HIV VACCINE TRIALS NETWORK

AERAS

PROTOCOL

HVTN 602 / AERAS A-042

A randomized, placebo-controlled, partially blinded phase 1b clinical trial to evaluate the safety and immunogenicity of BCG revaccination, H4:IC31, and H56:IC31 in healthy, HIV-1–uninfected adolescent participants

DAIDS DOCUMENT ID 12012

CLINICAL TRIAL SPONSORED BY

Aeras
Rockville, MD, USA

STUDY PRODUCT(S) PROVIDED BY

Sanofi Pasteur
Swiftwater, PA, USA

Statens Serum Institut
Copenhagen, Denmark

September 10, 2015
Amendment 2
HVTN 602 / AERAS A-042, Version 3.0, COPYING ONLY

Contents

1 Ethical considerations 5
1.1 Ethics and Regulatory Considerations 5
1.2 Institutional Review Board or Independent Ethics Committee Review 6
1.3 Informed Consent and Assent Forms and Processes 7

2 Overview 9
2.1 Protocol Team 12

3 Background 13
3.1 Introduction 13
3.2 Study rationale 14
3.3 H4:IC31 15
3.4 H56:IC31 15
3.5 BCG 16
3.6 Trial design rationale 16
3.7 Plans for future product development and testing 20
3.8 Preclinical safety studies 20
3.9 Preclinical immunogenicity studies 22
3.10 Clinical studies 22

4 Objectives and endpoints 31
4.1 Primary objectives and endpoints 31
4.2 Secondary objectives and endpoints 31
4.3 Exploratory objectives 33

5 Statistical considerations 35
5.1 Accrual and sample size calculations 35
5.2 Randomization 37
5.3 Blinding 37
5.4 Statistical analysis 38

6 Selection and withdrawal of participants 44
6.1 Inclusion criteria 44
6.2 Exclusion criteria 46
6.3 Participant departure from vaccination schedule or withdrawal 49

7 Study product preparation and administration 52
7.1 Vaccine regimen 52
7.2 Investigational product formulation 52
7.3 Preparation of study products 53
7.4 Administration 54
7.5 Acquisition of study products 55
7.6 Supplies 56
7.7 Receipt and Storage 56
7.8 Pharmacy records 57
7.9 Accountability 57
7.10 Final disposition of study products 57

8 Clinical procedures 58
8.1 Informed assent for adolescents, and Informed consent for parents and participants who turn 18 on study 58
8.2 Pre-randomization procedures 59
8.3 Enrollment and vaccination visits 61
8.4 Participant Diary and Daily Temperature Monitoring 62

8.5 Participant Follow-up and Contact 62
8.6 Follow-up visits 63
8.7 Assessments of reactogenicity 65
8.8 Visit windows and missed visits 66
8.9 Early termination visit 66
8.10 Follow-up of participants who become pregnant 67
8.11 Tuberculosis (TB) case evaluation 67

9 Laboratory 69
9.1 CRS laboratory procedures 69
9.2 Total blood volume 69
9.3 Primary immunogenicity timepoints 69
9.4 Endpoint assays: adaptive cellular responses 70
9.5 Endpoint assays: adaptive humoral responses 71
9.6 ESAT-6 free IGRA 71
9.7 Innate immunity assays 71
9.8 Exploratory studies 72
9.9 Other use of stored specimens 73
9.10 Biohazard containment 74

10 Safety monitoring and safety review 75
10.1 Safety monitoring and oversight 75
10.2 Definition of Adverse Events 77
10.3 Assessing Severity 78
10.4 Assessing Causal Relationship (Relatedness) 79
10.5 Definition of Adverse Reaction 80
10.6 Assessing "seriousness" and serious adverse events 80
10.7 Assessing Expectedness of Adverse Events 81
10.8 Definition of Suspected Unexpected Serious Adverse Reaction (SUSAR) 81
10.9 Safety reporting 81
10.10 Submission of safety forms to SDMC 84
10.11 Pausing and stopping rules 86

11 Protocol conduct 88
11.1 Social impacts 88
11.2 Emergency communication with study participants 88

12 Version history 89

13 Document references (other than literature citations) 91

14 Acronyms and abbreviations 93

15 Literature cited 95

Appendix A Sample informed consent form 100

Appendix B Approved birth control methods (for sample informed consent form) .. 115

Appendix C Sample consent form for use of samples and information in other studies 116

Appendix D Table of procedures (for sample informed consent and assent forms) ... 120

Appendix E Sample informed assent form 122

Appendix F Approved birth control methods (for sample informed assent form) 133

Appendix G Sample informed consent form for participants who turn 18 on study .. 134

Appendix H Sample assent form for use of samples and information in other studies 147

Appendix I Sample informed consent form for use of samples and information in other studies for participants who turn 18 on study 150

Appendix J Adverse Events of Special Interest 154

Appendix K Toxicity Table 156

Appendix L Laboratory procedures 162

Appendix M Procedures at CRS 165

1 Ethical considerations

1.1 Ethics and Regulatory Considerations

Multiple candidate TB vaccines will need to be studied simultaneously in different populations around the world before a successful TB preventive vaccine is found. It is critical that universally accepted ethical guidelines are followed at all sites involved in the conduct of these clinical trials. The HIV Vaccine Trials Network (HVTN) and Aeras, the study sponsor, have addressed ethical concerns in the following ways:

- This trial is designed and conducted to enhance the knowledge base necessary to find a preventive vaccine, using methods that are scientifically rigorous and valid, and in accordance with Good Clinical Practice (GCP) guidelines.

- This clinical trial will be conducted according to the ethical principles set forth in the Declaration of Helsinki, ICH-GCP, Protection of Human Subjects (45CFR 46 , South African Good Clinical Practice Guidelines, and other local regulatory requirements.

- Trials are reviewed by local and national regulatory bodies, the custodians responsible for ensuring the ethical conduct of research in each setting.

- The research is designed to minimize risk and maximize benefit to both study participants and their local communities. Protocols also include careful medical review of each research participant's health conditions and reactions to study products while in the study.

- The research will investigate preventive TB vaccines, so it will align with the local health needs of the communities where TB is common and will strengthen the capacity of the communities through training, support, shared knowledge, and equipment.

- Clinical trial scientists and operational staff are committed to substantive community input—into the planning, conduct, and follow-up of its research—to help ensure that locally appropriate cultural and linguistic needs of study populations are met. Community Advisory Boards (CAB) are required by DAIDS and supported at all HVTN research sites to ensure community input in accordance with Good Participatory Practices (GPP) and all local and national guidelines. For this study, input was received from the youth CAB at the Cape Town HVTN Clinical Research Site (CRS).

- Prior to implementation, trials are rigorously reviewed by scientists who are not involved in the conduct of the trials under consideration.

- Clinical trial staff are trained so that all participating sites similarly ensure fair participant selection, protect the privacy of research participants, and obtain meaningful informed consent. During the study, participants will have their wellbeing monitored, and to the fullest extent possible, their privacy protected. Participants may withdraw from the study at any time.
- A guidance document describing ethical and legal norms in adolescent HIV prevention research, developed by the Desmond Tutu HIV Foundation and the

European and Developing Countries Clinical Trials Partnership, was referenced in the development of the confidentiality language in the sample informed consent forms.

- Clinical trial staff counsel study participants routinely on how to reduce HIV risk. Participants who become HIV-infected during the trial are provided counseling on notifying their partners and about HIV infection according to local guidelines. Staff members will also counsel them about reducing their risk of transmitting HIV to others.

- Clinical trial staff counsel study participants on recognizing the symptoms of TB and assist them in obtaining medical care for it if necessary. Staff will also counsel participants that they may have a QFT-GIT conversion due to one of the vaccines but this may not mean they have a TB infection.

- Study participants will be compensated for their inconvenience and additional costs due to attendance at study visits.

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

- In this study, only some participants will be blinded to which study intervention they receive. After the study has been unblinded, the study participants will be informed which study intervention they received.

1.2 Institutional Review Board or Independent Ethics Committee Review

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 and assent forms 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 participants or when the modification involves only logistical or administrative aspects of the study. Such logistical or administrative modifications will be submitted to the IRB/IEC in writing by the investigator, and a copy of the correspondence to verify the submission will be maintained.

The investigator must inform the IRB/IEC of modifications to the informed assent and consent forms or any other documents previously submitted for review/approval, of any new information that may adversely affect the safety of the participants or the conduct of

the study, provide an annual update and/or request for re-approval, and advise the IRB/IEC when the study has been completed.

Any documents or forms to be provided to the participant or parent/legal guardian (eg, 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 participant or parent/legal guardian.

1.3 Informed Consent and Assent Forms and Processes

This protocol contains sample informed consent and assent forms for study participation and the storage of specimens for other uses in the Appendices. The Clinical Research Site (CRS) is responsible for developing site-specific consent/assent forms for local use, based on the sample consent forms. The sample informed consent/assent forms include instructions throughout for developing specific content.

The site-specific consent form(s) must be developed in accordance with requirements of the following:

- CRS's IRB/EC,
- CRS's institution and any applicable regulatory entities, and
- Elements of informed consent as described in Title 45, Code of Federal Regulations (CFR) Part 46, the International Conference on Harmonisation (ICH) E6, Good Clinical Practice (GCP): Consolidated Guidance 4.8, the current edition of the Declaration of Helsinki and South African GCPs.

Study sites are strongly encouraged to have their local Community Advisory Boards review their site-specific consent/assent forms. This review should include, but should not be limited to, issues of cultural competence, local language considerations, and the level of understandability.

Informed consent by the parent/legal guardian will be documented in writing on a consent form approved by the IRB/IEC. Similarly, participant informed assent will be documented in writing on an assent form approved by the IRB/IEC.

The informed consent and assent processes will be conducted in a private space at the study clinic or at home to maintain confidentiality. Consent and assent processes will be conducted in the participant's language of choice. All relevant information will be provided in both oral and written form in a way that is understandable to the participant and parent/legal guardian. Ample time and opportunity will be given for the participant and parent/legal guardian to inquire about details of the study. The investigator or the investigator's qualified designee will explain the nature of the study and inform the participant and parent/legal guardian that participation is voluntary and that the participant can leave the study at any time, without penalty or loss of benefits to which they are otherwise entitled. The participant and parent/legal guardian will be informed about the study's purpose including why the participant was selected to participate, study goals, expected benefits and risks, potential risks, and that some potential risks are

unforeseeable. The participant and parent/legal guardian will 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 participant and parent/legal guardian will 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. Participant and parent/legal guardian will be told who to contact for answers to any questions related to the study. The participant and parent/legal guardian will be told the extent of the confidentiality of participant records. For confidential matters that will not be shared with the parent/legal guardian, minor-participants will be encouraged to consult with a trusted adult (e.g., teacher, minister, relative).

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

2 Overview

Title

A randomized, placebo-controlled, partially blinded phase 1b clinical trial to evaluate the safety and immunogenicity of BCG revaccination, H4:IC31, and H56:IC31 in healthy, HIV-1–uninfected adolescent participants

Primary objectives

Primary objective 1:

To evaluate the safety and tolerability of the different vaccine regimens in adolescents.

Primary objective 2:

To evaluate the cellular immune responses of the different vaccine regimens in adolescents compared to those measured at baseline.

Study products and routes of administration

- **H4:IC31** is the H4 antigen (Ag85B + TB10.4) and the IC31® adjuvant, administered intramuscularly (IM).
- **H56:IC31** is the H56 antigen (Ag85B+ESAT-6+Rv2660C) and the IC31® adjuvant, administered IM.
- **BCG** is an attenuated, live culture of the Bacillus Calmette-Guérin, BCG Vaccine SSI, administered intradermally (ID).
- **Control:** is Sodium Chloride, 0.9% for injection, administered IM.

Table 2-1 Schema

				Injection schedule in months (days)	
Group	N	Dose	Volume	Day 0	Day 56
1	24	15 mcg H4/500 nmol IC31®	0.5 ml	H4:IC31	H4:IC31
2	24	5 mcg H56/500 nmol IC31®	0.5 ml	H56:IC31	H56:IC31
3	24	2-8 x 10^5 CFU	0.1 ml	BCG	-
4	12		0.5 ml	Control	Control
Total	84				

Participants

84 QFT-GIT negative, BCG vaccinated, healthy, HIV-1–uninfected volunteers aged 12 to 17 years; 72 vaccinees, 12 control recipients

Design

Single site, randomized, controlled, partially blinded trial

Duration per participant

8 months of scheduled clinic visits.

Estimated total study duration

Approximately 12 months (includes enrollment, and follow-up)

Study product providers

- H4: Sanofi Pasteur, Ltd, (Toronto, ON, Canada)
- IC31: Statens Serum Institut (Copenhagen, Denmark)
- H56:IC31: Statens Serum Institut (Copenhagen, Denmark)
- BCG: provided by the CRS and manufactured by Statens Serum Institut (Copenhagen, Denmark)

- Placebo control, Sodium Chloride, 09% for injection: Aeras (Rockville, MD, USA)

Core operations

HVTN Vaccine Leadership Group/Core Operations Center, Fred Hutchinson Cancer Research Center (FHCRC) (Seattle, Washington, USA)

Statistical and data management center (SDMC)

Aeras (Rockville, Maryland, USA)

Immunogenicity and statistical analysis center

Statistical Center for HIV/AIDS Research and Prevention (SCHARP), FHCRC (Seattle, Washington, USA)

Endpoint assay laboratories

- FHCRC/University of Washington (Seattle, Washington, USA)
- Cape Town HVTN Immunology Laboratory (Cape Town, South Africa)
- Aeras (Rockville, Maryland, USA)
- Duke Human Vaccine Institute, Duke University Medical Center (Durham, North Carolina, USA)

Study sites

HVTN Clinical Research Site in Cape Town, South Africa

Safety monitoring

HVTN 602 / AERAS A-042 Protocol Team; Aeras Safety Monitoring Committee (SMC)

2.1 Protocol Team

Protocol leadership

Chair	Linda-Gail Bekker Desmond Tutu HIV Center	*Medical officer*	Julia Hutter DAIDS, NIAID
Co-chair	Jim Kublin HVTN Core, FHCRC	*Sponsor representative*	Zhongkai Shi Aeras
Clinical research scientist and protocol development coordinator	Tracey Day HVTN Core, FHCRC	*HVTN Lab Lead*	Stephen DeRosa HVTN Laboratory Program, FHCRC
Statistician	Andrew Gartland SCHARP, FHCRC	*Co-chair*	Keren Middelkoop Desmond Tutu HIV Center

Other contributors to the original protocol

Vaccine developer representative	Robert Ryall Sanofi Pasteur	*Medical Monitor*	Dereck Tait Aeras
Vaccine developer representative	Carlos DiazGranados Sanofi Pasteur	*Clinical safety specialist*	Patricia Lese Aeras
Vaccine developer representative	Ingrid Kromann Statens Serum Institut	*Clinical trials manager*	Hanlie Bester Aeras
Vaccine developer representative	Morten Ruhwald Statens Serum Institut	*Aeras Immunology representative*	David Hokey Aeras
Laboratory Program representatives	John Hural HVTN Laboratory Program, FHCRC	*Aeras Regulatory affairs*	Marci Aderiye Aeras
	Nicole Frahm HVTN Laboratory Program, FHCRC		
	On Ho HVTN Laboratory Program, FHCRC	*Clinic coordinator*	Avril Masters Emavundleni HIV Research Center
HVTN Regulatory affairs	Liz Briesemeister HVTN Core, FHCRC	*Community Advisory Board (CAB) member*	Sinazo Peter Emavundleni HIV Research Center
Community engagement unit representative	Genevieve Meyer HVTN Core, FHCRC	*Community educator/recruiter*	Noxolo Mona Emavundleni HIV Research Center
Technical editor	Erik Schwab HVTN Core, FHCRC		

3 Background

3.1 Introduction

There were an estimated 8.6 million new cases of tuberculosis (TB) worldwide in 2012, (13% co-infected with HIV) and 1.3 million people died from TB, including 320,000 deaths among HIV-negative individuals [1]. Although new cases of TB have been falling worldwide for several years and the TB mortality rate has decreased 45% since 1990, Africa is not on track to reach TB mortality targets by 2015. South Africa ranks 3rd among the top 20 high TB burden countries worldwide for absolute incident cases, with 530,000 new TB cases each year, and second for TB rate after Swaziland [1]. Improved control of TB among young adults would have significant impact on the South African TB epidemic [2-4].

The currently available TB vaccine (bacillus Calmette-Guérin, or BCG) is relatively safe and inexpensive to produce, and is considered efficacious in protecting against tuberculous meningitis and miliary TB when given to infants. However, its efficacy in the prevention of pulmonary tuberculosis is highly variable [5-7], ranging from 80 percent in some studies to little or no efficacy in other studies. Although effective chemotherapy for drug-sensitive TB is available, strains of *Mycobacterium tuberculosis* (Mtb) resistant to antibiotics are rapidly emerging worldwide. Thus, the need for a more effective vaccine against TB has become increasingly evident.

There are a number of TB vaccines currently being studied in clinical trials. Some of these are in the late stages of clinical development, based on having demonstrated in smaller clinical trials that they elicit immune responses thought to be protective and have an acceptable safety profile. Since protective immune responses against TB are still not well understood, clinical development of a TB vaccine is still a costly and resource-intensive undertaking that can often lead to disappointing results [8]. Clinical development of TB vaccines is hampered by the lack of biologic correlates of protection or validated preclinical models, which provide evidence of likely efficacy for advancement into large scale trials.

The lack of correlates of protection (immune responses correlating with vaccine-induced protection) is a major impediment for the rational and expeditious development of TB vaccines. Pilot studies measuring a wide range of immune responses conducted in parallel with efficacy trials could provide a means to optimize and prioritize assays and immune response variables to prepare for correlates analysis in the parent efficacy trial [9].

The aims of the phase 1b trial described here are to facilitate identification of assays and immune responses that could then be evaluated as correlates of risk and correlates of protection in efficacy studies and ultimately to provide leads for biomarkers of protection against tuberculosis. This trial will complement 2 studies evaluating the prevention of *M. tuberculosis* infection using novel TB vaccines, H4:IC31 and H56:IC31 (also known previously as AERAS-404 and AERAS-456, respectively).

The H4:IC31 vaccine is being evaluated in the Aeras-sponsored study C-040-404, a randomized, placebo controlled, partially blinded phase 2 trial currently enrolling at the South African Tuberculosis Vaccine Initiative (SATVI) research site. The trial will

evaluate safety, immunogenicity, and prevention of Mtb infection (as determined by QuantiFERON®-TB Gold in-tube [QFT-GIT] conversion) of BCG revaccination and H4:IC31 in previously BCG vaccinated adolescents. QFT-GIT negative adolescents in the area where this study is being conducted have previously been shown to have very high rates of tuberculin skin test (TST) and QFT-GIT conversion, associated with increased risk of active TB disease in subsequent years [10,11].

Another phase 2 trial evaluating the H56:IC31 candidate TB vaccine for prevention of Mtb-infection is currently being developed by Aeras and Statens Serum Institut (SSI) [12,13]. The proposed study will also be a randomized, placebo controlled, partially blinded study in an adolescent cohort at relatively high risk for infection. Because the H56:IC31 vaccine contains ESAT-6, an antigen that is also present in the QFT-GIT assay, an alternative interferon-γ release assay (IGRA) will be included to assess prevention of infection (IGRA conversion). SSI has developed an ESAT-6 free diagnostic test that performs comparably to QFT-GIT. One of the additional aims of this phase 1b trial is to collect data on the performance of this ESAT-6 free IGRA. Further validation will likely be conducted in conjunction with the ongoing AERAS-C-040-404 trial.

3.2 Study rationale

This study proposes to further evaluate the safety and immunogenicity of the vaccines included in the 2 prevention of infection studies described above: H4:IC31, H56:IC31, and BCG revaccination. The study will be conducted in previously BCG vaccinated healthy adolescents, and will entail a thorough immunogenicity evaluation of these regimens incorporating unbiased systems vaccinology approaches and novel assessments of baseline and elicited responses that may impact vaccine responses. A major goal for this study is to generate immunological data on a wide range of immune responses using a variety of approaches including validated assessments, unbiased strategies, and novel exploratory assays to increase the likelihood of detecting responses correlating with risk or protection in the prevention of infection phase 2 studies. Investigators contributing to the proposed study have participated in a correlates analysis for an HIV vaccine exhibiting modest efficacy in which 2 correlates of risk were identified [9].

An additional aim of this study is to explore factors affecting vaccine induced responses that may also impact efficacy. For example, it is hypothesized that exposure to environmental mycobacteria may alter protection provided by BCG vaccination [14]. Reagents for evaluating levels of exposure to environmental mycobacteria are in development as part of a concurrent collaborative study. An exploratory objective for this trial is to apply these reagents to examine whether such exposures influence immune responses elicited by these regimens.

An exploratory objective will assess the diversity of the gut microbiome and investigate correlations of this diversity with vaccine induced immune responses. Variation in the microbial community of the human gut influences maturation of the immune system [15]. Probiotic bacteria have recently been used to modulate vaccine responses [16] and diversity of the gut microbiome appears to correlate with vaccine induced responses [17]. To understand whether differences in the human microbiome are correlated with immune responses, sampling of the stool in this study will coincide with first vaccination and peak immune response for participants opting to provide stool samples. Samples will be targeted for analysis of 16S rRNA sequences and compared to libraries of Bacterial and Archaea species.

3.3 H4:IC31

3.3.1 Description of H4:IC31 Vaccine

H4:IC31 (designated as AERAS-404 for Aeras-sponsored clinical development) has 2 components: the H4 antigen and the IC31® adjuvant, and is being jointly developed by Aeras and Sanofi Pasteur. The current formulation of H4:IC31 is a field-reconstituted vaccine with H4 antigen and IC31 adjuvant supplied in different vials. The components are dissolved in a sterile aqueous buffer containing tris-hydroxymethylaminomethane (Tris) and sodium chloride (NaCl).

3.3.2 H4 antigen

The H4 antigen is a fusion protein created from 2 Mtb antigens: antigen 85B (Ag85B) and TB10.4. Ag85B is also referred to as α-antigen and is a 30-kDa mycolyl transferase protein [18,19]. TB10.4 is 1 of 3 members of the very similar ESAT-6 group of proteins found in Mtb culture supernatants. TB10.4 induces broad immune responses (more epitopes recognized) in T cells isolated from TB participants compared to BCG-vaccinated donors and unvaccinated donors [20,21].

3.3.3 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 [22]. 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.

3.4 H56:IC31

3.4.1 Description of H56:IC31 vaccine

H56:IC31 (previously designated as AERAS-456 for Aeras-sponsored clinical development) has 2 components: the H56 antigen and the IC31® adjuvant, and is being jointly developed by Aeras and SSI. The vaccine is formulated in a sterile aqueous buffer containing glycine, Tris, and sodium chloride (NaCl).

3.4.2 H56 antigen

H56 is a fusion protein of the Ag85B, ESAT-6, RV2660 antigens. Ag85B was previously described above. 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 [23,24], cattle infected with M. bovis [25], and different strains of TB-infected mice [26]. 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 [27]. The late stage antigen Rv2660c is expressed at a higher level as Mtb adapts to persistence. *In vitro*, expression of Rv2660c has been found to be between 80 and 300 fold increased in nutrient starved cultures [28-30].

Rv2660c is selectively recognized by latently infected individuals as compared to individuals with active pulmonary TB [31].

Because the H56:IC31 vaccine contains ESAT-6, an antigen that is also present in the QFT-GIT assay, an alternative IGRA will be included to assess prevention of infection (IGRA conversion).

3.4.3 IC31 adjuvant

The adjuvant for the H56 vaccine, IC31, the same adjuvant used in H4:IC31 and is described above.

3.5 BCG

BCG Vaccine SSI is manufactured by SSI, Copenhagen, Denmark and imported and distributed in South Africa for the national immunization program by Biovac, Johannesburg, South Africa. BCG Vaccine SSI is registered in South Africa for prevention of TB in children and adults. BCG, an attenuated, live culture of the Bacillus Calmette-Guérin, was originally attenuated between 1906 and 1919 by serial passage of an M. bovis strain. SSI derives this vaccine from the Danish BCG strain 1331. SSI BCG is supplied by the manufacturer in amber 10-dose vials containing 0.75 mg lyophilized SSI BCG.

3.6 Trial design rationale

This clinical trial will evaluate the safety and immunogenicity of BCG revaccination, H4:IC31, and H56:IC31 in healthy, HIV-uninfected, QFT-GIT negative, adolescents who were BCG-vaccinated when they were infants in South Africa. The study arms reflect treatment arms of other ongoing or planned studies aimed at evaluating the efficacy of the vaccine regimens in the prevention of Mtb infection: a randomized, placebo-controlled, partially blinded phase 2 study evaluating safety, immunogenicity, and prevention of infection with Mtb of H4:IC31 and BCG revaccination in healthy adolescents is currently ongoing in South Africa (ClinicalTirals.gov NCT02075203, MCC trials reference number 20130828) (see Section 3.10.1); a similar prevention of infection trial with H56:IC31is currently in planning. In addition to safety evaluation and immunogenicity characterization, the proposed study will incorporate unbiased systems vaccinology approaches and novel assessment of baseline and postvaccination responses to generate immunological assay data to inform and correlate analyses of risk and potentially correlate of protection evaluations in the prevention of infection trials.

The randomized design aims for balanced and unbiased distribution of baseline characteristics across study arms. To maintain balanced distribution of postrandomization variables and unbiased assessments of study outcomes, the study is double-blinded for the experimental vaccines and placebo groups. Given that BCG is administered by a different route and is associated with well-characterized vaccination site reactions, the BCG arm will remain unblinded. A placebo group is required for optimal assessment of safety profiles, as well as for the assessment of longitudinal changes in immunogenicity parameters in the absence of active tuberculosis vaccination.

3.6.1 Dose (amount and number)

Dosing is informed by previous immunogenicity studies, presented in more detail in the Investigator's Brochures (IBs). In addition, the dosing used in this study reflects the dosing strategy used in the prevention of infection studies mentioned above. CD4 responses have consistently been induced by vaccination with H4:IC31. Overall, responses were higher after vaccinations of the 15 mcg /500 nmol dose compared to the higher and lower doses, and did not significantly increase with a third vaccination.

Study C-005-404 evaluated doses of H4 antigen (50 and 150 mcg) and doses of IC31 adjuvant (100 and 500 nmol) given as one vaccine injection or two vaccine injections separated by 56 days in BCG-vaccinated adults. The strongest responses were seen with the lower dose of H4 (50 mcg) and higher dose of IC131 (500 nmol) combination, given as a dose regimen.

Study C-006-404 evaluated 4 doses of H4 (5, 15, 50 and 150 mcg) and doses of IC131 adjuvant (100 and 500 nmol) given as two injections separated by 56 days in BCG-vaccinated adults. Responses were strongest among participants receiving IC31 at 500 nmol, with comparable responses between groups receiving 5, 15, and 50 mcg of H4.

Study C-011-404 evaluated 4 doses of H4 (5, 15, 50 and 150 mcg) combined with 500 nmol of IC131 adjuvant given as two injections separated by 56 days in BCG-vaccinated adults. Responses were highest in the group receiving 15 mcg of H4. No new T-cell responses were noted among participants receiving the highest level of antigen, 150 mcg H4. Mtb-specific CD4+ T-cell responses did not significantly change in the 5 or 50 mcg of H4:IC31 groups following the second dose of study vaccine, whereas a significant increase of Mtb-specific CD4+ T-cell responses was observed in the 15 mcg group. Responses were more polyfunctional in the 5 and 15 mcg than in the 50 and 150 mcg H4:IC31 groups.

The mentioned studies support the use of the H4 and IC31 combination dosed as 15 mcg and 500 nmol, respectively, both in the prevention of infection study (Aeras Study C-040-404) and the present study.

Study C-032-456 evaluated 50 mcg of H56 combined with 500 nmol of IC31 in QFT-GIT-negative healthy adults; this regimen was shown to elicit Ag85B-specific CD T cell responses which peaked after the second vaccination and did not increase after the 3rd dose. SSI Study THYB-04 performed with the H1 vaccine (considered very similar to H56 vaccine; see Figure 3-1, adapted from [13]) evaluated doses of H1 (15 mcg and 50 mcg) combined with 500 nmol of IC31. In this study, both arms induced similar level of ELISpot responses in QFT-GIT-negative adolescents which peaked after the second vaccination. Study C-035-456, an ongoing study at SATVI includes a dose-finding phase to evaluate 3 doses of H56 (5, 15, and 50 mcg) combined with 500 nmol of IC31 adjuvant given as two injections separated by 56 days in BCG-vaccinated QFT-GIT (-) healthy adults. Unblinded safety data through Study Day 84 and immunogenicity data through Study Day 70 have been reviewed by designated unblinded reviewers and the Data Monitoring Committee. Based on a pre-defined dose-selection algorithm, 5 mcg H56/500 nmol IC31 was recommended by the Data Monitoring Committee for the remaining C-035-456 study groups and will be the most appropriate dose to move forward both in the planned prevention of infection study and the present study.

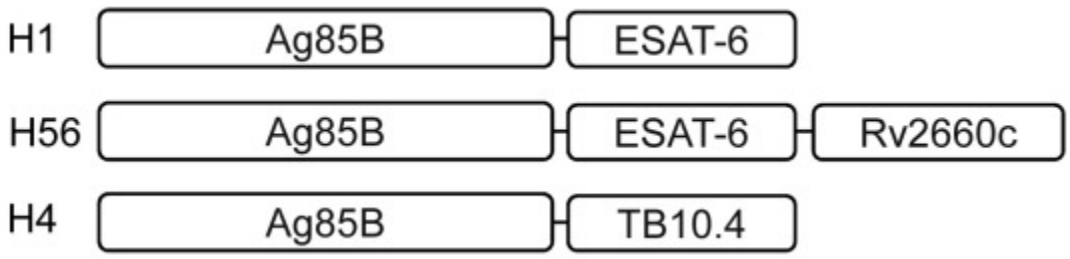


Figure 3-1 Schematic of antigens in H1, H56, and H4

3.6.2 Schedule

The schedule of vaccination proposed in this study is informed by previous immunogenicity studies and is reflective of the schedule used in the prevention of infection studies.

As described above, study C-005-404 evaluated doses of H4 antigen (50 and 150 mcg) and doses of IC31 adjuvant (100 and 500 nmol) given as one vaccine injection or two vaccine injections separated by 56 days in adults. The strongest responses were seen with the 2 doses of 50/500.

Study C-013-404 evaluated one dose of H4 (50 mcg) and one dose of IC31 adjuvant (500 nmol) given as 2 (Days 56 and 231) or 3 injections (Days 0, 56, 231) after recent BCG priming. Participants receiving 3 injections had a magnitude of Ag85B-specific CD4 T cell responses on Study Day 259 (28 days after the third dose) comparable to that seen on Study Day 84 (28 days after the second dose). The 3-injection regimen was seen to only slightly outperform the 2-injection regimen as measured by the magnitude of Ag85B-specific CD4+ T-cell responses.

Based on cumulative human data, two injections of the H4 and IC31 combination separated by 56 days was selected as the schedule for the ongoing prevention of infection study (Aeras Study C-040-404) and is therefore the schedule used in this study as well.

As mentioned above (Section 3.6.1), study C-032-456 evaluated 2-injection and 3-injection schedules with H56 adjuvanted with IC31 in adults. The study did not reveal any advantages from the third injection. A 2-injection schedule is also supported by SSI Study THYB-04, performed with H1 (H56-like vaccine) and IC31, which did not reveal added value of a third injection. Taken together, available data indicate moving forward with a two-injection schedule of H56 and IC31 separated by 56 days, both in the planned prevention of infection study and the present study.

The BCG revaccination arm will receive a single injection of BCG at Study Day 0.

3.6.3 Prime-boost regimen

Antigen 85B in both H4:IC31 and H56:IC31 and TB10.4 in H4:IC31 are present in BCG. All participants in the proposed study have been primed with BCG vaccination at least 5 years before enrollment. Therefore vaccination with H4:IC31 and H56:IC31 and revaccination with BCG are all considered distant booster immunizations for BCG vaccination.

3.6.4 Choice of control

Use of the placebo control and partially blind, randomized study design will allow for control of potential biases in the conduct of the study. The formulation buffer for both H4:IC31 and H56:IC31 has been used as placebo control for all completed human studies. Normal saline placebo is being used as the control in the ongoing H4:IC31 prevention of infection study (Aeras protocol C-040-404) and is proposed as the control for the planned H56:IC31 prevention of infection study. Normal saline will be used as the control in the present study.

3.6.5 Rationale for blood volumes and sample collection timepoints

This study is designed to examine innate and adaptive immune responses to vaccination at multiple timepoints. Data on samples collected at baseline will be important indicators of immune status, as well as for evaluating all assays measured at later timepoints; the blood volume required at the baseline visit reflects this requirement. Samples will be collected at 3 early (innate immune response) timepoints (D1, 3, 7) to characterize the kinetics of the initial response to the vaccines and the adjuvant. The timepoints will also ensure that measurements are made at a time that is near the peak innate response. The adaptive immune response timepoints (>=D14) are designed to detect the peak systemic and adaptive response for H4:IC31 (D14), H56:IC31 (D14) and BCG (D28), as well as to characterize the durability of the response (D63, D70, D168 for H4:IC31 and H56:IC31; D70, 168 for BCG). The blood volumes that will be collected at each timepoint reflect the minimum samples necessary for the endpoint assays. In accordance with recommended guidelines, the amount of blood collected from each participant will not exceed 4 mL/kg over any 8-week period [32]. To ensure that we do not exceed this recommendation, but still meet the assay blood volume requirements, we will not enroll any participant weighing less than 40 kg.

3.6.6 Relationship to the parent trials

As described in Section 3.1, a major aim for this study is to support two phase 2 trials evaluating the same study regimens for prevention of infection as assessed by IGRA conversion.

3.6.6.1 The rationale supporting HVTN 602 as a free-standing study

HVTN 602 is being developed as a free standing trial, as opposed to a sub-study for several reasons. Foremost is that, as a free standing trial, we can best achieve our major aim to identify and develop the most meaningful assays for the exploration of biomarkers as correlates of risk or protection while sparing the use of the precious specimens collected in the associated prevention of infection trials. Our goal is to use samples from HVTN 602 to develop and prioritize assays so that the specimens from the parent trials may be utilized most effectively to explore immune correlates, as described in Section 3.2.

There is a historic precedent for this approach from the HIV vaccine field. Following the results of the RV144 trial, which demonstrated modest efficacy of a vaccine regimen in the prevention of HIV infection [33], a large collaboration was established to investigate correlates of immunity for the HIV vaccine regimen. The actual assessment of correlates of immunity was preceded by intense pilot studies aimed at identifying the most

meaningful assays and biomarkers, which were subsequently tested as correlates of risk in the RV144 efficacy study [9].

HVTN 602 is analogous to the RV144 pilot studies, which provided key data to enable prioritization for assays to be included in the actual correlates analysis. These data were instrumental in the identification of correlates of risk that are considered a major advance for the HIV vaccine field [9]. Additional data supporting these immune responses as correlates of risk have been identified via alternative methods and continue to inform the HIV vaccine field.

