administered to HIV-negative, BCG-vaccinated adults with and without LTBI and with no history or evidence of TB disease.
- To select a dosage of AERAS-456 for testing of dose regimens.
- To evaluate kinetics of QFT responses following AERAS-456 vaccination in HIV-negative, BCG-vaccinated adults without LTBI and with no history or evidence of TB disease.

DESIGN:

This is a Phase I/IIa, double-blind, randomized, placebo-controlled, dose- and regimen-finding study in healthy adults with and without LTBI, who are BCG-vaccinated, HIV negative, and have no history or evidence of TB disease. The investigational product is AERAS-456 at 3 dose levels: 5, 15, and 50 µg of H56 antigen with 500 nmol IC31. The vaccine is administered by IM injection. The study will be conducted at sites in one or more countries in sub-Saharan Africa, including South Africa.

A total of 98 subjects will be enrolled in 2 phases into 4 groups based on LTBI status. The initial phase will be a dose ranging study of a 2-dose regimen at 3 dosage levels in LTBI(-) subjects, to select a dosage for the second phase. In the second phase, the study will be expanded to evaluate both 2-dose and 3-dose regimens and to include LTBI(+) subjects. In the first phase, 50 LTBI(-) subjects will be enrolled in Group 1 and randomized at a ratio of 3:3:3:1 to receive 2 doses of 5/500, 15/500, or 50/500 of AERAS-456, or placebo given at Study Days 0 and 56 (Table 0-1).

One dose level of AERAS-456 will be selected by the sponsor and SSI for the second phase of the study, based on analysis of unblinded safety and immunogenicity data through 28 days after the second dose in the first phase, in conjunction with safety and immunogenicity data from study C-032-456. The criteria for dose-selection will be specified in a statistical analysis plan to be finalized prior to the unblinded review. The selected dose, in conjunction with the unblinded safety and immunogenicity data, will be submitted to the SMC for review. In the second phase, 48 subjects will be enrolled concurrently into Group 2 (LTBI[-]) and into Groups 3 and 4 (LTBI[+], Table 0-2). In each of Groups 2 and 4, 16 subjects will be randomized at a ratio of 3:1 to receive 3 doses of AERAS-456 or placebo given at Study Days 0, 56, and 112. In Group 3, 16 subjects will be randomized at a ratio of 3:1 to receive 2 doses of AERAS-456 or placebo given at Study Days 0 and 56.

All subjects will stay on the study for 292 days after receiving the first vaccination. The subjects in Groups 1 and 3 will be followed up for 236 days after the second vaccination and subjects in Groups 2 and 4 will be followed up for 180 days after the third vaccination. The sample size for each study cohort was selected because it was judged to be adequate for preliminary safety and immunogenicity evaluations for a Phase I/IIa study rather than for statistical reasons. Given 12 and 15 subjects in individual AERAS-456 dosing groups, the study will have an 80% probability of detecting at least 1 specified event which occurs at a rate of 12.5% and 10.0%, respectively. If no such events are observed among 12 and 15 subjects receiving active study vaccine, an approximation to the upper one-sided 95% confidence bound on the rate of occurrence for that event would be 22% and 18%, respectively.

Table 0-1 Group 1 Treatment/Dose Assignments

Group	QFT Status	Number of Doses	Treatment Assignment				Total
			H56 μ g/IC31 nmol			Placebo	
			5/500	15/500	50/500		
1	-	2 (Days 0, 56)	15	15	15	5	50

Table 0-2 Groups 2, 3, and 4 Treatment/Dose Assignments

Group	QFT Status	Number of Doses	Treatment Assignment		N
			AERAS-456 (Dose Level TBD)	Placebo	
2	-	3 (Days 0, 56, 112)	12	4	16
3	+	2 (Days 0, 56)	12	4	16
4	+	3 (Days 0, 56, 112)	12	4	16
Total			36	12	48

ANALYSIS OF IMMUNOLOGY:

The immunogenicity of AERAS-456 in this study will be evaluated by PBMC ICS and IFN- γ ELISpot assays.

Assessment of immune response by PBMC ICS will be based on the percentage of cytokine producing CD4+ and CD8+ T cells in response to stimulation with H56 protein antigen and 1 of

3 antigenic peptide pools (Ag85B, ESAT-6, and Rv2660c) derived from and representing the entire amino acid sequences of the mycobacterial antigens Ag85B, ESAT-6 and Rv2660c, respectively. Median DMSO-subtracted cytokine responses and associated 95% confidence intervals (CIs) will be used to summarize the percentage of antigen-specific CD4 and CD8 T cell responses by study group and treatment assignment at all available time points. Summaries of T cell response will be presented by T cell type (CD4 and CD8), by stimulation antigen(s), and by cytokine profile. Summaries will include immune response at all available pre- and post-vaccination immunology time points.

Assessment of immune response by ELISpot will be based on the number of IFN- γ spot-forming units (SFU) per 10^6 PBMC in response to stimulation with H56 antigen or 1 of 3 antigenic peptide pools (Ag85B, ESAT-6, and Rv2660c) derived from and representing the entire amino acid sequences of the mycobacterial antigens Ag85B, ESAT-6, and Rv2660c, respectively. Geometric mean and associated 95% CIs will be used to summarize the number of IFN- γ SFU per 10^6 PBMC by study group and treatment assignment at all available time points. Summaries of the number of IFN- γ SFU per 10^6 PBMC will be presented by stimulation antigen(s) and will include immune response at all available pre- and post-vaccination immunology time points.

ANALYSIS OF SAFETY:

The safety profile will be described by study group and treatment assignment. The primary variable for evaluation of the safety profile will be the number and percentage of all unsolicited and solicited adverse events recorded post Day 0 vaccination. Additional summaries will also be presented based on type of adverse events (solicited or unsolicited) and reporting period of adverse events following each study vaccination.

The number (percentage) of subjects with adverse events will be summarized by MedDRA system organ class and preferred term. 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.

Listings will be provided for all subjects with serious adverse events.

Exploratory assessment of adverse events in relation to the degree of QFT positivity at study entry may also be performed.

1 INTRODUCTION

1.1 Background

Tuberculosis (TB) remains a major global health challenge. The available live tuberculosis vaccine, bacillus Calmette-Guérin (BCG), provides incomplete protection against pulmonary tuberculosis. For unknown reasons, a BCG revaccination or “booster” does not provide much additional protection. Although effective chemotherapy for drug-sensitive TB is available, inadequate diagnosis and treatment, plus approximately 450,000 cases of multiple drug-resistant TB, result in continued morbidity and death. A more effective booster vaccine would have a positive effect on the health of many people worldwide, and would be a crucial tool for controlling and ultimately eliminating TB as a global public health problem.

1.2 Description of AERAS-456

H56:IC31 (designated as AERAS-456 for Aeras-sponsored clinical development) contains a fusion protein (referred to as H56 antigen, or H56) of 3 mycobacterial antigens (the early secreted antigens Ag85B and ESAT-6, and the latency antigen Rv2660c) formulated in the Th1-stimulating IC31[®] adjuvant.

1.2.1 H56 Antigen

M. tuberculosis (*Mtb*) is the causative agent of tuberculosis. The H56 antigen is a fusion protein created from 3 *Mtb* antigens: antigen 85B (Ag85B), ESAT-6, and Rv2660c. Ag85B is also referred to as α -antigen and is a 30-kDa mycolyl transferase protein (Wiker et al, 1992; Belisle et al, 1997). Ag85B has been previously demonstrated in the guinea pig tuberculosis test system to induce a substantial protective immunity against aerosol challenge with the highly virulent Erdman strain of *Mtb* (Horwitz et al, 2000). ESAT-6 is a member of a secreted family of proteins that are virulence factors mediating the entry of mycobacteria into cells. ESAT-6 is well recognized in TB patients (Ravn et al, 1999; Ulrichs et al, 1998), cattle infected with *M. bovis* (Pollock and Anderson, 1997), and different strains of *Mycobacterium tuberculosis* (*Mtb*)-infected mice (Brandt et al, 1996). In mice vaccinated with an ESAT-6 subunit vaccine, strong ESAT-6-specific T-cell responses were seen that resulted in protective immunity to *Mtb* challenge at the same level as that provided by BCG (Brandt et al, 2000). The late stage antigen Rv2660c is expressed at a higher level as *Mtb* adapts to latency. In vitro, expression of Rv2660c has been found to be between 80 and 300 fold increased in nutrient starved cultures (Rustad et al, 2009; Betts et al 2002; Muttucumaru et al, 2004). Rv2660c is selectively recognized by latently infected individuals as compared to individuals with active pulmonary TB (Govender et al, 2010).

1.2.2 IC31 Adjuvant

IC31 is a 2-component adjuvant comprised of an oligodeoxynucleotide ODN1a and a polypeptide KLK. The first component, ODN1a contains alternating sequences of the unusual bases inosine and cytidine: oligo-d(IC)13. This motif is similar to CpG motifs that act as T-cell adjuvants (Kochenderfer, 2006). The second component KLK is a synthetic cationic

antimicrobial peptide composed of lysine (K) and leucine (L) in the sequence KLKLLLLLKLK. KLK is thought to enhance peptide specific immune responses by increasing uptake of the complexed antigen into antigen presenting cells. The negatively charged ODN1a and the positively charged KLK complex electrostatically. When IC31 is combined with H56, the adjuvant further complexes with the antigen to form AERAS-456.

The 2 components of IC31 may be combined in various ratios but a 25:1 molar ratio KLK to ODN1a is the ratio used for AERAS-456. Since the ratio of ODN1a and KLK is constant in AERAS-456, adjuvant amounts will be expressed in this document only as nmol of KLK for ease of presentation.

1.3 Nonclinical Experience with AERAS-456

H56:IC31 was immunogenic and protective in mouse and non-human primate animal models. The mycobacterial loads in the lungs of H56:IC31 vaccinated mice were significantly lower than those of mice vaccinated with BCG 12 to 24 weeks after challenge. The H56 antigen also protected against TB when given to mice post-exposure to *Mtb* (Aagaard et al, 2011). AERAS-456 as a boost to BCG vaccination delayed and reduced clinical disease in cynomolgus macaques challenged with *Mtb* (Lin et al 2012). No significant adverse reactions of an immunological nature were seen in animals vaccinated up to 8 times with H56:IC31. There was no evidence of a broader toxic effect of H56:IC31 administered to rabbits in a more intensive regimen than anticipated in the clinical program. All non-clinical information supports the suitability of H56:IC31 as a safe, immunogenic, and possibly effective vaccination to augment the immunity induced by a previous BCG vaccination and/or *Mtb* infection.

1.4 Clinical Experience with AERAS-456

The first in human study of AERAS-456, C-032-456, is a Phase I open-label study in 25 adults (17 with LTBI, 8 without LTBI) conducted at 1 site in South Africa. Eight LTBI(-) subjects have received 3 doses of intramuscular (IM) injections of 15/500 (15 µg H56/500 nmol IC31 KLK) AERAS-456 on Study Days 0, 56, and 112, and have completed 6 months follow up after last vaccination. Eight and 9 LTBI(+) subjects have completed 3 vaccinations with 15/500 and 50/500, respectively, of AERAS-456 on Study days 0, 56, and 112. All 17 LTBI(+) subjects have been followed up for at least 28 days after the last vaccination. No serious adverse events judged to be vaccine-related have been identified in this study. The last subject visit of Study C-032-456 is scheduled for December 2012.

1.5 Clinical Experience with Vaccines Related to AERAS-456

The Hybrid-1 (H1) vaccine, a vaccine closely related to AERAS-456, consists of the HYB-01 fusion protein of the *Mtb* antigens ESAT-6 and antigen 85B in combination with IC31, the same adjuvant used in AERAS-456. Three Phase I clinical trials have been completed by Statens Serum Institut: THYB-01, THYB-02, and THYB-03. These studies represent a combined population of 95 healthy, HIV(-) adults, of whom 80 received any dose of H1. Table 1-1 shows the dose matrix of the 3 studies. All 3 studies were open label and non-randomized. No serious adverse events were reported in these 3 studies. The expected local and systemic reactions

include Grade 1-2 stiffness and pain at the site of injection, headache, and influenza like symptoms such as fatigue, fever, and cough.

Table 1-1 Dose matrix of Studies THYB-01, THYB-02 and THYB-03

Trial #	Treatment Assignment			N
	50 µg Antigen Alone	50 µg Antigen 100 nmol IC31	50 µg Antigen 500 nmol IC31	
THYB-01	12	12	12	36
THYB-02			20	20
THYB-03	12		27	39
Total	24	12	59	95

1.6 Rationale for Study

BCG is currently the only licensed vaccine for the prevention of TB. There is currently no licensed vaccine for the prevention of TB disease in individuals with latent *Mtb* infection (LTBI). AERAS-456 is being developed to prevent TB disease in individuals with and without LTBI. AERAS-456 has 2 components: H56, a recombinant fusion protein of the *Mtb* antigens Ag85B, ESAT-6, and Rv2660c; and IC31, a novel oligonucleotide-based adjuvant designed to elicit a cell-mediated immune response. AERAS-456 is proposed to protect either against *Mtb* infection or against TB disease by boosting existing CD4 responses against *Mtb* antigens expressed by BCG (Ag85B) and by *Mtb* during both active disease (Ag85B and ESAT-6) and latency (Rv2660c). AERAS-456 has been demonstrated to be immunogenic and protective before and after TB exposure in mouse and non-human primate models (Aagaard et al 2011; Lin et al 2012).