Other advantages for HVTN 602 as a free standing trial include the ability to generate a more thorough characterization of vaccine-elicited immune responses for the study regimens. This free-standing design enables a large scope of assessments to be conducted without the risk of burdening the parent trials with complexities associated with increased enrollment needs, larger blood draw volumes, and additional study visits not included in the parent trials. In addition, the free-standing design provides the flexibility to include H56:IC31, and thereby support 2 prevention of infection trials and also provide data for the AEREAS C-040-404 trial in a timely manner.

3.6.6.2 Baseline characteristics as compared to parent trials

As HVTN 602 will be conducted in Cape Town, South Africa and the parent trials in and around this region, the baseline characteristics of HVTN 602 participants and those in the parent trials are expected to be very similar. Inclusion/exclusion criteria have been aligned to ensure that participants are as similar as possible in this trial and the parent trials.

3.7 Plans for future product development and testing

The ongoing H4:IC31 prevention of infection study and the planned H56:IC31 prevention of infection study, both in adolescents, will potentially provide proof of concept by demonstration of meaningful biological activity that justifies further development of both vaccines. If revaccination with BCG or vaccination with H4:IC31or H56:IC31 show sufficient efficacy in prevention of infection studies, additional larger scale studies examining the impact of the vaccine candidates on TB disease would be warranted.

3.8 Preclinical safety studies

Multiple toxicology studies of IC31 adjuvant alone, H4:IC31 or H56:IC31 have been evaluated in a variety of animal models (Table 3-1) showing an acceptable toxicity profile

Table 3-1 Summary of preclinical safety studies

Study Type	Vaccine/Adjuvant Administered	Dose	Species	Route of Administration	Study Number
Acute toxicity	IC31	3335nmol/kg body weight (bw)	CD1 mice	SC	17386/03 (LPT Hamburg)
Acute toxicity	IC31	3335nmol/kg bw	CD1 mice	IM	17387/03 (LPT Hamburg)
Repeat dose	IC31	up to 750nmol/kg bw	CD1 mice	SC	17388/03 (LPT Hamburg)
Repeat dose	IC31	up to 750nmol/kg bw	CD1 mice	IM	17389/03 (LPT Hamburg)
Local tolerance	IC31	500nmol	Himalayan rabbits	SC	17391/03 (LPT Hamburg)
Local tolerance	IC31	500nmol	Himalayan rabbits	IM	17392/03 (LPT Hamburg)
Repeat dose	H4:IC31	H4:150mcg IC31:500nmol	NZW rabbits	IM	63014 (LAB Scantox)
Repeat dose	H56:IC31	H56:100mcg IC31:500nmol	NZW rabbits	IM	71526 (LAB Scantox)

3.8.1 Toxicology of H4:IC31 in Study 63014

The repeated dose toxicity study (Study 63014) was performed to assess the acute and chronic toxicity of H4:IC31 administered to rabbits with and without a BCG priming vaccination approximately 70 days before exposure to the first dose of H4:IC31. Three intramuscular doses of 150/500 H4:IC31 were administered on Days 1, 15 and 29. Standard Good Laboratory Practice (GLP) measurements and observations included the following: mortality, clinical observations, food consumption, body temperature, ophthalmology, local tolerance (Days 1, 15 and 29), clinical pathology (after each dosing), necropsy and microscopic examination (3-6 and 13-14 days post final dose), and serology (Pre BCG and 1-3 days post each dose and prior to necropsy).Three intramuscular (IM) injections of H4:IC31 resulted in local reaction and minor fluctuations in white blood cell populations consistent with injection of a vaccine in both BCG pre-vaccinated and BCG non-vaccinated animals. Administration of H4:IC31 was not associated with overt signs of toxicity and did not demonstrate mortality, adverse clinical signs, effects on body weight or food consumption, body temperature, ophthalmology, or clinical pathology.

Additional details of toxicology studies in mouse and rabbits are available in the product Investigator's Brochure (IB).

3.8.2 Toxicology of H56:IC31 in Study 71526

The repeated dose toxicity study (Study 71526) was to assess the acute and chronic toxicity of H56:IC31 administered to rabbits without BCG priming. Four intramuscular does of 100 /500 H56:IC31 were administered on Days 1, 15, 29 and 43. Similar measurements and observations to H4:IC31 toxicology study (63014) were also

performed. Necropsy and microscopic examinations were performed 3 and 17 days post final dose. Four injections of H56:IC31 did not demonstrate mortality, adverse clinical signs, effects on body weight or food consumption, body temperature, ophthalmology, or clinical pathology. An increase in the absolute and relative number of neutrophils was observed on Day 56.

3.9 Preclinical immunogenicity studies

The H4 antigen used in H4:IC31 has been evaluated in mice at 0.5, 5.0 and 15 mcg and in guinea pigs at 20 mcg in combination with IC31 adjuvant. H4:IC31 is immunogenic in mice and induced a significant additive protective efficacy against subsequent aerosol challenge with Mtb compared to BCG alone [34,35]. Administration of 20 mcg of H4 antigen after BCG prime has been shown to protect guinea pigs from aerosol challenge with Mtb compared to BCG alone [36].

Additional details regarding the nonclinical experience with H4:IC31 can be found in the current version of the IB.

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 [37]. The H56 antigen also protected against TB when given to mice post exposure to Mtb [37]. H65:IC31 as a boost to BCG vaccination delayed and reduced clinical disease in cynomolgus macaques challenged with Mtb [38]. No significant adverse reactions of an immunological nature were seen in mice 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. For further details of nonclinical experience with H56, refer to the investigator's brochure

3.10 Clinical studies

3.10.1 Clinical studies of H4:IC31

H4:IC31 has been studied in 198 adults who were enrolled in 4 phase 1 studies as shown in Table 3-2. Participants received 2 or 3 doses of the vaccine or placebo at the doses indicated. Three studies were conducted in European adults (a TB non-endemic population) and one was conducted in a TB endemic population (South Africa). Participants were previously BCG-vaccinated, either in childhood or as part of a prime-boost regimen. All were Quantiferon (QFT) negative (non-latently infected) prior to enrollment.

Table 3-2 Clinical studies of H4:IC31 performed to date

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

Table 3-3 presents the number and percentage of participants in all 4 phase 1 studies of H4:IC31 experiencing adverse events related to H4:IC31 for events occurring in ≥5% of participants.

Table 3-3 Adverse events in the H4:IC31 treatment groups in adults with event rate of ≥5% and number of participants for each event

Related Adverse Event[a] (MedDRA Preferred Term[b,c])	H4:IC31 (N=198)[d] n[e] (%)
Injection site pain	109 (55.1)
Fatigue	95 (48.0)
Headache	82 (41.4)
Myalgia	76 (38.4)
Injection site erythema	29 (14.6)
Arthralgia	28 (14.1)
Injection site swelling	26 (13.1)
Pyrexia	26 (13.1)
Neutrophil count decreased	18 (9.1)
Vaccination site swelling	15 (7.6)
Tuberculin test positive[f]	14 (7.1)
Chills	13 (6.6)
Vaccination site erythema	13 (6.6)
Blood urine present	12 (6.1)
Diarrhea	12 (6.1)
Protein urine present	12 (6.1)
Hemoglobin decreased	11 (5.6)
Nausea	10 (5.1)

a. Adverse event data through the end of the study for C-005-404, C-006-404, C-011-404, and C-013-404; includes adverse events that occurred on or after the first dose of H4:IC31
b. Injection site AEs denote AEs at a placebo or H4:IC31 site. Vaccination site AEs denote AEs at the BCG site.
c. For the purpose of summarizing cumulative data across multiple studies, adverse events were coded using the same version of MedDRA, which may differ from the version of MedDRA that was used to code adverse events in an individual study.
d. N is number of participants who received at least 1 dose of H4:IC31
e. n is number of participants with at least 1 related adverse event for the preferred term in question
f. Tuberculin test positive refers to hypersensitivity reactions at the tuberculin skin test application site after administration of H4:IC31

No vaccine-related serious adverse events have occurred and the vaccine has generally been well tolerated at all doses and regimens evaluated. Injection site reactions (pain, swelling, and erythema) have been mild or moderate. Reactions have occurred at the site of tuberculin skin tests (TST) and BCG vaccination sites as described below. Systemic adverse reactions include mild to moderate fatigue, myalgia, headache, arthralgia, and mild to severe pyrexia. Asymptomatic transient isolated proteinuria was seen in participants receiving the vaccine and placebo in all 4 trials and was more frequent in South African adults, including at baseline (prior to vaccination). Proteinuria did not recur with revaccination and this finding is not considered to be clinically significant. One participant had a reactivation of Graves' disease, and 1 participant was diagnosed with celiac disease at the end of the study; neither event was considered related to study vaccine.

In study C-005-404 a TST result less than 10 mm was an eligibility requirement. H4:IC31 was shown to induce postvaccination hypersensitivity reactions (HSR) at the sites of recently administered TSTs, with 14/21 (66.7%) participants who received H4:IC31 after TST experiencing a postvaccination TST site HSR, characterized by warmth, erythema and induration, with onset from several hours to 2 days after Study Day 0 vaccination, and duration from 1 to 28 days, typically resolving without treatment. Two of the 14 participants also experienced pruritus at the TST site. For this reason, the TST was removed as a study procedure from C-005-404 and subsequent protocols.

Local BCG injection site reactions are well documented adverse events in participants who receive BCG. Induration appears at the site of the BCG injection which is followed by a local lesion (redness and swelling) that may ulcerate some weeks later. In study C-013-404 all study participants received BCG at Study Day -42, before receiving H4:IC31 or placebo at Study Days 0, 56, and 231. Redness was the most common local BCG injection site reaction with all participants (100%) experiencing redness after BCG vaccination. Redness persisted in the majority of the participants throughout the study, with at least 60% of participants in each group having redness at the final study assessment. Swelling was the second most common local BCG injection site reaction. None of the redness or swelling at the BCG injection site met protocol-specified toxicity grading scale criteria for a severe (Grade 3 or higher) adverse event. BCG injection site ulceration occurred after BCG vaccination in both placebo and H4:IC31 recipients. There was no dose-related increase in frequency of BCG site ulceration after receiving H4:IC31. Overall, there was similar incidence (regardless of severity) of BCG local reactions in participants who received H4:IC31 and placebo, with no dose response of BCG site reactions noted in participants who received 2 doses or 3 doses of H4:IC31 and no evidence of severe acute worsening of BCG site reactions after H4:IC31 administration.

CD4 responses have consistently been induced by vaccination with H4:IC31. Responses were higher after vaccinations with the 15/500 dose compared to the higher and lower doses, and did not significantly increase with a third vaccination. Among participants in study C-006-404, increases in ELISpot responses were seen among all groups receiving H4:IC31, and peaked at Day 84. Responses were strongest among participants receiving IC31 at 500 nmol, with comparable responses between groups receiving 5, 15, and 50 mcg H4. ICS responses showed Ag85B-specific cluster of differentiation 4 (CD4+) T-cells among all treatment groups, peaking at 2 or 4 weeks after the second dose of H4:IC31. The highest magnitude of responses was noted among the 5, 15, and 50 mcg H4 dose groups. CD4+ responses were predominantly bifunctional or polyfunctional. By comparison, TB10.4-specific CD4+ responses were lower in frequency and of lower magnitude compared to Ag85B-induced CD4+ responses.

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

In Study C-013-404, the dosage studied, 50/500 H4:IC31, was selected before 15/500 H4:IC31 was found to induce a more favorable immune profile in adults. Three doses of H4:IC31 boosted recent BCG priming and induced Ag85B antigen-specific CD4+ T cells above that seen with placebo control. These responses were primarily polyfunctional (IFN-γ, IL-2 and TNF-α) and bi-functional (IL-2 and TNF-α). In participants receiving 3 doses of H4:IC31, the magnitude of Ag85B-specific CD4 T cell responses on Study Day 259 (28 days after the third dose) was comparable to that seen on Study Day 84 (28 days after the second dose). The 3-dose regimen was seen to only slightly outperform the 2-dose regimen as measured by the magnitude of Ag85B-specific CD4+ T-cell responses. For further details of the clinical experience with H4:IC31, refer to the investigator's brochure.

There are 2 ongoing studies of H4:IC31. Study Aeras C-015-404/IMPAACT P1113, a phase 1/2 safety and immunogenicity study in BCG-primed infants, is conducted at 3 sites in South Africa in 229 infants aged between 64 days to 196 days in an age-de-escalation and dose-escalation manner (ClinicalTrials.gov identifier NCT01861730). Study Aeras C-040-404, a phase 2 study to evaluate safety, immunogenicity, and prevention of infection by Mycobacterium tuberculosis with H4:IC31 and BCG revaccination, is conducted at the South African Tuberculosis Vaccine Initiative (SATVI) site in 990 healthy adolescents (ClinicalTrials.gov identifier NCT02075203). In this study, participants are randomized in a 1:1:1 ratio to receive either 2 doses of H4:IC31, 2 doses of placebo, or 1 dose of BCG Vaccine SSI (Table 3-4). The primary Mtb infection endpoint will be QFT-GIT conversion from negative to positive test.

Table 3-4 Design of study Aeras C-040-404

Study cohort	Planned number of participants			
	Treatment group			
	Placebo	BCG	H4:IC31	Total
Safety and immunogenicity	30	30	30	90
Correlates	300	300	300	900
Total	330	330	330	990

As of February 4, 2015, 106 participants have been vaccinated in study C-015-404/IMPAACT P1113 and 603 participants have been vaccinated in study Aeras C-040-404, with neither vaccine-related serious adverse event (SAE) nor severe/grade 3 non-serious AE reported in these ongoing studies.

3.10.2 Clinical studies of H56:IC31

One phase 1 study (C-032-456) of H56:IC31 in 25 participants has been completed, and 1 phase 1/2a study (C-035-456) with 98 participants planned is ongoing at SATVI with results expected in third quarter of 2014. The dose of H56:IC31 in these studies ranges from 5 to 50 µg H56 with 500 nmol IC31. In C-032-456, H56:IC31 was associated with an acceptable safety profile in participants with and without LTBI, and no SAEs were reported. Vaccination with H56:IC31 was associated most commonly (≥10% of participants with a related adverse event) with mild to moderate injection site reactions of pain, warmth, and swelling; mild to moderate systemic adverse events of fatigue, bradycardia, myalgia, nausea, headache, arthralgia, and white blood cell count increased; and mild to severe hypertension (Table 3-5). H56:IC31 induced a dominant CD4+ T cell

response in both QFT-GIT (-) and QFT-GIT (+) participants. Responses were greater in QFT-GIT (+) participants than in QFT-GIT (-) participants. Responses to stimulation with Ag85B peptides among QFT-GIT (+) were similar for the 15/500 and 50/500 H56:IC31 dose levels. However, responses to stimulation with ESAT-6 peptides among QFT-GIT (+) participants were greater for the 15/500 compared to the 50/500 H56:IC31 dose level. Responses to stimulation with Rv2660c peptides were negligible (very weak) for both QFT-GIT (-) and QFT-GIT (+) participants. QFT conversion was reported in 2/8 (25%) participants in the QFT-GIT (-) 50/500 group at Study Day 182. In the ongoing double-blinded C-035-456 study, 50 QFT-GIT (-) participants have received 2 doses of H56:IC31 or adjuvant. An interim analysis of unblinded safety data through Day 84 was conducted by DMC with no safety issues identified. For further details of the clinical experience with H56:IC31, refer to the IB.

Table 3-5 Related adverse events in AERAS C-032-456

Related Adverse Event[a] (MedDRA Preferred Term)	H56:IC31 (AERAS-456) (N=25)[b] n[c] (%)
Injection site pain	18 (72.0)
Injection site warmth	17 (68.0)
Fatigue	9 (36.0)
Injection site swelling	7 (28.0)
Bradycardia	5 (20.0)
Myalgia	5 (20.0)
Nausea	5 (20.0)
Headache	4 (16.0)
Hypertension	4 (16.0)
Arthralgia	3 (12.0)
White blood cell count increased	3 (12.0)
Alanine aminotransferase increased	2 (8.0)
Chills	2 (8.0)
Dizziness	2 (8.0)
Hemoglobin decreased	2 (8.0)
Injection site erythema	2 (8.0)
Abdominal pain	1 (4.0)
Arthropod bite	1 (4.0)
Aspartate aminotransferase increased	1 (4.0)
Back pain	1 (4.0)
Epistaxis	1 (4.0)
Hot flush	1 (4.0)
Hypoesthesia	1 (4.0)
Injection site pruritus	1 (4.0)
Joint stiffness	1 (4.0)
Musculoskeletal pain	1 (4.0)
Neutrophil count decreased	1 (4.0)
Protein urine	1 (4.0)
Pyrexia	1 (4.0)
Red blood cells urine	1 (4.0)

a. Includes all related adverse events (solicited, unsolicited, serious) through the end of the study that occurred on or after the first dose of H56:IC31 (AERAS-456) for C-032-456
b. N is number of participants who received at least 1 dose of H56:IC31 (AERAS-456)
c. n is number of participants with at least 1 related adverse event for the preferred term in question

3.10.3 Clinical experience with H1 vaccine related to both H4:IC31 and H56:IC31

The Hybrid-1 (H1) vaccine, a vaccine closely related to H56:IC31, 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 H4:IC31 and H56:IC31. Five clinical trials have been completed by SSI: THYB-01, THYB-02, THYB-03, THYB-04 and THYB-05. These studies represent a combined population of 375 healthy, HIV(-) adolescents and adults and HIV(+) adults, of whom 240 are healthy adolescents with or without latent TB infection. Table 3-6 shows the dose matrix of the 4 studies. The first 3 studies were open

label and non-randomized. In THYB-01 and THYB-02 no vaccine related serious adverse events were reported. In THYB-03, 2 possible vaccine related SAEs were reported: volunteer number 29 had an increase in ALT/AST 48 hours after first vaccination and volunteer number 39 (a weight lifter) had an increase in CPK to 10.545 IU/l 24 hours post first vaccination and 11.025 IU/l 48 h post first vaccination. Both participants recovered without sequelae. 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. The majority of adverse events reported in THYB-04 adolescent study were mild to moderate. No serious adverse events considered related to study vaccine were reported. Most adverse events were mild immunization site reactions, mostly pain or tenderness. Mild or moderate tiredness, headache and myalgia accounted for the majority of systemic side effects. Immunization site and systemic reactions were self-limiting and of short duration. IFN-γ ELISpot responses to H1 antigen were greater in QFT-GIT (+) participants than in QFT-GIT (-) participants for both 15/500 and 50/500 dose levels. Responses to two doses of 50/500 H1:IC31 were higher than one dose of 50/500 in QFT-GIT (-) participants, and were also slightly higher than 2 doses of 15/500 H1:IC31 in QFT-GIT (-) participants. In THYB-05 no vaccine related SAEs were reported. Main solicited local AEs were pain, tenderness and erythema. Main solicited systemic AEs were headache, fatigue, myalgia and arthralgia. Main unsolicited AEs were blood/lymphatic disorders. H1/IC31® induced a strong, specific and long lasting, CD4+ T cell response. QFT conversion was reported in THYB-01 study with one individual remaining positive 32 weeks after vaccination and one individual remaining positive at 2. 5 years of follow up [39]. QFT conversion was also observed in THYB-05 study with 8 out the 23 (34.8%) QFT (-) vaccine recipients at study day 182 (personal communication from SSI).

Table 3-6 Clinical studies with H1

	Treatment Assignment				
Trial #	50 mcg antigen alone	50 mcg antigen 100 nmol IC31	15 mcg antigen 500 nmol IC31	50 mcg antigen 500 nmol IC31	N
THYB-01	12	12		12	36
THYB-02				20	20
THYB-03	12			27	39
THYB-04			120	120	240
THYB-05				40	40
Total	24	12	120	219	375

3.10.4 Clinical studies of BCG

There is considerable experience with BCG vaccine, which is licensed in South Africa for prevention of TB in children and adults. Local and systemic reactogenicity in various populations is well described [40-43]. Injection site and regional complications, such as extensive local ulceration, local subcutaneous abscesses, and suppurative lymphadenitis, occur in less than 1 per 5 million vaccinations. Induration with redness of the skin at the site of intradermal BCG vaccination typically develops within several days and represents a normal response to BCG vaccination. The induration gradually resolves over several days and is followed by a small local superficial ulcer. The ulcer opens and drains for 4 weeks on average, and spontaneously heals within 2-3 months, usually leaving a small scar. A brief period of minor, asymptomatic enlargement of the regional cervical and axillary lymph nodes (< 1 cm) is common. Systemic adverse events due to BCG

occur very infrequently. Fever, headache and non-injection site cutaneous manifestations occur in less than 1% of vaccinees. Severe systemic adverse events, such as osteitis and disseminated BCG disease, are rare (about 1 per 5 million vaccinations) and usually occur in immune-compromised infants. Severe local or systemic complications of BCG would not be expected in the healthy, HIV-uninfected, Mtb uninfected adolescents to be recruited in this trial.

Adolescents in this trial will have received prior BCG vaccination at birth. BCG revaccination appears to be safe and well tolerated in adolescents and young adults [44-46]. In a large trial of BCG revaccination in more than 7,000 Brazilian adolescents, only 25 of the participants had adverse reactions and no deaths or cases of disseminated BCG disease were reported [44,45]. Other published trials of BCG revaccination have concluded that BCG revaccination is not associated with significantly more AEs than primary BCG vaccination. In a trial of BCG revaccination in 2,997 14- to 15-year-old Swedish adolescents, open vaccination lesions were reported in 4% (mean diameter of open lesions 4mm) of adolescents receiving the Danish BCG vaccine that will be used in this trial [46]. South African Tuberculosis Vaccine Initiative (SATVI) data from an ongoing trial of BCG revaccination and isoniazid preventive therapy (South African National Clinical Trials Register DOH-27-0212-3995) indicate that BCG revaccination is safe and well tolerated in young adults. Eighty-two participants in this ongoing trial were enrolled with a TST ≥15mm. Data from these Mtb-infected young adults show that injection site reactions are mostly of mild intensity, with ulceration of the injection site resolving within 3 months. No BCG-related SAEs or major safety concerns have arisen.

4 Objectives and endpoints

4.1 Primary objectives and endpoints

Primary objective 1

- To evaluate the safety and tolerability of the different vaccine regimens in adolescents.

Primary endpoint 1

- The number and percentage of solicited and unsolicited adverse events (AEs), including SAEs, recorded postvaccination for all participants.

Primary objective 2

- To evaluate the cellular immune responses of the different vaccine regimens in adolescents compared to those measured at baseline.

Primary endpoint 2

- T-cell responses by flow cytometric intracellular cytokine staining (ICS) of CD4+ and CD8+ T cells after stimulation with a pool of mycobacterial peptides and/or PPD (purified protein derivative) using cryopreserved peripheral blood mononuclear cells (PBMC). The analysis will include monofunctional and polyfunctional T cells, different CD4+ T-cell subsets (TH1, TH2, TH17), and Treg responses.

4.2 Secondary objectives and endpoints

Secondary objective 1 (vaccine-elicited humoral responses)

- To evaluate humoral responses elicited by the different vaccine regimens.

Secondary endpoint 1

- Vaccine-specific binding antibodies elicited by the vaccine regimens as determined by multiplex antibody assay and/or ELISA.

Secondary objective 2 (innate and adaptive transcriptomics)

- To further evaluate the immune response elicited by the different vaccine regimens by measuring early (innate) vaccine induced peripheral blood transcription profiles, and to subsequently determine which of these responses are associated with antigen-specific adaptive responses. Changes in gene expression will be measured in longitudinally collected blood samples relative to samples collected at baseline. The transcriptional profiles will be correlated with antigen-specific adaptive responses measured in Primary objective 2.

- To further evaluate the adaptive immune response by performing transcriptional analysis of antigen-stimulated PBMC at 2 weeks post vaccination.

Secondary endpoints 2

- Use RNAseq to assess mRNA signatures in whole blood collected at baseline and multiple innate timepoints including 1, 3, and 7 days after vaccination.

- Use RNAseq to assess mRNA signatures of *ex vivo* antigen-stimulated PBMC at 2 weeks post vaccination (for Groups 1, 2 & 4) and 4 weeks post vaccination (for Group 3).

- Use Fluidigm to assess single cell RNA expression on a subset of participants. Choice of gene targets will be informed by outcome of gene expression data from stimulated PBMC (RNAseq).

Secondary objective 3 (innate cell enumeration)

- To evaluate changes in innate cells in response to the vaccine regimens.

Secondary endpoint 3

- Blood concentrations of innate immune cell populations including lymphocyte populations, dendritic cells, monocytes, and granulocytes before and after vaccination.

Secondary objective 4 (non-classical T-cell responses elicited by BCG revaccination)

- To measure non-classical MHC-restricted T-cell vaccine-induced responses, such as to mycobacterial lipids (CD1-restricted) and metabolites (MR1-restricted).

Secondary endpoints 4

- Frequency of CD4+, CD8+, and CD4/CD8 double-negative T-cell responses restricted by CD1 (recognizing specific Mtb lipids) before and after BCG revaccination in Group 3 participants.

- Frequency of mucosal-associated invariant T-cells (MAIT) restricted by MR1 (recognizing vitamin B metabolites) before and after BCG revaccination in Group 3 participants.

Secondary objective 5 (ESAT-6 free IGRA performance)

- To evaluate QFT-GIT and ESAT-6 free IGRA discordance and conversion/reversion rate during the course of the trial.

Secondary endpoints 5

- Magnitude and positivity of IFN-γ release using QFT-GIT ELISA in QFT-GIT tests.

- Magnitude and positivity of IFN-γ release using QFT-GIT ELISA in ESAT-6 free IGRAs.

4.3 Exploratory objectives

Exploratory objective 1 (adaptive cytokine/chemokine production)

- To evaluate the adaptive profile of secreted cytokines/chemokines elicited by the different vaccine regimens.

Exploratory endpoint 1

- Concentrations of soluble factors produced from *ex vivo* antigen-stimulated PBMC at the adaptive immune timepoints, measured by ELISA or multiplex array.

Exploratory objective 2 (pTfh and plasmablast phenotyping)

- To evaluate peripheral T-follicular helper cell and antibody-secreting B-cell (plasmablasts) responses elicited by the different vaccine regimens as measured in blood.

Exploratory endpoints 2

- Frequencies of peripheral T follicular helper cells (pTfh) in peripheral blood as determined by flow cytometry.

- Frequencies of antibody-secreting B cells (plasmablasts) in peripheral blood as determined by flow cytometry.

Exploratory objective 3 (innate cytokine/chemokine production)

- To evaluate the innate profile of secreted cytokines/chemokines elicited by the different vaccine regimens.

Exploratory endpoint 3

- Concentrations of soluble factors in serum at baseline and early, innate response timepoints (1, 3, and 7 days), measured by ELISA or multiplex array.

Exploratory objective 4 (baseline NTM-specific responses)

- To explore the potential influence of prior exposure to non-tuberculous mycobacteria (NTM) on vaccine-induced responses.

Exploratory endpoint 4

- Baseline NTM-specific responses by ELISpot or ICS from PBMCs after stimulation with NTM-specific peptides and vaccine-induced immunogenicity endpoints.

Exploratory objective 5 (PBMC mycobacterial growth inhibition)

- To evaluate the capacity of cryopreserved peripheral blood monocytes to inhibit the growth of mycobacteria.

Exploratory endpoint 5

- Magnitude of mycobacterial growth inhibition in PBMC specimens of vaccine recipients using a PBMC mycobacterial growth inhibition assay.

Exploratory objective 6

- To investigate additional elicited immune responses that may correlate with protection or risk of infection or disease.

Exploratory endpoint 6

- Immune responses that may include additional humoral response characterizations, proteomics analyses, CyTOF, or other analyses.

Exploratory objective 7

- Baseline variables associated or correlated with the vaccine immune responses.

Exploratory endpoint 7

- Baseline characteristics (clinical, demographic, laboratory measures) in conjunction with any of the postvaccination immune responses of interest (at the minimum the immune responses for the primary objectives).

Exploratory objective 8

- To evaluate the antigen specific release of cytokines and chemokines other than IFN-γ in surplus supernatants from QFT-GIT and ESAT-6 free IGRA.

Exploratory endpoint 8

- Alternative cytokines that may include IL-2 and IP-10 as measured by ELISA or multiplex array.

Exploratory objective 9

- To assess whether the diversity of gut microbiome correlates with vaccine responses using optionally provided stool specimens.

Exploratory endpoint 9

- Characterization of Bacterial and Archaea species in stool samples using 16S rRNA sequences or alternative assays.

5 Statistical considerations

5.1 Accrual and sample size calculations

Recruitment will target enrolling 84 QFT-GIT negative, BCG vaccinated, healthy, HIV-1–uninfected volunteers aged 12 to 17 years. Participants will be randomized to one of 3 treatment groups or the control group in a 2:2:2:1 ratio (treatment : treatment : treatment : control). Participants will be enrolled over an anticipated accrual period of 4 months and will be followed over 8 months of scheduled clinic visits.

Since enrollment is concurrent with receiving the first study vaccination, all participants will provide some safety data. However, for immunogenicity analyses, it is possible that data may be missing for various reasons, such as participants terminating from the study early, problems in shipping specimens, low cell viability of processed peripheral blood mononuclear cells (PBMCs) or high background. Prior experience with adolescent cohorts indicate that 10% is a reasonable estimate for the rate of missing data at the primary immunogenicity timepoint (day 28 for Groups 1, 2 and 4, day 56 for Group 3). For this reason, the sample size calculations in Section 5.1.2 account for 2 enrolled participants in each group having missing data for the primary immunogenicity endpoint.

5.1.1 Sample size calculations for safety

The goal of the safety evaluation for this study is to identify safety concerns associated with product administration. The ability of the study to detect serious adverse events (SAEs) (See Section 10) can be expressed by the true event rate above which at least 1 SAE would likely be observed and the true event rate below which no events would likely be observed. Specifically, for each vaccine arm of the study (n =24), there is a >90% chance of observing at least 1 event if the true rate of such an event is 9% or more; and there is >90% chance of observing no events if the true rate is 0.4% or less. For vaccine arms combined (n = 72), there is a 90% chance of observing at least 1 event if the true rate of such an event is 4% or more; and there is a 90% chance of observing no events if the true rate is 0.2% or less.

Probabilities of observing 0, 1 or more, and 2 or more events among arms of size 24 are presented in Table 5-1 for a range of possible true adverse event rates. These calculations provide a more complete picture of the sensitivity of this study design to identify potential safety problems with the vaccine.

Table 5-1 Probability of observing 0 events, 1 or more events, and 2 or more events, among arms of size 24 for different true event rates

True event rate (%)	Pr(0/24)	Pr(1+/24)	Pr(2+/24)
1	0.79	0.21	0.02
4	0.38	0.62	0.25
10	0.08	0.92	0.70
20	<0.01	0.99	0.97
30	<0.01	0.99	0.99
40	<0.01	0.99	0.99

An alternative way of describing the statistical properties of the study design is in terms of the 95% confidence interval for the true rate of an adverse event based on the observed data. Table 5-2 shows the 2-sided 95% confidence intervals for the probability of an event based on a particular observed rate. Calculations are done using the score test method [47]. If none of the 72 participants receiving a vaccine regimen experience a safety event, the 95% 2-sided upper confidence bound for the true rate of such events in the total vaccinated population is 5.1%. For each individual vaccine arm (n =24), the 2-sided upper confidence bound for this rate is 13.8%.

Table 5-2 Two-sided 95% confidence intervals based on observing a particular rate of safety endpoints for arms of size 24 or for the treatment arms overall (n = 72)

Observed event rate	**Confidence interval (%)**
0/24	[0.0, 13.8]
1/24	[0.7, 20.2]
2/24	[2.3, 25.8]
3/24	[4.3, 31.0]
0/72	[0.0, 5.1]
1/72	[0.2, 7.5]
2/72	[0.8, 9.6]
3/72	[1.4, 11.5]

5.1.2 Sample size calculations for immunogenicity

The primary objective of this trial regarding immunogenicity outcomes is to estimate T cell response rates based on data from flow cytometric intracellular cytokine staining (ICS) among participants in each vaccine group. Responses will be measured to 6 protein antigens or antigen pools based on staining for the presence of IFN-γ, TNF-α, IL-2, CD4, CD8 and other phenotypic markers. No adjustment for multiple comparisons will be made for the use of multiple antigens or cellular markers. The precision with which the true response rate can be estimated from the observed data depends on the true underlying response rate and the sample size. Two-sided 95% confidence intervals for the response rate based on observing a particular rate of responses in each vaccine group is shown in Table 5-3. Calculations are done using the score test method [47]. The n = 22 assumes a 8% loss of data.

Table 5-3 Two-sided 95% confidence intervals for the true response rate based on observing a particular rate of responses in each vaccine group (n = 22)

No. of responses	Observed response rate (%)	Confidence interval
10	45.5	[26.9, 65.3]
12	54.5	[34.7, 73.1]
14	63.6	[43.0, 80.3]
16	72.7	[51.8, 86.8]
18	81.8	[61.5, 92.7]
20	90.9	[72.2, 97.5]

5.2 Randomization

The randomization sequence will be obtained by computer-generated random numbers and provided to the CRS through the SDMC's Interactive Voice/Web Response System (IVRS/IWRS). The randomization schedule will be prepared by a statistician who will not be involved in the analysis of the study in order to maintain the blind of the study team. The randomization will be done in blocks to ensure balance across arms. At the CRS in Cape Town, South Africa, the pharmacist with primary responsibility for dispensing study products is charged with maintaining security of the treatment assignments. The day of enrollment for each participant will be Study Day 0. Participants who discontinue participation will not be replaced.

5.3 Blinding

Assignment of participants to the H4:IC31, H56:IC31 and placebo arms will be blinded. Participants and site staff (except for site pharmacists) will be blinded as to participant treatment arm assignments (eg, vaccine or control). Assignment of participants to the BCG Vaccine SSI arm will be unblinded (participants and clinical staff will be aware of study product allocation).

Study product assignments are accessible to those who are required to know this information in order to ensure proper trial conduct, including study vaccine manager (designated pharmacist, defined in Section 7), contract monitors, Aeras IP Manager(s) and SDMC staff. Any discussion of study product assignment between pharmacy staff and any other CRS staff is prohibited; however, the Protocol Team (safety subgroup, see Section 10.1.4) will review safety data blinded to groups 1, 2, and 4 and unblinded for group 3. The Aeras SMC members may be unblinded to treatment assignment in order to conduct review of trial safety.

When a participant leaves the trial prior to study completion, the participant will be told he or she must wait until all participants are unblinded to learn his or her treatment assignment.

Emergency unblinding decisions will be made by the site investigator in consultation with the principle investigator. If time permits, the HVTN 602 / AERAS A-042 Protocol Team should be consulted before emergency unblinding occurs. The principle investigator (in consultation with the medical monitor, if possible) will make a written request to the vaccine manager for urgent unblinding of a subject's treatment. The request must include the subject identification number, the date, a brief justification of the clinical requirement to the vaccine manager in the research pharmacy, and the

investigator's signature. The request will be kept in the pharmacy study file. Upon receipt of proper written request, the vaccine manager or designee will disclose the treatment assignment to the investigator. The sponsor or its designee must be notified immediately of any clinically required break of the study blind on an Immediately Reportable Event Form.

5.4 Statistical analysis

This section describes the final study analysis, unblinded as to treatment arm assignment. A participant is considered randomized after s/he gives consent/assent, meets eligibility criteria and is assigned a randomization number. A participant is considered enrolled after s/he is given a vaccination after randomization. Unblinded safety analyses will be performed on data from all participants based on the treatment they received (ie "as treated"), regardless of the treatment they were assigned, or the number of vaccinations they received. Immunogenicity analyses will be performed on data from all participants according to the initial randomization assignment regardless of how many vaccinations they received. This analysis is a modified intent-to-treat analysis in that individuals who are randomized but not enrolled do not contribute data and hence are excluded. Because of blinding and the brief length of time between randomization and enrollment—typically no more than 4 working days—very few such individuals are expected.

Analyses for primary endpoints will be performed using SAS and R. All other descriptive and inferential statistical analyses will be performed using SAS, StatXact, or R statistical software.

No formal multiple comparison adjustments will be employed for multiple safety endpoints, multiple primary immunogenicity endpoints, or secondary endpoints. However, multiplicity adjustments will be made for certain immunogenicity assays, as discussed below, when the assay endpoint is viewed as a collection of hypotheses (eg, testing multiple peptide pools to determine a positive response).

A detailed statistical analysis plan will be created and finalized prior to database lock in preparation of the final study report.

5.4.1 Analysis variables

The analysis variables consist of baseline participant characteristic, safety, and immunogenicity endpoints for the assessment of primary, secondary, and exploratory objectives.

5.4.2 Baseline comparability

Baseline participant characteristics will be summarized by treatment arm using descriptive statistics.

5.4.3 Safety/tolerability analysis

All subjects who received at least one vaccination will contribute to availability of safety data and will be included in the Safety analysis set.

5.4.3.1 Reactogenicity

The number and percentage of participants experiencing each type of reactogenicity sign or symptom will be tabulated by severity and treatment arm and the percentages displayed graphically by arm. For a given sign or symptom, each participant's reactogenicity will be counted once under the maximum severity for all injection visits and monitored for 7 days (see Section 8.7). In addition, to the individual types of events, the maximum severity of local pain or tenderness, induration or erythema, and of systemic symptoms will be calculated.

5.4.3.2 AEs and SAEs

AEs will be summarized using MedDRA System Organ Class and preferred terms. Tables will show by treatment arm the number and percentage of participants experiencing an AE within a System Organ Class or within preferred term category, overall and by severity or by relationship to study product. For the calculations in these tables, a participant with multiple AEs within a category will be counted once under the maximum severity or the strongest recorded causal relationship to study product. Formal statistical testing comparing arms is not planned since interpretation of differences must rely heavily upon clinical judgment.

A listing of SAEs reported to the Protocol Team (safety subgroup) will provide details of the events including severity, relationship to study product, time between onset and last vaccination, and number of vaccinations received.

5.4.3.3 Local laboratory values and Vital Sign Parameters

For each local laboratory measure and vital sign parameter, summary statistics will be presented by treatment arm and timepoint, as well as changes from baseline for postvaccination values. In addition, the number (percentage) of participants with local laboratory values recorded as meeting Grade 1 AE criteria or above as specified in the DAIDS AE Grading Table (see Section 8.7) will be tabulated by treatment arm for each postvaccination timepoint. Reportable clinical laboratory abnormalities without an associated clinical diagnosis will also be included in the tabulation of AEs described above.

5.4.3.4 Reasons for vaccination discontinuation and early study termination

The number and percentage of participants who discontinue vaccination and who terminate the study early will be tabulated by reason and treatment arm.

5.4.4 Immunogenicity analysis

5.4.4.1 General approach

For the statistical analysis of immunogenicity endpoints, data from enrolled participants will be used according to the initial randomization assignment regardless of how many injections they received. Additional analyses may be performed, limited to participants who received at least one dose or all scheduled injections per protocol. Also analyses may exclude assay results that are unreliable or from specimens collected outside of the visit window.

Discrete categorical assay endpoints (eg, response rates) will be analyzed by tabulating the frequency of positive response for each assay by antigen and treatment arm at each timepoint for which an assessment is performed. Crude response rates will be presented with their corresponding 95% confidence interval estimates calculated using the score test method [47]. Because of the small numbers of control participants in each group, no adjustment will be made to the vaccine arm estimates for the false positive rates in the control arms.

In addition to response rate estimates for each timepoint, the probability of observing at least 1 positive response by a given timepoint and the probability of observing more than 1 positive response by a given timepoint will be estimated, with corresponding confidence intervals, for each vaccine arm using maximum likelihood-based methods [48].

For quantitative assay data (eg, number of spot forming cells from the ELISpot assay or percentage of positive cells from the ICS assay), graphical and tabular summaries of the distributions by antigen, treatment arm, and timepoint will be made.

Based upon previous AIDS Vaccine Evaluation Group (AVEG) and HVTN trials, missing 10% of immunogenicity results for a specific assay is common due to study participants terminating from the study early, problems in shipping specimens, or low cell viability of processed peripheral blood mononuclear cells (PBMCs). To achieve unbiased statistical estimation and inferences with nonparametric tests and generalized linear models fit by generalized estimating equation (GEE) methods, missing data need to be missing completely at random (MCAR). MCAR assumes that the probability of an observation being missing does not depend upon the observed responses or upon any unobserved covariates but may depend upon covariates included in the model (eg, missing more among whites than nonwhites). When missing data are minimal (specifically if no more than 20% of participants are missing any values), then nonparametric tests and GEE methods will be used, because violations of the MCAR assumption will have little impact on the estimates and hypothesis tests. These models will include as covariates all available baseline predictors of the missing outcomes.

If a substantial amount of immunogenicity data are missing (at least 1 value missing from more than 20% of participants), then using the methods that require the MCAR assumption may give misleading results. In this situation, analyses of the immunogenicity endpoints at a specific timepoint will be performed using parametric generalized linear models fit by maximum likelihood. These methods provide unbiased estimation and inferences under the parametric modeling assumptions and the assumption that the missing data are missing at random (MAR). MAR assumes that the probability of an observation being missing may depend upon the observed responses and upon observed covariates, but not upon any unobserved factors. Generalized linear models for response rates will use a binomial error distribution and for quantitative endpoints, a normal error distribution. For assessing repeated immunogenicity measurement, linear mixed effects models will be used. If the immunological outcomes are left- and/or right- censored, then the linear mixed effects models of Hughes [49] will be used, because they accommodate the censoring. In addition, secondary analyses of repeated immunogenicity measurements may be done using weighted GEE [50] methods, which are valid under MAR. All of the models described above will include as covariates all available baseline predictors of the missing outcomes.

5.4.4.2 Analysis of CD4+ and CD8+ T-cell response as measured by the ICS assay

The analysis of CD4+ and CD8+ T-cell response rates as measured by the ICS assay will be evaluated as described under the general approach. For each T-cell subset, the positivity call for each peptide pool will include a multiple comparison adjustment for the number of peptide pools used in the assay using the discrete Bonferroni adjustment. The magnitude of response will be analyzed as described for quantitative data in the general approach section. For each T-cell subset, graphs will be used to display the background-subtracted magnitudes for each participant by protein, treatment arm and timepoint, with a box plot of data from positive responders superimposed on the individual data values. Statistical testing comparing the magnitudes will be based on positive responders only.

5.4.4.3 Assessing baseline variability of existing mycobacterial immunity

To quantify the existing immunity to TB antigens and to aid with validation for a given assay the Day 0 responses from all 4 treatment groups will be pooled for descriptive analyses. Data will be presented in tabular and graphical format with accompanying estimates and confidence intervals of the false-positivity rate for the assay in this cohort.

5.4.4.4 Characterizing innate response to IC31 adjuvant

To characterize the innate immune signature of the IC31 adjuvant that is present in the Group 1 and Group 2 treatment, results from innate immune assays will be pooled across the 2 groups in the analysis. These assays include whole blood gene expression, TruCount cell quantification and serum cytokines at the Day 0, 1, 3 and 7 timepoints.

5.4.4.5 Characterizing the QFT-GIT and ESAT-6 free IGRA assays

To characterize the operating characteristics of the QFT-GIT assay and the ESAT-6 free IGRA the positive response rates and accompanying confidence intervals will be presented for each assay and each treatment group at all timepoints for which data was collected (Visit 1, Day 70, 168, 224). We will pool for analysis the paired QFT-GIT and ESAT-6 free IGRA data from the following participant groups and timepoints: (1) all participants who contributed a sample at the screening visit (including those who were not subsequently enrolled), (2) participants enrolled in Groups 1, 3 and 4 (not H56:IC31) at all timepoints and (3) participants enrolled in Group 2 at Day 0 only. Discordance of the 2 assays will be quantified using Cohen's kappa coefficient and 95% confidence intervals. The kappa coefficient takes into account the probability of concordance by chance alone, however we note that estimates of kappa may be biased; since most participants will not be exposed to mycobacteria during this study, kappa may not reflect discordance on TB-positive samples. As a result of vaccination alone, Group 2 is expected to have a positive QFT-GIT assay and a negative ESAT-6 free assay on Days 70, 168 and 224 since ESAT-6 is present in the Group 2 treatment (H56:IC31) and the QFT-GIT assay, but not the ESAT-6 free IGRA. With these samples we will estimate a positivity rate and a reversion rate for the QFT-GIT assay in response to the H56:IC31 vaccine, however, this may be a biased estimate as some participants may have been exposed to mycobacteria in addition to the vaccine at these timepoints.

5.4.4.6 Identifying innate correlates of adaptive immune responses

One of the objectives of the trial is to identify innate responses that are correlated with the antigen-specific adaptive responses measured at later timepoints. Correlation analyses will treat innate measures as potential predictors and adaptive immune measures as outcomes. The low number of participants in each group will preclude use of a predictive training/validation analysis framework. However, we will use both pairwise Spearman's rank correlation in addition to more complex statistical learning methods to generate hypotheses that can be tested in the phase 2 parent trials. Emphasis will be placed on the descriptive nature of these analyses by using clustering and dimensionality reduction methods to summarize both the innate and adaptive immune measures and by visualizing correlations when possible. The Spearman's rank correlation between a continuous valued innate response (eg, fold-change differential gene expression or Day 1serum cytokine level) and a continuous valued adaptive response (eg, fold-change over baseline in percentage of IFN-γ releasing cells) has limited power to detect significant correlations. The 95% confidence interval for each of a range of possible Spearman's rank correlations is provided in Table 5-6 assuming n = 22.

Table 5-6 Two-sided 95% confidence intervals and associated P-values for a range of Spearman's rank correlations for n = 22

Spearman's rank correlation coefficient	95% confidence interval	P value*
0.3	[-0.15, 0.65]	0.17
0.4	[-0.04, 0.71]	0.065
0.5	[0.09, 0.77]	0.018
0.6	[0.23, 0.82]	0.003
0.7	[0.38, 0.87]	<0.001
0.8	[0.56, 0.92]	<0.001

*two-sided, unadjusted for multiple comparisons

5.4.4.7 Transcriptomics

Gene expression data will be collected using RNAseq with whole blood at baseline and innate timepoints (D0, 1, 3, 7) as well as antigen-stimulated PBMC at baseline and adaptive immune timepoints (D0, 14, 28, 70). The data will be analyzed according to methods that have been pioneered by several groups including Nakaya and Pulendran [51] and Zak and Aderem [52]. Log-fold changes in transcript abundance over baseline will be computed for every transcript at each timepoint. Differentially expressed genes (DEGs) are identified for each treatment group by computing a p-value for each gene (ANOVA across timepoints or t-test at individual timepoints) and using a false-discovery rate (FDR) adjusted q-value threshold of 0.2. Identification of DEGs will directly provide hypotheses for analyses of the phase 2 parent trials as well as immune variables that can be used to identify correlations with adaptive responses in this study. A list of DEGs will also be used to perform gene set enrichment analysis (GSEA) [53] and immune module analysis [54] to identify the gene regulatory networks that characterize the immune response.

5.4.5 Analyses prior to end of scheduled follow-up visits

Any analyses conducted prior to the end of the scheduled follow-up visits should not compromise the integrity of the trial in terms of participant retention or safety or

immunogenicity endpoint assessments. In particular, early unblinded analyses by treatment assignment require careful consideration and should be made available on a need to know basis only.

5.4.5.1 Safety

Ad hoc safety reports may be prepared for SMC review at the request of the HVTN 602 / AERAS A-042 Protocol Team. See Section 10.9 for details.

6 Selection and withdrawal of participants

Participants will be healthy, QFT-negative, HIV uninfected (seronegative) adolescents who comprehend the purpose of the study and have provided written informed assent and for whom a parent or legal guardian has provided written informed consent for their participation. Volunteers will be recruited and screened; those determined to be eligible, based on the inclusion and exclusion criteria, will be enrolled in the study. Final eligibility determination will depend on results of laboratory tests, medical history, physical examinations, and answers to self-administered and/or interview questions.

Investigators should always use good clinical judgment in considering a volunteer's overall fitness for trial participation. Some volunteers may not be appropriate for enrollment even if they meet all inclusion/exclusion criteria. Medical, psychiatric, occupational, or other conditions may make evaluation of safety and/or immunogenicity difficult, and some volunteers may be poor candidates for retention.

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

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

Determination of eligibility, taking into account all inclusion and exclusion criteria, must be made within 28 days prior to enrollment unless otherwise noted in Sections 6.1 and 6.2.

6.1 Inclusion criteria

General and Demographic Criteria

Participants must meet <u>all</u> of the following criteria at the time of randomization.

1. **Age** of 12 to ≤ 17 years at enrollment
2. **Minimum weight** ≥ 40 kg
3. **Access to the participating CRS** and willingness to be followed for the planned duration of the study
4. Written informed **consent** obtained from the participant's parent or legal guardian
5. Written **assent** obtained from the participant
6. **Assessment of understanding**: volunteer demonstrates understanding of this study; completes a questionnaire (prepared by the protocol team and provided to the site) prior to first vaccination with verbal demonstration of understanding of all questionnaire items answered incorrectly

7. **Agrees not to enroll in another** study of an investigational research agent prior to completion of last required protocol clinic visit

8. **Good general health** as shown by medical history, physical exam, and screening laboratory tests

TB-Related Criteria:

9. Previous BCG vaccination at least 5 years ago documented by scarification or medical card

10. No evidence of active TB disease, as determined by history, physical examination and, if deemed appropriate, sputum investigation and / or chest x-ray. Assessment for TB disease will be completed by a medical doctor as described in Section 8.2

11. Negative QFT-GIT test at screening, using the manufacturer's recommended threshold of 0.35 IU/mL

HIV-Related Criteria:

12. Willingness to receive **HIV test results**

13. Willingness to discuss HIV infection risks and amenable to HIV risk reduction counseling

14. Assessed by the clinic staff as being at low risk for HIV infection

Laboratory Inclusion Values

Hemogram/CBC

15. **Hemoglobin** ≥ 11.7 g/dL for volunteers who were born female, ≥ 12.5 g/dL for volunteers who were born male

16. **White blood cell count** ≥ lower limit of normal and ≤ upper limit of normal

17. **Total lymphocyte count** ≥ lower limit of normal

18. **Remaining differential** either within institutional normal range or with site physician approval

19. **Platelets** ≥ lower limit of normal and ≤ upper limit of normal

Chemistry

20. **Chemistry panel**: ALT, AST, and alkaline phosphatase <1.25 upper limit of normal; creatinine ≤ upper limit of normal

Virology

21. **Negative HIV-1 and -2 blood test**: Non-US sites may use locally available assays that have been approved by HVTN Laboratory Operations

Urine

22. **Normal urine**:

- Negative urine glucose, and
- Negative or trace urine protein, and
- Negative or trace urine hemoglobin (if trace hemoglobin is present on dipstick, a microscopic urinalysis with red blood cells levels within institutional normal range).

Reproductive Status

23. **Volunteers who were born female**: negative serum or urine beta human chorionic gonadotropin (β-HCG) pregnancy test performed prior to vaccination on the day of initial vaccination.

24. **Reproductive status**: A volunteer who was born female must:

- Agree to consistently use effective contraception (see Appendix A) for sexual activity that could lead to pregnancy from at least 20 days prior to enrollment through the last required protocol clinic visit. Effective contraception is defined as using the following methods (total of 2 methods):

Using 1 of the following methods:

  - Male or female condoms, or
  - Diaphragm or cervical cap;

PLUS 1 of the following methods:

  - Birth control drugs that prevent pregnancy—given by injections, pills, patches, vaginal rings, or inserts under the skin; or
  - Intrauterine device (IUD).

- Or be sexually abstinent.

25. **Volunteers who were born female must also agree not to seek pregnancy through alternative methods**, such as artificial insemination or *in vitro* fertilization until after the last required protocol clinic visit

6.2 Exclusion criteria

All participants must have none of the following at the time of randomization.

General

1. **Blood products** received within 120 days before first vaccination

2. **Investigational research agents** received within 182 days before first vaccination

3. **Intent to participate in another study** of an investigational research agent during the planned duration of the HVTN 602 / AERAS A-042 study

4. **Pregnant or breastfeeding**

5. **History of alcohol or drug abuse**

TB-related

6. A significant **contact with active TB disease:** for example, shared residency with an individual receiving anti-TB treatment, or with an individual known to have incompletely treated culture or smear positive TB

7. **TB prophylaxis** within 90 days prior to enrollment

8. History of treatment for active TB disease or latent Mtb infection

9. Positive and indeterminate QFT-GIT result

10. Received a tuberculin skin test (TST) within 90 days prior to enrollment

Vaccines and other Injections

11. **TB vaccine(s) other than BCG** received in a prior TB vaccine trial. For volunteers who have received control/placebo in a TB vaccine trial, the HVTN 602 / AERAS A-042 Protocol Team will determine eligibility on a case-by-case basis.

12. **Live attenuated vaccines** other than influenza vaccine received within 30 days before first vaccination or scheduled within 28 days after injection (eg, measles, mumps, and rubella [MMR]; oral polio vaccine [OPV]; varicella; yellow fever)

13. **Influenza vaccine or any vaccines that are not live attenuated vaccines** and were received within 14 days prior to first vaccination (eg, tetanus, pneumococcal, Hepatitis A or B)

14. **Allergy treatment with antigen injections** within 30 days before first vaccination or that are scheduled within 14 days after first vaccination

Immune System

15. **Immunosuppressive medications** received within 168 days before first vaccination. (Not excluded: [1] corticosteroid nasal spray; [2] inhaled corticosteroids; [3] topical corticosteroids for mild, uncomplicated dermatitis; or [4] a single course of oral/parenteral corticosteroids at doses < 2 mg/kg/day and length of therapy < 11 days with completion at least 30 days prior to enrollment.)

16. **Serious adverse reactions to vaccines** including history of anaphylaxis and related symptoms such as hives, respiratory difficulty, angioedema, and/or abdominal pain. (Not excluded: a volunteer who had a nonanaphylactic adverse reaction to pertussis vaccine as a child.)

17. **Immunoglobulin** received within 60 days before first vaccination

18. **Autoimmune disease** Not excluded: mild, well-controlled psoriasis

19. History of clinical or laboratory evidence of any past or present possible immunodeficiency state

Clinically significant medical conditions

20. **Clinically significant medical condition**, physical examination findings, clinically significant abnormal laboratory results, or past medical history with clinically significant implications for current health. A clinically significant condition or process includes but is not limited to:

    - A process that would affect the immune response,
    - A process that would require medication that affects the immune response,
    - Any contraindication to repeated injections or blood draws,
    - A condition that requires active medical intervention or monitoring to avert grave danger to the volunteer's health or well-being during the study period,
    - A condition or process for which signs or symptoms could be confused with reactions to vaccine, or
    - Any condition specifically listed among the exclusion criteria below.

21. **Any medical, psychiatric, occupational, or other condition** that, in the judgment of the investigator, would interfere with, or serve as a contraindication to, protocol adherence, assessment of safety or reactogenicity, or a volunteer's ability to give informed consent

22. **Psychiatric condition that precludes compliance with the protocol**. Specifically excluded are persons with psychoses within the past 3 years, ongoing risk for suicide, or history of suicide attempt or gesture within the past 3 years.

23. **Asthma exclusion criteria:**

    Asthma other than mild or moderate, well-controlled asthma. (Symptoms of asthma severity as defined in the most recent National Asthma Education and Prevention Program (NAEPP) Expert Panel report).

    Exclude a volunteer who:

    - Uses a short-acting rescue inhaler (typically a beta 2 agonist) daily, or
    - Uses high dose inhaled corticosteroids, or
    - In the past year has either of the following:
        - Greater than 1 exacerbation of symptoms treated with oral/parenteral corticosteroids;
        - Needed emergency care, urgent care, hospitalization, or intubation for asthma.

24. **Diabetes mellitus** type 1 or type 2, including cases controlled with diet alone. (Not excluded: history of isolated gestational diabetes.)

25. **Thyroidectomy, or thyroid disease** requiring medication during the last 12 months

26. **Bleeding disorder** diagnosed by a doctor (eg, factor deficiency, coagulopathy, or platelet disorder requiring special precautions)

27. **Malignancy** (Not excluded: Volunteer who has had malignancy excised surgically and who, in the investigator's estimation, has a reasonable assurance of sustained cure. or who is unlikely to experience recurrence of malignancy during the period of the study)

28. **Seizure disorder:** History of seizure(s) within past 3 years. Also exclude if volunteer has used medications in order to prevent or treat seizure(s) at any time within the past 3 years.

29. **Asplenia**: any condition resulting in the absence of a functional spleen

30. History of hereditary **angioedema**, acquired angioedema, or idiopathic angioedema.

6.3 Participant departure from vaccination schedule or withdrawal

This section concerns an individual participant's departure from the vaccination schedule. Pause rules for the trial as a whole are described in Section 10.11.

6.3.1 Delaying vaccinations for a participant

Under certain circumstances, a participant's scheduled vaccination will be delayed. The factors to be considered in such a decision include but are not limited to the following:

- Within 45 days prior to any study injection
    - Receipt of blood products or immunoglobulin
- Within 30 days prior to any study injection
    - Receipt of live attenuated vaccines other than influenza vaccine
    - Receipt of allergy treatment with antigen injections
    - Receipt of systemic steroids < 2 mg/kg
- Within 14 days prior to any study injection
    - Receipt of influenza vaccine or any vaccines that are not live attenuated vaccines (eg, pneumococcal)
- Pre-vaccination abnormal vital signs or clinical symptoms that may mask assessment of vaccine reaction.
- Investigation for active TB disease
- Any fever > Grade 1

- Acute illness

Vaccinations should not be administered outside the visit window period specified in the HVTN 602 / AERAS A-042 Study Specific Procedures.

In order to avoid vaccination delays and missed vaccinations, participants who plan to receive licensed vaccines, or allergy treatments, should be counseled to schedule receipt of these substances, when possible, outside the intervals indicated above. The effects of these substances on safety and immunogenicity assessments and their interactions with study vaccines are unknown. Therefore, if circumstances allow, these substances should also be avoided in the 28-day interval between the last study vaccination and completion of the 28-day postvaccination follow-up visit.

6.3.2 Participant departure from vaccination schedule

Every effort should be made to follow the vaccination schedule per the protocol. If a participant misses a vaccination and the visit window period for the vaccination has passed, that vaccination cannot be given. The participant should be asked to continue study visits. The participant should resume the vaccination schedule with the next vaccination unless there are circumstances that require further delay or permanent discontinuation of vaccination (see Sections 6.3.1 and 6.3.3).

6.3.3 Discontinuing vaccination for a participant

Under certain circumstances, an individual participant's vaccinations will be permanently discontinued. Specific events that will result in stopping a participant's vaccination schedule include:

- Co-enrollment in a study with an investigational research agent (rare exceptions allowing for the continuation of vaccinations may be granted with the unanimous consent of the HVTN 602 / AERAS A-042 Protocol Team).

- Clinically significant condition (ie, a condition that affects the immune system -- eg, start of immunosuppressive medication -- or for which continued vaccinations and/or blood draws may pose additional risk), including but not limited to the following:

    - Pregnancy (regardless of outcome);
    - Any vaccine-related SAE;
    - Any severe (≥ Grade 3) local or systemic reactogenicity symptom, lab abnormality, or AE that is subsequently considered to be related to vaccination (excluding local injection site redness, induration, or ulceration that follow the normal expected course of BCG vaccination);
    - Any grade 3 lab abnormality or other clinical AE (exception: fever or vomiting and subjective local and systemic symptoms) that is subsequently considered to be related to vaccination; or
    - Clinically significant type 1 hypersensitivity reaction associated with study vaccination. Consultation with the HVTN 602 / AERAS A-042 Protocol Team is required prior to subsequent vaccinations following any type 1 hypersensitivity reaction associated with study vaccination;

- Investigator determination in consultation with Protocol Team leadership (eg, for repeated nonadherence to study staff instructions).
- Diagnosis of active TB disease
- TST after first vaccination received
- Participant becomes HIV infected

Such participants should be counseled on the importance of continuing with the study and strongly encouraged to participate in follow-up visits and protocol-related procedures per the protocol for the remainder of the trial, unless medically contraindicated.

6.3.4 Participant termination from the study

Under certain circumstances, an individual participant may be terminated from participation in this study. Specific events that will result in early termination include:

- Participant, parent, or legal guardian refuses further participation,
- Participant relocates and remote follow-up is not possible,
- The CRS determines that the participant is lost to follow-up,
- Investigator decides, in consultation with Protocol Team leadership, to terminate participation (eg, if participant exhibits inappropriate behavior toward clinic staff or fails to comply with study requirements).
- Any condition where termination from the study is required by applicable regulations.

7 Study product preparation and administration

The protocol schema is shown in Table 2-1. See the Investigator's Brochures for further information about study products.

The investigator should document the assignment of responsibility for investigational product (IP) management and dose preparation to one primary individual. This person should be a licensed pharmacist, authorized to prepare medication doses per local regulations. This person will be referred to as the study vaccine manager. A Delegation of Authority Log will be maintained by the site and will identify the individual(s) authorized to function in the role of study vaccine manager.

7.1 Vaccine regimen

The schedule of vaccination is shown in Section 2 and additional information is given below.

Group 1

Treatment 1 (T1): H4:IC31 (15 mcg H4/500 nmol IC31®) to be administered IM as 0.5 mL in alternating deltoid at Days 0 and 56.

Group 2

Treatment 2 (T2): H56:IC31 (5 mcg H56/500 nmol IC31®) to be administered IM as 0.5 mL in alternating deltoid at Days 0 and 56.

Group 3

Treatment 3 (T3): BCG (2-8 x 10^5 CFU) to be administered ID as 0.1 mL in either deltoid at Day 0.

Group 4

Control 1 (C1): Sodium chloride 0.9% for injection to be administered IM as 0.5 mL in alternating deltoid at Days 0 and 56.

7.2 Investigational product formulation

7.2.1 H4:IC31

H4:IC31 is an investigational vaccine manufactured by Sanofi Pasteur (Toronto, Canada) and SSI (Copenhagen, Denmark). H4:IC31 has 2 components: the H4 antigen and the IC31 adjuvant. The reconstitution of the vaccine components, H4 antigen (manufactured at Sanofi Pasteur) and IC31 adjuvant (supplied by SSI), will take place at the study clinic.

H4 antigen is composed of a purified recombinant fusion protein. The H4 antigen is presented as a sterile, clear, colorless, or slightly yellow solution in single dose vials. Each 0.5 mL of H4 antigen solution contains 75 mcg of H4 antigen at a concentration of

150 mcg/mL. H4 antigen is formulated in a buffer consisting of 10 mmol/L Tris-HCl, pH 8.3. The presentation is 0.20 mL/vial (label claim) with an actual fill volume of 0.50 mL ± 0.05 mL. The antigen will be mixed further with adjuvant prior to injection (reconstitution).

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

H4 antigen and IC31 adjuvant must be stored at less than -15°C.

7.2.2 H56:IC31

H56:IC31 is an investigational vaccine manufactured by SSI (Copenhagen, Denmark). H56:IC31 has 2 components: the H56 antigen and the IC31 adjuvant.

H56:IC31 is formulated in a buffer consisting of 4 mM Glycine, 8 mM Tris and 135 mM NaCl (the vaccine is formulated using 1 part H56 concentrated bulk formulated in 20 mM glycine and 4 parts IC31 1.25 x concentration formulated in 10 mM Tris and 168.75 mM NaCl). The H56:IC31 vial contains 0.8 mL of greyish/colorless final formulated vaccine with an extractable volume >0.5 mL.

H56:IC31 must be stored between +2°C and +8°C.

7.2.3 BCG

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

BCG must be stored between +2°C and +8°C.

7.2.4 Control Product (Placebo)

Sodium chloride 0.9% for injection (control) will be supplied by Aeras in sterile vials. A separate vial will be used for each participant in order to ensure sterility. The sodium chloride 0.9% for injection must be stored at controlled room temperature.

7.3 Preparation of study products

Complete preparation, storage and disposition instructions will be provided in the study Vaccine Management Manual (VMM), provided under separate cover.

7.3.1 H4:IC31

A maximum 2-hour time period is allowed between the time the H4:IC31 is reconstituted and administered.

To ensure that the H4:IC31 has been administered within the 2-hour timeframe, the study vaccine manager and the clinic staff will need to coordinate their efforts to administer the H4:IC31 soon after it has been drawn into the syringe and within the 2-hour period.

Refer to the most recent version of the VMM for detailed instructions regarding H4:IC31 preparation.

H4:IC31 must be maintained at room temperature (as specified in the VMM) during transport from the pharmacy to the clinic.

7.3.2 H56:IC31

To ensure the study blind is maintained, the same 2 hour window for IP administration should be used as noted in the instructions for preparation of H4:IC31

Refer to the most recent version of the VMM for detailed instructions regarding H56:IC31 preparation.

H56:IC31 must be maintained at room temperature during transport from the pharmacy to the clinic.

7.3.3 BCG

A maximum 4-hour time period is allowed between the time the BCG is reconstituted and administered.

Refer to the package insert and the most recent version of the Vaccine Management Manual for detailed instructions regarding BCG preparation.

Refer to the most recent version of the VMM for additional instructions regarding BCG preparation.

BCG must be maintained at room temperature during transport from the pharmacy to the clinic.

7.3.4 Control

To ensure the study blind is maintained, the same 2 hour window for IP administration should be used as noted in the instructions for preparation of H4:IC31

Refer to the most recent version of the VMM for detailed instructions regarding placebo preparation

The sodium chloride 0.9% for injection (placebo) must be maintained at room temperature during transport from the pharmacy to the clinic.

7.4 Administration

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

7.4.1 BCG

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

7.4.2 H4:IC31, H56:IC31 and Placebo

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

The study vaccine manager will send the vaccines and placebo to the clinic as unit-dose syringes, which will be identified with the subject identification number (as detailed in the VMM), date and time of dose preparation, and the volume prepared. The date and time of expiration will be recorded on the IP Request Form. A medically qualified study team member must be present in the clinic at the time of all study injection administrations.

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

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

The study vaccine manager will refer to the VMM (provided under separate cover) for detailed instructions for vaccine and placebo storage and preparation.

7.5 Acquisition of study products

7.5.1 H4:IC31

H4:IC31 is manufactured by Sanofi Pasteur (Toronto, Canada) and SSI (Copenhagen, Denmark). The H4 antigen is manufactured and provided by Sanofi Pasteur and the IC31 adjuvant is manufactured and supplied by SSI.

7.5.2 H56:IC31

H56:IC31 is manufactured and supplied by SSI (Copenhagen, Denmark).

7.5.3 BCG

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

7.5.4 Control

The sodium chloride 0.9% for injection (control) will be supplied in sterile vials. A separate vial will be used for each participant in order to ensure sterility.

7.5.5 Syringe Overlays

Translucent colored labels to obscure differences in color and opacity between the products will be supplied by Aeras.

7.6 Supplies

Adequate quantities of the investigational products H4:IC31, H56:IC31and placebo (sodium chloride, 0.9% for injection) will be provided to the CRS. Supplies will be received in multiple shipments over the course of the trial.

- Aeras will notify the study vaccine manager of the pending shipments of H4 antigen from Sanofi Pasteur in Toronto, Canada, the IC31 adjuvant and H56:IC31 solution from SSI in Copenhagen, Denmark and the placebo (0.9% sodium chloride injection) from Aeras in Rockville, MD.

- H4 antigen and IC31 adjuvant will be shipped frozen on dry ice (-65°C or colder) with a temperature monitoring device.

- H56:IC31 will be shipped **cold (2 - 8°C)** with a temperature monitoring device.

- Placebo (sodium chloride, 0.9% for injection) will be shipped at ambient temperature with a temperature monitoring device.

The clinical site is responsible for sourcing adequate quantities of BCG vaccine.

7.7 Receipt and Storage

Upon receipt of study vaccine supplies, the study vaccine manager must immediately inspect all vials for damage. Supplies 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.

The investigational products must be stored protected from light at less than -15ºC for H4 and IC31, between +2ºC and +8ºC for H56:IC31 and BCG and at controlled room temperature for sodium chloride 0.9% for injection (control), in a secured location with no access for unauthorized personnel.

Complete storage instructions will be provided in the study VMM.

7.8 Pharmacy records

The study vaccine manager is required to maintain complete records of all study products. The pharmacist of record is responsible for maintaining randomization codes and randomization confirmation notices for each participant in a secure manner.

7.9 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

7.10 Final disposition of study products

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

8 Clinical procedures

The schedule of clinical procedures is shown in Appendix M.

8.1 Informed assent for adolescents, and Informed consent for parents and participants who turn 18 on study

A parent or legal guardian is required to provide the appropriate informed consent for participants under the age of 18 years. Therefore, both participant assent and parental/legal guardian consent is required for adolescent participation in this clinical research study. Any participant who turns 18 years old while on study will be consented as an adult at the next scheduled study visit.

The CRS staff will obtain informed assent/consent of participants and parent/legal guardians according to HVTN policies and procedures.

The informed assent/consent process continues throughout the study. Key study concepts should be reviewed periodically with the participant (and his/her parent/legal guardian or other responsible adult, if present) and the review should be documented. At each study visit, the CRS staff should consider reviewing the procedures and requirements for that visit and for the remaining visits. Additionally, if any new information is learned that might affect the participants' and parent/legal guardians' decisions to stay in the trial, this information will be shared with trial participants and their parent/legal guardian. If necessary, participants and parents/legal guardians will be asked to sign revised informed assent/consent forms.

The CRS may employ recruitment efforts prior to the participant assenting and the parent/guardian consenting. Participants and their parent/legal guardians must sign a protocol-specific assent/consent before any protocol specific procedures are performed.

8.1.1 Screening consent form

Without a general screening consent, screening for a specific study cannot take place until the site receives written approval from the sponsor to begin screening.

Screening of the first participant cannot occur until all site initiation procedures, regulatory requirements and sponsor approval have been completed.

8.1.2 Protocol-specific consent and assent forms

The sample protocol-specific consent/assent forms are included in this protocol as follows:

- Consent form for the study, to be signed by the parent/guardian, Appendix A.
- Assent form for the study, Appendix E
- Consent form for the study, to be signed by a participant who turns 18 on study, Appendix G

- Consent form for other uses of specimens, to be signed by the parent/guardian, Appendix C

- Assent form for other uses of specimens, Appendix H

- Consent form for other uses of specimens, to be signed by a participant who turns 18 on study, Appendix I

8.1.3 Assessment of Understanding

Study staff are responsible for ensuring that participants fully understand the study before enrolling them. This process involves reviewing the informed assent form with the participant, allowing time for the participant to reflect on the procedures and issues presented, and answering all questions completely.