Study C-035-456 is the second study of AERAS-456 to evaluate safety and immunogenicity in adults and will include subjects with and without LTBI. Dose selection for study C-035-456 is based on preclinical study results and preliminary safety data of the ongoing study C-032-456 (see Section 1.4 for further details of C-032-456). The selected highest dose of 50 µg AERAS-456 is below that administered in the toxicology study, and is the same as that shown to be protective against *Mtb* challenge in non-human primates. The 50 µg dose thus seems to be efficacious and non-toxic. The selected lowest dose of 5 µg AERAS-456 was protective against *Mtb* challenge in the mouse model. A 3-dose series is standard for many primary immunizing regimens, and is what was studied in the C-032-456 trial. Vaccination at intervals of 56 days is based on clinical data from AERAS-404 (which contains a fusion protein of Ag85B and TB10.4 with IC31 as adjuvant; Aeras unpublished data) and SSI H1 (van Dissel et al 2010; van Dissel et al 2011). Both vaccines elicited antigen-specific T cell responses when administered 56 days apart. The proposed study (C-035-456) will evaluate whether or not the magnitude and duration of immune responses are similar for 2 vs. 3 doses of AERAS-456. A 2-dose regimen has significant advantages in terms of delivery in the field, compliance with regimen completion, and cost. Preclinical studies also indicate that higher doses of AERAS-456 may inhibit immune responses and reduce protective efficacy. All subjects in Study C-035-456 will receive 500 nmol of the adjuvant IC31 based on results of clinical studies of AERAS-404 and H1.

For the purpose of C-035-456, LTBI will be diagnosed based on a positive QuantiFERON TB Gold test (QFT) at screening. Subjects will not receive a screening tuberculin skin test (TST), due to post-vaccination reactions at TST sites observed in previous clinical trials of other vaccines similar to AERAS-456, as well as the potentially confounding effects of the TST on baseline measurements of immune response to *Mtb* antigens.

This study is designed to select an optimal dose regimen of AERAS-456 for further clinical development. The optimal dose regimen will be selected based on safety and immunogenicity in an initial cohort of LTBI(-) subjects and expanded to LTBI(+) subjects. Dose selection for the initial cohort is based on preclinical study results and preliminary safety data from the ongoing Phase I study C-032-456. The rationale for conducting initial dose finding in LTBI(-) subjects is based on clinical data from prior H1 studies, in which the variability in baseline immunogenicity parameters was greater among LTBI(+) subjects compared to LTBI(-) subjects, making differences between vaccine-induced cellular responses easier to distinguish in the LTBI(-) population. Furthermore, cellular immune responses as measured by ELISpot were comparable between the LTBI(+) and LTBI(-) subjects for the same H1 dosage (van Dissel et al, 2010; van Dissel et al, 2011).

Previous BCG vaccination is a criterion for study entry for all subjects. AERAS-456 is being developed for use in countries with a high incidence of *Mtb*, many of which administer universal BCG at birth. Subjects with HIV co-infection or TB disease will be excluded from Study C-035-456; however, they may be included in future studies.

The lack of understanding of host defense mechanisms against *Mtb* infection and the lack of immune correlates of protection to TB vaccines are hampering the development of novel TB vaccines. There is an urgent need for clear immunological markers to predict and evaluate the immunogenicity and efficacy of these vaccines and to optimize vaccine regimens. Despite the lack of correlates, there are some clues that will help in the development of a vaccine for TB. Th1 responses appear to be very important for controlling infection, particularly IFN- γ and TNF. T cells are known to play an important role in controlling tuberculosis. Perhaps the greatest evidence for this in human populations is with HIV/TB coinfection. It is well-known that HIV infection greatly increases the risk of TB reactivation and infection. Prevalence of TB is directly correlated with the loss of CD4+ T cells in HIV-infected patients (Feller et al 2009). Studies have described the development of an animal model to mimic HIV/TB coinfection. In this model, cynomolgus macaques latently infected with TB are challenged with the highly pathogenic SIVmac251. TB reactivation in this model was shown to be correlated with peripheral T cell loss (Matilla et al 2011). In addition, loss of CD4+ T cells has been shown to result in increased tuberculosis-related mortality (Havlir et al 1999). Various CD4+ T cell effector subtypes have been identified, including those making only IL-2 or IFN- γ , as well as multifunctional cells expressing IL-2, IFN- γ , and TNF- α . The presence of these multifunctional cells has been associated with protection against some pathogens (Darrah et al 2007). Furthermore, multifunctional cells have been found at high frequency in tuberculosis patients (Winkler et al 2005) as well as in people in high incidence areas (Scriba et al 2008). In addition to CD4+ T cells, CD8+ T cells also play a critical role in the control of TB. Chen et al. demonstrated that depletion of CD8+ T cells in BCG-vaccinated macaques resulted in the loss of

control of TB infection (Chen et al 2009). Together the data clearly indicate that T cells are required for controlling infection and suggest that T cell immunity may also play an important role in preventing disease. In order to assess the immune responses to vaccination, Aeras will utilize two assays. The qualified ELISpot assay allows for very sensitive detection of the number of cells secreting the Th1 cytokine, IFN- γ , in response to vaccination. In addition, the ICS assay will be used to further assess the quality of the immune response. While the assay is not as sensitive as the ELISpot, it has the added benefit of examining multiple T cell functions (IFN- γ , IL-2, TNF- α , IL-17, CD107a, and CD154) and associating those functions with T cell phenotypes (CD3, CD4, CD8, and memory), resulting in a much more robust assessment of the immune responses generated by vaccination. These assays will allow for the assessment of the magnitude and quality of the immune responses to vaccination and also increase the likelihood that an immune correlate may be identified in future efficacy trials.

Due to the present lack of a biomarker for protection or surrogate marker for risk of TB disease, TB vaccine Phase IIb efficacy studies are tremendously costly and time consuming. For this reason there is a need to carefully select which vaccines are chosen to proceed to Phase IIb. Recent evidence suggests that the BCG vaccine is able to prevent *Mtb* infection as detected by the gamma interferon release assay (IGRA) (Eisenhut et al 2009). Thus the ability to further prevent infection with *Mtb* could be a positive selection criterion for a novel TB vaccine. Studies using prevention of TB infection as a biological outcome can be done with a much lower number of study participants and shorter study duration than prevention of TB disease efficacy studies. In line with this thinking, a prevention of *Mtb* infection study using QFT is currently in planning, and AERAS-456 is one of the vaccine candidates being considered for inclusion in the trial. One issue specific to the AERAS-456 vaccine is that it contains the *Mtb* antigen ESAT-6, which is an IFN- γ inducer in the QFT assay used for diagnosis of *Mtb* infection. This may potentially cause false positive QFT responses. Data from human TB vaccine studies so far show that QFT responses wane relatively quickly after vaccination with an ESAT-6 containing vaccine. Thus data suggests that this issue may very well be overcome (van Dissel et al 2011). However, more data are needed on the kinetics of QFT responses after AERAS-456 vaccination. QFT responses will therefore be measured at baseline, at 2 time points during the study, and at the end of the study in LTBI (-) subjects.

The reasons for conducting clinical trials of AERAS-456 in South Africa (and other sub-Saharan African countries) are as follows: 1) this vaccine is intended for licensing in South Africa and other sub-Saharan African countries. 2) South Africa has a very high rate of LTBI, particularly in the Western Cape Province, 50% overall, but up to 80% in the third decade of life (Wood et al, 2010). 3) Since the rate of LTBI and indeed active tuberculosis is so high, the utility of INH prophylaxis that has been found effective in low prevalence areas is not feasible in South Africa, and this has been so recognized in WHO recommendations (WHO, 1974). Thus, a vaccine that prevents reactivation from latency would have a significant positive impact on public health of South Africa. 4) The University of Cape Town and other organizations in the Republic are preeminent in the field of tuberculosis vaccinology. 5) The simultaneous presence of a high rate of LTBI, a high incidence of active TB, an unmet medical need, and a sophisticated research infrastructure make this clinical research study especially appropriate for South Africa.

2 STUDY OBJECTIVES AND DESIGN

2.1 Objectives

Primary Objective

The primary objective of this study is to evaluate the safety profile of multiple dosage levels and dosing regimens of AERAS-456 administered to HIV-negative, BCG-vaccinated adults with and without LTBI and with no history or evidence of TB disease.

Secondary Objectives

The secondary objectives of this study are:

- To evaluate the immunogenicity of multiple dosage levels and dosing regimens of AERAS-456 administered to HIV-negative, BCG-vaccinated adults with and without LTBI and with no history or evidence of TB disease.
- To select a dosage of AERAS-456 for testing of dose regimens.
- To evaluate kinetics of QFT responses following AERAS-456 vaccination in HIV-negative, BCG-vaccinated adults without LTBI and with no history or evidence of TB disease.

2.2 Design

This is a Phase I/IIa, double-blind, randomized, placebo-controlled, dose- and regimen-finding study in healthy adults with and without LTBI, who are BCG-vaccinated, HIV-negative, and have no history or evidence of TB disease. The investigational product is AERAS-456 at 3 dose levels: 5, 15, and 50 µg of H56 antigen with 500 nmol of IC31. The vaccine is administered by IM injection. The study will be conducted at sites in one or more countries in sub-Saharan Africa, including South Africa.

A total of 98 subjects will be enrolled in 2 phases into 4 groups based on LTBI status. The initial phase will be a dose ranging study of a 2-dose regimen at 3 dosage levels in LTBI(-) subjects, to select a dosage for the second phase. In the second phase, the study will be expanded to evaluate both 2-dose and 3-dose regimens and to include LTBI(+) subjects. In the first phase, 50 LTBI(-) subjects will be enrolled in Group 1 and randomized at a ratio of 3:3:3:1 to receive 2 doses of 5/500, 15/500, or 50/500 of AERAS-456, or placebo given at Study Days 0 and 56 (Table 2-1).

One dose level of AERAS-456 will be selected by the sponsor and SSI for the second phase of the study, based on analysis of unblinded safety and immunogenicity data through 28 days after the second dose in the first phase, in conjunction with safety and immunogenicity data from study C-032-456. The criteria for dose-selection will be specified in a statistical analysis plan to be finalized prior to the unblinded review. The selected dose, in conjunction with the unblinded safety and immunogenicity data, will be submitted to the SMC for review. In the second phase, 48 subjects will be enrolled concurrently into Group 2 (LTBI[-]) and into Groups 3 and 4 (LTBI[+]; Table 2-2). In each of Groups 2 and 4, 16 subjects will be randomized at a ratio of 3:1 to receive 3 doses of AERAS-456 or placebo given at Study Days 0, 56, and 112. In Group 3, 16

subjects will be randomized at a ratio of 3:1 to receive 2 doses of AERAS-456 or placebo given at Study Days 0 and 56.

All subjects will stay on the study for 292 days after receiving the first vaccination. The subjects in Groups 1 and 3 will be followed up for 236 days after the second vaccination and subjects in Groups 2 and 4 will be followed up for 180 days after the third vaccination. The sample size for each study cohort was selected because it was judged to be adequate for preliminary safety and immunogenicity evaluations for a Phase I/IIa study rather than for statistical reasons. Given 12 and 15 subjects in individual AERAS-456 dosing groups, the study will have an 80% probability of detecting at least 1 specified event which occurs at a rate of 12.5% and 10.0%, respectively. If no such events are observed among 12 and 15 subjects receiving active study vaccine, an approximation to the upper one-sided 95% confidence bound on the rate of occurrence for that event would be 22% and 18%, respectively.

Table 2-1 Group 1 Treatment/Dose Assignments

Group	QFT Status	Number of Doses	Treatment Assignment				Total
			H56 μ g/IC31 nmol			Placebo	
			5/500	15/500	50/500		
1	-	2 (Days 0, 56)	15	15	15	5	50

Table 2-2 Groups 2, 3, and 4 Treatment/Dose Assignments

Group	QFT Status	Number of Doses	Treatment Assignment		N
			AERAS-456 (Dose Level TBD)	Placebo	
2	-	3 (Days 0, 56, 112)	12	4	16
3	+	2 (Days 0, 56)	12	4	16
4	+	3 (Days 0, 56, 112)	12	4	16
Total			36	12	48

3 STUDY PROCEDURES

3.1 Schedule of Subject Evaluations

A Summary Schedule of Evaluations depicting all visit-specific procedures is provided in Table 3-1 (Groups 1 and 3) and Table 3-2 (Groups 2 and 4). See Appendix A for a more detailed description of the evaluations.

Table 3-1 Summary Schedule of Subject Evaluations for Groups 1 and 3

Evaluation	Screen	Study Days											
		0	3	7	14	28	56	59	63	70	84	146 ^g	292
Written informed consent	x												
Urine illicit drug screen	x												
QFT	x									x ^f		x ^f	x

Evaluation	Screen	Study Days																	
		0	3	7	14	28	56	59	63	70	84	112	115	119	126	140	210 ^g	292	
Hepatitis B, C; HIV-1	x																		
Vaccine eligibility verification		x					x					x							
Study Vaccination		X					X					X							
Urine βhCG	x	x					x					x							
Vital signs	x	x					x					x							
Urinalysis	x	x		x			x		x			x		x					
Serum chemistry ^a	x	x		x			x		x			x		x					
CBC with differential ^b	x	x		x			x		x			x		x					
PBMC ICS		x			x		x			x		x			x			x	
Whole blood ICS		x			x		x			x		x			x			x	
PBMC ELISpot		x			x		x			x		x			x			x	
Antibody ELISA		x					x					x				x			
RNA analysis		x	x						x				x	x	x				
BioVacSafe		x	x						x				x						
PBMC exploratory		x			x		x			x		x			x			x	
Distribute diary cards		x					x					x							
Review/collect diary cards			x	x					x	x				x	x				
Solicited adverse events		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x			
Unsolicited adverse events		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x			
Serious adverse events		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	x	
Examination of injection site(s)		x	x	x	x	x	x	x	x	x	x	x	x	x	x	x			
Blood Volume (mL) ^d	26	50	6	14	23	0	44	6	14	23	0	44	6	17	31	7	5	28	
Cumulative Blood (mL) ^d	26	76	82	96	119	119	163	169	183	206	206	250	256	273	304	311	316	344	

^aSerum chemistry includes: ALT, AST, total bilirubin, ALP, and creatinine.

^bCBC with differential includes: hemoglobin, hematocrit, white blood cell count, absolute lymphocyte count, absolute neutrophil count, platelet count.

^cSamples for whole blood ICS will be collected only at those sites capable of performing the analyses

^dBlood volumes are approximate

^eSATVI site only

^fQFT at Study Days 126 and 210 for subjects in Group 2 only

^gStudy Day 210 evaluations may be conducted by phone call for Group 4

3.2 Subject Selection

3.2.1 Recruitment and Informed Consent

Various methods of recruitment may be used such as advertising, referrals, or solicitation of subjects previously known to the clinical site. Interested subjects will be invited to participate in the informed consent process. Informed consent will be obtained by the use of a written consent form approved by the Institutional Review Board (IRB) or Independent Ethics Committee (IEC) and signed and dated by the subject at the time of consent. Potential subjects will be interviewed to ensure that the subjects meet all entry criteria relating to history. The clinical investigator or designee will conduct the consent discussion on an individual basis with each subject and will allow adequate time for all questions to be addressed. Written informed consent will be obtained

prior to conducting any study-related procedures. A copy of the signed consent form shall be given to the subject prior to Study Day 0.

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 web response system (IWRS) that consists of the last two digits of the Aeras product number (56), the last two digits of the protocol number (35), 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-035-456 would receive the number 563599-00001, where all except “-00001” were pre-assigned by Aeras.) This subject number will be used throughout the study.

Throughout the study, all screening activities will be captured and maintained in the IWRS. 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 entry into the study will be based on the inclusion and exclusion criteria described below. The investigator must document confirmation of eligibility prior to study entry.

3.2.3 Inclusion Criteria

Subjects must meet all of the following criteria prior to Study Day 0 vaccination:

1. Has completed the written informed consent process prior to the start of screening evaluations.
2. Is male or female.
3. Is age 18 through 50 years at the time of randomization.
4. Received BCG vaccination at least 5 years prior to randomization, documented through medical history or presence of a scar.
5. Females: Ability to avoid pregnancy during the trial: Women physically capable of pregnancy (not sterilized and still menstruating or within 1 year of the last menses if menopausal) in sexual relationships with men must avoid pregnancy with an acceptable method of avoiding pregnancy from 28 days prior to administration of the study vaccine through the end of the study. 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), the use of a condom or a diaphragm combined with spermicide.
6. Has general good health, confirmed by medical history and physical examination at screening.
7. Is able and willing to complete the full follow-up period of 292 days as required by the protocol.
8. Agrees to avoid elective surgery for the full duration of the study.

9. [Groups 1 and 2] Does not have LTBI, determined by a negative QFT at screening
or
[Groups 3 and 4] Has LTBI, determined by a positive QFT at screening.

3.2.4 Exclusion Criteria

Subjects must meet none of the following criteria prior to Study Day 0 vaccination:

1. Acute illness at the time of randomization.
2. Oral temperature $\geq 37.5^{\circ}\text{C}$ at the time of randomization.
3. Abnormal laboratory values (per local laboratory parameters) from blood collected within 21 days prior to Study Day 0 vaccination as follows:
 - hemoglobin, hematocrit, platelet count, absolute neutrophil count, or absolute lymphocyte count below lower limit of normal (LLN).
 - white blood cell count above upper limit of normal (ULN) or below LLN.
 - ALT, AST, total bilirubin, ALP, creatinine, above ULN.
4. Abnormal urinalysis that, in the opinion of the investigator, indicates systemic or local disease.
5. History or evidence of tuberculosis disease, including but not limited to pulmonary tuberculosis, pleural tuberculosis, lymph node tuberculosis or tuberculosis meningitis.
6. Received a TST within 21 days prior to a scheduled study vaccination.
7. Received investigational *Mtb* vaccine at any time prior to Study Day 0.
8. History or evidence of autoimmune disease.
9. History or laboratory evidence of HIV-1 infection at screening.
10. Positive test for hepatitis B surface antigen or hepatitis C antibody at screening.
11. Used immunosuppressive medication (other than inhaled or topical immunosuppressants) within 21 days prior to Study Day 0.
12. Received immunoglobulin or blood products within 21 days prior to Study Day 0.
13. Received any investigational product within 21 days prior to Study Day 0, or plans to participate in any other study involving administration of investigational product during the study period.
14. Inability to discontinue current chronic prescription medications, except contraceptives, inhaled or topical immunosuppressants, or nutritional supplements, during the study period.
15. Documented history of allergic reaction or hypersensitivity to any component of the study vaccine.
16. All female subjects: currently pregnant or lactating/nursing; or positive serum pregnancy test during screening; or positive urine pregnancy test on the day of any study vaccination.
17. History or evidence of any systemic disease or any acute or chronic illness that, in the opinion of the investigator, may compromise the safety of the subject in the study or interfere with the evaluation of the safety or immunogenicity of the vaccine.
18. History of dermatologic disease or skin features that, in the opinion of the investigator, may interfere with the assessment of injection site reactions.
19. History or evidence of any 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.

Note: A subject who fails to meet eligibility criteria because of self-limiting conditions expected to improve within the allowable window period (e.g., febrile illness) can return for repeat screening or, for vaccination subsequent to Study Day 0, have study vaccine administration deferred at the discretion of principal investigator.

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 Study Day 0.

Subjects will provide a detailed medical history and subjects will undergo a physical examination. The assessment will include the determination of any surgeries or medically significant procedures planned to occur during the entire study period. Demographic characteristics (date of birth, gender, and race) will also be collected. Any new abnormal findings will be discussed with the subject and referral will be made for follow-up care if necessary.

Screening laboratory tests (urinalysis, urine drug screen [opiates, cocaine, amphetamines], QFT, HIV serology, chemistry and hematology panels) 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 instructions for performing and interpreting QFT instructions will be maintained in the investigator's study binder.

For females of child-bearing potential, repeat urine β hCG must be performed on specimens obtained within 24 hours prior to study entry on Study Day 0.

3.3 Study Entry and Randomization

Subjects will be enrolled into a study group sequentially based on timing of completion of screening and LTBI status. Within study group, subjects will be randomized to receive either AERAS-456 or placebo based on a randomly-generated sequence of subject identification numbers on a randomization schedule, via an IWRS.

Within Group 1, a total of 50 LTBI(-) subjects will be randomized in a 3:3:3:1 ratio to receive a 2-dose regimen at 3 dosage levels of AERAS-456 (5/500, 15/500, or 50/500) or placebo given 56 days apart. The dosage of AERAS-456 to be administered in Groups 2, 3, and 4 will be selected based on the results from Study Group 1. A total of 48 subjects will be enrolled concurrently into Group 2 (LTBI[-] subjects) and into Groups 3 and 4 (LTBI[+] subjects). LTBI(-) subjects will be assigned to Group 2 and will be randomized in a 3:1 ratio to receive a 3-dose regimen of AERAS-456 at the selected dose level or placebo given 56 days apart. Within Group 3 and Group 4, LTBI(+) subjects will be randomized in a 3:1 ratio to receive 2 doses of AERAS-456 at

the selected dose level or placebo given 56 days apart (Group 3), or in a 3:1 ratio to receive 3 doses of AERAS-456 at the selected dose level or placebo given 56 days apart (Group 4).

In order to maintain the blind of the study team, the randomization schedule will be prepared by an unblinded statistician who will not be involved with the study conduct. The day on which a subject is randomized is Study Day 0. If a subject does not meet all study entry and vaccination eligibility criteria, entry or randomization of the subject should not occur on that day. If the situation permits, subjects may be reconsidered for entry or randomization at a later date, e.g., pending resolution of an acute illness or after a repeated clinical laboratory evaluation result is shown to be within normal limits, within the allowed time frame for complete screening evaluations.

A summary of the randomization procedure is as follows:

- Subject arrives in clinic for Study Day 0 visit.
 - Subjects must be present in the clinic at the time of randomization.
 - Pre-vaccination study procedures performed.
- Investigator reconfirms that subject meets all eligibility criteria for randomization and that the subject can receive study vaccine as soon as possible after randomization.
 - Some ineligible subjects may be reconsidered for randomization at a later date, e.g., pending resolution of an acute illness or after a repeated clinical laboratory evaluation result is shown to be within normal limits within the allowed time frame for complete screening evaluations.
- Investigator or designee (as assigned in the IWRS requirements specification document) randomizes the subject using the IWRS.
- Study vaccine manager accesses the IWRS to determine the randomization assignment for the subject, then prepares the appropriate study vaccine according to the IWRS treatment assigned to the subject and per the study Vaccine Management Manual prepared by the sponsor
- Study vaccine manager transfers the prepared unit-dose syringe to the designated study team member in the clinic for administration to the subject.

If a randomized subject withdraws consent or is removed from the study, that subject will not be replaced.

3.4 Blinding

The study is double blinded. At Aeras, the investigational product manager (and/or designee) will be the only unblinded person(/s) during the blinded periods of the study 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). All unblinded persons must 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 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.

Access to unblinded treatment assignment via the IWRS during the study will be provided only to the study vaccine manager (and designee), the study monitor, and the Aeras investigational product manager. Unblinded randomization list reports generated by the IWRS as well as all pharmacy source documents and dose preparation records that can link a subject identification number with a treatment assignment must remain secure (e.g., in the pharmacy with access limited to only unblinded persons) until notification from Aeras that the study has been unblinded.

Labels accompanying the syringes of prepared vaccine doses will not indicate which vaccine is in the syringe. Identical syringes and needles will be used for preparation and administration of each vaccine. Prior to transferring the prepared unit-dose syringe of study vaccine to the designated study team member in the clinic, the study vaccine manager will mask the syringe with a translucent colored label, in order to maintain the study blind by obscuring slight differences in color and opacity between active and placebo formulations, providing sufficient visibility of the volume of the syringe contents to guide correct dosing.

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 (directly or via the unblinded pharmacist) by following the "unblinding by site form call flow" process in the IWRS. The designated study management team will be notified of the unblinding immediately via the IWRS system alert. It is recommended that the principal investigator consults with the 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.

3.4.2 Unblinding for Safety Analysis by SMC

Unblinded safety data may be reviewed by the SMC and the independent statistician responsible for preparing these analyses according to the SMC charter as described in the Section 5.1.5.

3.4.3 Unblinding for Preliminary Data Review

A preliminary unblinded review of ICS immune response and safety data will be conducted following completion of the Study Day 84 visit for Group 1 as described in Section 7.7.1.

3.5 Study Vaccine Administration

On Study Day 0, subjects must receive their dose of study vaccine as soon as possible after randomization. If vaccination will not occur on the stated vaccination day the principal investigator or designee must notify the sponsor within 72 hours after deferral of vaccination and reschedule the subject to return to the clinic at another time. On Study Day 56 and/or Study Day

112, all subjects randomized who have not met any of the criteria for discontinuation of study injections (see Section 6.1) will receive a second and/or a third dose of study vaccine. In case of short term, reversible conditions, such as acute febrile or respiratory illness, the second and/or third dose of study vaccine should be deferred until the subject has recovered; the allowable window period for deferral of the second and/or third dose is 3 days.

The study vaccine manager will send the study vaccine to the clinic as a unit-dose syringe, which will be identified with the subject identification number, subject's initials, date and time of dose preparation, and the volume prepared. The syringe will contain 0.5 mL of the study vaccine. A medically qualified study team member must be present in the clinic at the time of all study vaccine administrations.

Before administering the injection, the study team member in the clinic who will be administering the vaccine must inspect the syringe and vaccine volume, checking that the syringe is identified with the correct subject identification number and initials and checking the date and time the dose was prepared. The vaccine manager must deliver the syringe to the clinic so that vaccine can be administered within 2 hours of being prepared. If circumstances result in a delay of administration beyond the allotted time, an explanation must be entered into the source documents and the expired syringe must be returned to the study vaccine manager, who will prepare a replacement syringe.

The study vaccine will be administered intramuscularly into the deltoid area using standard aseptic technique, and alternating arms for each vaccination. Administration of the study vaccine (including date, time and which arm was injected) must be documented in the subject's study records by the study team member who administered the vaccine.

3.6 Study Evaluations

3.6.1 Immunology Laboratory Evaluations

A summary of immunologic assays to be performed on blood specimens is shown in Table 3-3. Listed blood volumes are estimates. Blood will be collected for all assays. Required assays will be performed on available specimens from all listed subjects and time points. Exploratory assays may be performed on specimens from certain subjects and time points. Blood will also be collected for immunokinetics analysis and for participation in the BioVacSafe consortium (www.biovacsafe.eu) – an international collaboration between industry and academia aimed at developing cutting edge tools to speed up and improve the testing and monitoring of vaccine safety, both before and after release to the market (see Appendix E for further details).

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 for immunology assays.

Table 3-3 Summary of Immunology Specimens and Assays

Assay	Priority	Purpose of Assay	Location	Study Days		Blood Volume per time (Total)	
				Groups 1 and 3	Groups 2 and 4	Groups 1 and 3	Groups 2 and 4
13-color PBMC ICS Assay	Required	Determine cellular immune response to study vaccine	Aeras Maryland, USA	0, 14, 56, 70, 292	0, 14, 56, 70, 112, 126, 292	5 mL (25 mL)	5 mL (35 mL)
IFN- γ PBMC ELISpot	Required	Determine cellular immune response to study vaccine	Aeras Maryland, USA	0, 14, 56, 70, 292	0, 14, 56, 70, 112, 126, 292	5 mL (25 mL)	5 mL (35 mL)
Antigen-specific IgG (ELISA)	Exploratory	Determine humoral immune response to study vaccine	Aeras Maryland, USA	0, 56, 84	0, 56, 112, 140	7 mL (21 mL)	7 mL (28 mL)
Whole blood ICS Assay ^a	Exploratory	Determine cellular immune response to study vaccine	SATVI South Africa	0, 14, 56, 70, 292	0, 14, 56, 70, 112, 126, 292	5 mL (25 mL)	5 mL (35 mL)
RNA analysis (transcriptional profiling)	Exploratory	To perform a multivariate analysis of the innate immune responses following vaccination in order to identify early gene signatures that can predict immune responses	To be determined	0, 3, 59, 63, 70	0, 3, 59, 115, 119, 126	3 mL (15 mL)	3 mL (18 mL)
BioVacSafe Project	Exploratory	To perform a multivariate analysis in order to identify new biomarkers useful to identify warning signs that a candidate vaccine may be reactogenic	Max Planck Institute for Infection Biology, Berlin, Germany	0, 3, 59	0, 3, 59, 115	3 mL (9 mL)	3 mL (12 mL)
PBMC ^b	Exploratory	Future validation/ exploratory	SATVI South Africa	0, 14, 56, 70, 292	0, 14, 56, 70, 112, 126, 292	8 mL (40 mL)	8 mL (56 mL)

a. Samples for whole blood ICS will be collected only at those sites capable of performing the analyses

b. Samples to be collected at SATVI site only

The co-primary immunology endpoints will be ICS and ELISpot. A 13-color ICS assay using frozen PBMC will be performed at Aeras' immunology laboratory, where the reagents, equipment, and training of personnel have all been standardized to provide consistent results. The IFN- γ ELISpot assay using frozen PBMC will also be conducted at Aeras' immunology laboratory using a qualified assay. Conducting both the ICS and IFN- γ ELISpot assays in the same laboratory using the same material will reduce the assay variability for this multi-site study, particularly when determining the correlation between the 2 assays. Currently there are no laboratories in South Africa that have a highly standardized, qualified, and controlled multi-parameter flow cytometry assay or IFN- γ ELISpot assay conducted by trained operators. This is, in part, why the consortium of Aeras, the US HIV Vaccine Trials Network (HVTN), and the US National Institutes of Health (NIH) are currently considering building a new Immunology Central Endpoint Laboratory in Cape Town, which will be staffed with local expertise.