An Assessment of Understanding is used to document the participant's understanding of key concepts in this TB vaccine trial. The participant must complete the Assessment of Understanding before enrollment. Staff may provide assistance in reading and understanding the questions and responses, if necessary. Participants must verbalize understanding of all questions answered incorrectly. This process and the participant's understanding of the key concepts should be recorded in source documentation at the site.

EC and any applicable Regulatory Entity (RE) may require that a participant has signed either a screening or protocol-specific assent document prior to administering the Assessment of Understanding. The assent process (including the use of the Assessment of Understanding) should be explained thoroughly to the EC and any applicable RE, whose recommendations should be followed.

8.2 Pre-randomization procedures

After informed consent/assent is obtained, participants will be screened to assess eligibility for the study. For identification purposes each participant will be assigned a unique 10-digit participant number by an interactive voice/web response system (IVRS/IWRS) that consists of a 3-digit study number, a 2-digit site number assigned by Aeras, followed by a 5-digit number sequentially assigned by the system. (For example, the first subject enrolled on the A-042 study at site 14 would receive the number 042-14-00001, where all except "-00001" were pre-assigned by Aeras.) This participant number will be used throughout the study.

Eligibility for entry into the study will be based on the inclusion and exclusion criteria described in Section 6.1. The investigator must document confirmation of eligibility prior to randomization/study entry.

Screening may occur over the course of several contacts/visits, up to and including before randomization. All inclusion and exclusion criteria must be assessed within 28 days before enrollment, unless otherwise specified in the eligibility criteria (or below in this section).

After the appropriate informed consent and assent have been obtained and before randomization, the following procedures are performed:

- Medical history, documented in the case history record;
- Complete physical examination, including height, weight, vital signs, and clinical assessments of head, ears, eyes, nose, and throat; neck; lymph nodes; heart; chest; abdomen; extremities; neurological function; and skin (including BCG scar);
- Assessment of concomitant medications the volunteer is taking, including prescription and nonprescription drugs, vitamins, topical products, alternative/complementary medicines (eg, herbal and health food supplements), recreational drugs, vaccinations, and allergy shots (record the complete generic name for all medications);
- Assessment of whether the volunteer is at low risk for HIV infection;
- Counseling on HIV testing and risk reduction, as described in Section 8.6.1;
- Laboratory tests as defined in the inclusion and exclusion criteria, including:
    - Urinalysis
    - Complete blood count (CBC) with differential, platelets
    - Serum chemistry (including ALT, AST, alkaline phosphatase (ALP) and creatinine)
    - HIV-1 and 2 blood test
    - Urine and/or serum β-HCG pregnancy test, for volunteers who are female
- Other laboratory tests:
    - QFT-GIT
    - As deemed appropriate to exclude active TB according to local practices, following guidelines for the diagnosis of TB in children from the WHO Guidance for national tuberculosis programs on the management of tuberculosis in children (2006). Local practices utilize a symptoms-based approach according to the South African childhood tuberculosis guidelines (available at http://reference.sabinet.co.za/webx/access/electronic_journals/mp_sajei/mp_sajei_v24_n3_a5.pdf), which are based on the WHO 2006 guidance.
- Obtaining of volunteer demographics in compliance with the NIH Policy on Reporting Race and Ethnicity Data: Subjects in Clinical Research, Aug. 8, 2001 (available at http://grants.nih.gov/grants/guide/notice-files/NOT-OD-01-053.html); and
- Discussion of pregnancy prevention. A pregnant or breastfeeding person may not be enrolled in this trial. Specific criteria and assessment of contraception and pregnancy status are described in study inclusion criteria. Discussion of pregnancy prevention includes advising a participant who was born female and who reports no current sexual activity that could lead to that participant becoming pregnant to have a plan to begin adequate birth control. This plan

would be put to use if, during the study, the participant becomes sexually active in a way that could lead to that participant becoming pregnant.

8.3 Enrollment and vaccination visits

Enrollment is simultaneous with first vaccination. The time interval between randomization and enrollment should not exceed 4 working days. The CRS randomizes the participant via the Interactive Voice/Web Response System (IVRS/IWRS), and receives the randomization assignment.

At all vaccination visits, the following procedures are performed before vaccination:

- Abbreviated physical examination, including weight, vital signs, and a symptom-directed evaluation by history and/or appropriate physical exam based on participant self-reported symptoms or complaints;
- Assessment of baseline reactogenicity parameters;
- Assessment of concomitant medications (as described in Section 8.2);
- Assessment of any new or unresolved AEs/intercurrent illnesses; and
- Urine or serum pregnancy test (for participants who were born female).
- Specimen collection (including optional stool collections)

Following completion of all procedures in the preceding list, and if results indicate that vaccination may proceed, vaccination is prepared and administered (see Sections 7.3 and 7.4).

Immediately following vaccination, the participant remains in the clinic under close observation. An initial reactogenicity assessment is made at least 30 minutes after injection. Before leaving the clinic, the participant has vital signs taken and is given the diary card and is instructed on how to complete it. The site will make arrangements to obtain daily reports of reactogenicity events from the participant during the reactogenicity period (as described in Section 8.7).

The following procedures will be performed at all vaccination visits. These procedures may be performed prior to or following vaccination:

- HIV and TB risk reduction counseling (as described in Section 8.6.1 and 8.6.2);
- Pregnancy prevention assessment (as described in Section 6.1 and 8.2); and
- Assessment of new or unresolved social impacts (site staff will ask participant about the status of any unresolved social impacts and if s/he has experienced any new social impacts as a result of the trial participation).
- Additional procedures will be performed at scheduled visits as specified in Appendix M:

- Confirm that participants received HIV test results from previous visit. If not, provide test results and post-test counseling as appropriate; and

- Specimen collection (should be completed prior to vaccination)

8.4 Participant Diary and Daily Temperature Monitoring

Participants/parents or legal guardians of participants will receive, and be instructed in, the operation of a daily adverse event diary and a digital thermometer to be used during the specified postvaccination diary period after vaccine administration. 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 (who is medically qualified and is a study team member) at which time any clinical details required for complete understanding of the information recorded will be obtained. If possible, diaries not brought to the scheduled visit should be obtained before adverse event assessment can be performed and events discussed with the principal investigator or designee. If a diary is lost, the principal investigator or designee will discuss the occurrence of any solicited adverse events with the participant/parent or legal guardian and document the discussion. Information on the diary card will not be directly recorded onto eCRFs. The diary card will be considered source documentation and adverse event information obtained from the diary card will be recorded and completely assessed on the adverse event eCRF. Body temperatures below 38°C will not be considered fevers.

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

8.5 Participant Follow-up and Contact

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

Participants/parents or legal guardians will be instructed to contact a study team member to report new diagnoses or new or worsening adverse events and will be referred for medical attention as applicable. 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.

During each clinic visit, participants/parents or legal guardians will be reminded to notify a study team member of the following:

- The occurrence of AEs and SAEs during the respective reporting periods

- Receipt of any concomitant medications during the applicable reporting period

- Plans to move or if contact information changes

- If participant/parent or legal guardian has decided to withdraw from the study

- Change in general health status
- Any other change in status that may affect the participant's participation (eg, plan to participate in another investigational study)

All participant-level deviations from protocol procedures, evaluations, and/or visits must be documented according to eCRF completion guidelines. When possible, missed visits and procedures must be rescheduled and performed at the nearest possible timepoint to the original schedule.

8.6 Follow-up visits

The following procedures are performed at scheduled follow-up visits as indicated Appendix M:

- Risk reduction counseling (as described in Section 8.6.1);
- Pregnancy prevention assessment (as described in Section 6.1 and 8.2);
- Assessment of new or unresolved social impacts (site staff will ask participant about the status of any unresolved social impacts and if s/he has experienced any new social impacts as a result of the trial participation);
- Assessment of new or continuing concomitant medications (as described in Section 8.2);
- Assessment of new or unresolved AEs/intercurrent illnesses.
- TB exposure/infection assessment: symptoms, contacts, etc.
- Additional procedures will be performed at scheduled follow-up visits as specified in Appendix M:
- Administration of the social impact assessment questionnaire (types of impacts assessed involve personal relationships, health insurance, life insurance, educational or employment opportunities, housing, immigration, or travel);
- HIV infection assessment including pre-test counseling. A subsequent follow-up contact is conducted to provide post-test counseling and to report results to participant;
- Confirm that participants received HIV test results from previous visit. If not, provide test results and post-test counseling as appropriate;
- Abbreviated physical examination including weight, vital signs, and a symptom-directed evaluation by history and/or appropriate physical exam based on participant self-reported symptoms or complaints;
- Complete physical examination, including weight, vital signs, and clinical assessments of head, ears, eyes, nose, and throat; neck; lymph nodes; heart; chest; abdomen; extremities; neurological function; and skin;

- Specimen collection;
- Clinical laboratory tests including:
    - CBC with differential and platelet count
    - Chemistry panel (see Section 8.2)
    - Urine dipstick (urinalysis if appropriate; see Section 8.2)
    - Urine or serum pregnancy test (for participants who were born female). Persons who are NOT of reproductive potential due to having undergone total hysterectomy with bilateral oophorectomy (verified by medical records), are not required to undergo pregnancy testing.

8.6.1 HIV counseling and testing

Participants will be counseled routinely during the trial on the avoidance of HIV infection.

Potential participants identified as being HIV infected during screening are not enrolled. Potential and enrolled participants identified as HIV infected will be referred for medical treatment, counseling, and management of the HIV infection. Enrolled participants identified as HIV infected may continue on study. These individuals may also be referred to appropriate ongoing clinical trials or observational studies.

8.6.2 TB Counseling and Referral

Participants will be counseled on risk reduction for TB including infection control and prevention, general facts about TB (active vs. latent), signs and symptoms, and exposure. Staff will also counsel participants that they may have a QFT-GIT conversion due to one of the vaccines. All participants who are found to be QFT-GIT positive (including excluded volunteers at screening) will be educated on the symptoms of TB and the benefits of early diagnosis and treatment. Screening for symptoms compatible with TB disease will therefore be performed on all participants who QFT-GIT-convert. Those consistent with TB will be followed with the necessary laboratory and clinical investigations. See Section 8.11 of the protocol for further information about TB evaluation.

If it is suspected that a participant has TB he/she will be referred for further testing, diagnosis and treatment within the National TB Control Program for standard of care treatment. Clinical staff may use their own materials or fact sheets provided by the CDC for TB Counseling and referral. Materials provided to participants must be reviewed and approved by the local EC.

8.6.3 Contraception/sexual activity status

Contraception status is assessed and documented at every scheduled clinic visit for a participant who was born female and who is sexually active in a way that could cause that participant to become pregnant. Prior to enrollment and throughout the study, staff will ask participants to verbally confirm their use of adequate contraceptive methods. A participant who was born female and is sexually active in a way that could cause that participant to become pregnant should be reminded at all scheduled clinic visits of the importance of using contraception and should be referred to specific counseling,

information, and advice as needed. (Specific contraception requirements are listed in Section 6.1). This reminder should be documented in the participant's study record.

8.7 Assessments of reactogenicity

For all participants, baseline assessments are performed before and reactogenicity assessments are performed after each vaccination. All reactogenicity symptoms are followed until resolution and graded according to the Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events (DAIDS AE Grading Table), Version 1.0, December 2004 (Clarification August 2009; Appendix K).

The reactogenicity assessment period is 7 days following each vaccination per the assessment schedule shown in Table 8-1. Participants are instructed to record symptoms using a diary card. The CRS will collect diary cards at the Day 7 visit after each vaccination. Clinic staff will follow new or unresolved reactogenicity symptoms present at Day 7 to resolution. Participants are instructed to contact the clinic for events that arise during the period between vaccination and the next scheduled visit.

Local injection site reactions must be noted, measured, and recorded in the source documents by the study nurse for assessment by the investigator based on the written report. If the local reaction is ≥ Grade 3 (severe) or the observed reaction is out of the ordinary or concerning in any way, the study nurse will call the investigator to assess the participant at the clinic. In addition, in the rare event of local reaction that looks unusual, study staff may photograph the observed reaction (asking the participant's permission — and parent's or legal guardian's permission if needed — and documenting permissions in a chart note).

Reactogenicity events are reported using CRFs that correspond to the time of assessment in Table 8-1. Reactogenicity assessments include assessments of systemic and local symptoms, vaccine-related lesions, and lymph nodes.

Injection site reactions that meet adverse event criteria according the toxicity table in Appendix K will be recorded as adverse events.

Table 8-1 Schedule of reactogenicity assessments

Day	Time	Performed by
0[a]	Baseline: before vaccination	CRS staff
	Early: 25-60 minutes after vaccination	CRS staff
	Between early assessment and 11:59pm day 0	CRS staff or participant
1	Between 12:00am and 11:59pm day 1	CRS staff or participant
2	Between 12:00am and 11:59pm day 2	CRS staff or participant
3	Between 12:00am and 11:59pm day 3	CRS staff or participant
7[b]	assessment at clinic visit day 7	CRS staff or participant

[a] Day of vaccination

[b] New or unresolved reactogenicity symptoms present on day 7 are followed until resolution

8.7.1 Assessment of systemic and local symptoms

Systemic symptoms include pyrexia; myalgia; arthralgia; fatigue; headache; anorexia; hives; and chills. Local symptoms include pain proximal to the injection site, redness, and

swelling at site of injection. The daily maximum severity reached for each symptom during the assessment period is reported.

Body temperature is measured by axillary or infrared thermometry and reported in degrees Celsius. A measurement is taken once daily during the assessment period and should be repeated if participant is feeling feverish.

8.7.2 Assessment of injection site

Typical injection site reactions are erythema/induration/swelling/edema. The maximum horizontal and maximum vertical measurements for all injection site reactions will be collected at each visit.

Solicited adverse events of local injection site reactions (ie, pain, erythema, swelling) will be considered causally related to study injection (adverse reaction).

All injection site reactions are monitored until resolution. Areas greater than 25 cm^2 are followed daily; otherwise, the frequency of follow-up is based on clinician judgment.

8.7.3 Assessment of lymph nodes

This assessment is required only when reactogenicity assessments are performed by CRS staff, not by the participant.

Only the proximally draining lymph nodes are assessed (eg, axillary nodes on the same side of the body for injections given in the deltoid). Lymph nodes are first evaluated for enlargement and tenderness. If they are found to be enlarged, measurements are taken to determine the size (widest diameter) of the enlarged node(s).

8.8 Visit windows and missed visits

Visit windows are defined in HVTN 602 / AERAS A-042 Study Specific Procedures. For a visit not performed within the protocol-specified window period, the CRS staff will complete regular visit CRFs and protocol deviation form as required. If the missed visit is one that required safety assessments or local safety labs, CRS staff should attempt to bring the participant in for an interim visit as soon as possible.

Procedures performed at an interim visit are usually toxicity/safety assessments (including local safety labs) and HIV testing. With the exception of HIV testing, these procedures are performed only if they were required at the missed visit or if clinically indicated. HIV testing may be performed as deemed appropriate by the study staff.

If a missed visit required vaccination, please refer to Section 6.3.2 and Section 6.3.3 for resolution.

8.9 Early termination visit

In the event of early participant termination, site staff should consider if the following assessments are appropriate: a final physical examination, clinical laboratory tests

(including urine dipstick, CBC with differential, platelet count, and chemistry panel), and immunogenicity including QFT-GIT.

8.10 Follow-up of participants who become pregnant

If a participant becomes pregnant during the study, she will not receive any further doses of study vaccine, but 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 medical monitor of the pregnancy immediately (even if already known to have resulted in spontaneous or elective abortion) by completing the electronic Pregnancy Notification Form in the EDC system. At a minimum, the estimated date of conception, the estimated due date, and the date the participant received the study vaccine should be provided.

If a participant becomes pregnant, she will not have any interventions done as normally mandated by the protocol. The participant 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 medical monitor after delivery, using an electronic Pregnancy Notification Form. If delivery occurs before the final study visit, the participant 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 participant 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 a serious adverse event.

A congenital anomaly or birth defect (ie, an adverse finding in a child or fetus of a participant 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 participant became pregnant during the study, the participant should notify the investigator. The pregnancy must be reported to the medical monitor and the status of the mother and child after delivery will be obtained and reported, when possible.

8.11 Tuberculosis (TB) case evaluation

Participants who develop TB will be evaluated according to standard of care. Local practices utilize a symptoms-based approach according to the South African childhood tuberculosis guidelines (available at http://reference.sabinet.co.za/webx/access/electronic_journals/mp_sajei/mp_sajei_v24_n3_a5.pdf), which are based on the WHO 2006 guidance.

The currently practiced local diagnostic approach consists of the following:

- Persistent, non-remitting cough or wheeze for >2 weeks (not responding to broad-spectrum antibiotic therapy);
- Documented loss of weight or failure to thrive during the past 3 months (excluding other common causes)
- Fatigue or reduced activity
- Persistent fever >2 weeks
- A painless enlarged mass of matted lymph nodes (>2x2 cm) in the neck (without a visible local cause on the scalp or response to a course of antibiotics)

If >2 symptoms are found then the adolescent will be considered a "TB suspect." The principal investigator or designated investigator will refer these patients to the national TB program for care.

QFT-GIT conversion is known to be associated with increased risk of active TB disease. Although most participants who convert in this study may do so as a result of the H56 vaccine, symptom screening for early diagnosis and treatment of TB disease will be a safety priority, and all participants who are found to be QFT-GIT positive (including excluded volunteers at screening) will be educated on the symptoms of TB and the benefits of early diagnosis and treatment. Screening for symptoms compatible with TB disease will therefore be performed on all participants who QFT-GIT-convert. Those consistent with TB will be followed with the necessary laboratory and clinical investigations.

The QFT-GIT is not currently part of TB diagnostic procedures in South Africa, nonetheless, participants will be instructed to contact the site in the event that they test positive for QFT-GIT in the future so that they may be assessed and counselled for TB risk, as described above.

9 Laboratory

9.1 CRS laboratory procedures

The HVTN Site Lab Reference Manual provides further guidelines for operational issues concerning the clinical and processing laboratories. The manual includes guidelines for general specimen collection, special considerations for phlebotomy, specimen labeling, whole blood processing, TB screening/diagnostic testing, and general screening and safety testing.

Tube types for blood collection are specified in Appendix L. For tests performed locally, the local lab may assign appropriate tube types.

In specific situations, the blood collection tubes may be redirected to another laboratory or may require study-specific processing techniques. In these cases, laboratory special instructions will be posted on the protocol-specific section of the HVTN website.

9.2 Total blood volume

Required blood volumes per visit are shown in Appendix L. The FHCRC laboratory will further specify the tube type and collection volumes in special instructions posted to the protocol-specific section of the HVTN website. Not shown is any additional blood volume that would be required if a safety lab needs to be repeated, or if a serum pregnancy test needs to be performed. The additional blood volume would likely be minimal.

The blood volumes that will be collected at each timepoint reflect the minimum sample necessary for the endpoint assays. In accordance with recommended guidelines, the amount of blood collected from each participant will not exceed 4 mL/kg over any 8-week period [32]. To ensure that we do not exceed this recommendation, but still meet the assay blood volume requirements, we will not enroll any participant weighing less than 40 kg.

9.3 Primary immunogenicity timepoints

The primary immunogenicity timepoints in this study for Groups 1, 2 and 4 are at visits 6 and 10 (day 14 and 70) (ie, 2 weeks after the first and second (last) vaccination visit). The primary immunogenicity timepoint in this study for Group 3 is at visits 7 and 10 (day 28 and 70) (ie, 4 weeks after vaccination and 2.5 months after vaccination). Endpoint assays for humoral and cellular responses are performed on participants at the primary immunogenicity timepoints and will be performed at baseline. Depending on the number of responders observed, assays for humoral and cellular responses may be performed on participants at other timepoints; the schedule is shown in Appendix L.

9.4 Endpoint assays: adaptive cellular responses

9.4.1 Flow cytometry

Flow cytometry will be used to examine vaccine-specific CD4+ and CD8+ T-cell responses following stimulation of PBMCs with synthetic TB peptides that span the proteins encoded by the vaccine construct. Assessment of TH1, TH2, and TH17 subsets will likely be included. Other functional markers and markers to assess regulatory T cells may also be included. Data will be reported as percentages of CD4+ or CD8+ T cells responding to a specific peptide pool. Analysis may also include polyfunctional T cell function.

9.4.2 RNA expression in stimulated PBMC

RNA expression from stimulated PBMC will be evaluated using RNA sequencing (RNAseq) or microarray to determine which expression profiles are associated with antigen-specific T-cell responses. Changes in gene expression signatures will be evaluated in longitudinally collected blood samples from vaccine recipients and compared to placebo recipients. The transcriptional profiles will be correlated with antigen-specific T-cell responses measured by ICS. The antigens to use for the stimulation will be chosen based on results from the ICS. The time period for stimulation will be chosen based on optimization experiments.

9.4.3 Single cell gene expression (Fluidigm)

Single cell transcriptomics can reveal transcriptional networks and candidate biomarkers that can be missed in bulk specimen analyses. Using a Fluidigm platform, targeted gene expression may be evaluated on the single cell level utilizing PBMC specimens. The Fluidigm platform enables analysis of 96 genes for each plate and up to 96 individual cells for each plate. Individual antigen-specific T cells can be sorted by flow cytometry. For CD4+ T cells, the most likely T cell induced by these vaccine candidates, PBMC will be stimulated with antigen (chosen based on the ICS results), and the cells will be sorted based on up-regulation of CD40L (CD154). The choice of which 96 genes to examine will be based on results of the stimulated PBMC RNA expression.

9.4.4 Non-classical T-cell responses

This assay enumerates and characterizes polyclonal CD1-restricted T cells. The assay uses K562 cells, which are a human myelogenous leukemic cell line that does not express CD1 and expresses very low levels of MHC Class I and Class II. When stably transfected with single isoforms of human CD1 proteins, these cells are capable of lipid antigen presentation to T-cell clones derived after long-term *in-vitro* culture, and to T cells directly *ex vivo*. Briefly, cryopreserved PBMC are thawed, washed, and rested overnight. At the same time, K562 cells (mock or stably transfected with CD1) are plated separately and loaded with lipid antigen. The following day, PBMC are harvested, counted, and added to wells with lipid loaded K562 cells at a ratio of 5:1. As a positive control, PBMC that have been spiked with a specific T-cell clone are included. The inclusion of this control in every batch of processed samples will account for variation in CD1 expression on K562 cells, stability of the lipid, processing and presentation of lipid, and the antibody staining cocktail. Cultures are stimulated, stored at 4 ^{0}C overnight, and stained for ICS with a staining panel similar to the standard PBMC ICS assay. Because mycobacterial cell wall lipids are not part of H4 or H56, only samples from the individuals in the BCG

vaccination arm will be assayed. To start, only 1 or 2 lipid antigens presented by CD1b (mycolic acid and glucose monomycolate) will be profiled. Depending on cell yields, up to a total of 5 lipid antigens may be included.

The MHC Class I-related molecule (MR1) is a non-classical antigen-presenting molecule that presents small molecules, such as metabolites of vitamin B, to T cells. T cells restricted by MR1 are known as mucosal associated invariant T (MAIT) cells and can kill *M. tuberculosis* infected cells in an MR1-dependent manner. Because MAIT cells express a semi-invariant T-cell receptor (TCR), they can be identified by staining with a specific antibody against this TCR segment (Vα7.2). MAIT cells also express high levels of CD161, which has been associated with a subset of CD8+ T cells that are important in mucosal immunity and express IL-17A and IL-22. Thus, MAITs will be quantified in PBMC by first gating on CD3+ events and then staining against Vα7.2 and CD161 to determine whether MAIT cells are expanded after vaccination with BCG [55,56].

9.5 Endpoint assays: adaptive humoral responses

9.5.1 Multiplex antibody assay

Total binding IgG antibodies to TB antigens will be assessed on plasma/serum samples from study participants taken at the primary immunogenicity timepoints and baseline. Specimens from other timepoints as well as other antibody isotypes may also be assayed based on the results of the initial assay.

9.5.2 Binding antibodies by ELISA

Presence of H4 and H56 antigen-specific antibodies in the serum samples will be tested at a single dilution (1:100) in triplicate wells following indirect ELISA method.

9.6 ESAT-6 free IGRA

Because the H56:IC31 vaccine contains ESAT-6, an antigen that is also present in the QFT-GIT assay, an alternative IGRA will be included to assess prevention of infection (IGRA conversion).

9.7 Innate immunity assays

9.7.1 Enumeration and phenotyping of cell populations

Phenotyping of DCs, monocytes, NK cells, B cells, T cells or other leukocytes for lineage, maturation and activation markers may be performed on whole blood soon after collection. Trucount tubes include a known number of counting beads that enable quantification by flow cytometry of these subsets per µl of blood. Alternatively, cryopreserved PBMC from the same blood draw may be examined by flow cytometry for these subsets. Data will be reported as cell concentrations per microliter of blood and as percent of cells positive for each maker at the various timepoints.

9.8 Exploratory studies

Samples may be used for other testing and research related to furthering the understanding of TB immunology or vaccines. In addition, cryopreserved samples may be used to perform additional assays to support standardization and validation of existing or newly developed methods.

9.8.1 PBMC multiplex cytokine secretion assay

Multiplex cytokine secretion assays may be performed to examine cytokines, chemokines, and other immunomodulatory factors that emerge following stimulation of PBMCs with synthetic TB peptides that span the proteins encoded by the vaccine construct at the primary immunogenicity timepoints and baseline. Panel selection for analytes will be based on the analytes established or potential importance as immune correlates or biomarkers of protection from TB infection or disease progression. The assay will either use the multiplex bead array (Luminex) platform or the Meso-Scale Discovery (MSD) platform, depending on the availability of analytes and performance of analytes in the 2 platforms.

9.8.2 Peripheral Tfh phenotyping

Follicular helper T cells (Tfh) cells are thought to play a primary role in the induction of durable antibody responses. It is possible to detect Tfh-like cells in peripheral circulation, referred to as peripheral Tfh (pTfh), and these may be related to the Tfh in germinal centers. We will assess the presence of pTfh at baseline and 7 days post vaccination. Identification of pTfh will be based on expression of CXCR5 and PD-1 on CD4+ T cells, and may include additional markers. For example, assessment of IL-21 as a functional marker may be included.

9.8.3 B cell plasmablasts

Typically, antibody-secreting plasma cells are localized in the bone marrow. But, for a brief period following vaccination, antibody-secreting plasma blasts are detectable in the peripheral circulation. Plasmablasts are characterized by high expression of CD27 and CD38 on CD19+ B cells. Additional markers to characterize these cells may also be included. Phenotyping of B cell plasmablasts will be conducted on PBMC samples collected at baseline and post vaccination to assess the effects of vaccination on the bulk plasmablast population.

9.8.4 Soluble factors in serum or plasma

Multiplex cytokine analysis (Luminex or MSD platforms) and/or enzyme-linked immunosorbent assay (ELISA) may be used to measure soluble cytokines, chemokines, and other immunomodulatory factors in the serum or plasma. Analytes will be selected based on prior innate studies following vaccination and based on factors or particular interest for TB.

9.8.5 Mass cytometry (CyTOF)

Mass cytometry, or CyTOF, is a variation on flow cytometry that enables more cell markers to be analyzed within a single specimen (currently up to 34 parameters, but likely 50 parameters in the near future). Antibodies are labeled with heavy metals and

binding to cells is evaluated via time-of-flight mass spectrometry. Since the different metals and isotopes can be uniquely identified by mass, this technology largely overcomes the limitations of spectral overlap in fluorescence-based flow cytometry. By employing a large number of markers in a single specimen, it is possible to analyze more immune cell subsets and potentially identify cellular populations of interest. The staining panel can include a variety of cell phenotyping markers along with functional markers, and thus is a variation of the ICS assay that can be used to examine immunophenotyping or intracellular cytokine expression. The staining panel can also include various peptide-MHC multimer reagents as another method to identify TB-specific T cells.

9.8.6 NTM assay

Identification of antigens to enable specific detection of environmental non-tuberculous mycobacterial (NTM) responses is currently on-going in a research study with collaborators. This study will likely identify individual epitopes within NTM proteins. Peptides encompassing these epitopes will be synthesized and pooled. These will be used to measure responses from PBMC collected in the current study at baseline using an intracellular cytokine staining flow cytometric assay or IFN-γ ELISpot assay.

9.8.7 Microbiome analysis

Samples of stools that are collected will be shipped to a central laboratory. Specimens are processed to enable nucleic acid sequencing. 16s rRNA sequences will then be determined using pyro-sequencing approaches or other methods.

9.9 Other use of stored specimens

The HVTN stores specimens from all study participants indefinitely, unless a participant requests that specimens be destroyed or if required by EC, or RE.

Other use of specimens is defined as studies not described in the protocol.

This research may relate to TB, vaccines, the immune system, and other diseases. This could include limited genetic testing and, potentially, genome-wide studies. This research is done only to the extent authorized in each study site's informed consent form, or as otherwise authorized under applicable law. Other testing on specimens will occur only after review and approval by the HVTN, the EC of the researcher requesting the specimens, and the CRS's ECs if required.

The protocol sample informed consent and assent forms are written so that the participant and their parent/guardian either explicitly allows or do not allow their samples to be used in other research when they sign the forms. Participants and parents/guardians who initially agree to other use of their samples may rescind their approval once they enter the study; such participants will remain in this study and their samples will only be used for the studies described in this protocol. If a participant or parent/guardian decides against allowing other research using the participant's samples, or at any time rescinds prior approval for such other use, the study site investigator or designee must notify HVTN Regulatory Affairs in writing. In either case, HVTN Regulatory Affairs directs the HVTN Lab Program not to use samples from these participants for such other uses.

CRSs must notify HVTN Regulatory Affairs if institutional or local governmental requirements pose a conflict with or impose restrictions on other use of specimens.

9.10 Biohazard containment

As the transmission of blood-borne pathogens can occur through contact with contaminated needles, blood, and blood products, appropriate precautions will be employed by all personnel in the drawing of blood and shipping and handling of all specimens for this study, as currently recommended by the US Centers for Disease Control (CDC), the NIH, or other applicable agencies.

All dangerous goods materials, including Biological Substances, Category A or Category B, must be transported according to instructions detailed in the International Air Transport Association Dangerous Goods Regulation.

10 Safety monitoring and safety review

10.1 Safety monitoring and oversight

10.1.1 Responsibilities for Ensuring the Safety of Trial Participants

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

10.1.2 Principal Investigator

The principal investigator has a personal responsibility to closely monitor trial participants and an inherent authority to take whatever measures necessary to ensure their safety. The principal investigator has the authority to terminate, suspend or require changes to a clinical trial for safety concerns and may delay 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 participant at significant risk. The principal investigator determines severity and causality with respect to the study vaccine for each adverse event. For blinded studies the principal investigator is blinded, in which case the study vaccine may consist of a placebo, an active control, or the investigational product.

Responsibilities of the principal investigator may be assigned to a designee who is a medically qualified team member; however the accountability for the specific task remains with the principal investigator.

10.1.3 Study Sponsor

The sponsor (Aeras) also has an institutional responsibility to ensure participant safety. This responsibility is vested in the medical monitor and a safety monitoring committee (SMC).

10.1.4 Protocol Team (safety subgroup)

The HVTN 602 / AERAS A-042 Protocol Team (safety subgroup) will consist at a minimum of the following members:

- AERAS sponsor medical monitor,
- DAIDS medical officer representative,
- Protocol chair and co-chair,
- Clinical research scientist,
- Clinical safety specialist,
- AERAS sponsor representative, and

- Study product provider

The clinician members of HVTN 602 / AERAS A-042 Protocol Team (safety subgroup) are responsible for decisions related to participant safety.

The Protocol Team clinic coordinator, project manager, vaccine developer representative, clinical trial manager, and others may also be included in HVTN 602 / AERAS A-042 Protocol Team (safety subgroup) meetings.

The protocol team will review and discuss blinded summaries of safety data, as prepared by the statistician with the Aeras designated CRO data management team, on conference calls at least monthly. Conference calls will also be scheduled as needed in response to any adverse event that requires the immediate attention of the protocol team. Notification of team members will be by e-mail, phone or fax, depending on time differences. Data on accrual and all toxicities will be reviewed

10.1.5 SMC

The Aeras SMC is a multidisciplinary group consisting of biostatisticians, clinicians, and experts in vaccine research that, collectively, has experience in the conduct and monitoring of vaccine trials. Members of the SMC are not directly affiliated with the protocols under review.

The SMC will operate according to its charter. There are no scheduled safety reviews for the SMC in this study.

If study vaccine administration is paused by the Protocol Team or the principal investigator, the SMC will be convened. Based on its review and the protocol stopping rules (Section 10.11) the SMC will make recommendations in the SMC minutes to the sponsor regarding further conduct of the study and further administration of study vaccine. 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. The SMC may unblind any amount of safety information needed to conduct their assessment. All procedures associated with this review, including objectives, data handling, and elements to be included for review will be documented in SMC minutes. The SMC may recommend suspension or resumption of enrollment and study vaccine administration after review of safety data. However, the COG (see Section 10.1.6) will make the final decision to suspend or resume study activities. The protocol team will be notified of the decision of the COG by a written memorandum. The protocol team will then forward the memorandum to the sponsor. The sponsor will be committed to following the decision of the COG. The recommendations of the SMC, along with the COG's decision, will be communicated to the investigators, EC, and the national regulatory authorities. The sponsor or its designee agrees to abide by any directives issued by the national regulatory authorities or the EC.

Study sites will receive SMC summary minutes and are responsible for forwarding them to their EC and any applicable RE.

10.1.6 Collaboration Oversight Group (COG)

The COG is composed of representatives from Aeras, Sanofi Pasteur, SSI, NIH, and HVTN members not involved with conducting the study but responsible for overseeing the collaborations and for making decisions on specific issues.

10.1.7 Institutional Review Boards and Ethics Committees

The EC has institutional responsibility for the safety of participants in clinical trials. The EC has the authority to terminate, suspend or require changes to a clinical trial.

10.1.8 National Regulatory Authority

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

10.1.9 Aeras team roles and responsibilities in safety monitoring

The roles and responsibilities of the Aeras Team review in relation to safety monitoring include:

- Maintaining a central database management system for Aeras clinical data
- Providing reports of clinical data to appropriate groups such as the HVTN 602 / AERAS A-042 Protocol Team and Aeras SMC

10.1.10 Aeras medical monitor roles and responsibilities in safety monitoring

- Ongoing monitoring of clinical data for events that meet the safety pause and HVTN 602 / AERAS A-042 Protocol Team AE review criteria (see Section 10.11);
- Notifying study CRSs and other groups when safety pauses or planned holds are instituted and lifted (see Section 10.11);
- Querying study CRSs for additional information regarding reported clinical data; and
- Providing support to the HVTN 602 / AERAS A-042 Protocol Team.