3.6.2 Safety Evaluations

3.6.2.1 Pre-vaccination and Post-Vaccination Monitoring of Subjects

Subjects will have vital signs (pulse, respiratory rate, temperature, and blood pressure) taken prior to each study vaccination. Subjects will remain in the clinic under close observation for at least 60 minutes after receiving study vaccine. As with any vaccine, allergic reactions to dose administration 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.

3.6.2.2 Clinical Assessments and Laboratory Tests

Subjects will have their vital signs measured before each study vaccination and 60 minutes after each vaccination on Study Days 0, 56 and/or 112. An assessment of the sites of injection and axilla(e) will be performed during each scheduled clinic visit and any adverse events will be recorded on the AE case report form (CRF). Female must have a negative urine pregnancy test on Days 0, 56 and/or 112 before each vaccination. All laboratory samples will be processed according to SOPs provided by the local clinical laboratory. Subjects will be retested for QFT on Study Days 70 and 146 for Group 1, Study Days 126 and 210 for Group 2, and on the last clinic visit at Study Day 292 for Groups 1 through 4.

Clinical assessments and laboratory tests results obtained on the study must be reviewed by the investigator (or a designee who is a medically qualified study team member) within 72 hours of receiving the results to determine if abnormalities exist, with the exception of urine β hCG on vaccination days, the results of which must be reviewed and confirmed to be negative before administration of study vaccine.

Abnormal Clinical Assessments and Laboratory Tests

Laboratory abnormalities that have increased in toxicity grade from Study Day 0 will be graded as adverse events according to a pre-specified toxicity table (see Appendix D for toxicity grading scales), except for visually interpreted urine dipstick findings, which will be graded according to the clinical judgment of the principal investigator (PI) or designee. Abnormal laboratory tests must be repeated promptly 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 local 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.

3.6.2.3 Adverse Events

The collection periods for adverse events are:

Solicited adverse events: 28 days after each study vaccination
Unsolicited adverse events: 28 days after each study vaccination
Serious adverse events: Entire study period (i.e., 292 days after first study vaccination)

Solicited adverse events to be collected include the following:

Local: injection site pain, injection site erythema, injection site swelling

Systemic: fatigue, myalgia, arthralgia, pyrexia, chills, nausea

Adverse events will be followed to resolution (see Section 5.3).

3.6.2.4 Concomitant Medications

The collection of information on concomitant medications used by subjects following vaccination will coincide with the collection period of adverse events. The collection period for concomitant medications associated with the treatment of adverse events will be 28 days following each vaccination. The collection period for concomitant medications associated with the treatment of serious adverse events (SAE) will be Study Days 0–292.

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.

3.6.3 Subject Follow-up and Contact

All subjects who 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. All sites will have study staff available for consultation by study participants for any health concerns that they may have.

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 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 investigational study)

All deviations from protocol procedures, evaluations, and/or visits must be categorized and documented as they occur. Each deviation must be documented on a Protocol Deviation Form. When possible, missed visits and procedures must be rescheduled and performed at the nearest possible time point to the original schedule.

3.6.4 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 Study Day 292 visit date, then a determination of "lost to follow-up" can be made.

4 STUDY VACCINES

4.1 Supplies

Study vaccine manager will be provided with adequate quantities of AERAS-456 and placebo. The product will be manufactured and shipped by Statens Serum Institut (SSI) located in Copenhagen, Denmark, in accordance with good manufacturing practices (GMP).

AERAS-456 vaccine will be supplied as 3 fixed-dose combinations of H56 and IC31. A description of the contents of the vials follows:

- **AERAS-456 5/500:** 0.8 mL containing 5 µg/0.5 mL H56 antigen and 500 nmol/0.5 mL IC31 adjuvant. The liquid material may appear as a greyish/colorless solution
- **AERAS-456 15/500:** 0.8 mL containing 15 µg/0.5 mL H56 antigen and 500 nmol/0.5 mL IC31 adjuvant. The liquid material may appear as a greyish/colorless solution.
- **AERAS-456 50/500:** 0.8 mL containing 50 µg/0.5 mL H56 antigen and 500 nmol/0.5 mL IC31 adjuvant. The liquid material may appear as a greyish/colorless solution.

Placebo will be supplied in vials containing 0.8 mL sterile buffer consisting of 10 mM Tris and 169 mM NaCl at pH 7.4. The buffer solution for placebo is colorless and similar to the buffer solution in which AERAS-456 is formulated. The buffers are isotonic.

Both AERAS-456 and placebo will be stored and prepared for administration as described in the Vaccine Management Manual, provided under separate cover.

4.2 Accountability

The study vaccine manager is required to maintain accurate study vaccine accountability records. Instructions and required 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 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-456 and placebo 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.

Both AERAS-456 and placebo must be stored at between +2°C and +8°C in a secured location with no access for unauthorized personnel.

Complete storage instructions will be provided in the study Vaccine Management Manual.

4.4 Vaccine Preparation

Preparation of the study vaccine for injection should not begin before a subject is confirmed eligible on Study Day 0 and re-confirmed eligible on Study Day 56 and, if applicable, on Study Day 112. The study vaccine manager will follow the detailed instructions provided in the study Vaccine Management Manual to prepare the dose of AERAS-456 vaccine or placebo. Aseptic technique must be utilized.

Preparation of the vaccine including date and time must be documented in the study vaccine management records by the study vaccine manager who prepares the doses.

Request for preparation of the second vaccination on Study Day 56, and, for subjects in Groups 2 and 4, the third vaccination on Study Day 112 will be made by the investigator in a similar fashion as on Study Day 0. The study vaccine manager will prepare the appropriate study vaccine according to the treatment assignment indicated for that subject identification number per the randomization schedule.

4.5 Disposal of Unused Supplies

Upon completion of the study, Aeras must provide authorization for any unused study vaccine and supplies to be disposed of according to the facility's SOPs. Any disposing of study vaccine conducted at the clinical site will be documented in the study file.

5 SAFETY

5.1 Responsibilities for Ensuring the Safety of Trial Subjects

The national regulatory authority, the vaccine 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. The principal investigator determines severity and causality with respect to the study vaccine for each adverse event. The principal investigator is blinded in this study, in which case the study vaccine may consist of a placebo, 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 two medical monitors (one local medical monitor and one global medical monitor) and an independent safety monitoring committee (SMC).

5.1.3 Local Medical Monitor

The local medical monitor is the sponsor's representative and is a licensed 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 may make a sponsor's assessment of severity and causality for adverse events that may upgrade the degree of severity and causality determined by the principal investigator. The local medical monitor, like the principal investigator, is blinded for this study.

5.1.4 Global Medical Monitor

The purpose of the Global Medical Monitor (GMM) is to authorize SUSAR (suspected unexpected serious adverse reaction) reporting to regulatory agencies without unblinding the sponsor, protocol team or the clinical sites. Aeras retains a GMM through the contract research organization PPD, Inc., located in Cambridge, UK. The GMM reviews and unblinds all SUSAR reports and, if he or she deems it necessary may review and unblind selected SAE reports. The GMM may contact study sites directly if additional information is needed for assessment of the SUSAR. For all SUSARS the GMM will make available to sponsor and the clinical trial sites a completed blinded CIOMS II so that the sites and sponsor may remain blinded.

5.1.5 Safety Monitoring Committee

If study vaccine administration is paused (see safety pausing rules in Section 6.2) by the principal investigator, the local medical monitor, or the global medical monitor, an SMC will be convened. The SMC composition is described in an SMC charter. The voting members cannot be directly involved with the conduct of the study. Voting members cannot be employees of Aeras. Additional subject area experts may be present to provide expertise if requested by the SMC. The SMC 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. Only the SMC and the independent statistician responsible for preparing these analyses would be unblinded during these reviews. All procedures associated with this review, including objectives, data handling, and elements to be included for review will be documented in SMC minutes.

Based on its review and the protocol stopping rules (Section 6) the SMC will make recommendations in the SMC minutes to Aeras regarding further conduct of the study and further administration of study vaccine. The conclusions of the SMC will be communicated to the investigators and the Institutional Review Boards/Ethics Committees and the national regulatory authority. The sponsor agrees to abide by the decision of its SMC and any directives issued by the national regulatory authority, the Institutional Review Board or Ethics Committee.

5.1.6 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.

5.1.7 National Regulatory Authority

Since the national regulatory authority (such as the Medicines Control Council) 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 SMC will review safety data on an as needed basis.

5.3 Definition of Adverse Event

An adverse event (AE) is defined as any unfavorable or unintended sign, symptom, disease, syndrome, abnormal laboratory finding, or concurrent illness that emerges or worsens relative to

the subject's pretreatment baseline, whether or not it is considered to be related to the medicinal 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 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 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 blinded studies, of

whether AERAS-456 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 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 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:

Local: injection site pain, injection site erythema, injection site swelling

Systemic: fatigue, myalgia, arthralgia, pyrexia, chills, nausea

Solicited adverse events of local injection site reactions 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 will be provided a diary card to record temperature and information regarding occurrences of these specific events for the first 7 days of the solicited adverse event reporting period (see further details in Section 5.15).

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) in adults and adolescents are considered to be adverse events that are causally related to the study vaccine and are of special interest, but do not constitute solicited adverse events. Site of injection ulceration (including presence of drainage) and axillary lymphadenopathy will be actively evaluated during each clinic visit through the end of the study. These events will be recorded on the Adverse Event CRF.

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 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.

5.9 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 conjunction with the local medical monitor, determines expectedness. If the assessment is that the adverse event is **expected** no further action is required. If assessment is that the adverse event is **unexpected**, then the event may represent a SUSAR (see Sections 5.10 and 5.11).

5.10 Definition of Suspected Unexpected Serious Adverse Reaction (SUSAR)

When an adverse event is judged to be related to an investigational product, such as AERAS-456, and also is judged to be serious and unexpected, it is a SUSAR (suspected unexpected serious adverse reaction) and is subject to expedited reporting.

5.11 Reporting of Serious Adverse Events

Serious adverse events, which include SUSARs, are reported to the sponsor, SSI, 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 Adverse Event CRF and any supplemental reporting forms to be used. Serious adverse events will be reported on the Adverse Event CRF using a recognized medical term or diagnosis that accurately reflects the event.

Serious adverse events will be assessed by the investigator and the local medical monitor according to their roles (as described in Sections 5.1.1 and 5.1.3) for severity, causal relationship to the study vaccine, and expectedness. The onset and resolution dates of the event and the action taken in response to the event will be documented. If the event has not resolved by the final study visit, it will be documented as “ongoing” on the CRF, however, follow-up of the SAE must continue until SAE is resolved (or stabilization of the chronic conditions). Information recorded on the CRF must be substantiated in the source documents.

The SAE Report form completed for that event must be faxed by the principal investigator or his/her designee, within 1 business day of the clinical site becoming aware of the event, to the local medical monitor and to the World-Wide Safety Center at PPD. The AE CRF should be completed with all information known at the time; the Supplemental SAE Report (paper form) should be completed and both forms faxed (even if all information concerning the event is not yet known) within 1 business day of awareness of the event.

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 PPD World Wide Safety Center to execute its responsibilities for 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 submits additional information requested as soon as it becomes available.

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

5.12 Other Events Requiring Immediate Reporting

The investigator must report the following events by faxing the appropriate form to the local medical monitor within 1 business day of becoming aware of the event:

- Withdrawal of consent during the study (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)

5.13 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 source documents and 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.

5.14 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 faxing the Pregnancy Notification Form to the medical monitor and PPD World Wide Safety Center. 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 PPD World Wide Safety Center and the status of the mother and child after delivery will be obtained and reported, when possible.

5.15 Subject Diary and Daily Temperature Monitoring

Subjects 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 PI 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. Diaries not brought to the scheduled visit should be obtained before adverse event assessment can be performed and events discussed with the PI and/or designee. Lost diaries will be reconstructed as possible on a new diary booklet by the subject from memory on the closest clinic visit and labeled as a 'reconstructed diary.' Adverse events obtained from the diary, as determined by the PI or designee, will be recorded and completely assessed on 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.

In circumstances where subject illiteracy is a factor, study sites can choose to have a study staff member verbally administer the question on the diary card to obtain this data.

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 an individual, between individuals within a single dose group, and between dose groups.

6.1 Rules for Discontinuing Study Injections in an Individual Subject

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

- A 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
- Fever (oral temperature $\geq 102.1^{\circ}\text{F}/39^{\circ}\text{C}$) within 1 week following study injection which is associated with constitutional symptoms (myalgia, arthralgia, fatigue, headache, anorexia, hives, chills) AND is judged to be possibly, probably, or definitely related to study injection
- Injection site reactogenicity that involves Grade 3 or Grade 4 induration, erythema, or tenderness as defined in the protocol toxicity table
- Development of active tuberculosis
- Development of autoimmune disease or immunosuppression
- Receipt of investigational drug therapy or investigational vaccine (other than study injections received as part of this study)
- Missed study injection
- Any event that in the opinion of the principal investigator precludes administration of any further study injections
- Pregnancy

6.2 Safety Pausing Rules

The principal investigator and/or local medical monitor will pause administration of study vaccine in the trial if:

1. It is determined that a suspected unexpected serious adverse reaction (SUSAR) occurred
OR
2. It is determined that a serious adverse event *OR* a severe (toxicity Grade 3 or 4) adverse event (except for visually interpreted urine dipstick findings) *OR* an adverse event pattern of concern occurred *AND* is judged to be POSSIBLY, PROBABLY or DEFINITELY related to study vaccine

If the principal investigator and/or local medical monitor pauses administration of study vaccine in the study, he or she will record this in a memorandum to the study file and, within 24 hours, notify Aeras who will then convene the SMC. If additional clinical information becomes available that reduces the principal investigator's assessment of causality, severity or toxicity grade such that study pausing is no longer required, then the principal investigator, with the agreement of the local medical monitor, may resume study vaccine administration, record this in a memorandum to the study file, and notify the sponsor within 24 hours.

Since the global medical monitor is unblinded and reviews data from all Aeras trials, he or she may become aware of an adverse event pattern of concern not appreciated by the principal investigator or local medical monitor. If the global medical monitor independently determines that an adverse event pattern of concern that is judged to be POSSIBLY, PROBABLY or DEFINITELY related to study vaccine has occurred, the global medical monitor will pause administration of study vaccine in the trial, record the study pause in a memorandum to the study file, and notify, within 24 hours, the local medical monitor, principal investigator and sponsor who will convene the SMC.

6.3 Safety Stopping Rules

The rules for stopping further enrollment and study vaccine administration by the SMC are below:

- Death in any subject unless the SMC determines it is UNRELATED to AERAS-456
- An anaphylactic reaction to AERAS-456 in any subject
- A severe (Toxicity Grade 3 or 4) or serious adverse event (except for Grade 3 local injection site reactions and visually interpreted urine dipstick findings) unless the SMC determines it is UNRELATED to AERAS-456
- A potentially life-threatening adverse event unless the SMC determines it is UNRELATED to AERAS-456
- A pattern of significant symptoms, physical findings or laboratory abnormalities (adverse events) that, although individually minor, collectively represent a safety concern in the opinion of the investigator or the medical monitor and are judged by the SMC to be DEFINITELY, PROBABLY or POSSIBLY related to AERAS-456

The SMC may recommend resumption of enrollment if it judges that changes to the study protocol will eliminate or greatly reduce the safety risks specified in the stopping rules.

If a decision to resume study enrollment and study vaccine administration is made the SMC will record their judgment in a memorandum to the study file and notify Aeras, within 24 hours. The SMC memorandum will be forwarded to the medical monitors and principal investigators. 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 preliminary unblinded review, and for preparation of the final study report (see Section 7.7).

7.1 Subject Populations

The safety population will consist of all subjects who received at least one dose of study vaccine.

7.2 Demographics and Protocol Compliance

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

As subject enrollment to each treatment group will be conducted based on timing of completion of study eligibility requirements, any imbalance in baseline characteristics between study groups will also be examined.

Listings of subjects who missed any dose of study vaccine and of subjects with protocol deviations (to be defined in the statistical analysis plan) will be provided.

7.3 Efficacy Analyses

There will be no efficacy analyses performed in this study.

7.4 Immunogenicity and Other Immunology Analyses

All immunogenicity analyses will be based on subjects who received at least one dose of study vaccine. Immunogenicity will be summarized for all time points as collected and as available. No imputation for missing data will be performed. Data will be transformed as appropriate prior to analysis.

Additional analyses may be performed based on subjects who complete all scheduled vaccinations, on LTBI status, and the magnitude of QFT positivity at study screening.

7.4.1 Required Immunology Analyses

The primary variables of interest for assessment of immune response to vaccine will be the percentage of CD4+ and CD8+ T cells that produce any of selected Th-1 and Th-2 cytokines. The immunogenicity of AERAS-456 in this study will be evaluated by PBMC ICS and IFN- γ ELISpot. Due to the exploratory nature of immunogenicity endpoints, the primary evaluation will be based on descriptive summaries and no formal hypothesis testing will be performed.

7.4.1.1 PBMC ICS Assay

Assessment of immune response by PBMC ICS will be based on the percentage of cytokine producing CD4+ and CD8+ T cells in response to stimulation with H56 protein antigen and 1 of 3 antigenic peptide pools (Ag85B, ESAT-6, and Rv2660c) derived from and representing the entire amino acid sequences of the mycobacterial antigens Ag85B, ESAT-6 and Rv2660c, respectively. ICS parameters will include cytokines such as IFN- γ , IL-2 and TNF- α , and may include other cytokines to identify T cells of specific functionality (such as IL-17). Additional cell surface markers, cytokines, or functional markers may also be analyzed. Median DMSO-subtracted cytokine responses and associated 95% confidence intervals (CIs) or other descriptive statistics as appropriate will be used to summarize the percentage of antigen-specific CD4 and CD8 T cell responses by study group and treatment assignment at all available time points. Summaries of T cell response will be presented by T cell type (CD4 and CD8), by stimulation antigen(s), and by cytokine profile. Summaries will include immune response at all available pre- and post-vaccination immunology time points. Exploratory assessment of antigen specific CD4+ and CD8+ T cell responses within the LTBI(+) groups (i.e., Groups 3 and 4) in relation to the magnitude of QFT positivity at study screening may also be performed.

7.4.1.2 IFN- γ ELISPOT

Assessment of immune response by ELISpot will be based on the number of IFN- γ spot-forming units (SFU) per 10^6 PBMC in response to stimulation with H56 antigen or 1 of 3 antigenic peptide pools (Ag85B, ESAT-6, and Rv2660c) derived from and representing the entire amino acid sequences of the mycobacterial antigens Ag85B, ESAT-6, and Rv2660c, respectively. Geometric mean and associated 95% CIs will be used to summarize the number of IFN- γ SFU per 10^6 PBMC by study group and treatment assignment at all available time points. Summaries of the number of IFN- γ SFU per 10^6 PBMC will be presented by stimulation antigen(s) and will include immune response at all available pre- and post-vaccination immunology time points.

7.4.1.3 QuantiFERON-TB Gold Test

Results for the QuantiFERON-TB Gold test at each available time point will be summarized using subject count (percentage) summaries and descriptive statistics.

7.4.2 Exploratory Assays

7.4.2.1 12 Hour Whole Blood ICS Assay

Assessment of immune response by 12 hour whole blood ICS will be based on the percentage of cytokine producing CD4 and CD8 T cells in response to stimulation with H56 protein antigen

and 1 of 3 antigenic peptide pools (Ag85B, ESAT-6, and Rv2660c) derived from and representing the entire amino acid sequences of the mycobacterial antigens Ag85B, ESAT-6 and Rv2660c, respectively. Median DMSO-subtracted cytokine responses and associated 95% CIs will be used to summarize the percentage of antigen-specific CD4+ and CD8+ T cell responses by study group at all available time points. Summaries of T cell response will be presented by T cell type (CD4 and CD8), by stimulation antigen(s), and by cytokine profile. Summaries will include immune response at all available pre- and post-vaccination immunology time points.

7.4.2.2 RNA Analysis

As an exploratory endpoint for examining RNA gene expression signatures, RNA expression will be performed. RNA will be isolated from whole blood. Signatures of gene expression changes after vaccination may be determined using computational systems biology tools.

7.4.2.3 Antibody ELISA

Geometric mean titers for antibody ELISA to Ag85B, ESAT-6, and Rv2660c and associated 95% CIs will be summarized at all pre- and post-vaccination immunology time points through Study Day 292.

7.4.2.4 BioVacSafe Project

Details of the analysis for the BioVacSafe Project are in Appendix E.

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 study group for all subjects in the safety population.

7.5.1 Adverse Events

The safety profile of AERAS-456 will be described by study group and treatment assignment. 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.

In addition, dose-safety curves may be produced to examine the proportion of subjects who experience adverse events by study group and treatment assignment.

Serious adverse events will be recorded through the final study visit for all subjects. Listings will be provided for subjects with serious adverse events.

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 D) will be tabulated at each post-vaccination time point and overall. Clinical laboratory and vital sign abnormalities will also be reported as adverse events and will be included in the summary of adverse events.

Exploratory assessment of adverse events in relation to the degree of QFT positivity at study entry may also be performed.

7.5.2 Clinical Laboratory and Vital Sign Parameters

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

7.6 Sample Size Considerations

The sample size for this study was selected as adequate for a review of the safety profile of AERAS-456 in HIV-negative adults with and without LTBI, rather than for statistical reasons. Given 12 and 15 subjects in individual AERAS-456 dosing groups, the study will have an 80% probability of detecting at least 1 specified event which occurs at a rate of 12.5% and 10.0%, respectively. If no such events are observed among 12 and 15 subjects receiving active study vaccine, an approximation to the upper one-sided 95% confidence bound on the rate of occurrence for that event would be 22% and 18%, respectively.

7.7 Plan for Statistical Summaries and Analyses

7.7.1 Preliminary Data Reviews

A preliminary unblinded review of ICS immune response and safety data, in conjunction with safety and immunogenicity data from study C-032-456, will be conducted by the sponsor following completion of Study Day 84 visit for all subjects in Group 1. The database will be locked through Study Day 84 and the study will be unblinded by treatment group for the preparation of this review. The purpose of this review is to obtain preliminary immunogenicity and safety data for use in the decision-making process regarding vaccine dose level to be employed in the second phase of this study. The criteria for dose-selection will be specified in a

statistical analysis plan to be finalized prior to the unblinded review. This review will include adverse events and serious adverse events through Study Day 84, and ICS and ELISpot immune response data, through Study Day 84 for Group 1. The selected dose, in conjunction with the unblinded safety and immunogenicity data, will be submitted to the SMC for review. Study procedures and monitoring practices will not change following this preliminary review. No study stopping rules will be stipulated. Statistical summaries for this preliminary analysis will be prepared by a statistician who is not the study statistician and who is not involved in the study conduct. Data identified for inclusion in the safety summaries will be cleaned prior to statistical analysis. Personnel at the research site and at the immunology laboratory will remain blinded to all study results and to treatment assignments until after the Study Day 292 data have been collected, reviewed and queries resolved.

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 for all Groups. The database will be locked prior to 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. Any source document templates provided by Aeras or its designee will serve as supplements to the subject's study record.

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. Completed, original CRFs will be retrieved by Aeras or its designee and a copy of each completed CRF will be retained at the clinical site as part of the study records.

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.). 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), and local regulatory requirements.

The protocol and informed consent form will be reviewed and approved by the IRB or IEC of each participating 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. Aeras will also have an independent IRB review and approval of the protocol and informed consent form.

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

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. Clinical information will not be released 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.

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 consent form 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 (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.

9.3 Informed Consent

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 will be documented in writing on a consent form approved by the IRB/IEC.

All relevant information should be provided in both oral and written form in a way that is understandable to the subject. Ample time and opportunity must be given for the subject to inquire about details of the study. The written consent document will embody the elements of informed consent as described in the Declaration of Helsinki and will also comply with local regulations.

The investigator or the investigator's qualified designee will explain the nature of the study and inform the subject 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 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 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 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. Subjects 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 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 must be informed that his/her signature on the informed 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 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 consent form for each subject will be maintained by the investigator as part of the subject's study records. A copy of the signed informed consent form will be provided to each subject.

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 and SSI for purposes of publications. The principal investigator, SSI personnel, and study staff must send all manuscripts, abstracts, and presentations using data from this study to Aeras for review prior to their submission. Aeras and SSI reserve the right to delete any part or parts of such materials deemed to be confidential or proprietary.

No data from the clinical trial may be published, presented or communicated, except to regulatory authorities, prior to the release of the study report, unless approved by Statens Serum Institut in writing.

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; 10/May/2013)

Section Number (Title)	Version 1.0	Version 2.0
Title page	Change/rationale: The name of the sponsor's authorized representative was changed.	
	Robert Walker, MD Vice President, Clinical Development	Ann Ginsberg, MD, PhD Vice President, Scientific Affairs, and Acting Chief Medical Officer
Study Abstract	Change/rationale: The study abstract was updated to reflect the changes described below.	
Section 1.6 (Rationale for Study)	Change/rationale: Text was added to this section to describe the rationale for measuring QFT at multiple time points during the study.	
	<i>Not present</i>	Due to the present lack of a biomarker for

Section Number (Title)	Version 1.0	Version 2.0
		<p>protection or surrogate marker for risk of TB disease, TB vaccine Phase IIb efficacy studies are tremendously costly and time consuming. For this reason there is a need to carefully select which vaccines are chosen to proceed to Phase IIb. Recent evidence suggests that the BCG vaccine is able to prevent <i>Mtb</i> infection as detected by the gamma interferon release assay (IGRA) (Eisenhut et al 2009). Thus the ability to further prevent infection with <i>Mtb</i> could be a positive selection criterion for a novel TB vaccine. Studies using prevention of TB infection as a biological outcome can be done with a much lower number of study participants and shorter study duration than prevention of TB disease efficacy studies. In line with this thinking, a prevention of <i>Mtb</i> infection study using QFT is currently in planning, and AERAS-456 is one of the vaccine candidates being considered for inclusion in the trial. One issue specific to the AERAS-456 vaccine is that it contains the <i>Mtb</i> antigen ESAT-6, which is an IFN-γ inducer in the QFT assay used for diagnosis of <i>Mtb</i> infection. This may potentially cause false positive QFT responses. Data from human TB vaccine studies so far show that QFT responses wane relatively quickly after vaccination with an ESAT-6 containing vaccine. Thus data suggests that this issue may very well be overcome (van Dissel et al 2011). However, more data are needed on the kinetics of QFT responses after AERAS-456 vaccination. QFT responses will therefore be measured at baseline, at 2 time points during the study, and at the end of the study in LTBI (-) subjects.</p>
Section 2.1 (Objectives)	<p>Change/rationale: A new secondary objective was added regarding evaluation of the kinetics of QFT responses.</p> <p><i>Not present</i></p>	<ul style="list-style-type: none"> • To evaluate kinetics of QFT responses following AERAS-456 vaccination in HIV-negative, BCG-vaccinated adults without LTBI and with no history or evidence of TB disease.