10.2 Definition of Adverse Events

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

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

Any adverse change from the participant's baseline condition (determined from screening evaluations conducted to confirm study eligibility) that occurs following the administration of the 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 or irritability 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 participant, signs and symptoms noted by the participant/participant's caregiver, concomitant disease with onset or increased severity after study vaccine administration, and changes in laboratory safety parameters occurring after study vaccine administration.

Adverse events will be reported using a recognized medical term or diagnosis that accurately reflects the event. Adverse event evaluations will be reviewed by the principal investigator or by a designated medically qualified practitioner. AE information is to be completed by members of the study team designated in writing by the principal investigator. The onset and resolution dates of the event and action taken in response to the event will be documented.

10.3 Assessing Severity

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 principal investigator or medical qualified designee who is a study team member 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: mild, moderate, or 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 eCRF, Toxicity Table Grade 1 events will be considered mild in severity, Toxicity Table Grade 2 events will be considered moderate in severity, and both Toxicity Table Grade 3 and 4 events will be considered as severe. 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 participant.

- "**Moderate**" events generally have some impact on an individual's normal activities and may require general symptomatic medical intervention by a healthcare professional or by the participant.

- "**Severe**" adverse events may be incapacitating, leading to suspension of normal daily activities, and would generally require more immediate medical evaluation and intervention by a healthcare professional.

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

10.4 Assessing Causal Relationship (Relatedness)

For all adverse events, the investigator will determine a **causal relationship** to the study vaccine without knowledge, of whether H4:IC31, H56:IC31, or placebo, was administered. Note: the BCG study arm is unblinded.

For all serious adverse events, the investigator and the sponsor (the medical monitor) will determine a causal relationship.

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. It is expected that communication and consultation may occur in the assessment of the causality of adverse events.

Causality of all AEs should be assessed by the investigator using the following question:

"Is there a reasonable possibility that the AE may have been caused by the investigational vaccine/product?"

YES (related): There is a reasonable possibility that the vaccine(s) contributed to the AE.

NO (not related): There is no reasonable possibility that the AE is causally related to the administration of the study vaccine(s). There are other, more likely causes and administration of the study vaccine(s) is not suspected to have contributed to the AE.

Every effort should be made by the investigator to determine the existence of any pre-existing conditions (eg, headache, rashes on Study Day 0 with onset prior to study vaccination) that must be taken into consideration when assessing causal relationship of an adverse event. Pre-existing conditions should be recorded in the eCRF as baseline history and substantiated by appropriate source documentation. Intermittent conditions such as headaches or irritability 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.

10.5 Definition of Adverse Reaction

An adverse reaction is an adverse event judged to be related to study vaccine.

10.6 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 medical monitor. If either principal investigator or 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** (ie, 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** (ie, the AE placed the participant at immediate risk of dying. It does not refer to an event which hypothetically may have led to death if it were more severe).

- It required inpatient **hospitalization** or prolonged hospitalization beyond the expected length of stay. Hospitalizations for scheduled treatments and elective medical/surgical procedures related to a pre-existing condition that did not increase in severity or frequency following receipt of study vaccine, are **not** serious by this criterion. Hospitalization is defined as a hospital admission or an emergency room visit for a period greater than 24 hours.

- It resulted in a persistent or significant **disability/incapacity** (ie, substantial reduction of the participant's ability to carry out activities of daily living).

- It resulted in a **congenital anomaly or birth defect** (ie, an adverse finding in a child or fetus of a participant exposed to the study vaccine prior to conception or during pregnancy).

- Other **medically important conditions** that may not result in death, threaten life or require hospitalization (ie, the AE does not meet any of the above serious criteria) may be considered a serious adverse event when, based on appropriate medical judgment, they may jeopardize the participant and require medical or surgical intervention to prevent one of the serious outcomes listed in these criteria (eg, 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.

10.7 Assessing Expectedness of Adverse Events

Expected adverse events are adverse events consistent with the applicable product information provided by the sponsor (the investigator's brochure for an investigational product). The sponsor determines expectedness of related serious adverse events.

10.8 Definition of Suspected Unexpected Serious Adverse Reaction (SUSAR)

When an adverse event is judged to be related to an investigational product, such as H4:IC31 and H56:IC31, to be serious and unexpected, and is in a participant who received active vaccine, it is a SUSAR (suspected unexpected serious adverse reaction) and is subject to expedited reporting.

10.9 Safety reporting

10.9.1 Reporting of serious adverse events

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

Serious adverse events will be assessed for severity, causal relationship to the study vaccine, and expectedness by the investigator and the medical monitor according to their roles (as described in Section 10.1). The onset and resolution dates of the event and medical care taken in response to the event will be documented. If the event has not resolved by the final study visit, it will be documented as "ongoing" on the eCRF, however, follow-up of the SAE must continue until resolved or the condition has stabilized. Information recorded on the eCRF must be substantiated in the source documents.

The SAE eCRF for that event must be completed by the principal investigator, within one business day of the clinical site becoming aware of the event. The SAE eCRF should be completed with all information known at the time. In case the eCRF cannot be completed, the Supplemental SAE Report (paper form) should be completed by the principal investigator, and scanned and emailed, or faxed to the World Wide Safety Center and to the medical monitor.

Fatal or life-threatening serious adverse events that the investigator suspects are related to the study vaccine should be telephoned to the medical monitor immediately upon the investigator's awareness of the event. If the 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 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
- Participant ID number (and initials and date of birth, if available)
- Date participant received study vaccine
- Serious adverse event(s) and date of event onset
- Current status of participant

Aeras will notify the SMC, the manufacturers of H4:IC31 and H56:IC31, and DAIDS of all SUSARs and SAEs within 3 working days of becoming aware of an event and will provide all follow-up information in a timely manner.

10.9.2 Expedited reporting of serious adverse events

Cases of serious adverse events determined to be both related to study vaccine and unexpected should be unblinded. Unblinding for the purpose of expedited reporting will occur based on the regulatory requirements. Such serious adverse events in participants who received active vaccine are SUSARs and are subject to expedited reporting, while such cases in participants who received placebo are not subject to expedited reporting (see Appendix J).

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

10.9.3 Other events requiring immediate reporting

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

- Withdrawal of consent during the study for safety reasons
- Emergency unblinding
- Protocol violation affecting the safety of a participant or involving the vaccination process

- 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)

- In case of pregnancy, the investigator must complete the Pregnancy Notification eCRF within one business day of becoming aware of the event.

10.9.4 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 eCRF.

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

The investigator will continue follow-up on adverse events, including laboratory abnormalities and solicited adverse events, until the event has resolved, is otherwise satisfactorily explained, or the participant completes the study. The resolution date will be recorded on the eCRF as the last date on which the participant experienced the adverse event. If an adverse event resolution date is uncertain the principal investigator should estimate the completion date based on medical judgment and interview of the participant/participant's caregiver. 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 eCRF must be substantiated in the source documents. If an adverse event evolves into a condition that becomes "serious," it will be designated as serious on the AE eCRF and a Supplemental SAE Report (SAER) form will be completed.

Follow-up for serious adverse events must continue until resolution and the outcome reported to Aeras, even if this extends beyond the serious adverse event reporting period (ie, 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.

10.10 Submission of safety forms to SDMC

Sites must submit all safety forms (eg, reactogenicity, adverse experience, urinalysis, local lab results, and concomitant medications) before the end of the next business day after receiving the information. The forms should not be held in anticipation of additional information at a later date. If additional information is received at a later date, the forms should be updated and refaxed before the end of the next business day after receiving the new information.

10.10.1 Toxicity management

The toxicity table in Appendix K will be used for grading the severity of adverse events.

Management of adverse experiences will be according to the best clinical practice and the judgment of the principal investigator. Alternate explanations for clinical and laboratory abnormalities must be sought.

The toxicity management guidelines are for events for which a relationship to study vaccine cannot be excluded. Clinical or laboratory AEs that are definitely unrelated to study vaccine may not result in study vaccine interruption.

10.10.2 Adverse events

The collection periods for adverse events are shown in Table 10-1.

Table 10-1 Adverse event collection periods

Type of Event	Collection Period
Unsolicited adverse events	28 days post each vaccination[a]
Solicited adverse events	7 days post each vaccination (with diary cards to be used for 7 days after each vaccination)
Solicited injection site reaction adverse events	BCG Group: 84 days post vaccination H4:IC31/Placebo Group: 28 days post each vaccination[a] H56:IC31/Placebo Group: 28 days post each vaccination[a]
Serious adverse events, adverse events of special interest, and SUSARs	Entire study period
a. Adverse events will be collected through the end of the study visit window for each adverse event collection period (ie, 28 days after each vaccination + 5-day window = 33 days)	

10.10.3 AE reporting

10.10.3.1 Unsolicited adverse events

Unsolicited adverse events will be collected for 28 days post vaccination (adverse events will be collected through the end of the study visit window for each adverse event collection period [ie, 28 days after each vaccination + 5-day window = 33 days]).

10.10.3.2 Solicited Adverse Events

Solicited adverse events will be collected for 7 days (with diary cards to be used for 7 days after each vaccination) post vaccination.

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

- Injection site reactions
    - Solicited injection site reaction adverse events include pain, erythema, swelling, and axillary lymphadenopathy.
- Systemic adverse events
    - Solicited systemic adverse events include pyrexia, myalgia, arthralgia, fatigue, headache, nausea, diarrhea, and chills.

Solicited adverse events of injection site reactions will be considered causally related to study vaccine.

The solicited adverse event reporting period begins with vaccination. All participants/parents or legal guardians 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.

10.10.3.3 Adverse Events of Special Interest

Adverse events of special interest will be collected throughout the entire study period.

Adverse events of special interest represent a subset of AEs that include autoimmune diseases and other systemic disorders of interest which could potentially have an immune etiology. Adverse events of special interest are listed in Appendix J. The principal investigator should use clinical and scientific judgment in deciding whether other adverse events (ie, events not listed in Appendix J) could have an autoimmune origin and should therefore be reported as AEs of special interest.

10.10.3.4 Serious Adverse Events

Serious adverse events will be collected throughout the entire study period.

10.10.3.5 Concomitant Medications

The collection of information on concomitant medications used by participants following vaccination will coincide with the collection period of adverse events. The collection period for concomitant medications associated with the treatment of AEs will be 28 days following each vaccination. The collection period for concomitant medications associated with the treatment of SAEs and AESIs will be throughout study follow-up.

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 participant's medical history, or be noted as prophylaxis, eg, dietary supplement.

10.11 Pausing and stopping rules

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

10.11.1 Rules for the suspension of the entire study

The following rules will trigger pausing by the principal investigator, Protocol Team or the sponsor of further enrollment and study vaccine administration, and SMC review of unblinded safety data:

One or more SAE(s) judged related to vaccination occurs

OR

Anaphylaxis or bronchospasm within 4 hours of injection, indicative of an immediate hypersensitivity reaction to the study injection

OR

It is determined that a SUSAR occurred

OR

> 15% of participants experience a Grade 3 or higher event judged related to study vaccine*, excluding local injection site reactions that decrease to < Grade 3 within 24 hours

OR

an adverse event pattern of concern occurred

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

If the principal investigator, Protocol Team, or sponsor pauses administration of study vaccine in the study, the decision will be recorded in a memorandum to the study file and will trigger SMC review. If a recommendation to resume study enrollment and study vaccine administration is made, the SMC will record their judgment in a memo with a recommendation to the sponsor (See Section 10.1.5). The SMC memorandum will be forwarded to the principal investigator. The SMC may recommend resumption of enrollment with changes to the protocol if it judges that such changes will eliminate or

greatly reduce the safety risks specified in the stopping rules. However, the final decision to resume study activities or amend the protocol will be made by the COG. The clinical site will be allowed to resume activities upon receipt of written notification from Aeras.

11 Protocol conduct

This protocol and all actions and activities connected with it will be conducted in compliance with the principles of GCP (ICHE6), and consistent with DAIDS, HVTN, and Aeras policies and procedures.

11.1 Social impacts

Participants in this study risk experiencing discrimination or other personal problems, resulting from the study participation itself. The CRS is obliged to provide advocacy for and assistance to participants regarding these negative social impacts associated with the vaccine trial. If CRS staff have questions regarding ways to assist a participant dealing with a social impact, a designated NIAID or HVTN Core representative can be contacted.

11.2 Emergency communication with study participants

As in all clinical research, this study may generate a need to reach participants quickly to avoid imminent harm, or to report study findings that may otherwise concern their health or welfare.

When such communication is needed, the CRS will request that the EC and any applicable RE expedite review of the notification to the participant. If this review cannot be completed in a timeframe consistent with the urgency of the required communication, the site should contact the participant first, and then notify the EC and any applicable RE of the matter as soon as possible.

12 Version history

The Protocol Team may modify the original version of the protocol. Modifications are made to HVTN protocols via clarification memos, letters of amendment, or full protocol amendments. The version history of, and modifications to, Protocol HVTN 602 / AERAS A-042 are described below.

Protocol history and modifications

Date: 9/10/2015

Protocol version: 3.0

Protocol modification: Full Protocol Amendment 2

Item 1 Revised in Section 6.1, Inclusion criteria, Item 20, the acceptable range of ALT, AST, and alkaline phosphatase results for enrollment eligibility

Date: 6/12/2015

Protocol version: 2.0

Protocol modification: Clarification Memo 2

Item 1 Revised in Section 4.2, *Secondary objectives and endpoints*, the second bullet under Secondary endpoints 2

Item 2 Revised in Appendix L, Laboratory procedures, the allocation of 8.5mL of blood drawn at M2.5 for Group 3

Date: 2/24/2015

Protocol version: 2.0

Protocol modification: Full Protocol Amendment 1

Item 1 Changes to Section 2.1, Protocol Team

Item 2 Addition of second optional stool collection to coincide with peak immunogenicity time point

Item 3 Updates number of participants vaccinated to date

Item 4 Removal of Secondary Endpoint #5, whole blood mycobacterial inhibition assay

Item 5 Updated the unbinding procedure to align with current process.

Item 6 Clarification to approved birth control method, 2 methods required

Item 7 Clarified that limited genetic testing is part of the study in sample informed consents Item 2 Addition of second optional stool collection to coincide with peak immunogenicity time point

Item 8 Fixed minor errors where language in informed concent should have referenced the child as the study participant

Item 9 Added language required by University of Cape Town Research Ethics Committee regarding litigation and settlement for study-related injuries

Item 10 Added language to clarify that agreement with specimen use in other studies is not required for participation in study

Item 11 Added language to meet H3Africa guidelines for informed consent documents

Item 12 Multiple inconsistencies, minor changes and typographical errors corrected in Appendix L

Item 13 Multiple inconsistencies, minor changes and typographical errors corrected in Appendix M

Item 14 Corrected typographical errors and minor inconsistencies Item 1 Changes to Section 2.1, Protocol Team

Item 15 Increase screening period from 21 to 28 days

Date: 8/21/2014

Protocol version: 1.0

Original Protocol

13 Document references (other than literature citations)

Other documents referred to in this protocol, and containing information relevant to the conduct of this study, include:

- Assessment of Understanding
- Division of AIDS (DAIDS) Clinical Research Policies and Standard Procedures Documents. Available at http://www3.niaid.nih.gov/research/resources/DAIDSClinRsrch/
- HVTN 602 / AERAS A-042 Study Specific Procedures. Accessible through the HVTN website.
- Vaccine Management Manual
- HVTN Laboratory Manual of Operations. Accessible through the HVTN website.
- Strode, A. & Slack, C. (2013). Selected ethical-legal norms in child and adolescent HIV prevention research: Consent, confidentiality and mandatory reporting [revised]. NIH, Desmond Tutu HIV Foundation (DTHF) and European and Developing Countries Clinical Trials Partnership (EDCTP): 2013.
- Childhood tuberculosis guidelines of the Southern African Society for Paediatric Infectious Diseases. Available at http://reference.sabinet.co.za/webx/access/electronic_journals/mp_sajei/mp_sajei_v24_n3_a5.pdf
- Dangerous Goods Regulations (updated annually), International Air Transport Association. Available for purchase at http://www.iata.org/ps/publications/dgr/Pages/index.aspx.
- International Conference on Harmonisation (ICH) E6 (R1), Guideline for Good Clinical Practice: Section 4.8, Informed consent of trial subjects. Available at http://www.ich.org/fileadmin/Public_Web_Site/ICH_Products/Guidelines/Efficacy/E6_R1/Step4/E6_R1__Guideline.pdf
- NIH Policy on Reporting Race and Ethnicity Data: Subjects in Clinical Research. Available at http://grants1.nih.gov/grants/guide/notice-files/NOT-OD-01-053.html.
- Requirements for Source Documentation in DAIDS Funded and/or Sponsored Clinical Trials. Available at https://phacs.nichdclinicalstudies.org/publicDocs/DAIDS_SourceDocPolicy.pdf
- Title 45, Code of Federal Regulations, Part 46. Available at http://ecfr.gpoaccess.gov/cgi/t/text/text-idx?c=ecfr&sid=2e2429c70115b7df5635f222901ae8f7&rgn=div5&view=text&node=45:1.0.1.1.25&idno=45

See Section 15 for literature cited in the background and statistics sections of this protocol.

14 Acronyms and abbreviations

Ab	antibody
AE	adverse event
Ag85b	antigen 85b
ALT	alanine aminotransferase
ANOVA	analysis of variance
AST	aspartate aminotransferase
AVEG	AIDS Vaccine Evaluation Group
BCG	bacillus Calmette-Guérin
β-HCG	beta human chorionic gonadotropin
BMI	body mass index
CAB	Community Advisory Board
CBC	complete blood count
CDC	US Centers for Disease Control and Prevention
cfu	colony forming units
CFR	Code of Federal Regulations
CIOMS	Council for International Organizations of Medical Sciences
CI	confidence intervals
COG	Collaboration Oversight Committee
CRF	case report form
CRS	clinical research site
EC	Ethics Committee
ELISA	enzyme-linked immunosorbent assay
ELISpot	enzyme-linked immunospot
FHCRC	Fred Hutchinson Cancer Research Center
GCP	Good Clinical Practice
GEE	generalized estimating equation
HIV	human immunodeficiency virus
HVTN	HIV Vaccine Trials Network
IB	Investigator's Brochure
ICH	International Conference on Harmonisation
ICS	intracellular cytokine staining
IFN-γ	interferon gamma
IGRA	interferon-γ release assay
IUD	intrauterine device
mcg	microgram
Mtb	*Mycobacterium tuberculosis*
MAR	missing at random
MMR	measles, mumps, and rubella
NIAID	National Institute of Allergy and Infectious Diseases (US NIH)
NICD	National Institute for Communicable Diseases (Johannesburg, South Africa)
NIH	US National Institutes of Health

ODN1	oligodeoxynucleotide 1
OPV	oral polio vaccine
PBMC	peripheral blood mononuclear cell
PTE	potential T-cell epitope
QFT	QuantiFERON
QFT-GIT	QuantiFERON-TB Gold in-tube
RE	regulatory entity
SATVI	South African Tuberculosis Vaccine Initiative
SAE	serious adverse event
SCHARP	Statistical Center for HIV/AIDS Research and Prevention
SDMC	statistical and data management center
SMC	Safety Monitoring Committee
SSI	Statens Serum Institut
TB	tuberculosis
TST	tuberculin skin test

15 Literature cited

1. WHO Global Tuberculosis Report 2013. **2014**.

2. Dye C, Williams BG. Eliminating human tuberculosis in the twenty-first century. J R Soc Interface **2008**;5:653-62.

3. Abu-Raddad LJ, Sabatelli L, Achterberg JT, Sugimoto JD, Longini IM, Jr., Dye C, Halloran ME. Epidemiological benefits of more-effective tuberculosis vaccines, drugs, and diagnostics. Proc Natl Acad Sci U S A **2009**;106:13980-5.

4. Lawn SD, Bekker LG, Middelkoop K, Myer L, Wood R. Impact of HIV infection on the epidemiology of tuberculosis in a peri-urban community in South Africa: the need for age-specific interventions. Clin Infect Dis **2006**;42:1040-7.

5. Fine PE. The BCG story: lessons from the past and implications for the future. Rev Infect Dis **1989**;11 Suppl 2:S353-S359.

6. Colditz GA, Brewer TF, Berkey CS, Wilson ME, Burdick E, Fineberg HV, Mosteller F. Efficacy of BCG vaccine in the prevention of tuberculosis. Meta-analysis of the published literature. JAMA **1994**;271:698-702.

7. Rodrigues LC, Diwan VK, Wheeler JG. Protective effect of BCG against tuberculous meningitis and miliary tuberculosis: a meta-analysis. Int J Epidemiol **1993**;22:1154-8.

8. Tameris MD, Hatherill M, Landry BS, Scriba TJ, Snowden MA, Lockhart S, Shea JE, McClain JB, Hussey GD, Hanekom WA, Mahomed H, McShane H. Safety and efficacy of MVA85A, a new tuberculosis vaccine, in infants previously vaccinated with BCG: a randomised, placebo-controlled phase 2b trial. Lancet **2013**;381:1021-8.

9. Haynes BF, Gilbert PB, McElrath MJ, Zolla-Pazner S, Tomaras GD, Alam SM, Evans DT, Montefiori DC, Karnasuta C, Sutthent R, Liao HX, DeVico AL, Lewis GK, Williams C, Pinter A, Fong Y, Janes H, DeCamp A, Huang Y, Rao M, Billings E, Karasavvas N, Robb ML, Ngauy V, de Souza MS, Paris R, Ferrari G, Bailer RT, Soderberg KA, Andrews C, Berman PW, Frahm N, De Rosa SC, Alpert MD, Yates NL, Shen X, Koup RA, Pitisuttithum P, Kaewkungwal J, Nitayaphan S, Rerks-Ngarm S, Michael NL, Kim JH. Immune-correlates analysis of an HIV-1 vaccine efficacy trial. N Engl J Med **2012**;366:1275-86.

10. Machingaidze S, Verver S, Mulenga H, Abrahams DA, Hatherill M, Hanekom W, Hussey GD, Mahomed H. Predictive value of recent QuantiFERON conversion for tuberculosis disease in adolescents. Am J Respir Crit Care Med **2012**;186:1051-6.

11. Mahomed H, Hawkridge T, Verver S, Abrahams D, Geiter L, Hatherill M, Ehrlich R, Hanekom WA, Hussey GD. The tuberculin skin test versus QuantiFERON TB Gold(R) in predicting tuberculosis disease in an adolescent cohort study in South Africa. PLoS One **2011**;6:e17984.

12. Billeskov R, Christensen JP, Aagaard C, Andersen P, Dietrich J. Comparing adjuvanted H28 and modified vaccinia virus ankara expressingH28 in a mouse and a non-human primate tuberculosis model. PLoS One **2013**;8:e72185.

13. Hoang T, Aagaard C, Dietrich J, Cassidy JP, Dolganov G, Schoolnik GK, Lundberg CV, Agger EM, Andersen P. ESAT-6 (EsxA) and TB10.4 (EsxH) based vaccines for pre- and post-exposure tuberculosis vaccination. PLoS One **2013**;8:e80579.

14. Brandt L, Feino CJ, Weinreich OA, Chilima B, Hirsch P, Appelberg R, Andersen P. Failure of the Mycobacterium bovis BCG vaccine: some species of environmental mycobacteria block multiplication of BCG and induction of protective immunity to tuberculosis. Infect Immun **2002**;70:672-8.

15. Mazmanian SK, Liu CH, Tzianabos AO, Kasper DL. An immunomodulatory molecule of symbiotic bacteria directs maturation of the host immune system. Cell **2005**;122:107-18.

16. Bjorksten B. Diverse microbial exposure - consequences for vaccine development. Vaccine **2012**;30:4336-40.

17. Eloe-Fadrosh EA, McArthur MA, Seekatz AM, Drabek EF, Rasko DA, Sztein MB, Fraser CM. Impact of oral typhoid vaccination on the human gut microbiota and correlations with s. Typhi-specific immunological responses. PLoS One **2013**;8:e62026.

18. Horwitz MA, Harth G, Dillon BJ, Maslesa-Galic' S. Recombinant bacillus calmette-guerin (BCG) vaccines expressing the Mycobacterium tuberculosis 30-kDa major secretory protein induce greater protective immunity against tuberculosis than conventional BCG vaccines in a highly susceptible animal model. Proc Natl Acad Sci U S A **2000**;97:13853-8.

19. Belisle JT, Vissa VD, Sievert T, Takayama K, Brennan PJ, Besra GS. Role of the major antigen of Mycobacterium tuberculosis in cell wall biogenesis. Science **1997**;276:1420-2.

20. Skjot RL, Oettinger T, Rosenkrands I, Ravn P, Brock I, Jacobsen S, Andersen P. Comparative evaluation of low-molecular-mass proteins from Mycobacterium tuberculosis identifies members of the ESAT-6 family as immunodominant T-cell antigens. Infect Immun **2000**;68:214-20.

21. Skjot RL, Brock I, Arend SM, Munk ME, Theisen M, Ottenhoff TH, Andersen P. Epitope mapping of the immunodominant antigen TB10.4 and the two homologous proteins TB10.3 and TB12.9, which constitute a subfamily of the esat-6 gene family. Infect Immun **2002**;70:5446-53.

22. Kochenderfer JN, Chien CD, Simpson JL, Gress RE. Synergism between CpG-containing oligodeoxynucleotides and IL-2 causes dramatic enhancement of vaccine-elicited CD8+ T cell responses. J Immunol **2006**;177:8860-73.

23. Ravn P, Demissie A, Eguale T, Wondwosson H, Lein D, Amoudy HA, Mustafa AS, Jensen AK, Holm A, Rosenkrands I, Oftung F, Olobo J, von RF, Andersen P.

Human T cell responses to the ESAT-6 antigen from Mycobacterium tuberculosis. J Infect Dis **1999**;179:637-45.

24. Ulrichs T, Munk ME, Mollenkopf H, Behr-Perst S, Colangeli R, Gennaro ML, Kaufmann SH. Differential T cell responses to Mycobacterium tuberculosis ESAT6 in tuberculosis patients and healthy donors. Eur J Immunol **1998**;28:3949-58.

25. Pollock JM, Andersen P. Predominant recognition of the ESAT-6 protein in the first phase of interferon with Mycobacterium bovis in cattle. Infect Immun **1997**;65:2587-92.

26. Brandt L, Oettinger T, Holm A, Andersen AB, Andersen P. Key epitopes on the ESAT-6 antigen recognized in mice during the recall of protective immunity to Mycobacterium tuberculosis. J Immunol **1996**;157:3527-33.

27. Brandt L, Elhay M, Rosenkrands I, Lindblad EB, Andersen P. ESAT-6 subunit vaccination against Mycobacterium tuberculosis. Infect Immun **2000**;68:791-5.

28. Rustad TR, Sherrid AM, Minch KJ, Sherman DR. Hypoxia: a window into Mycobacterium tuberculosis latency. Cell Microbiol **2009**;11:1151-9.

29. Betts JC, Lukey PT, Robb LC, McAdam RA, Duncan K. Evaluation of a nutrient starvation model of Mycobacterium tuberculosis persistence by gene and protein expression profiling. Mol Microbiol **2002**;43:717-31.

30. Muttucumaru DG, Roberts G, Hinds J, Stabler RA, Parish T. Gene expression profile of Mycobacterium tuberculosis in a non-replicating state. Tuberculosis (Edinb) **2004**;84:239-46.

31. Govender L, Abel B, Hughes EJ, Scriba TJ, Kagina BM, de KM, Walzl G, Black G, Rosenkrands I, Hussey GD, Mahomed H, Andersen P, Hanekom WA. Higher human CD4 T cell response to novel Mycobacterium tuberculosis latency associated antigens Rv2660 and Rv2659 in latent infection compared with tuberculosis disease. Vaccine **2010**;29:51-7.

32. Howie SR. Blood sample volumes in child health research: review of safe limits. Bull World Health Organ **2011**;89:46-53.

33. Rerks-Ngarm S, Pitisuttithum P, Nitayaphan S, Kaewkungwal J, Chiu J, Paris R, Premsri N, Namwat C, de SM, Adams E, Benenson M, Gurunathan S, Tartaglia J, McNeil JG, Francis DP, Stablein D, Birx DL, Chunsuttiwat S, Khamboonruang C, Thongcharoen P, Robb ML, Michael NL, Kunasol P, Kim JH. Vaccination with ALVAC and AIDSVAX to prevent HIV-1 infection in Thailand. N Engl J Med **2009**;361:2209-20.

34. Billeskov R, Elvang TT, Andersen PL, Dietrich J. The HyVac4 subunit vaccine efficiently boosts BCG-primed anti-mycobacterial protective immunity. PLoS One **2012**;7:e39909.

35. Dietrich J, Aagaard C, Leah R, Olsen AW, Stryhn A, Doherty TM, Andersen P. Exchanging ESAT6 with TB10.4 in an Ag85B fusion molecule-based tuberculosis

subunit vaccine: efficient protection and ESAT6-based sensitive monitoring of vaccine efficacy. J Immunol **2005**;174:6332-9.

36. Skeiky YA, Dietrich J, Lasco TM, Stagliano K, Dheenadhayalan V, Goetz MA, Cantarero L, Basaraba RJ, Bang P, Kromann I, McMclain JB, Sadoff JC, Andersen P. Non-clinical efficacy and safety of HyVac4:IC31 vaccine administered in a BCG prime-boost regimen. Vaccine **2010**;28:1084-93.

37. Aagaard C, Hoang T, Dietrich J, Cardona PJ, Izzo A, Dolganov G, Schoolnik GK, Cassidy JP, Billeskov R, Andersen P. A multistage tuberculosis vaccine that confers efficient protection before and after exposure. Nat Med **2011**;17:189-94.

38. Lin PL, Dietrich J, Tan E, Abalos RM, Burgos J, Bigbee C, Bigbee M, Milk L, Gideon HP, Rodgers M, Cochran C, Guinn KM, Sherman DR, Klein E, Janssen C, Flynn JL, Andersen P. The multistage vaccine H56 boosts the effects of BCG to protect cynomolgus macaques against active tuberculosis and reactivation of latent Mycobacterium tuberculosis infection. J Clin Invest **2012**;122:303-14.

39. van Dissel JT, Arend SM, Prins C, Bang P, Tingskov PN, Lingnau K, Nouta J, Klein MR, Rosenkrands I, Ottenhoff TH, Kromann I, Doherty TM, Andersen P. Ag85B-ESAT-6 adjuvanted with IC31 promotes strong and long-lived Mycobacterium tuberculosis specific T cell responses in naive human volunteers. Vaccine **2010**;28:3571-81.

40. FitzGerald JM. Management of adverse reactions to bacille Calmette-Guerin vaccine. Clin Infect Dis **2000**;31 Suppl 3:S75-S76.

41. Hoft DF, Leonardi C, Milligan T, Nahass GT, Kemp B, Cook S, Tennant J, Carey M. Clinical reactogenicity of intradermal bacille Calmette-Guerin vaccination. Clin Infect Dis **1999**;28:785-90.

42. Lotte A, Wasz-Hockert O, Poisson N, Dumitrescu N, Verron M, Couvet E. BCG complications. Estimates of the risks among vaccinated subjects and statistical analysis of their main characteristics. Adv Tuberc Res **1984**;21:107-93.

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

44. Pereira SM, Barreto ML, Pilger D, Cruz AA, Sant'Anna C, Hijjar MA, Ichihara MY, Santos AC, Genser B, Rodrigues LC. Effectiveness and cost-effectiveness of first BCG vaccination against tuberculosis in school-age children without previous tuberculin test (BCG-REVAC trial): a cluster-randomised trial. Lancet Infect Dis **2012**;12:300-6.

45. Rodrigues LC, Pereira SM, Cunha SS, Genser B, Ichihara MY, de Brito SC, Hijjar MA, Dourado I, Cruz AA, Sant'Anna C, Bierrenbach AL, Barreto ML. Effect of BCG revaccination on incidence of tuberculosis in school-aged children in Brazil: the BCG-REVAC cluster-randomised trial. Lancet **2005**;366:1290-5.

46. Bottiger M, de VC, Lind A, Beskow R, Linden G, Granath B. A comparative study of Danish (Statens Seruminstitut), Glaxo and Behringwerke vaccines--revaccination of schoolchildren. J Biol Stand **1983**;11:1-12.

47. Agresti A, Coull BA. Approximate is better than "exact" for interval estimation of binomial proportions. Am Stat **1998**;52:119-26.

48. Hudgens MG. Estimating cumulative probabilities from incomplete longitudinal binary responses with application to HIV vaccine trials. Statistics in Medicine **2003**;22:463-79.

49. Hughes JP. Mixed effects models with censored data with application to HIV RNA levels. Biometrics **1999**;55:625-9.

50. Rotnitzky A, Robins J. Analysis of semi-parametric regression models with non-ignorable non-response. Stat Med **1997**;16:81-102.

51. Nakaya HI, Pulendran B. Systems vaccinology: its promise and challenge for HIV vaccine development. Curr Opin HIV AIDS **2012**;7:24-31.

52. Zak DE, Aderem A. Systems biology of innate immunity. Immunol Rev **2009**;227:264-82.

53. Subramanian A, Tamayo P, Mootha VK, Mukherjee S, Ebert BL, Gillette MA, Paulovich A, Pomeroy SL, Golub TR, Lander ES, Mesirov JP. Gene set enrichment analysis: a knowledge-based approach for interpreting genome-wide expression profiles. Proc Natl Acad Sci U S A **2005**;102:15545-50.

54. Chaussabel D, Baldwin N. Democratizing systems immunology with modular transcriptional repertoire analyses. Nat Rev Immunol **2014**;14:271-80.

55. Dusseaux M, Martin E, Serriari N, Peguillet I, Premel V, Louis D, Milder M, Le BL, Soudais C, Treiner E, Lantz O. Human MAIT cells are xenobiotic-resistant, tissue-targeted, CD161hi IL-17-secreting T cells. Blood **2011**;117:1250-9.

56. Martin E, Treiner E, Duban L, Guerri L, Laude H, Toly C, Premel V, Devys A, Moura IC, Tilloy F, Cherif S, Vera G, Latour S, Soudais C, Lantz O. Stepwise development of MAIT cells in mouse and human. PLoS Biol **2009**;7:e54.

Appendix A Sample informed consent form

Title: A randomized, placebo-controlled, partially blinded phase 1b clinical trial to evaluate the safety and immunogenicity of BCG revaccination, H4:IC31, and H56:IC31 in healthy, HIV-1–uninfected adolescent participants

HVTN protocol number: HVTN 602 / AERAS A-042

Site:

Thank you for your interest in our research study. Please read this consent form or ask someone to read it to you. If you decide that you would like your child to join the study, we will ask you to sign this form. We will offer you a copy to keep. We will ask you questions to see if we have explained everything clearly. You can also ask us questions about the study.

We will give your child an assent form with similar information. We will ask him/her to read it or have it read to them. We will discuss the study with them and we will ask them questions to make sure we have explained it clearly to them. Your child can ask us questions about the study. If your child wants to join the study, he/she will sign the assent form. Both you and your child need to agree in order for him/her to be enrolled.

In this study, we may do limited genetic testing on your child's samples. Your child cannot be in this study unless you and your child agree to this. We will tell you more about this in section 13 below. By signing this consent form, you agree to limited genetic testing on your child's samples. Please do not sign this form if you do not agree to this.