Section Number (Title)	Version 1.0	Version 2.0
Section 3.1 (Schedule of Subject Evaluations)	Change/rationale: In order to determine the degree to which AERAS-456 vaccination influences QFT response, additional measurements of QFT were added at Study Days 70 and 146 for Group 1, and at Study Days 126 and 210 for Group 2. In addition, a footnote was added to Table 3-1 to indicate that the evaluations for Study Day 146 for Group 3 and Study Day 210 for Group 4 may be conducted by phone call.	
Section 3.2.2 (Screening) Section 3.3 (Study Entry and Randomization) Section 3.4 (Blinding) Section 3.4.1 (Unblinding for Clinical Emergencies)	Change/rationale: This section was revised to reflect that an IWRS will be used.	
Section 3.2.4 (Exclusion Criteria)	Change/rationale: Exclusion criterion #2 was revised to change ‘axillary temperature’ to ‘oral temperature’ 2. Axillary temperature $\geq 37.5^{\circ}\text{C}$ at the time of randomization.	2. Oral temperature $\geq 37.5^{\circ}\text{C}$ at the time of randomization.
Section 3.6.2.2 (Clinical Assessments and Laboratory Tests)	Change/rationale: This section was revised to reflect the addition of QFT measurements, and that visits at Study Day 146 (Group 3) and Study Day 210 (Group 4) may be conducted by phone call, as described for Section 3.1.	
Section 7.7.1 (Preliminary Data Reviews)	Change/rationale: This section was revised to reflect changes to parameters that will be reviewed through Study Day 84 for subjects in Group 1. A preliminary unblinded review of ICS immune response and safety data, in conjunction with safety and immunogenicity data from study C-032-456, will be conducted by the sponsor following completion of Study Day 84 visit for Group 1. The database will be locked and the study will be unblinded by treatment group for the preparation of this review. The purpose of this review is to obtain preliminary immunogenicity and safety data for use in the decision-making process regarding vaccine dose level to be employed in the second phase of this study. The criteria for dose-selection will be specified in a statistical analysis plan to be finalized prior to the unblinded review. This review will include all safety data, ICS immune response data, clinical assessments, and concomitant medications through Study Day 84. The selected dose, in conjunction with the unblinded safety and immunogenicity data, will be submitted to the SMC for review. Study procedures and monitoring practices will not change following this preliminary	A preliminary unblinded review of ICS immune response and safety data, in conjunction with safety and immunogenicity data from study C-032-456, will be conducted by the sponsor following completion of Study Day 84 visit for all subjects in Group 1. The database will be locked through Study Day 84 and the study will be unblinded by treatment group for the preparation of this review. The purpose of this review is to obtain preliminary immunogenicity and safety data for use in the decision-making process regarding vaccine dose level to be employed in the second phase of this study. The criteria for dose-selection will be specified in a statistical analysis plan to be finalized prior to the unblinded review. This review will include adverse events and serious adverse events through Study Day 84, and ICS and ELISpot immune response data, through Study Day 84 for Group 1. The selected dose, in conjunction with the unblinded safety and immunogenicity data, will be submitted to the SMC for review. Study procedures

Section Number (Title)	Version 1.0	Version 2.0
	review. No decision cut points or stopping rules will be stipulated. No formal hypothesis testing will be performed.....	and monitoring practices will not change following this preliminary review. No study stopping rules will be stipulated.....
Appendix A (Detailed Description of Study Visits)	Change/rationale: This section was revised to reflect the addition of QFT measurements as described for Section 3.1.	

13 REFERENCES

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APPENDIX A Detailed Description of Study Visits**Screening**

Unless otherwise noted, all screening evaluations must be completed within 21 days prior to Study Day 0. More than one clinic visit may be required to complete the screening process. The screening process will include:

1. Written informed consent
2. Assignment of screening number
3. Medical history (confirm methods of contraception for females)
4. Physical examination including vital signs (blood pressure, pulse, and temperature), height, and weight
5. Blood collection for:
 - Hepatitis B & C
 - HIV-1
 - QuantiFERON[®]-TB Gold In Tube (process per package insert)
 - Serum chemistry: includes ALT, AST, total bilirubin, ALP, and creatinine
 - Hematology: includes hemoglobin, hematocrit, white blood cell count, absolute lymphocyte count, absolute neutrophil count, and platelet count
6. Urine collection for:
 - Urine β -hCG (pregnancy test) for all females of child-bearing potential
 - Illicit drug screen
 - Urinalysis: includes dipstick, microscopic analysis, and urine hemosiderin
7. Confirmation that study entry eligibility criteria are met

Study Day 0***Pre-vaccination:***

1. Confirm absence of acute illness and significant symptomatic infection
2. Update medical history including use of medications
3. Examine *planned* SD0 injection site
4. Vital signs:
 - Blood pressure
 - Pulse
 - Oral temperature, must be $< 37.5^{\circ}\text{C}$
5. Blood collection for (refer to Aeras Specimen Management Manual):
 - Serum chemistry: includes ALT, AST, total bilirubin, ALP, and creatinine
 - Hematology: includes hemoglobin, hematocrit, white blood cell count, absolute lymphocyte count, absolute neutrophil count, and platelet count
 - Whole blood for 12 hour ICS assay
 - PBMC for ICS and ELISpot assay
 - PBMC for future validation/exploratory
 - Serum for antibody ELISA
 - Whole blood for RNA analysis and BioVacSafe assay
6. Urine collection for:
 - Urinalysis: includes dipstick, microscopic analysis, and urine hemosiderin
7. Confirm continued eligibility for vaccination including negative β -hCG (urine pregnancy test) for all females of child-bearing potential (must be done within 24 hours prior to vaccination)
8. Assign subject ID number (randomize)

9. Complete Vaccine Request Form; send to study vaccine manager for preparation of study vaccine

Vaccination:

10. Inspect syringe and vaccine volume, confirm subject identification number, check subject initials, date and time of dose preparation
11. Administer study vaccine into deltoid area by intramuscular (IM) injection. Record date and time of vaccination and which arm was vaccinated

Post-vaccination:

12. Monitor subject for acute adverse events for at least 60 minutes after vaccination
13. Obtain 30±5 minutes and 60±10 minutes post-immunization vital signs (blood pressure, pulse, oral temperature)
14. Examine site of SD0 injection 60±10 minutes post-immunization
15. Record any adverse events, including solicited and serious adverse events (including concomitant medications) reported
16. Distribute and review diary card and diary card instructions with subject

Study Day 3 (*Study Day 0 visit date + 3 days*)

1. Review/collect diary card entries for completeness and accuracy
2. Record any adverse events including solicited and serious adverse events occurring since previous evaluation; record any concomitant medications taken associated with treatment of the adverse event
3. Examine site of SD0 injection
4. Blood collection for RNA analysis and BioVacSafe assay

Study Day 7

Allowable window for clinic visit is 7 ±1 day from Study Day 0 visit date.

1. Review/collect diary card entries for completeness and accuracy
2. Record any adverse events including solicited and serious adverse events occurring since previous evaluation; record any concomitant medications taken associated with treatment of the adverse event
3. Examine site of SD0 injection
4. Urine collection for urinalysis, including dipstick, microscopic analysis, and urine hemosiderin
5. Blood collection for:
 - Serum chemistry: includes ALT, AST, total bilirubin, ALP, and creatinine
 - Hematology: includes hemoglobin, hematocrit, white blood cell count, absolute lymphocyte count, absolute neutrophil count, and platelet count

Study Day 14

Allowable window for clinic visit is 14 ±3 days from Study Day 0 visit date.

1. Record any adverse events including solicited and serious adverse events occurring since previous evaluation; record any concomitant medications taken associated with treatment of the adverse event
2. Examine site of SD0 injection
3. Blood collection for:
 - Whole blood for 12 hour ICS assay
 - PBMC for ICS assay and ELISpot assay

- PBMC for future validation/exploratory

Study Day 28

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

1. Record any adverse events including solicited and serious adverse events occurring since previous evaluation; record any concomitant medications taken associated with treatment of the adverse event
2. Examine site of SD0 injection

Study Day 56

Allowable window for clinic visit is 56 ±3 days from Study Day 0 visit date.

Pre-vaccination:

1. Confirm continued eligibility for vaccination including negative β -hCG (urine pregnancy test) for all females of child-bearing potential (must be done within 24 hours prior to vaccination)
2. Physical examination
3. Examine *planned* SD56 injection site
4. Vital signs:
 - Blood pressure
 - Pulse
 - Oral temperature, must be $< 37.5^{\circ}\text{C}$
5. Blood collection for (refer to Aeras Specimen Management Manual):
 - Serum chemistry: includes ALT, AST, total bilirubin, ALP, and creatinine
 - Hematology: includes hemoglobin, hematocrit, white blood cell count, absolute lymphocyte count, absolute neutrophil count, and platelet count
 - Whole blood for 12 hour ICS assay
 - PBMC for ICS assay and ELISpot assay
 - PBMC for future validation/exploratory
 - Serum for antibody ELISA
6. Urine collection for:
 - Urinalysis: includes dipstick, microscopic analysis, and urine hemosiderin
7. Complete Vaccine Request Form; send to study vaccine manager for preparation of study vaccine

Vaccination:

8. Inspect syringe and vaccine volume, confirm subject identification number, check subject initials, date and time of dose preparation
9. Administer study vaccine into deltoid area by intramuscular (IM) injection. Record date and time of vaccination and which arm was vaccinated

Post-vaccination:

10. Monitor subject for acute adverse events for at least 60 minutes after vaccination
11. Obtain 30±5 minutes and 60±10 minutes post-immunization vital signs (blood pressure, pulse, oral temperature)
12. Examine site of SD56 injection 60±10 minutes post-immunization
13. Record any adverse events, including solicited and serious adverse events (including concomitant medications) reported
14. Distribute and review diary card and diary card instructions with subject

Study Day 59 (*Study Day 56 visit date + 3 days*)

1. Review/collect diary card entries for completeness and accuracy
2. Record any adverse events including solicited and serious adverse events occurring since previous evaluation; record any concomitant medications taken associated with treatment of the adverse event
3. Examine site of SD56 injection
4. Blood collection for RNA analysis and BioVacSafe assay

Study Day 63

Allowable window for clinic visit is 7 ±1 day from Study Day 56 visit date.

1. Review/collect diary card entries for completeness and accuracy
2. Record any adverse events including solicited and serious adverse events occurring since previous evaluation; record any concomitant medications taken associated with treatment of the adverse event
3. Examine site of SD56 injection
4. Urine collection for urinalysis, including dipstick, microscopic analysis, and urine hemosiderin
5. Blood collection for:
 - Serum chemistry: includes ALT, AST, total bilirubin, ALP, and creatinine
 - Hematology: includes hemoglobin, hematocrit, white blood cell count, absolute lymphocyte count, absolute neutrophil count, and platelet count
 - [Groups 1 and 3 only] RNA analysis

Study Day 70

Allowable window for clinic visit is 14 ±3 days from Study Day 56 visit date.

1. Record any adverse events including solicited and serious adverse events occurring since previous evaluation; record any concomitant medications taken associated with treatment of the adverse event
2. Examine site of SD56 injection
3. Blood collection for:
 - Whole blood for 12 hour ICS assay
 - PBMC for ICS assay and ELISpot assay
 - PBMC for future validation/exploratory
 - [Group 1 only] QuantiFERON[®]-TB Gold In Tube (process per package insert)
 - [Groups 1 and 3 only] RNA analysis

Study Day 84

Allowable window for clinic visit is 28 ±3 days from Study Day 56 visit date.

1. Record any adverse events including solicited and serious adverse events occurring since previous evaluation; record any concomitant medications taken associated with treatment of the adverse event
2. Examine site of SD56 injection
3. [Groups 1 and 3 only] Blood collection for (refer to Aeras Specimen Management Manual):
 - Serum for antibody ELISA

Study Day 112 for Groups 2 and 4 only

Allowable window for clinic visit is 56 ±3 days from Study Day 56 visit date.

Pre-vaccination:

1. Confirm continued eligibility for vaccination including negative β -hCG (urine pregnancy test) for all females of child-bearing potential (must be done within 24 hours prior to vaccination)
2. Physical examination
3. Examine *planned* SD112 injection site
4. Vital signs:
 - Blood pressure
 - Pulse
 - Oral temperature, must be $< 37.5^{\circ}\text{C}$
5. Blood collection for (refer to Aeras Specimen Management Manual):
 - Serum chemistry: includes ALT, AST, total bilirubin, ALP, and creatinine
 - Hematology: includes hemoglobin, hematocrit, white blood cell count, absolute lymphocyte count, absolute neutrophil count, and platelet count
 - Whole blood for 12 hour ICS assay
 - PBMC for ICS assay and ELISpot assay
 - PBMC for future validation/exploratory
 - Serum for antibody ELISA
6. Urine collection for:
 - Urinalysis: includes dipstick, microscopic analysis, and urine hemosiderin
7. Complete Vaccine Request Form; send to study vaccine manager for preparation of study vaccine

Vaccination:

8. Inspect syringe and vaccine volume, confirm subject identification number, check subject initials, date and time of dose preparation
9. Administer study vaccine into deltoid area by intramuscular (IM) injection. Record date and time of vaccination and which arm was vaccinated

Post-vaccination:

10. Monitor subject for acute adverse events for at least 60 minutes after vaccination
11. Obtain 30±5 minutes and 60±10 minutes post-immunization vital signs (blood pressure, pulse, oral temperature)
12. Examine site of SD112 injection 60±10 minutes post-immunization
13. Record any adverse events, including solicited and serious adverse events (including concomitant medications) reported
14. Distribute and review diary card and diary card instructions with subject

Study Day 115 for Groups 2 and 4 only (Study Day 112 visit date + 3 days)

1. Review/collect diary card entries for completeness and accuracy
2. Record any adverse events including solicited and serious adverse events occurring since previous evaluations; record any concomitant medications taken associated with treatment of the adverse event
3. Examine site of SD112 injection
4. Whole blood for RNA analysis and BioVacSafe assay

Study Day 119 for Groups 2 and 4 only

Allowable window for clinic visit is 7 ±1 day from Study Day 112 visit date.

1. Review/collect diary card entries for completeness and accuracy
2. Record any adverse events including solicited and serious adverse events occurring since previous evaluation; record any concomitant medications taken associated with treatment of the adverse event
3. Examine site of SD112 injection
4. Urine collection for urinalysis, including dipstick, microscopic analysis, and urine hemosiderin
5. Blood collection for:
 - Serum chemistry: includes ALT, AST, total bilirubin, ALP, and creatinine
 - Hematology: includes hemoglobin, hematocrit, white blood cell count, absolute lymphocyte count, absolute neutrophil count, and platelet count
 - RNA analysis

Study Day 126 for Groups 2 and 4 only

Allowable window for clinic visit is 14 ±3 days from Study Day 112 visit date.

1. Record any adverse events including solicited and serious adverse events occurring since previous evaluation; record any concomitant medications taken associated with treatment of the adverse event
2. Examine site of SD112 injection
3. Blood collection for:
 - Whole blood for 12 hour ICS assay
 - PBMC for ICS assay and ELISpot assay
 - PBMC for future validation/exploratory
 - [Group 2 only] QuantiFERON[®]-TB Gold In Tube (process per package insert)
 - RNA analysis

Study Day 140 for Groups 2 and 4 only

Allowable window for clinic visit is 28 ±3 days from Study Day 112 visit date.

1. Record any adverse events including solicited and serious adverse events occurring since previous evaluation; record any concomitant medications taken associated with treatment of the adverse event
2. Examine site of SD112 injection
3. Blood collection for:
 - Serum for antibody ELISA

Study Day 146 for Groups 1 and 3 only

Allowable window for clinic visit is 90 ±5 days from Study Day 56 visit date.

Evaluations may be conducted by phone call for Group 3

1. Record any serious adverse events occurring since previous evaluation; record any concomitant medications taken associated with treatment of the serious adverse event
2. Blood collection for:
 - [Group 1 only] QuantiFERON®-TB Gold In Tube (process per package insert)

Study Day 210 for Groups 2 and 4 only

Allowable window for clinic visit is 210 ±14 days from Study Day 0 visit date.

Evaluations may be conducted by phone call for Group 4

1. Record any serious adverse events occurring since previous evaluation; record any concomitant medications taken associated with treatment of the serious adverse event
2. Blood collection for:
 - [Group 2 only] QuantiFERON®-TB Gold In Tube (process per package insert)

Study Day 292 (End of Study)

Allowable window for clinic visit is 292 ±14 days from Study Day 0 visit date.

1. Review medical history
2. Review physical examination
3. Record any serious adverse events occurring since previous evaluation; record any concomitant medications taken associated with treatment of the serious adverse event
4. Blood collection for:
 - QuantiFERON®-TB Gold In Tube (process per package insert)
 - Whole blood for 12 hour ICS assay
 - PBMC for ICS assay and ELISpot assay
 - PBMC for future validation/exploratory

APPENDIX B Sample Subject Diary and Instructions**Daily Diary
Aeras Protocol C-035-456 for Investigational TB Vaccine****General Instructions to Subjects for Completing Your Daily Diaries**

1. Use a pen to complete the diary.
2. The first day for completing the diary will be the day you receive a vaccination. All the Study Days and dates you should be completing the diary until your next clinic visit have already been filled in for you.
3. You will need to take your temperature every day, and report on the specific symptoms that are listed on your daily diary. Do not skip any of the specific symptoms. If you did not have the symptom, check “No” on the diary for that day.
4. The time of day that you take your temperature and evaluate your symptoms should be about the same time each day. You may find it helpful to use “just before bedtime” as your time to do this, but choose a time that will be convenient for you. If you are concerned that you may have a fever, you may take your temperature more often than once daily. Please report the highest temperature that you record on any day when multiple temperatures are taken.
5. Use the section at the back of each diary page to describe any medications you may have taken to treat the listed symptom(s) that day, or use it to record any other symptoms that you may have had that day.
6. You will need to take your completed diaries with you to the clinic on your next scheduled visit.
7. At the clinic, a study team member will review with you the entries you have made in each of your daily diaries. You may be asked questions so that the study team member in the clinic understands any notes you may have recorded.
8. You will then be asked to sign the diary card to confirm that it is accurate and that it has been reviewed with you.

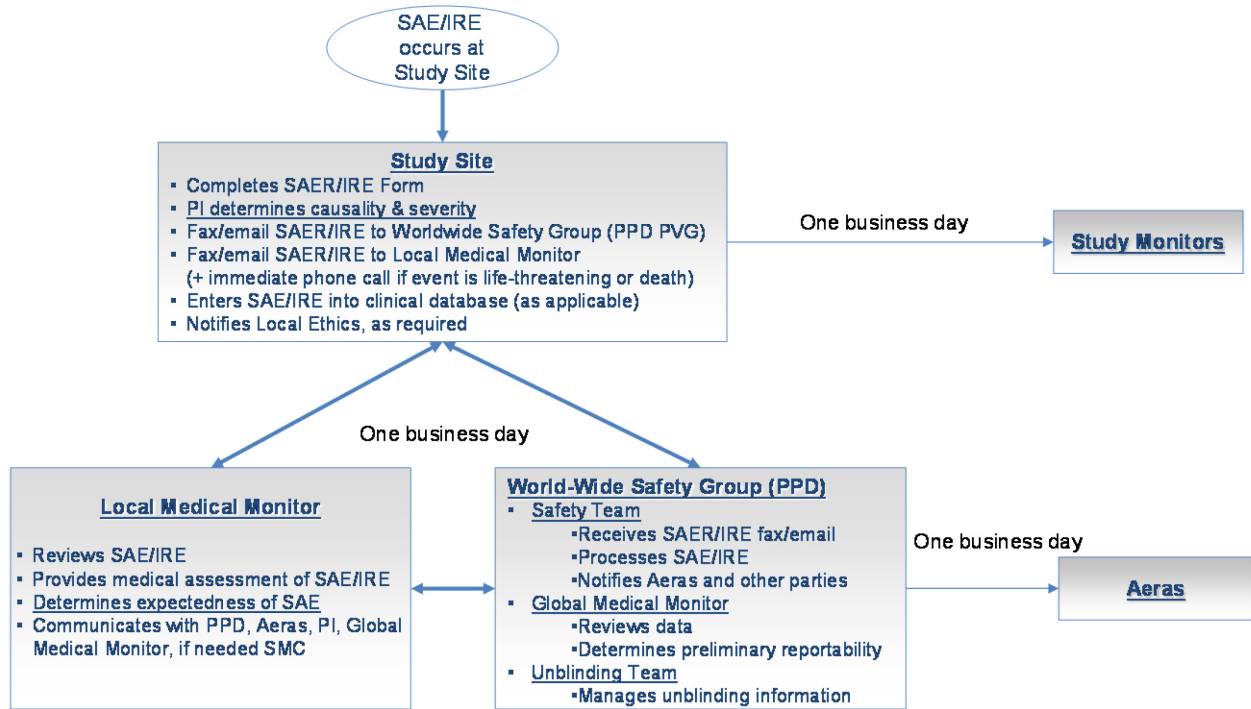
Subject ID: _____ - _____ Initials: _____	
STUDY DAY _____ Date: _____	
Return your completed diary on your next scheduled clinic visit: _____ <div style="text-align: right;"><i>(date of your next scheduled clinic visit)</i></div>	
My temperature today was _____ . _____ °C	
Symptoms	Check the one that describes the worst a symptom was today
Did you have pain at the injection site?	<input type="checkbox"/> No <input type="checkbox"/> Yes, but it did not interfere with my usual daily activities <input type="checkbox"/> Yes, and it interfered with my usual daily activities <input type="checkbox"/> Yes, and it prevented me from doing my usual daily activities
Did you have redness at the injection site?	<input type="checkbox"/> No <input type="checkbox"/> Yes, and the redness measured _____ . _____ cm at the widest point
Did you have swelling at the injection site?	<input type="checkbox"/> No <input type="checkbox"/> Yes, and the swelling measured _____ . _____ cm at the widest point
Did you have fatigue (feeling tired)?	<input type="checkbox"/> No <input type="checkbox"/> Yes, but it did not interfere with my usual daily activities <input type="checkbox"/> Yes, and it interfered with my usual daily activities <input type="checkbox"/> Yes, and it prevented me from doing my usual daily activities
Did you have myalgia (muscle aches)?	<input type="checkbox"/> No <input type="checkbox"/> Yes, but it did not interfere with my usual daily activities <input type="checkbox"/> Yes, and it interfered with my usual daily activities <input type="checkbox"/> Yes, and it prevented me from doing my usual daily activities
Did you have arthralgia (joint aches)?	<input type="checkbox"/> No <input type="checkbox"/> Yes, but it did not interfere with my usual daily activities <input type="checkbox"/> Yes, and it interfered with my usual daily activities <input type="checkbox"/> Yes, and it prevented me from doing my usual daily activities
Did you have chills?	<input type="checkbox"/> No <input type="checkbox"/> Yes, but it did not interfere with my usual daily activities <input type="checkbox"/> Yes, and it interfered with my usual daily activities <input type="checkbox"/> Yes, and it prevented me from doing my usual daily activities
Did you have nausea?	<input type="checkbox"/> No <input type="checkbox"/> Yes, but it did not interfere with my usual daily activities <input type="checkbox"/> Yes, and it interfered with my usual daily activities <input type="checkbox"/> Yes, and it prevented me from doing my usual daily activities

Subject's signature: _____ Date: _____

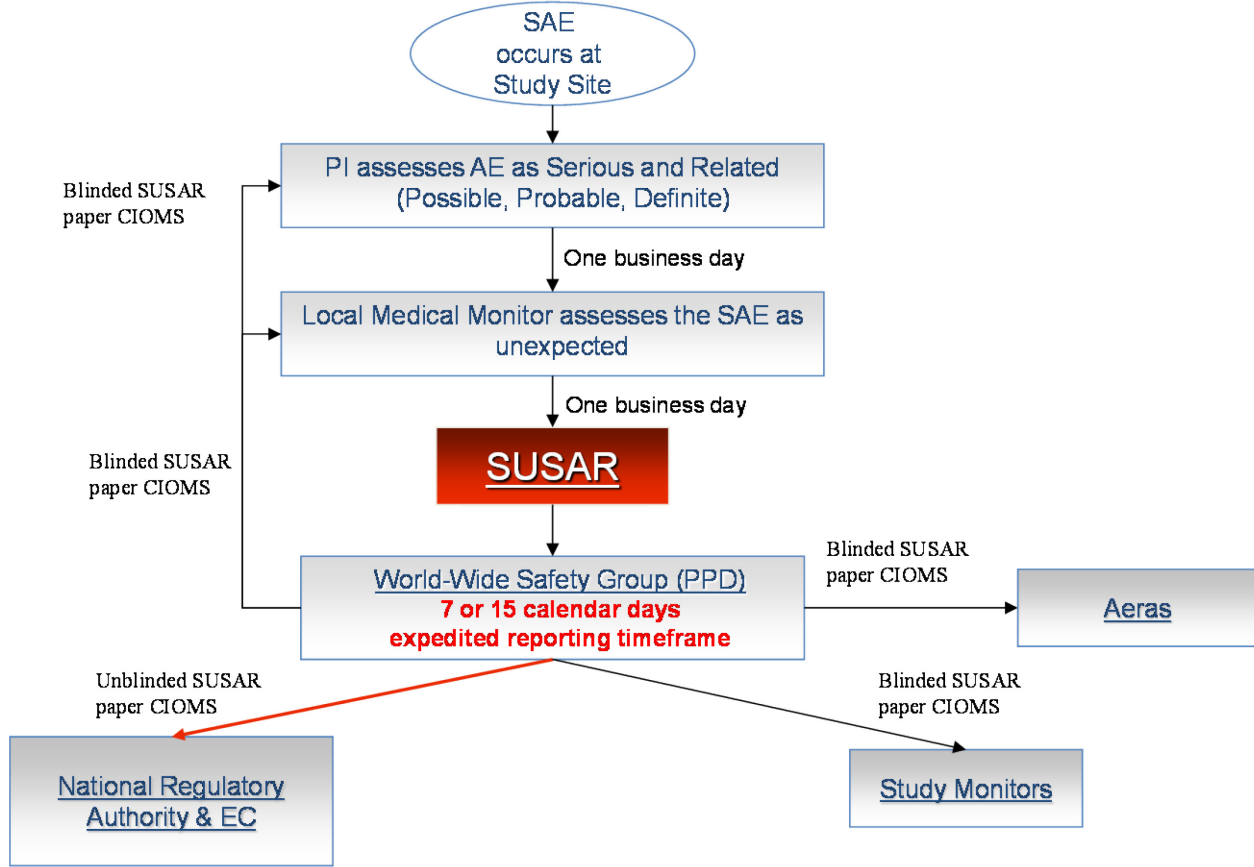
Clinic staff's signature: _____ Date: _____

APPENDIX C Reporting Schemes for SAEs, Immediately Reportable Events, and SUSARs

Reporting Scheme for SAEs and Immediately Reportable Events (IREs)



Reporting Scheme for SUSARs



APPENDIX D Toxicity Table

Note: From final US FDA guidance: Toxicity Grading Scale for Healthy Adult and Adolescent Subjects Enrolled in Preventive Vaccine Clinical Trials (September 2007); laboratory values are in conventional and SI units. The laboratory values provided in the tables serve as guidelines and are dependent upon institutional normal parameters. Institutional normal reference ranges should be provided to demonstrate that they are appropriate.