About the study

Aeras, in partnership with the HIV Vaccine Trials Network (HVTN) and the Desmond Tutu HIV Center (DTHC) are doing a research study to test tuberculosis (TB) vaccines. Before a vaccine can be used by the public, the vaccine maker must do a lot of research and tests to make sure the vaccine is safe and it works. This study is one of many that are being done to try and make a better TB vaccine that is safe and works.

About 84 people will take part in this study. The researcher in charge of this study at this clinic is Prof. Linda-Gail Bekker. The US National Institutes of Health (NIH), Aeras, Sanofi Pasteur and the Staten Serum Institut (SSI) are paying for the study.

TB is a common infection in South Africa. It is caused by a germ that is spread through the air when an infected person coughs or spits near others. TB causes illness in the lungs. In most cases, TB can be treated successfully.

Most teenagers in South Africa got a TB vaccine as babies. This vaccine, called BCG, stops very bad TB in most babies but it may not protect teenagers or adults very well. A better vaccine to prevent TB is needed.

Research is not the same as treatment or medical care. The purpose of a research study is to answer scientific questions.

1. **We are doing this study to answer several questions.**

    - Are the study vaccines safe to give to young people between 12 and 17 years of age?

    - Are young people able to take the study vaccines without becoming too uncomfortable?

    - How do young people's immune systems respond to the study vaccines? (The immune system protects the body from disease.)

2. **The study vaccines cannot give your child TB.**

    The study vaccines are not made from actual TB. It is impossible for the study vaccines to give your child TB. Also, they cannot cause him/her to give TB to someone else.

3. **Two of the study vaccines are experimental. The third is a licensed vaccine given in an experimental way.**

    When we say that study vaccines are experimental, it means that the study vaccines are not approved by the South African Medicines Control Council (MCC). When we say that a vaccine is given in an experimental way, it means that it has an approved use, but the study will be using it in a way that is not approved by MCC.

    The experimental study vaccines are H4:IC31 and H56:IC31. From now on we will call them the H4 vaccine and the H56 vaccine.

    The H4 vaccine has been given to 198 adults in research studies in Europe and South Africa. It is also being given to 490 babies and teenagers in South Africa in two different studies. As of June 2014, 76 babies and 157 teenagers are enrolled in the studies. The vaccine has not caused serious health problems and people have been able to take it without serious side effects. However, studies with a small number of people do not tell us everything about the safety of the study vaccines. This vaccine is made by Sanofi Pasteur.

    The H56 vaccine has been given to 25 adults in a research study in South Africa. It did not cause serious health problems and people were able to take it without serious side effects. However, studies with a small number of people do not tell us everything about the safety of the study vaccines. Another study which will give the H56 vaccine to 98 more people in South Africa has been started. This vaccine is made by SSI.

    The licensed vaccine given in an experimental way in this study is BCG. It is registered in South Africa for the prevention of TB in children and adults. BCG is commonly given to children shortly after birth, but the vaccine's protection may not last. We know that some teenagers and adults who received the BCG vaccine at birth still get TB. BCG is not approved to be given to a person more than once so its use in this study is experimental. This vaccine is also made by SSI.

    Risks of the study vaccines:

This section lists the side effects we know about. There may be others that we don't yet know about, even serious ones. We will tell you and your child if we learn about any new side effects.

Some people who get these study vaccines may have problems where the needle went into the arm. The problems we have seen in other studies of these vaccines are: mild pain, redness, mild swelling, or a mildly swollen and painful gland, usually in the arm pit.

In the first few days after getting a study vaccine, some people may have symptoms of the flu like fatigue, muscle and/or joint pains, headache and/or fever. They may also experience chills, loose stools and feeling sick. These symptoms usually get better in a few days on their own.

For a short time after getting a study vaccine, some people may have changes in their blood or urine that can only be seen in laboratory tests. These changes usually go away on their own in a few days and rarely cause any problems. A few people may feel dizzy, have a lower heart rate, or have a small increase or decrease in blood pressure for a short time after we take blood from them or give them an injection.

People who get these study vaccines and then have a TB skin test (known also as a Mantoux Test) up to 90 days later may have an allergic reaction to the TB skin test. Their arm may become very swollen, painful, red and irritated. Rarely, a small open sore may develop that takes a while to go away. If your child has a TB skin test done outside this clinic within 90 days of a study injection, they should tell the person giving the TB skin test about this or ask them to call this clinic to learn more. We will also give your child a note to explain this.

People who get the BCG vaccine may have a small bump where the needle went into the upper arm. This may scab over after a few days. A small open sore may develop that may take up to 3 months to go away.

If your child receives the H56 vaccine it is possible that it could interfere with the results of a TB blood test (the Quantiferon test) he or she might have in the future. However, doctors can determine if your child has TB by using other ways such as chest x-rays. We will make sure that participants who have a positive TB blood test or TB symptoms are examined to make sure that they do not have TB. If a doctor determines that your child has TB, we will help your child get the proper care.

Joining the study

4. It is completely up to you and your child whether or not to join the study.

Take your time in deciding. If it helps, talk to people you trust, such as your doctor, friends or family. If you decide that you do not want your child to join this study, or if you change your mind after he/she has joined, your child's other care at this clinic and the benefits or rights he/she would normally have will not be affected.

If your child joins this study, he/she may not be allowed to join other TB vaccine studies now or in the future. Also during the study, your child should not donate blood or tissue.

If your child chooses not to join this study, he/she could join another study if one is available and he/she is able to join.

Site: Remove item 5 if you use a separate screening consent that covers these procedures.

5. If you and your child decide that he/she will join the study, we will screen him/her to see if he/she is eligible.

Screening involves asking questions about your child's medical history and a physical exam. A physical exam includes the following tests:

- checking his/her height, weight, temperature, heart rate, breathing rate and blood pressure
- looking in his/her ears, eyes, mouth and throat
- listening to her/his heart and lungs
- feeling his/her abdomen (stomach and liver), throat and neck

We will also take blood from your child's arm and a urine sample from him/her. We will use this blood and urine to do some tests. These tests will tell us about some key aspects of his/her health. We will ask your child if he/she has ever been exposed to TB. Not everyone who is exposed to TB or is positive for TB develops active disease. We may ask your child to spit into a cup or we may do a chest x-ray to help us learn if your child has active TB. If your child is found to have or is suspected of having TB, he/she will be referred to an appropriate clinic who may then diagnose TB. Your child cannot join this study if he/she has active TB.

We will ask him/her about medicines, traditional treatments or drugs he/she may be taking. We will ask him/her about behaviors that might put him/her at risk for getting HIV and test him/her for HIV. Your child cannot join this study if he/she has HIV. If your child is female, we will test her for pregnancy. Your child cannot join this study if she is pregnant. Your child cannot be in another research study where he/she receives a study product and be enrolled in this study.

We will review the screening results with your child, and offer him/her counseling and referral if he/she needs medical care. We will not pay for this medical care. The screening results may show that your child cannot join the study, even if he/she wants to. We will not tell you the reason unless your child wants us to. If your child is not eligible to join this study, we will still use his/her blood sample in this study. We will use it only to learn more about how one of the screening tests is working. We are doing this for research purposes only, not to learn about your child's health, so we will not give this result to you or your child.

6. If your child is female and could become pregnant, she must agree to use birth control to join this study.

Site: List approved birth control methods here if you do not want to hand out the separate Approved Birth Control Methods sheet (Appendix B).

Your child should not become pregnant during the study because we do not know how the study vaccines could affect the developing baby. Your child must agree to use effective birth control from 20 days before her first injection until her last study visit. We will talk to her about effective birth control methods. They are listed on a handout that we will give to your child. If she joins the study, we will test her urine for pregnancy at some visits, including before each study injection.

If your child becomes pregnant while in the study, she must contact the study doctor immediately. The study doctor will advise your child regarding her and her baby's future medical care.

Being in the study

If your child meets the study requirements and wants to join, here is what will happen:

Site: Insert number of visits and range of visit lengths. (There is site-specific variation in screening protocols and in the number of possible follow-up visits between protocol-mandated visits.)

7. **He/she will come to the clinic for scheduled visits about [#] times over 8 months.**

   Visits can last from [#] to [#] hours.

   Your child may have to come for more visits if he/she has an abnormal test result or health issue.

   We may contact your child after the main study ends (for example, to tell your child about the study results).

8. **We will give your child [Site: Insert compensation] for each study visit he/she completes.**

   This amount is to cover the costs of [Site: Insert text].

   If you come to a study visit with your child, we will give you [Site: Insert compensation] to cover the costs of [Site: Insert text].

   Your child does not have to pay anything to be in this study.

9. **We will give your child one of the study vaccines or a placebo.**

   Not everyone in this study will get one of the study vaccines. Some people will get a placebo, a substance that does not contain a vaccine product. We will compare the results from people who got the placebo with results from people who got the different study vaccines. In this study, the placebo is sterile salt water.

   Your child has a 6-in-7 chance of receiving one of the study vaccines. This means that over 85% of the participants will receive one of the study vaccines.

   Whether your child gets the study vaccines or the placebo is completely random, like flipping a coin.

The clinic staff has no say in whether your child gets the study vaccines or the placebo. The participants who get the BCG vaccine will be the only ones in the study who know what study product they are getting. This is because they will only get 1 injection while everyone else will get 2.

Your child will have to wait until all participants complete their final study visits to find out whether they got the other study vaccines or the placebo. This could be several years. But, if your child has a serious medical problem and needs to know what he/she got before the end of the study, we can tell him/her.

10. We will give your child the study products on a schedule.

Your child will be in one of 4 groups. If he/she is in group 1, 2 or 4, we will not tell them which group they are in. The children in these 3 groups will get 2 injections. The first injection is at the enrollment visit. The second injection will be 56 days later. These injections will be given in the upper arm.

If he/she is in group 3, they will know because they will only get one injection during the study, at the enrollment visit. This injection will be given in the upper arm.

Injection Schedule

Group	Enrollment visit	56 days later
1	H4	H4
2	H56	H56
3	BCG	
4	Placebo	Placebo

Your child will have to wait in the clinic for about 30 minutes after each injection to see if there are any problems. Then for that night and for seven more days, he/she will need to write down how he/she is feeling and if he/she has any symptoms. Clinic staff will show her/him how to do this in the diary card provided to them. We ask that you help your child complete the diary card and contact us, if he/she needs help. You or your child should contact the clinic staff if there are any issues or concerns after receiving an injection. If he/she has a problem, we will continue to check on him/her until it goes away. We will discuss with your child the best way to contact him/her.

11. In addition to giving your child the study products, we will:

- Do regular HIV testing on your child, as well as counseling on the results and on how to avoid getting HIV;
- Do physical exams on your child;
- Take blood and urine samples from your child;
- Do pregnancy tests if your child is female;

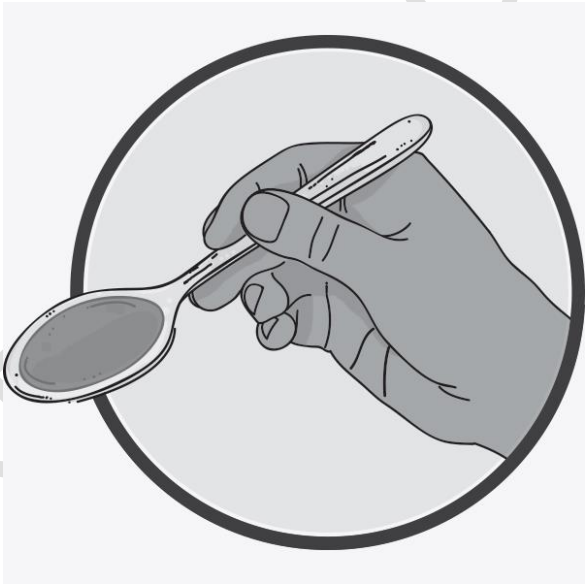
- Ask your child questions about his/her health, including medications he/she may be taking;

- Ask your child questions about any personal problems or benefits he/she may have from being in the study; and

- Teach your child about the symptoms of TB, and ask them if they have any of these symptoms. If we think your child might have TB we will help them get medical care.

When we take blood, the amount will depend on the lab tests we need to do. It will be some amount between 7 mL and 57 mL (about 1/2 tablespoon to almost 4 tablespoons) at some visits. Your child's body will make new blood to replace the blood we take out. We will check your child's blood to watch your child's health. Researchers will also look at your child's blood to learn about the immune response to the study vaccines, and to develop some new ways to look at it.

This drawing shows an example of a tablespoon of blood.



We will review the results of these procedures and tests with your child at his/her next visit, or sooner if necessary. If any of the results are important to your child's health, we will tell him/her. We will also offer your child counseling and referral for needed care.

12. If you and your child agree, we will also collect small samples of your child's stool (poop).

If you and your child agree for your child to give these samples, we will give your child more information about how we will collect them. We will do this twice during the study.

13. We will test your child's samples for this study.

We will send your child's samples (without his/her name) to a lab to see how your child's immune system responds to the study products. The researchers may:

- Take cells from your child's samples and grow more of them. We may grow more of your child's cells over time, so that they can continue to contribute to this study.

- Do limited genetic testing. Your genes are passed to you from your birth parents. They affect how you look and how your body works. The differences in people's genes can help explain why some people get a disease while others do not. Limited genetic testing involves only some of your child's genes, not all of your child's genes (your child's genome). The researchers will not look at all of your child's genes, only the genes related to the immune system and diseases.

- If your child gives stool samples, we will look at the bacteria living in his/her stomach. We want to learn if your child's immune response to the study vaccines is influenced by these bacteria.

In order to join the main study, you and your child must agree to this limited genetic testing. These tests are for research purposes only. The lab will not give the results to your child or this clinic, and the results will not become part of your child's study record.

14. We will do our best to protect your child's private information.

Your child's study records and samples will be kept in a secure location. We will label all of your child's samples and most of his/her records with a code number, not his/her name or other personal information. However, it is possible to identify your child, if necessary. We will not share your child's name with the lab that does the tests on your child's samples, or with anyone else who does not need to know your child's name.

Clinic staff will have access to your child's study records. Your child's records may also be reviewed by groups who watch over this study to see that we are protecting your rights, keeping you safe, and following the study plan. These groups include:

- The US National Institutes of Health,

- Aeras, and its study monitors,

- [Site: insert name of your IRB/EC],

- South African Medicines Control Council,

- Sanofi Pasteur and Statens Serum Institut and people who work for them,

- The HVTN and people who work for them,

- Aeras Safety Monitoring Committee, and

- The US Office for Human Research Protections.

All reviewers will take steps to keep your child's records private.

We cannot guarantee absolute privacy. At this clinic, we have to report the following information:

[Site: Include any public health or legal reporting requirements. Bulleted examples should include all appropriate cases (reportable communicable disease, risk of harm to self or others, etc.).]

A summary of this study (in English) is on the website *http:/www.ClinicalTrials.gov* or *http:/www.sanct.gov.za*. This website does not include information that could identify anyone participating in this study. You can search this website at any time.

We will do medical examinations and tests on your child and ask questions about their sexual activity. We will generally keep information about your child's participation confidential. We will not tell you, unless your child wants us to:

- Anything your child tells us about his/her sexual behavior. If he/she is sexually active, we will give him/her counseling and support to help him/her practice safer sex.
- If your child gets contraceptives or contraceptive information.
- The results of tests and treatment that he/she has consented to.

In some cases, we will help your child to tell a trusted adult about difficult situations he/she may face. We need your child to tell a trusted adult (not necessarily you):

- If she becomes pregnant and/or has a termination of pregnancy (abortion or miscarriage).
- If the results of his/her HIV or TB tests are positive.
- If he/she is not going to school. (We will never ask your child to attend the clinic during school hours).
- If your child tells us he/she is abusing drugs.

In all of the above cases, we will help your child get counseling and support.

We will need to report some types of harm to the appropriate social services. If we need to report, we will tell your child, but we may not tell you. We need to report:

- If your child tells us that he/she is being abused in a manner causing physical injury, sexually abused or deliberately neglected.
- If your child tells us that he/she has been the victim of a sexual offense, like rape.
- If your child tells us they are harming or thinking of harming themselves or others.

In all the above cases, we will help your child get counseling and support. We may also help him/her to tell a trusted adult (not necessarily you).

We will not report, nor will we tell you:

- If your child informs us that he/she is abusing substances.

- If he/she is not going to school. (We will never ask your child to attend the clinic during school hours.)

In all the above cases, we will help your child get counseling and support.

15. We may stop your child's injections or take him/her out of the study at any time.

We may do this even if you want your child to stay in the study. If your child is taken out of the study, we will not tell you the reason unless your child wants us to. This may happen if:

- he/she does not follow instructions,
- the researcher thinks that staying in the study might harm your child,
- your child gets HIV,
- your child gets active TB disease,
- your child becomes pregnant,
- your child is in prison (arrested),
- your child enrolls in a different research study where he/she receives another study product, or
- the study is stopped for any reason.

If we stop your child's injections, we may ask him/her to stay in the study to complete other study procedures.

16. If your child becomes pregnant during the study, we will continue with some procedures but not injections.

If your child leaves the study while she is still pregnant, we will contact her after her due date to ask some questions about her pregnancy. We will save this information in our safety database.

Risks

17. There are other risks to being in this study.

This section describes the risks and restrictions we know about. There may also be unknown risks, even serious ones. We will tell you and your child if we learn anything new that may affect your willingness to have them stay in the study or his/her willingness to stay in the study.

Risks of routine medical procedures:

In this study, we will do some routine medical procedures. These are taking blood and giving injections. These procedures can cause bruising, pain, fainting, soreness, redness, swelling, itching, and (rarely) muscle damage or infection where the needle was inserted.

Taking blood can cause a low blood cell count (anemia), and may make your child feel tired.

Personal problems/discrimination:

Family or friends may worry, get upset or angry, or assume that your child is infected with TB or HIV or at high risk and treat you unfairly as a result.

Risks of disclosure of your child's personal information:

We will take several steps to protect your child's personal information. Although the risk is very low, it is possible that your child's personal information could be given to someone who should not have it. If that happened, your child could face discrimination, stress, and embarrassment. We can tell you more about how we will protect your child's personal information if you would like it.

Unknown risks:

We do not know if the study vaccines will increase, decrease, or not change your child's risk of getting TB disease if your child is exposed to it.

We do not know if getting these study vaccines will affect how your child responds to any future approved TB vaccine.

We do not know how the study vaccines will affect a pregnant participant or a developing baby.

Benefits

18. The study may not benefit your child.

Getting the study vaccines will probably not benefit your child.. However, being in the study might still help your child in some ways. The screening tests and counseling that he/she gets as part of the study may help him/her avoid getting TB and HIV. The lab tests and physical exams that he/she gets while in this study might detect health problems your child does not yet know about.

Your child's participation in this study may help in the search for a vaccine to prevent TB. However, if any of the study vaccines later becomes approved and sold, there are no plans to share any money with you or your child.

Your child's rights and responsibilities

19. If your child joins the study, he/she has rights and responsibilities.

Your child's duties as a study participant are listed below. We ask that you help your child to do these things, if he/she needs help to do them.

- Come to all Study Visits when he/she is told to. Give complete and truthful information about his/her medical history and any signs of illness he/she may have during the study.

- Give complete and truthful information about all medicines he/she takes (or stops taking) during the study, including medicines from other doctors, herbal medicines, and medicine he/she buys for himself/herself.
- Give blood and urine samples to be tested.
- Complete the daily diary and remember to bring it to the study clinic for review;
- Tell the study doctor if he/she no longer wants to be in the study.

Leaving the study

20. Your child should tell us if he/she decides to leave the study.

Your child is free to leave the study at any time and for any reason. His/her care at this clinic and legal rights will not be affected, but it is important for him/her to let us know.

We will ask your child to come back to the clinic one last time for a physical exam, and we may ask to take some blood and urine samples. We will also ask about any personal problems or benefits your child may have experienced from being in the study. We believe these steps are important to protecting your child's health, but it is up to your child whether to complete them.

Injuries

21. If your child gets sick or injured during the study, he/she must contact us immediately.

Your child's health is important to us. We will help you and your child get the medical care he/she needs.

We call an injury or illness study-related if it occurs as a direct result of the administration of the study products or study-related procedures. The clinic staff will treat your child for study-related problems or tell you and your child where to get the treatment he/she needs. If a study-related injury occurs you and your child have not waived any of the legal rights which you otherwise would have by signing this form.

If your child gets sick or injured because of the study vaccines, insurance has been purchased to cover your child's medical treatment. This policy will follow the guidelines for payment of study-related illness or injury approved by the Association of the British Pharmaceutical Industry ("ABPI Guidelines"). You can get a copy of these ABPI Guidelines from us if you wish.

If your child's injuries are more severe or chronic, the insurance funds may not be enough. If needed, Aeras has agreed to pay the cost of medical expenses that arise from injuries caused by the study products subject to certain limitations.

In addition, the HVTN also has limited funds from the U.S. government to pay for your child's treatment for study related injuries.

Some injuries are not physical. For example, someone might be harmed psychologically or emotionally by being in a TB vaccine study. Or they might lose wages from injuries because they could not go to work. No funds have been set aside to pay for nonphysical injuries, even if they are related to participation in the study.

Your child's usual healthcare provider and/or his/her health insurance carrier will continue to be responsible for the cost of your child's usual medical care outside this study, and for medical expenses that are determined not directly related to study procedures or products.

Medicines Control Council, South Africa -- MCC

If you have questions about this study you should first discuss them with the study doctor or the ethics committee (contact details as provided on this form). After you have consulted your doctor or the ethics committee and if they have not provided you with answers to your satisfaction, you should write to the Medicines Control Council (MCC) South Africa at:
The Registrar of Medicine
Medicines Control Council
Department of Health
Private Bag X828
Pretoria
0001
Fax: (021) 395 9201

Ethical approval

This clinical study protocol has been submitted to the Human Research Ethics Committee of the University of Cape Town and written approval has been granted by that committee.

The study has been written to follow the guidelines of the Declaration of Helsinki (last updated: October 2008), which deals with the recommendations guiding doctors in biomedical research involving human participants, and the Ethics in Health Research: Principles, Structures and Processes (2004) and Guidelines for Good Practice in the Conduct of Clinical Trials in Human Participants in South Africa.

We can provide you copies if you wish to review them.

Questions

22. If you or your child have questions or problems at any time during your child's participation in this study, use the following important contacts.

If you have questions about this study, contact
[name and telephone number of the investigator or other study staff].

If your child has any symptoms that he/she thinks may be related to this study, contact
[name and telephone number of the investigator or other study staff].

If you have questions about your child's rights as a research participant, or problems or concerns about how he/she is being treated in this study, contact [name/title/phone of person on IRB or other appropriate organization].

If you want your child to leave this study, contact [name and telephone number of the investigator or other study staff].

Your signature

23. In Section 12 of this form, we told you about collecting stool (poop) samples from your child. Please write your initials or make your mark in the box next to the option you choose.

☐ I allow you to collect stool (poop) samples from my child.

☐ I do not allow you to collect stool (poop) samples from my child.

24. If you allow your child to join this study, you will need to sign or make your mark below. Before you sign or make your mark on this consent form, make sure of the following:

- You have read this consent form, or someone has read it to you.
- You feel that you understand what the study is about and what will happen to your child if he/she joins. You understand what the possible risks and benefits are.
- You have had your questions answered and know that you can ask more.
- You agree for your child to join this study.

_____
Participant's name

Parent/Legal Guardian's name (print)	Parent/Legal Guardian's signature or mark	Date	Time

Clinic staff conducting consent discussion (print)	Clinic staff signature	Date	Time

For parent/legal guardian who is unable to read or write, a witness should complete the signature block below:

Witness's name (print)	Witness's signature	Date	Time

*Witness is impartial and was present for the consent process.

Appendix B Approved birth control methods (for sample informed consent form)

Your child should not become pregnant during the study because we do not know how the study vaccines could affect the developing baby.

If your child is female and is sexually active in a way that could lead to pregnancy, she must agree to use effective birth control, starting at 20 days before her first injection until her last study visit.

This means using 1 of the following methods every time she has sex:

- Male or female condoms, or
- Diaphragm or cervical cap;

PLUS 1 of the following methods:

- Birth control drugs that prevent pregnancy—given by injections, pills, patches, vaginal rings, or inserts under the skin; or
- Intrauterine device (IUD).

If your child is female and is not sexually active, she must have a plan to use effective birth control if she does become sexually active. We will help her to make this plan.

Appendix C Sample consent form for use of samples and information in other studies

Title: A randomized, placebo-controlled, partially blinded phase 1b clinical trial to evaluate the safety and immunogenicity of BCG revaccination, H4:IC31, and H56:IC31 in healthy, HIV-1–uninfected adolescent participants

HVTN protocol number: HVTN 602 / AERAS A-042

Site:

When samples are no longer needed for this study, the HVTN and Aeras wants to keep them for use in other studies. We will call these "extra samples."

This form gives you information so you can decide if you want your child's extra samples and information used in other studies. You will mark your decision at the end of the form. If you have any questions, please ask.

1. Do I have to agree?

No. Your child can join the study even if you do not agree to the use of their extra samples and information in other studies. You are free to say yes or no, or to change your mind after you sign this form. We will only use your child's extra samples in other studies if both you and your child agree to this. At your request, we will destroy all extra samples from your child that we have. Your decision will not affect your child's being in this study or have any negative consequences here.

2. Where are the samples stored?

Extra samples are stored in locked freezers in a secure central place called a repository. *[Site: insert specific information if your regulatory authority requires it.]* The central repositories for the HVTN and Aeras are located in the United States and South Africa.

3. How long will the samples be stored?

There is no limit on how long your child's extra samples will be stored. *[Site: insert limits if your regulatory authority imposes them.]*

4. Will I be paid for the use of my child's samples?

No. Also, a researcher may make a new scientific discovery or product based on the use of your child's samples. If this happens, there is no plan to share any money with you or your child. The researcher is not likely to ever know who your child is.

5. Will I benefit from allowing my child's samples to be used in other studies?

Probably not. Results from these other studies are not given to your child, this clinic, or your doctor. They are not needed for your child's medical care. They are not part of your child's medical record. The studies are only being done for research purposes.

6. Will the HVTN or Aeras sell my child's samples and information?

No, but the HVTN and Aeras may share your child's samples with other researchers. Once we share your child's samples and information, we will not be able to get them back.

7. How do other researchers get my child's samples and information?

The other researchers could be from universities, hospitals, companies that make and sell medicines, government agencies or other institutions. When a researcher wants to use your child's samples and/or information, their research plan must be approved by the HVTN and Aeras. Also, the researcher's institutional review board (IRB) or ethics committee (EC) will review their plan. *[Site: insert review by your institution's IRB/EC, if applicable.]* IRBs/ECs protect the rights and well-being of people in research. The HVTN and Aeras keep track of your decision and your child's decision about how your child's samples and information can be used.

8. What information is shared with other researchers?

The samples and limited information will be labeled with a code number. Your child's name will not be part of the information. However, some information that we share may be personal, such as your child's race, ethnicity, gender, health information from the study, and HIV status. We may share information about the study product your child received and how your child's body responded to the study product.

9. What kind of studies might be done with my child's extra samples and information?

The studies will be related to TB, vaccines, the immune system, and other diseases. The researchers may:

- Take cells from your child's samples and grow more of them. This means the researchers may keep your child's cells growing over time.
- Do limited genetic testing, which involves only looking at some of your child's genes, not all of your child's genes.

If you agree, your child's samples could also be used for genome wide studies. In these studies, researchers will look at all of your child's genes (your child's genome). The researchers compare the genomes of many people, looking for common patterns of genes that could help them understand diseases. The researchers may put the information from the genome-wide studies into a protected database so that other researchers can access it. Usually, no one would be able to look at your child's genome and link it to your child as a person. However, if another database exists that also has information on your child's genome and your child's name, someone might be able to compare the databases and identify your child. If others found out, it could lead to discrimination or other problems. The risk of this is very small.

10. What are the risks of genetic testing?

The genetic testing could show your child may be at risk for certain diseases. If others found out, it could lead to discrimination or other problems. However, it is almost impossible for your child or others to know your child's test results from the genetic

testing. The results are not part of your child's study records and are not given to you or your child.

11. Who will have access to my child's information in studies using my child's extra samples?

People who may see your child's information are:

- Researchers who use your child's stored samples and limited information for other research
- Government agencies that fund or monitor the research using your child's samples or information
- The researcher's Institutional Review Board or Ethics Committee
- The people who work with the researcher

All of these people will do their best to protect your child's information. The results of any new studies that use your child's extra samples or information may be published. No publication will use your child's name or identify your child personally.

Questions

12. If you have questions or problems about allowing your child's samples and information to be used in other studies, use the following important contacts.

If you have questions about the use of your child's samples or information or if you want to change your mind about their use, contact
[name and telephone number of the investigator or other study staff].

If you think your child may have been harmed because of studies using your child's samples or information, contact
[name and telephone number of the investigator or other study staff].

If you have questions about your child's rights as a research participant, contact
[name/title/phone of person on IRB or other appropriate organization].

13. Please write your initials or make your mark in the box next to the option you choose.

☐ I allow my child's extra samples combined with limited information to be used for other studies related to TB, vaccines, the immune system, and other diseases. This may include limited genetic testing and keeping my child's cells growing over time.

OR

☐ I agree to the option above and also to allow my child's extra samples combined with limited information to be used in genome wide studies.

OR

☐ I do not allow my child's extra samples to be used in any other studies. This includes not allowing limited genetic testing, growing more of my child's cells, or genome wide studies.

_____
Participant's name

Parent/Legal Guardian's name (print)	Parent/Legal Guardian's signature or mark	Date	Time

Clinic staff conducting consent discussion (print)	Clinic staff signature	Date	Time

For parent/legal guardian who is unable to read or write, a witness should complete the signature block below:

Witness's name (print)	Witness's signature	Date	Time

*Witness is impartial and was present for the consent process.

Appendix D Table of procedures (for sample informed consent and assent forms)

Groups 1, 2, and 4

Procedure	Screening visit(s)	First injection visit	Time after 1st injection visit (in days and *months*) 1	3	7	14	56	63	70	168	224
							2 mo	*2¼ mo*	*2½ mo*	*6 mo*	*8mo*
Injection		√					√				
Medical history	√										
Complete physical	√										√
Brief physical		√	√	√	√	√	√	√	√	√	
Stool sample container (optional)*		(√)	(√)	(√)	(√)			(√)	(√)		
Urine test	√				√			√			
Blood drawn	√	√	√	√	√	√	√	√	√	√	√
Pregnancy test (participants born female)	√	√					√				
HIV testing and pretest counseling	√								√	√	√
Risk reduction counseling	√								√	√	√
Interview/questionnaire	√	√	√	√	√	√	√	√	√	√	√

Not shown in this table is a time after all study participants have completed their last scheduled visit when you can find out what products you received.

* If you choose to give the 2 optional stool samples, we would like to collect the first within 7 days of your first injection and the second 7 to 14 days after your second injection.

Group 3

Procedure	Screening visit(s)	Injection visit	Time after 1st injection visit (in days and *months*) 1	3	7	28 *1 mo*	70 *2½ mo*	168 *6 mo*	224 *8mo*
Injection		√							
Medical history	√								
Complete physical	√								√
Brief physical		√	√	√	√	√	√	√	
Stool sample container (optional)*		√				√			
Urine test	√				√				
Blood drawn	√	√	√	√	√	√	√	√	√
Pregnancy test (participants born female)	√	√							
HIV testing and pretest counseling	√						√	√	√
Risk reduction counseling	√						√	√	√
Interview/questionnaire	√	√	√	√	√	√	√	√	√

Not shown in this table is a time after all study participants have completed their last scheduled visit when you can find out what products you received.

* If you choose to give the 2 optional stool samples, we would like to collect the first within 7 days of your injection visit and the second either 1 month or 2 1/2 months after your injection.

Appendix E Sample informed assent form

Title: A randomized, placebo-controlled, partially blinded phase 1b clinical trial to evaluate the safety and immunogenicity of BCG revaccination, H4:IC31, and H56:IC31 in healthy, HIV-1–uninfected adolescent participants

HVTN protocol number: HVTN 602 / AERAS A-042

Site:

Thank you for your interest in our research study. Please read this assent form or ask someone to read it to you. Assent means agreeing to join this research study. If you decide that you would like to join the study, we will ask you to sign this form. We will offer you a copy to keep. We will ask you questions to see if we have explained everything clearly. You can also ask us questions about the study.

We will also review a form like this with your parent or guardian. We will ask him or her to sign it if they agree for you to join this study. Both you and your parent/guardian need to agree in order for you to join the study. We encourage you to talk with your parent/guardian about the study.

In this study, we may do limited genetic testing on your samples. You cannot be in this study unless you and your parent/guardian agree to this. We will tell you more about this in section 13 below. By signing this consent form, you agree to limited genetic testing on your samples. Please do not sign this form if you do not agree to this.

About the study

Aeras, the HIV Vaccine Trials Network (HVTN) and the Desmond Tutu HIV Center (DTHC) are doing a research study to test tuberculosis (TB) vaccines. Before a vaccine can be used by the public, the vaccine maker must do a lot of research and tests to make sure the vaccine is safe and it works. This study is one of many that are being done to try and make a better TB vaccine that is safe and works.

About 84 people will take part in this study. The researcher in charge of this study at this clinic is Prof. Linda-Gail Bekker. The US National Institutes of Health (NIH), Aeras, Sanofi Pasteur and the Staten Serum Institut (SSI) are paying for the study.

TB is a common infection in South Africa. It is caused by a germ that is spread through the air when an infected person coughs or spits near others. TB causes illness in the lungs. In most cases, TB can be cured.

Most teenagers in South Africa got a TB vaccine as babies. This vaccine is called BCG. It stops very bad TB in most babies but it may not protect teenagers or adults very well. A better vaccine to prevent TB is needed.

Research is not the same as treatment or medical care. The purpose of a research study is to answer scientific questions.

1. **We are doing this study to answer several questions.**
    - Are the study vaccines safe to give to young people between 12 and 17 years of age?

- Are young people able to take the study vaccines without becoming too uncomfortable?

- How do young people's immune systems respond to the study vaccines? (Your immune system protects you from disease.)

2. The study vaccines cannot give you TB.

The study vaccines are not made from actual TB. It is not possible for the study vaccines to give you TB. Also, they cannot cause you to give TB to someone else.

3. Two of the study vaccines are experimental. The third is a licensed vaccine given in an experimental way.

The experimental study vaccines are H4:IC31 and H56:IC31. "Experimental" means they are still being tested and we do not know if they work. From now on we will call them the H4 vaccine and the H56 vaccine. They have been given to people in other studies. They have not caused serious health problems or serious side effects.

We are also using the BCG vaccine in this study. BCG is a licensed vaccine but we are giving it in a different way. Babies usually get it just after they are born. In this study, we will give it to teenagers who also got it as babies. BCG is not approved to be given to a person more than once. This is why the way we are using it is experimental. In other studies, teenagers who got the BCG vaccine as babies were given the BCG vaccine again. This did not cause serious health problems or serious side effects in them.

Risks of the study vaccines:

This section lists the side effects we know about. There may be others that we don't yet know about, even serious ones. We will tell you and your parent/guardian if we learn about any new side effects.