Local Site of Injection Symptoms	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Pain	Does not interfere with activity	Repeated use of non-narcotic pain reliever > 24 hours or interferes with activity	Any use of narcotic pain reliever or prevents daily activity	ER visit or hospitalization
Tenderness	Mild discomfort to touch	Discomfort with movement	Significant discomfort at rest	ER visit or hospitalization
Erythema/Redness *	2.5 - 5 cm	5.1 - 10 cm	>10 cm	Necrosis (ulceration) or exfoliative dermatitis
Induration/Swelling **	2.5 - 5 cm and does not interfere with activity	5.1 - 10 cm or interferes with activity	>10 cm or prevents daily activity	Necrosis (ulceration)

* In addition to grading the local reaction at the greatest single diameter, the measurement should be recorded as a continuous variable.

** Induration/Swelling should be evaluated and graded using the functional scale as well as the actual measurement.

Vital Signs*	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Fever**	38.0 – 38.4°C 100.4 – 101.1°F	38.5 - 38.9°C 101.2 - 102.0°F	39.0 - 40°C 102.1 - 104°F	>40°C >104°F
Tachycardia – beats per minute	101 – 115	116 – 130	>130	ER visit or hospitalization for arrhythmia
Bradycardia – beats per minute	50 – 54	45 – 49	<45	ER visit or hospitalization for arrhythmia
Hypertension (systolic) – mm Hg	141 – 150	151 – 155	>155	ER visit or hospitalization for malignant hypertension
Hypertension (diastolic) – mm Hg	91 – 95	96 – 100	>100	ER visit or hospitalization for malignant hypertension
Hypotension (systolic) – mm Hg	85 - 89	80 – 84	<80	ER visit or hospitalization for hypotensive shock
Respiratory rate – breaths per minute	17 – 20	21 – 25	>25	Intubation

* Subject should be at rest for all vital sign measurements. ** Oral temperature; no recent hot or cold beverages or smoking.

Systemic (General)	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Nausea/vomiting	No interference with activity or 1 - 2 episodes/24 hours	Some interference with activity or >2 episodes/24 hours	Prevents daily activity, requires outpatient IV hydration	ER visit or hospitalization for hypotensive shock
Diarrhea	2 - 3 loose stools or < 400 grams/ 24 hours	4 - 5 stools or 400 - 800 grams/24 hours	6 or more watery stools or > 800 grams/24 hours or requires outpatient IV hydration	ER visit or hospitalization for hypotensive shock
Headache	No interference with activity	Repeated use of non-narcotic pain reliever > 24 hours or some interference with activity	Significant; any use of narcotic pain reliever or prevents daily activity	ER visit or hospitalization
Fatigue	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization
Myalgia	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization

Systemic (General)	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Illness or clinical adverse event (as defined according to applicable regulations)	No interference with activity	Some interference with activity not requiring medical intervention	Prevents daily activity and requires medical intervention	ER visit or hospitalization

Serum	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Sodium – hyponatremia mEq/L or mmol/L:	132 – 134	130 – 131	125 – 129	<125
Sodium – hypernatremia mEq/L or mmol/L:	144 – 145	146 – 147	148 – 150	>150
Potassium – hyperkalemia mEq/L or mmol/L:	5.1 – 5.2	5.3 – 5.4	5.5 – 5.6	>5.6
Potassium – hypokalemia mEq/L or mmol/L:	3.5 – 3.6	3.3 – 3.4	3.1 – 3.2	<3.1
Glucose – hypoglycemia mg/dL: mmol/L:	65 – 69 3.6 – 3.8	55 – 64 3.0 – 3.5	45 – 54 2.5 – 2.9	<45 <2.5
Glucose – hyperglycemia Fasting - mg/dL: mmol/L: Random – mg/dL: mmol/L:	100 – 110 5.5 – 6.0 110 – 125 6.1 – 6.8	111 – 125 6.1 – 6.8 126 – 200 6.9 – 11.0	>125 >6.8 >200 >11.0	Insulin requirement or hyperosmolar coma
Blood urea nitrogen (BUN) – mg/dL: mmol/L:	23 – 26 8.3 – 9.5	27 – 31 9.6 – 11.2	>31 >11.2	Requires dialysis
Creatinine – elevated mg/dL: umol/L:	1.5 – 1.7 121 – 145	1.8 – 2.0 146 – 170	2.1 – 2.5 171 – 208	>2.5 or requires dialysis >208 or requires dialysis
Calcium – hypocalcemia mg/dL: mmol/L:	8.0 – 8.4 2.00 – 2.10	7.5 – 7.9 1.87 – 1.99	7.0 – 7.4 1.75 – 1.86	<7.0 <1.75
Calcium – hypercalcemia mg/dL: mmol/L:	10.5 – 11.0 2.63 – 2.76	11.1 – 11.5 2.77 – 2.88	11.6 – 12.0 2.89 – 3.00	>12.0 >3.00
Magnesium – hypomagnesemia mg/dL: mmol/L:	1.3 – 1.5 0.52 – 0.62	1.1 – 1.2 0.43 – 0.51	0.9 – 1.0 0.37 – 0.42	<0.9 <0.37
Phosphorus – hypophosphatemia mg/dL: mmol/L:	2.3 – 2.5 0.73 – 0.80	2.0 – 2.2 0.63 – 0.72	1.6 – 1.9 0.51 – 0.62	<1.6 <0.51
Albumin – hypoalbuminemia g/dL: g/L:	2.8 – 3.1 28 – 31	2.5 – 2.7 25 – 27	<2.5 <25	---- ----
Total protein – hypoproteinemia g/dL: g/L:	5.5 – 6.0 55 – 60	5.0 – 5.4 50 – 54	<5.0 <50	---- ----
Alkaline phosphatase (ALP) – increased	1.1 – 2.0 x ULN	2.1 – 3.0 x ULN	3.1 – 10 x ULN	>10 x ULN
Liver Function Tests (LFT): AST, ALT – increased	1.1 – 2.5 x ULN	2.6 – 5.0 x ULN	5.1 – 10 x ULN	>10 x ULN
Lactate dehydrogenase (LDH), – increased	1.1 – 2.5 x ULN	2.6 – 5.0 x ULN	5.1 – 10 x ULN	>10 x ULN
Bilirubin (with any increase in LFT) - increased	1.1 – 1.25 x ULN	1.26 – 1.5 x ULN	1.51 – 1.75 x ULN	>1.75 x ULN
Bilirubin (with normal LFT) - increased	1.1 – 1.5 x ULN	1.6 – 2.0 x ULN	2.1 – 3.0 – x ULN	>3.0 x ULN
Cholesterol – increased mg/dL: mmol/L:	201 – 210 6.0 – 6.3	211 – 225 6.4 – 6.7	>226 >6.7	---- ----

Serum	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Pancreatic enzymes: amylase, lipase – increased	1.1 – 1.5 x ULN	1.6 – 2.0 x ULN	2.1 – 5.0 x ULN	>5.0 x ULN

ULN (upper limit of normal) dependent on normal reference ranges per institutional parameters.

Hematology	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Hemoglobin (Female) – g/dL: g/L:	11.0 – 12.0 110 – 120	9.5 – 10.9 95 – 109	8.0 – 9.4 80 – 94	<8.0 <80
Hemoglobin (Male) – g/dL: g/L:	12.5 – 13.5 125 – 135	10.5 – 12.4 105 – 124	8.5 – 10.4 85 – 104	<8.5 <85
WBC – increased cells/mm ³ : cells x 10 ⁹ /L:	10,800 – 15,000 10.8 – 15.0	15,001 – 20,000 15.1 – 20.0	20,001 – 25,000 20.1 – 25.0	>25,000 >25.0
WBC – decreased cells/mm ³ : cells x 10 ⁹ /L:	2,500 – 3,500 2.5 – 3.5	1,500 – 2,499 1.5 – 2.4	1,000 – 1,499 1.0 – 1.4	<1,000 <1.0
Lymphocytes – decreased cells/mm ³ : cells x 10 ⁹ /L:	750 – 1,000 0.8 – 1.0	500 – 749 0.5 – 0.7	250 – 499 0.3 – 0.4	<250 <0.3
Neutrophils – decreased cells/mm ³ : cells x 10 ⁹ /L:	1,500 – 2,000 1.5 – 2.0	1,000 – 1,499 1.0 – 1.4	500 – 999 0.5 – 0.9	<500 <0.5
Eosinophils – increased cells/mm ³ : cells x 10 ⁹ /L:	650 – 1,500 0.7 – 1.4	1,501 – 5,000 1.5 – 5.0	>5,000 >5.0	Hyper eosinophilic
Platelets – decreased cells/mm ³ : cells x 10 ⁹ /L:	125,000 – 140,000 125 – 140	100,000 – 124,000 100 – 124	25,000 – 99,000 25 – 99	<25,000 <25
Prothrombin time (PT) – increased	>1.0 – 1.10 x ULN**	1.11 – 1.20 x ULN	1.21 – 1.25 x ULN	>1.25 x ULN
Partial thromboplastin time (PTT) – increased	>1.0 – 1.2 x ULN	1.21 – 1.4 x ULN	1.41 – 1.5 x ULN	>1.5 x ULN
Fibrinogen – increased mg/dL: g/L:	400 – 500 4.00 – 5.00	501 – 600 5.01 – 6.00	>600 >6.00	---- ----
Fibrinogen – decreased mg/dL: g/L:	150 - 200 1.50 – 2.00	125 – 149 1.25 – 1.49	100 - 124 1.00 – 1.24	<1.0 or associated with gross bleeding or disseminated intravascular coagulation (DIC)

ULN (upper limit of normal) dependent on normal reference ranges per institutional parameters.

Urine	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Protein	Trace	1+	2+	Hospitalization or dialysis
Glucose	Trace	1+	2+	Hospitalization for hyperglycemia
Blood (microscopic) – red blood cells per high power field (rbc/hpf)	1+ or 1 – 10 rbc/hpf	2+ or 11 – 50 rbc/hpf	3+ or 4+ or >50 rbc/hpf and/or gross blood	Hospitalization or packed red blood cells (PRBC) transfusion
Hemosiderin	Not applicable	Positive	Not applicable	Not applicable

APPENDIX E BioVacSafe Project**BioVacSafe Consortium****Background**

Vaccines are widely acknowledged to be one of the most efficient and cost-effective ways to combat infectious diseases in both developed and developing countries. With billions of doses of vaccines administered globally every year, vaccine safety remains a top priority for pharmaceutical companies, regulators and the public. To develop cutting edge tools to accelerate and improve the testing and monitoring of vaccine safety before and after commercial release, a consortium, “Biomarkers for Enhanced Vaccines Immunosafety (BioVacSafe)” was established in March 2012 (www.biovacsafe.eu). The BioVacSafe Consortium consists of three of Europe’s leading vaccine producing companies (Novartis, GSK, and Sanofi Pasteur), selected major academic institutions, and small and medium-sized enterprises (SMEs) including Statens Serum Institut (SSI). SSI’s participation in the Consortium will focus on human clinical vaccine safety, animal models of vaccine safety, and transcriptomic and cytokine/chemokine profiling of blood samples provided from clinical trials.

Rationale for Including C-035-456 in the BioVacSafe Project

Some vaccines are thought to trigger innate inflammatory responses to induce antigen-specific adaptive immunity, but excessive inflammation may lead to serious inflammatory complications. A lack of reliable biomarkers predicting severe inflammation has halted development of several exploratory vaccines, and led to the withdrawal of some licensed vaccines, some of which were associated with inflammatory complications, albeit at low frequency. Specific objectives of the BioVacSafe project include the characterization of early inflammation induced by vaccines and the identification and validation of biomarkers of early inflammation and allergic responses. AERAS-456, which contains both novel antigens and a novel adjuvant, is early in clinical development. Study C-035-456 is the second study in humans, and the assessment of safety is the primary objective. By incorporating samples from the C-035-456 study into the BioVacSafe project, biomarkers correlated to standard clinical readouts and to inflammatory markers assessed in natural infections could be identified and potentially used in future studies to predict acceptable safety for this and other experimental vaccines.

Types of Assays and Analyses to be Performed

Whole blood samples harvested in a stabilizing reagent, such as PAXgene tubes for storage and transport, will be transferred to the Max Planck Institute for Infection Biology (MPIIB) in Berlin for RNA isolation and transcriptional profiling in the institute’s Microarray Core Facility. Transcriptome data will be analyzed using a combination of classical gene expression expanded with pathway and functional association analyses, including Gene Ontology (GO) analysis, KEGG pathway, and STRING functional network analysis. A new bioinformatics approach developed at MPIIB, using intra-individual expression correlations, will be incorporated within the limitations of sample numbers. Time course data points from the clinical trials and animal models will be analyzed for the same subject and cumulative integration of intra-host profiles. Transcriptome data have been successfully applied in previous studies at MPIIB in infectious disease and vaccine trial monitoring (Maertzdorf et al 2011; Weiner et al 2012)

Sample Identification and Handling

Because the planned analyses will compare baseline (day 0) with post-vaccination time points to assess changes in expression patterns, subject identifier codes (not names) and study visit dates will be linked with the samples. The confidentiality of records that could identify subjects will be protected at all times in accordance with the Declaration of Helsinki and the convention of the Council of Europe on Human Rights and Biomedicine, the ICH-GCP guidelines, and applicable national guidelines.

SSI will be responsible for the samples committed to the BioVacSafe project and will receive, log, monitor, and store samples according to SSI's established sample management system at their facilities in Copenhagen, Denmark. SSI will also be responsible for shipping samples to the testing and analysis site at MPIIB in Berlin, Germany.

Subjects must provide written informed consent in order for their blood samples to be sent to and tested by the BioVacSafe Consortium. Consent for BioVacSafe assays is not required for participation in study C-035-456.

References

Maertzdorf J, Ota M, Repsilber D, et al. Functional correlations of pathogenesis-driven gene expression signatures in tuberculosis. [PLoS One](#). 2011;6:e26938.

Weiner J 3rd, Parida SK, Maertzdorf J, et al. Biomarkers of inflammation, immunosuppression and stress with active disease are revealed by metabolomic profiling of tuberculosis patients. [PLoS One](#). 2012;7:e40221.

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