Some people who get these study vaccines may have problems where the needle went into the arm. The problems we have seen in other studies of these vaccines are: mild pain, redness, mild swelling, or a mildly swollen and painful gland, usually in the arm pit.

Some people who get a study vaccine may have symptoms of the flu like feeling tired, muscle and/or joint pains, headache and/or fever. They may also experience chills, loose stools and feeling sick. This usually happens in the first few days after the injection. These symptoms usually get better in a few days on their own.

Some people who get a study vaccine may have changes in their blood or urine that can only be seen in laboratory tests. These changes usually go away on their own in a few days. Giving blood or getting an injection may make people feel dizzy or change their blood pressure for a short time.

People who get these study vaccines and then have a TB skin test (known also as a Mantoux Test) up to 90 days later may have an allergic reaction to the TB skin test. Their arm may become very swollen, painful, red and irritated. Rarely, a small open sore may develop that takes a while to go away. If you have a TB skin test done outside this clinic within 90 days of a study injection, you should tell the person giving the TB skin test

about this or ask them to call this clinic to learn more. We will also give you a note to explain this.

People who get the BCG vaccine may have a small bump where the needle went into the upper arm. This may scab over after a few days. A small open sore may develop that may take up to 3 months to go away.

If you receive the H56 vaccine it is possible that it could interfere with the results of a TB blood test (the Quantiferon test) you might have in the future. However, doctors can determine if you have TB by using other ways such as chest x-rays. We will make sure that participants who have a positive TB blood test or TB symptoms are examined to make sure that they do not have TB. If a doctor determines that you have TB, we will help you get the proper care.

Joining the study

4. **It is completely up to you and your parent/guardian whether or not to join the study.**

   You can decide not to join this study. *You can* leave it after you have joined. *We* will not treat you any differently.

   Site: Remove item 5 if you use a separate screening consent that covers these procedures.

5. **If you and your parent/guardian would like for you to join the study, we will screen you to see if you can be in the study.**

   In screening we will ask you questions about your medical history and do a physical exam. In the physical exam, we will:

   - check your height, weight, temperature, heart rate, breathing rate and blood pressure
   - look in your ears, eyes, mouth and throat
   - listen to your heart and lungs
   - feel your abdomen (stomach and liver), throat and neck

   We will also take blood from your arm and a urine sample from you. We will test your blood and urine to learn some important things about your health. We will ask if you have ever been exposed to TB. Not everyone who is exposed to TB develops active TB. We may ask you to spit into a cup or we may do a chest x-ray to help us learn if you have active TB. You cannot join this study if you have TB.

   We will ask you about medicines, traditional treatments or drugs you are taking. We will ask you about behaviors that might put you at risk for getting HIV and test you for HIV. You cannot join this study if you have HIV. If you are female, we will test you for pregnancy. You cannot join this study if you are pregnant.

The screening visit will be done in private without your parent/guardian. If you are not eligible to join, we will tell you the reason why. We will not tell your parent/guardian unless you want us to.

If you are not eligible to join this study, we will still use your blood sample in this study. We will use it only to learn more about how one of the screening tests is working. We are doing this for research purposes only, not to learn about your health. We will not give this result to you or your parent/guardian.

6. **If you could become pregnant, you must agree to use birth control to join this study.**

   Site: List approved birth control methods here if you do not want to hand out the separate Approved Birth Control Methods sheet (Appendix F).

   We do not know how the study vaccines could affect the developing baby. You must agree to use effective birth control from 20 days before your first injection until your last study visit. We will talk to you about effective birth control methods and give you a list of them.

   If you become pregnant while in the study, contact the study doctor immediately. The study doctor will advise you about medical care.

Being in the study

If you are in this study, here is what will happen:

Site: Insert number of visits and range of visit lengths. (There is site-specific variation in screening protocols and in the number of possible follow-up visits between protocol-mandated visits.)

7. **You will come to the clinic about [#] times over 8 months.**

   Visits can last from [#] to [#] hours.

8. **We will give you [Site: Insert compensation] for each study visit you complete.**

   This amount is to cover the costs of [Site: Insert text].

   If your parent/guardian comes to a study visit with you, we will give them [Site: insert compensation] to cover the costs of [Site: Insert text].

   You do not have to pay anything to be in this study.

9. **We will give you one of the study vaccines or a placebo.**

   Not everyone in this study will get one of the study vaccines. Some people will get a placebo. A placebo is a substance that does not contain vaccine. We will compare the results from people who got the placebo with results from people who got the different study vaccines. The placebo is sterile salt water.

You have a 6-in-7 chance of getting one of the study vaccines. This means that over 85% of the participants will get one of the study vaccines.

Whether you get one of the study vaccines or the placebo is completely random, like flipping a coin.

10. We will give you the study products on a schedule.

You will be in one of 4 groups. If you are in group 1, 2 or 4, we will not tell you which group you are in. Everyone in these 3 groups will get 2 injections. The first injection will be at the enrollment visit. The second injection will be 56 days later. These injections will be given in the upper arm.

If you are in group 3, you will know because you will only get one injection during the study. It will be at the enrollment visit. This injection will be given in the upper arm.

Injection Schedule

Group	Enrollment visit	56 days later
1	H4	H4
2	H56:	H56
3	BCG	
4	Placebo	Placebo

You will have to wait in the clinic for about 30 minutes after each injection. We want to see if there are any problems. Then for that night and for seven more days, you will need to write down how you are feeling and if you have any symptoms. We will give you a diary card and show you how to complete it. You should contact the clinic staff if you have any issues or concerns after getting an injection. We have asked your parent/guardian to help you complete the diary card and to contact us, if you need help with these things.

11. In the study, we will also:

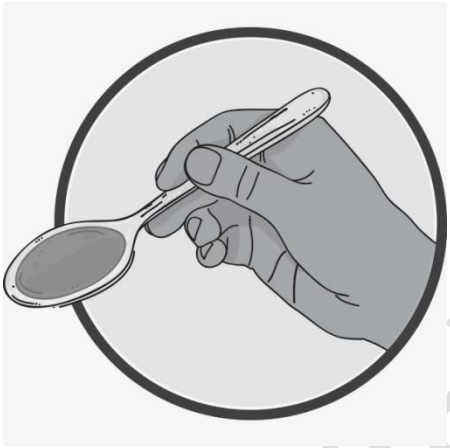
- Teach you about the symptoms of TB, and ask you if you have any of these symptoms. If we think you might have TB we will help you get medical care.
- Do regular HIV testing and counseling on the test results and on how to avoid getting HIV;
- Do physical exams;
- Take blood and urine samples;
- Do pregnancy tests if you are female;

- Ask you questions about any personal problems or benefits you may have from being in the study;

- Ask you questions about your health, including medications you may be taking;

We will take blood from you at some visits. It will be some amount between 7 mL and 57 mL (about 1/2 tablespoon to almost 4 tablespoons). Your body will make new blood to replace the blood we take out. We will check your blood to watch your health. Researchers will also look at your blood to learn about the immune response to the study vaccines, and to develop some new ways to look at it.

This drawing shows an example of a tablespoon of blood.



12. If you agree, we will also collect small samples of your stool (poop).

If you and your parent/guardian agree for you to give these samples, we will give you more information about how we will collect them. We will do this twice during the study.

13. We will test your samples for this study.

We will send your samples (without your name) to a lab. The lab will look at how your immune system responds to the study products. The researchers may:

- Take cells from your samples and grow more of them.

- Do limited genetic testing. Your genes are passed to you from your birth parents. They affect how you look and how your body works. The differences in people's genes can help explain why some people get a disease while others do not. Limited genetic testing looks at only some of your genes, not all of your genes (your genome). The researchers will not look at all of your genes. They will only look at the genes related to the immune system and diseases.

- If you give stool samples, the researchers will be able to look at the bacteria living in your stomach. They want to learn if your immune response to the study vaccine is influenced by these bacteria.

In order to join the main study, you must agree to this limited genetic testing. These tests are for research purposes only. The lab will not give the results to you or this clinic. The results will not become part of your study record.

14. We will do our best to protect your private information.

We will keep your records and samples safe. We will label most of them with a code number instead of your name. We will not share your name with the lab that tests your samples or with anyone else who does not need to know your name.

There are some groups who watch over this study to see that we are protecting your rights, keeping you safe, and following the study plan. They may look at your study records but they will keep them private.

We will do medical examinations and tests on you and ask questions about your sexual activity. We will generally keep information about you confidential. We will not tell your parent/guardian, unless you want us to:

- Anything you tell us about your sexual behavior. If you are sexually active, we will give you counseling and support to help you practice safer sex.
- If you get contraceptives or contraceptive information.
- The results of tests and treatment that you have consented agreed to.

In some cases, we will help you to tell a trusted adult about difficult situations you may face. We need you to tell a trusted adult (not necessarily your parent/guardian):

- If you become pregnant and/or have a termination of pregnancy (abortion or miscarriage).
- If the results of your HIV or TB tests are positive.
- If you are not going to school. (We will never ask you to attend the clinic during school hours).
- If you tell us you are abusing drugs.

In all of the above cases, we will help you get counseling and support.

We will need to report some types of harm to the appropriate social services. If we need to report, we will tell you, but we may not tell your parent/guardian. We need to report:

- If you tell us that you are being abused in a manner causing physical injury, sexually abused or deliberately neglected.
- If you tell us that you has been the victim of a sexual offense, like rape.
- If you tell us that you are harming or thinking of harming yourself or others.

In all the above cases, we will help you get counseling and support. We may also help you to tell a trusted adult (not necessarily your parent/guardian).

We will not tell the authorities, nor will we tell your parent/guardian:

- If you inform us that you are abusing substances.
- If you are not going to school. (We will never ask you to attend the clinic during school hours.)

In all the above cases, we will help you get counseling and support.

15. We may stop your injections or take you out of the study at any time.

We may do this if:

- you do not follow instructions,
- your parent/guardian withdraws their permission for you to be in this study,
- the researcher thinks that staying in the study might harm you,
- you get HIV,
- you get active TB disease,
- you become pregnant,
- you are in prison (arrested),
- you enroll in a different research study where you receive another study product, or
- the study is stopped for any reason.

If we stop your injections, we may ask you to stay in the study to complete other study procedures.

16. If you become pregnant during the study, we will continue with some procedures but not injections.

If you leave the study while you are still pregnant, we will contact you after your due date to ask some questions about your pregnancy. We will save this information in our safety database.

Risks

17. There are other risks to being in this study.

We list below the ones we know about. There may also be unknown risks, even serious ones. We will tell you if we learn anything new that might change how you feel about staying in the study.

Risks of taking blood and giving injections:

These can cause bruising, pain, fainting, soreness, redness, swelling, itching and (rarely) muscle damage or infection where the needle went into the arm. Taking blood can cause a low blood cell count (anemia), and may make you feel tired.

Risk of personal problems/discrimination:

Family or friends may worry or get upset or angry. They may think that you are infected with TB or HIV. They may treat you unfairly because of this.

Risks of other people learning your personal information:

We will protect your personal information. It is very unlikely that your personal information could be given to someone who should not have it. If that happened you could be treated unfairly. You could feel stressed or embarrassed.

Unknown risks:

We do not know if the study vaccines will increase, decrease, or not change your risk of getting TB disease if you are exposed to it.

We do not know if getting the study vaccines will affect how you respond to any future approved TB vaccine.

We do not know how the study vaccines will affect a pregnant participant or a developing baby.

Benefits

18. The study may not benefit you.

Getting the study vaccines will probably not benefit you. The counseling that you get in the study may help you avoid getting HIV. The lab tests and physical exams you get in the study might find health problems you do not yet know about.

Your participation in this study may help in the search for a vaccine to prevent TB. If any of the study vaccines are later approved and sold, there are no plans to share any money with you or your parent/guardian.

Your rights and duties

19. If you join the study, you have rights and duties.

Your duties as a study participant are listed below. We are asking your parent/guardian to help you do these things if needed.

- Come to all study visits when you are told to. Give complete and truthful information about your medical history and any signs of illness you may have during the study.

- Give complete and truthful information about all medicines you take (or stop taking) during the study. This means medicines from other doctors, herbal medicines, and medicine you buy for yourself.

- Give blood and urine samples to be tested.
- Complete the daily diary and remember to bring it to the study clinic for review.
- Tell us if you want to leave the study.

Leaving the study

20. You are free to leave the study at any time and for any reason.

We will not treat you any differently. We will ask you to come back to the clinic one last time. We would like to do a physical exam. We may also ask to take some blood and urine samples.

Injuries

21. If you get sick or injured during the study, contact us right away.

Your health is important to us. If you are injured by the study products or study procedures, we will give you the medical care you need or tell you where you can get it.

Questions

22. If you have questions or problems at any time during the study, here is who you can contact.

If you have questions about this study, contact
[name and telephone number of the investigator or other study staff].

If you have any symptoms that you think may be related to this study, contact
[name and telephone number of the investigator or other study staff].

If you have questions about your rights as a research participant, or problems or concerns about how you are being treated in this study, contact
[name/title/phone of person on IRB or other appropriate organization].

If you want to leave this study, contact
[name and telephone number of the investigator or other study staff].

Your signature

23. In Section 12 of this form, we told you about collecting stool (poop) samples from you. Please write your initials or make your mark in the box next to the option you choose.

☐ I allow you to collect samples of my stool (poop).

☐ I do not allow you to collect samples of my stool (poop).

24. If you agree to join this study, you will need to sign or make your mark below. Before you sign or make your mark on this assent form, make sure of the following:

- You have read this assent form, or someone has read it to you.
- You feel that you understand what the study is about and what will happen to you if you join. You understand what the possible risks and benefits are.
- You have had your questions answered and know that you can ask more.
- You agree to join this study.

_____
Parent/Legal Guardian's name

Participant's name (print)	Participant's signature or mark	Date	Time

Clinic staff conducting consent discussion (print)	Clinic staff signature	Date	Time

For participant who is unable to read or write, a witness should complete the signature block below:

Witness's name (print)	Witness's signature	Date	Time

*Witness is impartial and was present for the consent process.

Appendix F Approved birth control methods (for sample informed assent form)

You should not become pregnant during the study because we do not know how the study vaccines could affect the developing baby. If you are sexually active in a way that could lead to pregnancy, you must agree to use effective birth control, starting at 20 days before your first injection until your last study visit.

This means using any 1 of the following methods every time you have sex:

- Male or female condoms, or
- Diaphragm or cervical cap;

PLUS 1 of the following methods:

- Birth control drugs that prevent pregnancy—given by injections, pills, patches, vaginal rings, or inserts under the skin; or
- Intrauterine device (IUD).

If you are female and are not sexually active, you must have a plan to use effective birth control if you do become sexually active. We will help you to make this plan.

Appendix G Sample informed consent form for participants who turn 18 on study

Title: A randomized, placebo-controlled, partially blinded phase 1b clinical trial to evaluate the safety and immunogenicity of BCG revaccination, H4:IC31, and H56:IC31 in healthy, HIV-1–uninfected adolescent participants

HVTN protocol number: HVTN 602 / AERAS A-042

Site:

Thank you for your interest in our research study. Please read this consent form or ask someone to read it to you. If you decide that you would like to join the study, we will ask you to sign this form. We will offer you a copy to keep. We will ask you questions to see if we have explained everything clearly. You can also ask us questions about the study.

In this study, we may do limited genetic testing on your samples. You cannot be in this study unless you agree to this. We will tell you more about this in section 13 below. By signing this consent form, you agree to limited genetic testing on your samples. Please do not sign this form if you do not agree to this.

About the study

Aeras, in partnership with the HIV Vaccine Trials Network (HVTN) and the Desmond Tutu HIV Center (DTHC) are doing a research study to test tuberculosis (TB) vaccines. Before a vaccine can be used by the public, the vaccine maker must do a lot of research and tests to make sure the vaccine is safe and it works. This study is one of many that are being done to try and make a better TB vaccine that is safe and works.

About 84 people will take part in this study. The researcher in charge of this study at this clinic is Prof. Linda-Gail Bekker. The US National Institutes of Health (NIH), Aeras, Sanofi Pasteur and the Staten Serum Institut are paying for the study.

TB is a common infection in South Africa. It is caused by a germ that is spread through the air when an infected person coughs or spits near others. TB causes illness in the lungs. In most cases, TB can be treated successfully.

Most teenagers in South Africa got a TB vaccine as babies. This vaccine, called BCG, stops very bad TB in most babies but it may not protect teenagers or adults very well. A better vaccine to prevent TB is needed.

Research is not the same as treatment or medical care. The purpose of a research study is to answer scientific questions.

1. **We are doing this study to answer several questions.**
    - Are the study vaccines safe to give to young people between 12 and 17 years of age?
    - Are young people able to take the study vaccines without becoming too uncomfortable?

- How do young people's immune systems respond to the study vaccines? (The immune system protects the body from disease.)

2. The study vaccines cannot give you TB.

The study vaccines are not made from actual TB. It is impossible for the study vaccines to give you TB. Also, they cannot cause you to give TB to someone else.

3. Two of the study vaccines are experimental. The third is a licensed vaccine given in an experimental way.

When we say that study vaccines are experimental, it means that the study vaccines are not approved by the South African Medicines Control Council (MCC). When we say that a vaccine is given in an experimental way, it means that it has an approved use, but the study will be using it in a way that is not approved by MCC.

The experimental study vaccines are H4:IC31 and H56:IC31. From now on we will call them the H4 vaccine and the H56 vaccine.

The H4 vaccine has been given to 198 adults in research studies in Europe and South Africa. It is also being given to 490 babies and teenagers in South Africa in two different studies. As of June 2014, 76 babies and 157 teenagers are enrolled in the studies. The vaccine has not caused serious health problems and people have been able to take it without serious side effects. This vaccine is made by Sanofi Pasteur.

The H56 vaccine has been given to 25 adults in a research study in South Africa. It did not cause serious health problems and people were able to take it without serious side effects. Another study which will give the H56 vaccine to 98 more people in South Africa has been started. This vaccine is made by SSI.

The licensed vaccine given in an experimental way in this study is BCG. It is registered in South Africa for the prevention of TB in children and adults. BCG is commonly given to children shortly after birth, but the vaccine's protection may not last. We know that some teenagers and adults who received the BCG vaccine at birth still get TB. BCG is not approved to be given to a person more than once so its use in this study is experimental. This vaccine is also made by SSI.

Risks of the study vaccines:

This section lists the side effects we know about. There may be others that we don't yet know about, even serious ones. We will tell you if we learn about any new side effects.

Some people who get these study vaccines may have problems where the needle went into the arm. The problems we have seen in other studies of these vaccines are: mild pain, redness, mild swelling, or a mildly swollen and painful gland, usually in the arm pit.

In the first few days after getting a study vaccine, some people may have symptoms of the flu like fatigue, muscle and/or joint pains, headache and/or fever. They may also experience chills, loose stools and feeling sick. These symptoms usually get better in a few days on their own.

For a short time after getting a study vaccine, some people may have changes in their blood or urine that can only be seen in laboratory tests. These changes usually go away on their own in a few days and rarely cause any problems. A few people may feel dizzy or have a small increase or decrease in blood pressure for a short time after we take blood from them or give them an injection.

People who get these study vaccines and then have a TB skin test (known also as a Mantoux Test) up to 90 days later may have an allergic reaction to the TB skin test. Their arm may become very swollen, painful, red and irritated. Rarely, a small open sore may develop that takes a while to go away. If you have a TB skin test done outside this clinic within 90 days of a study injection, you should tell the person giving the TB skin test about this or ask them to call this clinic to learn more. We will also give you a note to explain this.

People who get the BCG vaccine may have a small bump where the needle went into the upper arm. This may scab over after a few days. A small open sore may develop that may take up to 3 months to go away.

If you receive the H56 vaccine it is possible that it could interfere with the results of a TB blood test (the Quantiferon test) you might have in the future. However, doctors can determine if you have TB by using other ways such as chest x-rays. We will make sure that participants who have a positive TB blood test or TB symptoms are examined to make sure that they do not have TB. If a doctor determines that you have TB, we will help you get the proper care.

Joining the study

4. It is completely up to you whether or not to join the study.

Take your time in deciding. If it helps, talk to people you trust, such as your doctor, friends or family. If you decide that you do not want to join this study, or if you change your mind after you have joined, your other care at this clinic and the benefits or rights you would normally have will not be affected.

If you join this study, you may not be allowed to join other TB vaccine studies now or in the future. Also during the study, you should not donate blood or tissue.

If you choose not to join this study, you could join another study if one is available and you are eligible to join.

Site: Remove item 5 if you use a separate screening consent that covers these procedures.

5. If you decide to join the study, we will screen you to see if you are eligible.

Screening involves asking questions about your medical history and a physical exam. A physical exam includes the following tests:

- checking your height, weight, temperature, heart rate, breathing rate and blood pressure
- looking in your ears, eyes, mouth and throat

- listening to your heart and lungs
- feeling your abdomen (stomach and liver), throat and neck

We will also take blood from your arm and a urine sample from you. We will use this blood and urine to do some tests. These tests will tell us about some key aspects of your health. We will ask if you have ever been exposed to TB. Not everyone who is exposed to TB or is positive for TB develops active disease. We may ask you to spit into a cup or we may do a chest x-ray to help us learn if you have active TB. If you are found to have or are suspected of having TB, you will be referred to an appropriate clinic who may then diagnose TB. You cannot join this study if you have active TB.

We will ask you about medicines, traditional treatments or drugs you may be taking. We will ask you about behaviors that might put you at risk for getting HIV and test you for HIV. You cannot join this study if you have HIV. If you are female, we will test you for pregnancy. You cannot join this study if you are pregnant. You cannot be in another research study where you receive a study product and be enrolled in this study.

We will review the screening results with you and offer you counseling and referral if you need medical care. We will not pay for this medical care. The screening results may show that you cannot join the study, even if you want to.

If you are not eligible to join this study, we will still use your blood sample in this study. We will use it only to learn more about how one of the screening tests is working. We are doing this for research purposes only, not to learn about your health, so we will not give this result to you.

6. **If you are female and could become pregnant, you must agree to use birth control to join this study.**

Site: List approved birth control methods here if you do not want to hand out the separate Approved Birth Control Methods sheet (Appendix B).

You should not become pregnant during the study because we do not know how the study vaccines could affect the developing baby. You must agree to use effective birth control from 20 days before her first injection until her last study visit. We will talk to you about effective birth control methods. They are listed on a handout that we will give to you. If you join the study, we will test your urine for pregnancy at some visits, including before each study injection.

If you become pregnant while in the study, you must contact the study doctor immediately. The study doctor will advise you regarding your medical care.

Being in the study

If you meet the study requirements and wants to join, here is what will happen:

Site: Insert number of visits and range of visit lengths. (There is site-specific variation in screening protocols and in the number of possible follow-up visits between protocol-mandated visits.)

7. You will come to the clinic for scheduled visits about [#] times over 8 months.

Visits can last from [#] to [#] hours.

You may have to come for more visits if you have an abnormal test result or health issue.

We may contact you after the main study ends (for example, to tell you about the study results).

8. We will give you [Site: Insert compensation] for each study visit you complete.

This amount is to cover the costs of [Site: Insert text].

You do not have to pay anything to be in this study.

9. We will give you one of the study vaccines or a placebo.

Not everyone in this study will get one of the study vaccines. Some people will get a placebo, a substance that does not contain a vaccine product. We will compare the results from people who got the placebo with results from people who got the different study vaccines. In this study, the placebo is sterile salt water.

You have a 6-in-7 chance of receiving one of the study vaccines. This means that over 85% of the participants will receive one of the study vaccines.

Whether you get the study vaccines or the placebo is completely random, like flipping a coin.

The clinic staff has no say in whether you get the study vaccines or the placebo. The participants who get the BCG vaccine will be the only ones in the study who know what study product they are getting. This is because they will only get 1 injection while everyone else will get 2.

You will have to wait until all participants complete their final study visits to find out whether you got the other study vaccines or the placebo. This could be several years. But, if you have a serious medical problem and need to know what you got before the end of the study, we can tell you.

10. We will give you the study products on a schedule.

You will be in one of 4 groups. If you are in group 1, 2 or 4, we will not tell you which group you are in. The people in these 3 groups will get 2 injections. The first injection is at the enrollment visit. The second injection will be 56 days later. These injections will be given in the upper arm.

If you are in group 3, you will know because you will only get one injection during the study, at the enrollment visit. This injection will be given in the upper arm.

Injection Schedule		
Group		
	Enrollment visit	56 days later
1	H4	H4
2	H56	H56
3	BCG	
4	Placebo	Placebo

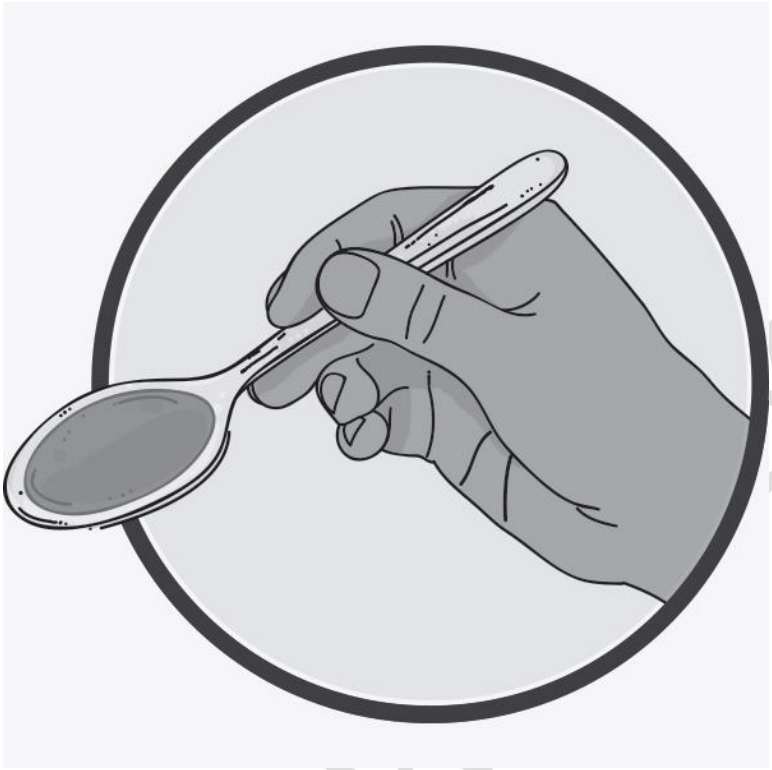
You will have to wait in the clinic for about 30 minutes after each injection to see if there are any problems. Then for that night and for seven more days, you will need to write down how you are feeling and if you have any symptoms. Clinic staff will show you how to do this in the diary card they will give you. You should contact the clinic staff if you have any issues or concerns after receiving an injection. If you have a problem, we will continue to check on you until it goes away. We will ask you the best way to contact you.

11. In addition to giving you the study products, we will:

- Do regular HIV testing, as well as counseling on the results and on how to avoid getting HIV;
- Do physical exams;
- Take blood and urine samples;
- Do pregnancy tests if you are female;
- Ask questions about your health, including medications you may be taking;
- Ask questions about any personal problems or benefits you may have from being in the study; and
- Teach you about the symptoms of TB, and ask if you have any of these symptoms. If we think you might have TB we will help you get medical care.

When we take blood, the amount will depend on the lab tests we need to do. It will be some amount between 7 mL and 57 mL (about 1/2 tablespoon to almost 4 tablespoons) at some visits. Your body will make new blood to replace the blood we take out. We will check your blood to watch your health. Researchers will also look at your blood to learn about the immune response to the study vaccines, and to develop some new ways to look at it.

This drawing shows an example of a tablespoon of blood.



We will review the results of these procedures and tests with you at your next visit, or sooner if necessary. If any of the results are important to your health, we will tell you. We will also offer you counseling and referral for needed care.

12. If you agree, we will also collect small samples of your stool (poop).

If you agree to give theses samples, we will give you more information about how we will collect them. We will do this twice during the study.

13. We will test your samples for this study.

We will send your samples (without your name) to a lab to see how your immune system responds to the study products. The researchers may:

- Take cells from your samples and grow more of them. We may grow more of your cells over time, so that they can continue to contribute to this study.

- Do limited genetic testing. Your genes are passed to you from your birth parents. They affect how you look and how your body works. The differences in people's genes can help explain why some people get a disease while others do not. Limited genetic testing involves only some of your genes, not all of your genes (your genome). The researchers will not look at all of your genes, only the genes related to the immune system and diseases.

- If you give stool samples, we will look at the bacteria living in your stomach. We want to learn if your immune response to the study vaccines is influenced by these bacteria.

In order to join the main study, you must agree to this limited genetic testing. These tests are for research purposes only. The lab will not give the results to you or this clinic, and the results will not become part of your study record.

14. We will do our best to protect your private information.

Your study records and samples will be kept in a secure location. We will label all of your samples and most of your records with a code number, not your name or other personal information. However, it is possible to identify you, if necessary. We will not share your name with the lab that does the tests on your samples, or with anyone else who does not need to know your name.

Clinic staff will have access to your study records. Your records may also be reviewed by groups who watch over this study to see that we are protecting your rights, keeping you safe, and following the study plan. These groups include:

- The US National Institutes of Health,
- Aeras, and its study monitors,
- [Site: insert name of your IRB/EC],
- South African Medicines Control Council,
- Sanofi Pasteur and Statens Serum Institut and people who work for them,
- The HVTN and people who work for them,
- Aeras Safety Monitoring Committee, and
- The US Office for Human Research Protections.

All reviewers will take steps to keep your records private.

We cannot guarantee absolute privacy. At this clinic, we have to report the following information:

[Site: Include any public health or legal reporting requirements. Bulleted examples should include all appropriate cases (reportable communicable disease, risk of harm to self or others, etc.).]

A summary of this study (in English) is on the website *http:/www.ClinicalTrials.gov* or http*:/www.sanct.gov.za*. This website does not include information that could identify anyone participating in this study. You can search this website at any time.

15. We may stop your injections or take you out of the study at any time.

We may do this even if you want to stay in the study. This may happen if:

- you do not follow instructions,
- the researcher thinks that staying in the study might harm you,

- you get HIV,
- you get active TB disease,
- you become pregnant,
- you are in prison (arrested),
- you enroll in a different research study where you receive another study product, or
- the study is stopped for any reason.

If we stop your injections, we may ask you to stay in the study to complete other study procedures.

16. If you become pregnant during the study, we will continue with some procedures but not injections.

If you leave the study while you are still pregnant, we will contact you after your due date to ask some questions about your pregnancy. We will save this information in our safety database.

Risks

17. There are other risks to being in this study.

This section describes the risks and restrictions we know about. There may also be unknown risks, even serious ones. We will tell you if we learn anything new that may affect your willingness to stay in the study.

Risks of routine medical procedures:

In this study, we will do some routine medical procedures. These are taking blood and giving injections. These procedures can cause bruising, pain, fainting, soreness, redness, swelling, itching and (rarely) muscle damage or infection where the needle was inserted. Taking blood can cause a low blood cell count (anemia), and may make your child feel tired.

Personal problems/discrimination:

Family or friends may worry, get upset or angry, or assume that you are infected with TB or HIV or at high risk and treat you unfairly as a result.

Risks of disclosure of your personal information:

We will take several steps to protect your personal information. Although the risk is very low, it is possible that your personal information could be given to someone who should not have it. If that happened, you could face discrimination, stress, and embarrassment. We can tell you more about how we will protect your personal information if you would like it.

Unknown risks:

We do not know if the study vaccines will increase, decrease, or not change your risk of getting TB disease if you are exposed to it.

We do not know if getting these study vaccines will affect how you respond to any future approved TB vaccine.

We do not know how the study vaccines will affect a pregnant participant or a developing baby.

Benefits

18. The study may not benefit you.

Getting the study vaccines will probably not benefit you. However, being in the study might still help you in some ways. The screening tests and counseling that you get as part of the study may help you avoid getting TB and HIV. The lab tests and physical exams that you get while in this study might detect health problems you do not yet know about.

Your participation in this study may help in the search for a vaccine to prevent TB. However, if any of the study vaccines later becomes approved and sold, there are no plans to share any money with you.

Your rights and responsibilities

19. If you join the study, you have rights and responsibilities.

Your duties as a study participant are listed below.

- Come to all Study Visits when you are told to. Give complete and truthful information about your medical history and any signs of illness you may have during the study.
- Give complete and truthful information about all medicines you take (or stop taking) during the study, including medicines from other doctors, herbal medicines, and medicine you buy for yourself.
- Give blood and urine samples to be tested.
- Complete the daily diary and remember to bring it to the study clinic for review;
- Tell the study doctor if you no longer want to be in the study.

Leaving the study

20. You should tell us if you decide to leave the study.

You are free to leave the study at any time and for any reason. Your care at this clinic and legal rights will not be affected, but it is important for you to let us know.

We will ask you to come back to the clinic one last time for a physical exam, and we may ask to take some blood and urine samples. We will also ask about any personal problems or benefits you may have experienced from being in the study. We believe these steps are important to protecting your health, but it is up to you whether to complete them.

Injuries

21. If you get sick or injured during the study, contact us immediately.

Your health is important to us. We will help you get the medical care you need.

We call an injury or illness study-related if it occurs as a direct result of the administration of the study products or study-related procedures. The clinic staff will treat you for study-related problems or tell you where to get the treatment you need. If a study-related injury occurs you have not waived any of the legal rights which you otherwise would have by signing this form.

If you get sick or injured because of the study vaccines, insurance has been purchased to cover your medical treatment. This policy will follow the guidelines for payment of study-related illness or injury approved by the Association of the British Pharmaceutical Industry ("ABPI Guidelines"). You can get a copy of these ABPI Guidelines from us if you wish.

If your injuries are more severe or chronic, the insurance funds may not be enough. If needed, Aeras has agreed to pay the cost of medical expenses that arise from injuries caused by the study products subject to certain limitations.

In addition, the HVTN also has limited funds from the U.S. government to pay for your treatment for study related injuries.

Some injuries are not physical. For example, someone might be harmed psychologically or emotionally by being in a TB vaccine study. Or they might lose wages from injuries because they could not go to work. No funds have been set aside to pay for nonphysical injuries, even if they are related to participation in the study.

Your usual healthcare provider and/or your health insurance carrier will continue to be responsible for the cost of your usual medical care outside this study, and for medical expenses that are determined not directly related to study procedures or products.

Medicines Control Council, South Africa -- MCC

If you have questions about this study you should first discuss them with the study doctor or the ethics committee (contact details as provided on this form). After you have consulted your doctor or the ethics committee and if they have not provided you with answers to your satisfaction, you should write to the Medicines Control Council (MCC) South Africa at:
The Registrar of Medicine
Medicines Control Council
Department of Health
Private Bag X828
Pretoria

0001
Fax: (021) 395 9201

Ethical approval

This clinical study protocol has been submitted to the Human Research Ethics Committee of the University of Cape Town and written approval has been granted by that committee.

The study has been written to follow the guidelines of the Declaration of Helsinki (last updated: October 2008), which deals with the recommendations guiding doctors in biomedical research involving human participants, and the Ethics in Health Research: Principles, Structures and Processes (2004) and Guidelines for Good Practice in the Conduct of Clinical Trials in Human Participants in South Africa.

We can provide you copies if you wish to review them.

Questions

22. If you have questions or problems at any time during your participation in this study, use the following important contacts.

If you have questions about this study, contact
[name and telephone number of the investigator or other study staff].

If you have any symptoms that you think may be related to this study, contact
[name and telephone number of the investigator or other study staff].

If you have questions about your rights as a research participant, or problems or concerns about how you are being treated in this study, contact
[name/title/phone of person on IRB or other appropriate organization].

If you want to leave this study, contact
[name and telephone number of the investigator or other study staff].

Your signature

23. In Section 12 of this form, we told you about collecting stool (poop) samples from you. Please write your initials or make your mark in the box next to the option you choose.

☐ I allow you to collect samples of my stool (poop).

☐ I do not allow you to collect samples of my stool (poop).

24. If you agree to join this study, you will need to sign or make your mark below. Before you sign or make your mark on this consent form, make sure of the following:

- You have read this consent form, or someone has read it to you.
- You feel that you understand what the study is about and what will happen to you if you join. You understand what the possible risks and benefits are.
- You have had your questions answered and know that you can ask more.
- You agree to join this study.

Participant's name (print)	Participant's signature or mark	Date	Time

Clinic staff conducting consent discussion (print)	Clinic staff signature	Date	Time

For participant who is unable to read or write, a witness should complete the signature block below:

Witness's name (print)	Witness's signature	Date	Time

*Witness is impartial and was present for the consent process.

Appendix H Sample assent form for use of samples and information in other studies

Title: A randomized, placebo-controlled, partially blinded phase 1b clinical trial to evaluate the safety and immunogenicity of BCG revaccination, H4:IC31, and H56:IC31 in healthy, HIV-1–uninfected adolescent participants

HVTN protocol number: HVTN 602 / AERAS A-042

Site:

You have agreed to be in a research study. We will take samples from you in this study. When we are done using your samples for this study, the HVTN and Aeras want to keep the extra ones to do other studies. During the study we also get information about you. Some of this information can be helpful in the other studies. We want your permission to use it.

You don't have to let us use your extra samples and information. It is OK to say yes or no. You can say no and still be in the study. It is OK to change your mind later. If you tell us to, we will destroy all extra samples from you that we have. We will not treat you any differently. You can ask us any questions you want to, anytime.

We will also give a form like this to your parent/guardian. We will ask them if they agree to have your extra samples and information used in other studies. We will only use your extra samples and information in other studies if both you and your parent/guardian agree to it.

1. Other studies

The other studies will be related to TB, vaccines, the immune system, and other diseases. The researchers may:

- Take cells from your samples and grow more of them. This means the researchers may keep your cells growing over time.
- Do limited genetic testing. This means looking at just some of your genes, not all of your genes.
- Do genome-wide studies. This means looking at all of your genes (your genome). In genome-wide studies, researchers compare the genomes of many people. They look for common patterns of genes that could help them understand diseases. The researchers may put the information from these studies into a protected database to share it with other researchers.

2. Risks

It is very unlikely that using your samples and information in other studies will cause problems for you. We will not put your name on them. Instead, we will put a code number on them. There is a very small chance that someone could learn something about you from your samples or information and use it to hurt you. We will only share your samples and information with the researchers who do the other studies and with groups

who pay for or watch over the other studies. All of these people will keep your information private.

3. Benefits

We will not pay you for letting us use your extra samples in other studies. The other studies will not help you, but they might help us learn more about TB and other diseases and how the body fights diseases.

4. Information

Your name will not be part of the information that we share. We may share information such as your race, ethnicity, gender, and information about your health during the study.

5. Storing the samples

We will store them in a safe place in South Africa. Some may be sent to the US for some tests, including genetic tests. There is no limit on how long we will store your extra samples. *[Site: insert limits if your regulatory authority imposes them.]*

6. Using the samples and information

We will not sell your samples or information. We may share them with other researchers. When a researcher wants to use your samples and/or information, their research plan must be approved by the HVTN and Aeras. Also, the researcher's institutional review board (IRB) or ethics committee (EC) will review their plan. *[Site: insert review by your institution's IRB/EC, if applicable.]* IRBs/ECs protect the rights and well-being of people in research.

7. Questions

If you have questions about the use of your samples or information or if you want to change your mind about their use, contact
[name and telephone number of the investigator or other study staff].

If you think you may have been harmed because of studies using your samples or information, contact [name and telephone number of the investigator or other study staff].

If you have questions about your rights as a research participant, contact
[name/title/phone of person on IRB or other appropriate organization].

Please write your initials or make your mark in the box next to the option you choose.

☐ I allow my extra samples and information to be used for other studies related to TB, vaccines, the immune system, and other diseases. This may include limited genetic testing and keeping my cells growing over time.

OR

☐ I agree to the option above and also to allow my extra samples and limited information to be used in genome wide studies.

OR

☐ I do not allow my extra samples to be used in any other studies. This includes not allowing limited genetic testing, growing more of my cells, or genome wide studies.

_____

Parent/guardian's name (print)

Participant's name (print)	Participant's signature or mark	Date	Time
Clinic staff conducting consent discussion (print)	Clinic staff signature	Date	Time

For participants who are unable to read or write, a witness should complete the signature block below:

Witness's name (print)	Witness's signature	Date	Time

*Witness is impartial and was present for the consent process.

Appendix I Sample informed consent form for use of samples and information in other studies for participants who turn 18 on study

Title: A randomized, placebo-controlled, partially blinded phase 1b clinical trial to evaluate the safety and immunogenicity of BCG revaccination, H4:IC31, and H56:IC31 in healthy, HIV-1–uninfected adolescent participants

HVTN protocol number: HVTN 602 / AERAS A-042

Site:

When samples are no longer needed for this study, the HVTN and Aeras wants to keep them for use in other studies. We will call these "extra samples."

This form gives you information so you can decide if you want your extra samples and information used in other studies. You will mark your decision at the end of the form. If you have any questions, please ask.

1. Do I have to agree?

No. You are free to say yes or no, or to change your mind after you sign this form. You can join the study even if you do not agree to the use of your extra samples and information in other studies. At your request, we will destroy all extra samples from you that we have. Your decision will not affect your being in this study or have any negative consequences here.

2. Where are the samples stored?

Extra samples are stored in locked freezers in a secure central place called a repository. *[Site: insert specific information if your regulatory authority requires it.]* The central repositories for the HVTN and Aeras are located in the United States and South Africa.

3. How long will the samples be stored?

There is no limit on how long your extra samples will be stored. *[Site: insert limits if your regulatory authority imposes them.]*

4. Will I be paid for the use of my samples?

No. Also, a researcher may make a new scientific discovery or product based on the use of your samples. If this happens, there is no plan to share any money with you. The researcher is not likely to ever know who you are.

5. Will I benefit from allowing my samples to be used in other studies?

Probably not. Results from these other studies are not given to you, this clinic, or your doctor. They are not needed for your medical care. They are not part of your medical record. The studies are only being done for research purposes.

6. Will the HVTN or Aeras sell my samples and information?

No, but the HVTN and Aeras may share your samples with other researchers. Once we share your samples and information, we will not be able to get them back.

7. How do other researchers get my samples and information?

The other researchers could be from universities, hospitals, companies that make and sell medicines, government agencies or other institutions. When a researcher wants to use your samples and/or information, their research plan must be approved by the HVTN and Aeras. Also, the researcher's institutional review board (IRB) or ethics committee (EC) will review their plan. *[Site: insert review by your institution's IRB/EC, if applicable.]* IRBs/ECs protect the rights and well-being of people in research. The HVTN and Aeras keep track of your decision about how your samples and information can be used.

8. What information is shared with other researchers?

The samples and limited information will be labeled with a code number. Your name will not be part of the information. However, some information that we share may be personal, such as your race, ethnicity, gender, health information from the study, and HIV status. We may share information about the study product you received and how your body responded to the study product.

9. What kind of studies might be done with my extra samples and information?

The studies will be related to TB, vaccines, the immune system, and other diseases. The researchers may:

- Take cells from your samples and grow more of them. This means the researchers may keep your cells growing over time.

- Do limited genetic testing, which involves only looking at some of your genes, not all of your genes.

If you agree, your samples could also be used for genome wide studies. In these studies, researchers will look at all of your genes (your genome). The researchers compare the genomes of many people, looking for common patterns of genes that could help them understand diseases. The researchers may put the information from the genome-wide studies into a protected database so that other researchers can access it. Usually, no one would be able to look at your genome and link it to you as a person. However, if another database exists that also has information on your genome and your name, someone might be able to compare the databases and identify you. If others found out, it could lead to discrimination or other problems. The risk of this is very small.

10. What are the risks of genetic testing?

The genetic testing could show you may be at risk for certain diseases. If others found out, it could lead to discrimination or other problems. However, it is almost impossible for you or others to know your test results from the genetic testing. The results are not part of your study records and are not given to you.

11. Who will have access to my information in studies using my extra samples?

People who may see your information are:

- Researchers who use your stored samples and limited information for other research
- Government agencies that fund or monitor the research using your samples or information
- The researcher's Institutional Review Board or Ethics Committee
- The people who work with the researcher

All of these people will do their best to protect your information. The results of any new studies that use your extra samples or information may be published. No publication will use your name or identify you personally.

Questions

12. If you have questions or problems about allowing your samples and information to be used in other studies, use the following important contacts.

If you have questions about the use of your samples or information or if you want to change your mind about their use, contact
[name and telephone number of the investigator or other study staff].

If you think you may have been harmed because of studies using your samples or information, contact [name and telephone number of the investigator or other study staff].

If you have questions about your rights as a research participant, contact [name/title/phone of person on IRB or other appropriate organization].

13. Please write your initials or make your mark in the box next to the option you choose.

☐ I allow my extra samples combined with limited information to be used for other studies related to TB, vaccines, the immune system, and other diseases. This may include limited genetic testing and keeping my cells growing over time.

OR

☐ I agree to the option above and also to allow my extra samples combined with limited information to be used in genome wide studies.

OR

☐ I do not allow my extra samples to be used in any other studies. This includes not allowing limited genetic testing, growing more of my cells, or genome wide studies.

Participant's name (print)	Participant's signature or mark	Date	Time

Clinic staff conducting consent discussion (print)	Clinic staff signature	Date	Time

For participant who is unable to read or write, a witness should complete the signature block below:

Witness's name (print)	Witness's signature	Date	Time

*Witness is impartial and was present for the consent process.

Appendix J Adverse Events of Special Interest

Adverse events of special interest represent a subset of AEs that include autoimmune diseases and other systemic disorders of interest which could potentially have an autoimmune etiology. Adverse events of special interest are listed below. The principal investigator should use clinical and scientific judgment in deciding whether other adverse events (ie, events not listed here) could have an autoimmune origin and should therefore be reported as AEs of special interest.

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

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

Appendix K Toxicity Table

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

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

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Unintentional weight loss	NA	5 – 9% loss in body weight from baseline	10 – 19% loss in body weight from baseline	≥ 20% loss in body weight from baseline OR Aggressive intervention indicated [eg, tube feeding or total parenteral nutrition (TPN)]
INFECTION				
Infection (any other than HIV infection)	Localized, no systemic antimicrobial treatment indicated AND Symptoms causing no or minimal interference with usual social and functional activities	Systemic antimicrobial treatment indicated OR Symptoms causing greater than minimal interference with usual social and functional activities	Systemic antimicrobial treatment indicated AND Symptoms causing inability to perform usual social and functional activities OR Operative intervention (other than simple incision and drainage) indicated	Life-threatening consequences (eg, septic shock)
INJECTION SITE REACTIONS				
Injection site pain (pain without touching) Or Tenderness (pain when area is touched)	Pain/tenderness causing no or minimal limitation of use of limb	Pain/tenderness limiting use of limb OR Pain/tenderness causing greater than minimal interference with usual social and functional activities	Pain/tenderness causing inability to perform usual social and functional activities	Pain/tenderness causing inability to perform basic self-care function OR Hospitalization (other than emergency room visit) indicated for management of pain/tenderness
Injection site reaction (localized)				
Adult > 15 years	Erythema OR Induration of 5x5 cm – 9x9 cm (or 25 cm^2 – 81cm^2)	Erythema OR Induration OR Edema > 9 cm any diameter (or > 81 cm^2)	Ulceration OR Secondary infection OR Phlebitis OR Sterile abscess OR Drainage	Necrosis (involving dermis and deeper tissue)
Pediatric ≤ 15 years	Erythema OR Induration OR Edema present but ≤ 2.5 cm diameter	Erythema OR Induration OR Edema > 2.5 cm diameter but < 50% surface area of the extremity segment (eg, upper arm/thigh)	Erythema OR Induration OR Edema involving ≥ 50% surface area of the extremity segment (eg, upper arm/thigh) OR Ulceration OR Secondary infection OR Phlebitis OR Sterile abscess OR Drainage	Necrosis (involving dermis and deeper tissue)
Pruritus associated with injection See also Skin: Pruritus (itching - no skin lesions)	Itching localized to injection site AND Relieved spontaneously or with < 48 hours treatment	Itching beyond the injection site but not generalized OR Itching localized to injection site requiring ≥ 48 hours treatment	Generalized itching causing inability to perform usual social and functional activities	NA

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
SKIN – DERMATOLOGICAL				
Cutaneous reaction – rash	Localized macular rash	Diffuse macular, maculopapular, or morbilliform rash OR Target lesions	Diffuse macular, maculopapular, or morbilliform rash with vesicles or limited number of bullae OR Superficial ulcerations of mucous membrane limited to one site	Extensive or generalized bullous lesions OR Stevens-Johnson syndrome OR Ulceration of mucous membrane involving 2 or more distinct mucosal sites OR Toxic epidermal necrolysis (TEN)
Pruritis (itching – no skin lesions) (See also Injection Site Reactions: Pruritis associated with injection)	Itching causing no or minimal interference with usual social and functional activities	Itching causing greater than minimal interference with usual social and functional activities	Itching causing inability to perform usual social and functional activities	NA
CARDIOVASCULAR				
Hypertension				
Adult > 17 years (with repeat testing at same visit)	140 – 159 mmHg systolic OR 90 – 99 mmHg diastolic	160 – 179 mmHg systolic OR 100 – 109 mmHg diastolic	**≥** 180 mmHg systolic OR **≥** 110 mmHg diastolic	Life-threatening consequences (eg, malignant hypertension) OR Hospitalization indicated (other than emergency room visit)
Pediatric ≤ 17 years (with repeat testing at same visit)	NA	91st – 94th percentile adjusted for age, height, and gender (systolic and/or diastolic)	≥ 95th percentile adjusted for age, height, and gender (systolic and/or diastolic)	Life-threatening consequences (eg, malignant hypertension) OR Hospitalization indicated (other than emergency room visit)
Hypotension	NA	Symptomatic, corrected with oral fluid replacement	Symptomatic, IV fluids indicated	Shock requiring use of vasopressors or mechanical assistance to maintain blood pressure
GASTROINTESTINAL				
Anorexia	Loss of appetite without decreased oral intake	Loss of appetite associated with decreased oral intake without significant weight loss	Loss of appetite associated with significant weight loss	Life-threatening consequences OR Aggressive intervention indicated [eg, tube feeding or total parenteral nutrition (TPN)]
Comment: Please note that, while the grading scale provided for Unintentional Weight Loss may be used as a guideline when grading anorexia, this is not a requirement and should not be used as a substitute for clinical judgment.				

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
Diarrhea	Transient or intermittent episodes of unformed stools OR Increase of ≤ 3 stools over baseline per 24-hour period	Persistent episodes of unformed to watery stools OR Increase of 4 – 6 stools over baseline per 24-hour period	Bloody diarrhea OR Increase of ≥ 7 stools per 24-hour period OR IV fluid replacement indicated	Life-threatening consequences (eg, hypotensive shock)
Nausea	Transient (< 24 hours) or intermittent nausea with no or minimal interference with oral intake	Persistent nausea resulting in decreased oral intake for 24 – 48 hours	Persistent nausea resulting in minimal oral intake for > 48 hours OR Aggressive rehydration indicated (eg, IV fluids)	Life-threatening consequences (eg, hypotensive shock)
Vomiting	Transient or intermittent vomiting with no or minimal interference with oral intake	Frequent episodes of vomiting with no or mild dehydration	Persistent vomiting resulting in orthostatic hypotension OR Aggressive rehydration indicated (eg, IV fluids)	Life-threatening consequences (eg, hypotensive shock)
Headache	Symptoms causing no or minimal interference with usual social and functional activities	Symptoms causing greater than minimal interference with usual social and functional activities	Symptoms causing inability to perform usual social and functional activities	Symptoms causing inability to perform basic self-care functions OR Hospitalization indicated (other than emergency room visit) OR Headache with significant impairment of alertness or other neurologic function
Insomnia	NA	Difficulty sleeping causing greater than minimal interference with usual social and functional activities	Difficulty sleeping causing inability to perform usual social and functional activities	Disabling insomnia causing inability to perform basic self-care functions
RESPIRATORY				
Bronchospasm (acute)	FEV1 or peak flow reduced to 70 **–** 80%	FEV1 or peak flow 50 **–** 69%	FEV1 or peak flow 25 **–** 49%	Cyanosis OR FEV1 or peak flow < 25% OR Intubation
Dyspnea or respiratory distress				
Adult ≥ 14 years	Dyspnea on exertion with no or minimal interference with usual social and functional activities	Dyspnea on exertion causing greater than minimal interference with usual social and functional activities	Dyspnea at rest causing inability to perform usual social and functional activities	Respiratory failure with ventilatory support indicated
Pediatric < 14 years	Wheezing OR minimal increase in respiratory rate for age	Nasal flaring OR Intercostal retractions OR Pulse oximetry 90 – 95%	Dyspnea at rest causing inability to perform usual social and functional activities OR Pulse oximetry < 90%	Respiratory failure with ventilatory support indicated

PARAMETER	GRADE 1 MILD	GRADE 2 MODERATE	GRADE 3 SEVERE	GRADE 4 POTENTIALLY LIFE-THREATENING
MUSCULOSKELETAL				
Arthralgia See also Arthritis	Joint pain causing no or minimal interference with usual social and functional activities	Joint pain causing greater than minimal interference with usual social and functional activities	Joint pain causing inability to perform usual social and functional activities	Disabling joint pain causing inability to perform basic self-care functions
Arthritis See also Arthralgia	Stiffness or joint swelling causing no or minimal interference with usual social and functional activities	Stiffness or joint swelling causing greater than minimal interference with usual social and functional activities	Stiffness or joint swelling causing inability to perform usual social and functional activities	Disabling joint stiffness or swelling causing inability to perform basic self-care functions
Myalgia (non-injection site)	Muscle pain causing no or minimal interference with usual social and functional activities	Muscle pain causing greater than minimal interference with usual social and functional activities	Muscle pain causing inability to perform usual social and functional activities	Disabling muscle pain causing inability to perform basic self-care functions

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

LABORATORY				
PARAMETER	**GRADE 1 MILD**	**GRADE 2 MODERATE**	**GRADE 3 SEVERE**	**GRADE 4 POTENTIALLY LIFE-THREATENING**
CHEMISTRIES	***Standard International Units are listed in italics***			
Alkaline Phosphatase	1.25 – 2.5 x ULN†	2.6 – 5.0 x ULN†	5.1 – 10.0 x ULN†	> 10.0 x ULN†
ALT (SGPT)	1.25 – 2.5 x ULN	2.6 – 5.0 x ULN	5.1 – 10.0 x ULN	> 10.0 x ULN
AST (SGOT)	1.25 – 2.5 x ULN	2.6 – 5.0 x ULN	5.1 – 10.0 x ULN	> 10.0 x ULN
Blood urea nitrogen (BUN)	23 – 26 mg/dL 8.3 – 9.5 mmol/L	27 – 31 mg/dL 9.6 – 11.2 mmol/L	>31 mg/dL >11.2 mmol/L	Requires dialysis
Creatinine – elevated	1.5 – 1.7 mg/dL 121 - 145 umol/L	1.8 – 2.0 mg/dL 146 – 170 umol/L	2.1 – 2.5 mg/dL 171 – 208 umol/L	>2.5 mg/dL or requires dialysis >208 umol/L or requires dialysis
Bilirubin (Total)	1.1 – 1.5 x ULN	1.6 – 2.5 x ULN	2.6 – 5.0 x ULN	> 5.0 x ULN
URINALYSIS	***Standard International Units are listed in italics***			
Hematuria (microscopic)	6 – 10 RBC/HPF	> 10 RBC/HPF	Gross, with or without clots OR with RBC casts	Transfusion indicated
Proteinuria, random collection	1 +	2 – 3 +	4 +	NA
Glucosuria	Trace	1 +	2 +	NA
Adult and Pediatric ≥ 10 years	200 – 999 mg/24 h *0.200 – 0.999 g/d*	1,000 – 1,999 mg/24 h *1.000 – 1.999 g/d*	2,000 – 3,500 mg/24 h *2.000 – 3.500 g/d*	> 3,500 mg/24 h *> 3.500 g/d*

Appendix L Laboratory procedures

Groups 1, 2, and 4

					Tube volume (mL)												
				Visit:	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	
				Day:	Screening	D0	D1	D3	D7	D14	D28	D56	D63	D70	D168	D224	
				Month:	visit[3]	M0			M0.25	M0.5	M1	M2	M2.25	M2.5	M6	M8	
Description	**Ship to[1]**	**Assay location[2]**	**Tube[4]**	**Tube size (vol capacity)[4]**		**AERAS-404 OR H56IC OR Placebo**						**AERAS-404 OR H56IC OR Placebo**					**Total**
BLOOD COLLECTION																	
Screening or diagnostic assays																	
QuantiFERON TB Gold-in tube	Local Lab	Local Lab	QFT-GIT	1mL	3	—	—	—	—	—	—	—	—	3	3	3	**12.0**
ESAT-6 free IGRA	Local Lab	Local Lab	ESAT-6 free IGRA	1mL	2	—	—	—	—	—	—	—	—	2	2	2	**8.0**
HIV test[8]	Local Lab	Local Lab	EDTA	2mL	2	—	—	—	—	—	—	—	—	2	2	2	**8.0**
Safety labs																	
CBC/ Diff/ platelets	Local Lab	Local Lab	EDTA	2mL	2	—	—	—	2	—	—	—	2	—	2	—	**8.0**
Chemistry Panel[5]	Local Lab	Local Lab	SST	3.5mL	3.5	—	—	—	3.5	—	—	—	3.5	—	3.5	—	**14.0**
Immunological assays[6]																	
Adaptive Immunity																	
PBMC ICS	CSR	FHCRC/CHIL	ACD	8.5mL	—	17	—	—	—	***17***	—	—	—	***17***	17	—	**68.0**
PBMC Cytokine Multiplex Assay	CSR	FHCRC/CHIL	ACD	8.5mL	—	w	—	—	—	***w***	—	—	—	***w***	w	—	**-**
Phenotyping - pTfh	CSR	FHCRC/CHIL	ACD	8.5mL	—	w	—	—	8.5	—	—	8.5	8.5	—	—	—	**25.5**
Phenotyping - Plasmablasts	CSR	FHCRC/CHIL	ACD	8.5mL	—	w	—	—	w	—	—	w	w	—	—	—	**-**
Stimulated PBMC Transcription (RNA Seq)	CSR	FHCRC/CHIL	ACD	8.5mL	—	8.5	—	—	—	—	—	—	—	***8.5***	—	—	**17.0**
Fluidigm	CSR	FHCRC/CHIL	ACD	8.5mL	—	w	—	—	—	***w***	—	—	—	***w***	—	—	**-**
ELISA / BAMA	CSR	AERAS/Duke-DHVI	ACD	—	—	y	—	—	—	***y***	—	—	—	***y***	y	—	**-**
Innate Immunity																	
TruCount	CSR	BARC/CHIL	ACD	3.5mL	—	w	3.5	3.5	w	—	—	—	—	—	—	—	**7.0**
Serum cytokines	CSR	FHCRC/CHIL	SST	3.5mL	—	3.5	3.5	3.5	3.5	—	—	—	—	—	—	—	**14.0**
Whole Blood Gene Expression (RNA Seq)	CSR	FHCRC/CHIL	Tempus	3mL	—	3	3	3	3	—	—	—	—	3	—	—	**15.0**
Additional assays																	
NTM assay	CSR	FHCRC/CHIL	ACD	6mL	—	6	—	—	—	—	—	—	—	—	—	—	**6.0**
PBMC mycobacterial growth-inhibition assay	CSR	TBD[10]	ACD	8.5mL	—	w	—	—	—	***w***	—	—	—	***w***	—	—	**-**
CyTOF	CSR	FHCRC/CHIL	ACD	8.5mL	—	w	—	—	—	—	—	—	—	***w***	—	—	**-**
Storage																	
PBMC storage	CSR	—	ACD	8.5mL	—	8.5	—	—	—	8.5	—	—	—	8.5	8.5	—	**34.0**
Plasma storage	CSR	—	ACD	—	—	y	—	—	—	y	—	—	—	y	y	—	**-**
Maximum Total					**12.5**	**46.5**	**10.0**	**10.0**	**20.5**	**25.5**	—	**8.5**	**14.0**	**44.0**	**38.0**	**7.0**	**236.5**
Maximum 28-Day total					**12.5**	**59.0**	**69.0**	**79.0**	**99.5**	**125.0**	—	**8.5**	**22.5**	**66.5**	**38.0**	**7.0**	
URINE COLLECTION																	
Urinalysis	Local Lab	Local Lab	—	—	X	—	—	—	X	—	—	—	X	—	—	—	
Pregnancy test[7]	Local Lab	Local Lab	—	—	X	X	—	—	—	—	—	X	—	—	—	—	
STOOL COLLECTION (OPTIONAL)																	
Stool[9]	CSR	FHCRC	—	—	—	(X)	(X)	(X)	(X)	—	—	—	(X)	(X)	—	—	

Footnotes are located after the following table.

Group 3

Description	Ship to[1]	Assay location[2]	Tube[4]	Tube size (vol capacity)[4]	Tube volume (mL)												
Visit:					1	2	3	4	5	6	7	8	9	10	11	12	
Day:					Screening	D0	D1	D3	D7	D14	D28	D56	D63	D70	D168	D224	
Month:					visit[3]	M0			M0.25	M0.5	M1	M2	M2.25	M2.5	M6	M8	
						BCG											**Total**
BLOOD COLLECTION																	
Screening or diagnostic assays																	
QuantiFERON TB Gold-in tube	Local Lab	Local Lab	QFT-GIT	1mL	3	—	—	—	—	—	—	—	—	3	3	3	**12.0**
ESAT-6 free IGRA	Local Lab	Local Lab	ESAT-6 free IGRA	1mL	2	—	—	—	—	—	—	—	—	2	2	2	**8.0**
HIV test[8]	Local Lab	Local Lab	EDTA	2mL	2	—	—	—	—	—	—	—	—	2	2	2	**8.0**
Safety labs																	
CBC/ Diff/ platelets	Local Lab	Local Lab	EDTA	2mL	2	—	—	—	2	—	—	—	—	—	2	—	**6.0**
Chemistry Panel[5]	Local Lab	Local Lab	SST	3.5mL	3.5	—	—	—	3.5	—	—	—	—	—	3.5	—	**10.5**
Immunological assays[6]																	
PBMC ICS	CSR	FHCRC/CHIL	ACD	8.5mL	—	17	—	—	—	—	***17***	—	—	***17***	17	—	**68.0**
PBMC Cytokine Multiplex Assay	CSR	FHCRC/CHIL	ACD	8.5mL	—	w	—	—	—	—	***w***	—	—	***w***	w	—	**-**
Phenotyping - Tfh	CSR	FHCRC/CHIL	ACD	8.5mL	—	w	—	—	8.5	—	—	—	—	—	—	—	**8.5**
Phenotyping - Plasmablasts	CSR	FHCRC/CHIL	ACD	8.5mL	—	w	—	—	w	—	—	—	—	—	—	—	**-**
Non-classical T cells	CSR	UW	ACD	8.5mL	—	8.5	—	—	—	—	***8.5***	—	—	***8.5***	—	—	**25.5**
Stimulated PBMC Transcription	CSR	FHCRC/CHIL	ACD	8.5mL	—	8.5	—	—	—	—	***w***	—	—	—	—	—	**8.5**
Fluidigm	CSR	FHCRC/CHIL	ACD	8.5mL	—	w	—	—	—	—	***w***	—	—	***w***	—	—	**-**
ELISA / BAMA	CSR	AERAS/Duke-DHVI	ACD	—	—	y	—	—	—	—	***y***	—	—	***y***	y	—	**-**
Innate Immunity																	
TruCount	CSR	BARC/CHIL	ACD	3.5mL	—	w	3.5	3.5	w	—	—	—	—	—	—	—	**7.0**
Serum cytokines	CSR	FHCRC/CHIL	SST	3.5mL	—	3.5	3.5	3.5	3.5	—	—	—	—	—	—	—	**14.0**
Whole Blood Gene Expression	CSR	FHCRC/CHIL	Tempus	3mL	—	3	3	3	3	—	—	—	—	***3***	—	—	**15.0**
Additional assays																	
NTM assay	CSR	FHCRC/CHIL	ACD	6mL	—	6	—	—	—	—	—	—	—	—	—	—	**6.0**
PBMC mycobacterial growth-inhibition assay	CSR	TBD[10]	ACD	8.5mL	—	w	—	—	—	—	***w***	—	—	***w***	—	—	**-**
CyTOF	CSR	FHCRC/CHIL	ACD	8.5mL	—	w	—	—	—	—	***w***	—	—	—	—	—	**-**
Storage																	
PBMC storage	CSR	—	ACD	8.5mL	—	8.5	—	—	—	—	8.5	—	—	17	8.5	—	**42.5**
Plasma storage	CSR	—	ACD	—	—	y	—	—	—	—	y	—	—	y	y	—	**-**
Maximum Total					**12.5**	**55.0**	**10.0**	**10.0**	**20.5**	—	**34.0**	—	—	**52.5**	**38.0**	**7.0**	**239.5**
Maximum 28-Day total					**12.5**	**67.5**	**77.5**	**87.5**	**108.0**	—	**142.0**	—	—	**52.5**	**38.0**	**7.0**	
URINE COLLECTION																	
Urinalysis	Local Lab	Local Lab	—	—	X	—	—	—	X	—	—	—	—	—	—	—	
Pregnancy test[7]	Local Lab	Local Lab	—	—	X	X	—	—	—	—	—	—	—	—	—	—	
STOOL COLLECTION (OPTIONAL)																	
Stool[9]	CSR	FHCRC	—	—	—	(X)	(X)	(X)	(X)	—	(X)	—	—	(X)	—	—	

Footnotes are located on the following page.

The following footnotes apply to both the preceding tables.
Grayed out columns = visit 7 not required for Groups 1, 2, and 4, and visits 6, 8, and 9 not required for Group 3.
note: QFIT: 1mL blood for each of 3 tubes for ppt (includes controls)
w = PBMC collected for other assays and/or storage will cover specimen needs; no separate blood draw is needed
y= up to 5mL of plasma will be harvested during ACD blood processing for PBMCs; no separate blood collection required

[1] CSR = central specimen repository

[2] HVTN Laboratory Program includes laboratories at Duke-DHVI, FHCRC, CHIL. Duke-DHVI = Duke Human Vaccine Institute, Duke University Medical Center (Durham, North Carolina, USA); FHCRC = Fred Hutchinson Cancer Research Center (Seattle, Washington, USA);
CHIL = Cape Town HVTN Immunology Laboratory (Cape Town, South Africa).
Non-HVTN laboratories: Aeras = Aeras (Rockville, Maryland, USA); UW = University of Washington (Seattle, Washington, USA).

[3] Screening may occur over the course of several contacts/visits up to and including day 0 prior to vaccination.

[4] Local labs may assign appropriate alternative tube types for locally performed tests.

[5] Chemistry panels are defined in Section 8.2 (pre-enrollment) and Section 8.6 (post-enrollment).

[6] Immunogenicity assays will be performed at M0, M0.5 (for Groups 1, 2, and 4), M1 (for Group 3), and M2.5. Based on the number of responders observed at these timepoints, lab assays may be performed on participants for humoral and cellular responses at other time points.

[7] Pregnancy test may be performed on blood specimens.

[8] At an early termination visit for a withdrawn or terminated participant (see Section 8.9), blood should be drawn for HIV diagnostic testing, as shown for visit 12 above.

[9] Optional stool specimens may be collected at visit 2, 3, 4, or 5 (between enrollment and 7 days post vaccination 1), and at visit 9 or 10 (between 7 and 14 days post vaccination 2, for Groups 1, 2, and 4) or visit 7 or 10 (for Group 3), from study participants who agree to this procedure.

[10] Assay location to be determined.

Appendix M Procedures at CRS

Groups 1, 2, and 4 Visit[a]:	**01[b]**	**02**	**03**	**04**	**05**	**06**	**07**	**08**	**09**	**10**	**11**	**12**	**Early study discontinuation**	**Post**
Day:		D0	D1	D3	D7	D14	D28	D56	D63	D70	D168	D224		
Month:		M0			M0.25	M0.5	M1	M2	M2.25	M2.5	M6	M8		
Procedure	Scr.	H4:IC31 or H56:IC31or Placebo						H4:IC31 or H56:IC31or Placebo						
Study procedures[c]														
Signed screening consent (if used)	X	—	—	—	—	—	—	—	—	—	—	—		—
Assessment of understanding	X	—	—	—	—	—	—	—	—	—	—	—		—
Signed protocol consent and extended safety surveillance consent	X	—	—	—	—	—	—	—	—	—	—	—		—
Medical history	X	—	—	—	—	—	—	—	—	—	—	—	X	—
Complete physical exam	X	—	—	—	—	—	—	—	—	—	—	X		—
Abbreviated physical exam	—	X	X	X	X	X	—	X	X	X	X	—	X	—
Stool sample collection - optional[d]	—	(X)	(X)	(X)	(X)	—	—	—	(X)	(X)	—	—		—
Risk reduction counseling	X	—	—	—	—	—	—	—	—	X	X	X		—
Pregnancy prevention assessment[e]	X	X	—	—	—	X	—	X	—	X	X	X		—
Confirm eligibility, obtain demographics, randomize	X	—	—	—	—	—	—	—	—	—	—	—		—
Social impact assessment	—	X	X	X	X	X	—	X	X	X	X	X		—
Concomitant medications	X	X	X	X	X	X	—	X	X	X	X	X		—
Intercurrent illness/adverse experience	—	X	X	X	X	X	—	X	X	X	X	X		—
HIV infection assessment[f]	X	—	—	—	—	—	—	—	—	X	X	X		—
Confirm HIV test results provided to participant	—	X	—	—	—	—	—	—	—	—	X	X		X
Local lab assessment[g]														
Urine dipstick	X	—	—	—	X	—	—	—	X	—	—	—	X	—
Pregnancy (urine or serum HCG)[h]	X	X	—	—	—	—	—	X	—	—	—	—		—
CBC, differential, platelet	X	—	—	—	X	—	—	—	X	—	X	—	X	—
Chemistry panel	X	—	—	—	X	—	—	—	X	—	X	—	X	—
Vaccination[i]	—	X	—	—	—	—	—	X	—	—	—	—		—
Reactogenicity assessments[j]	—	X	—	—	—	—	—	X	—	—	—	—		—
QFT-GIT	X	—	—	—	—	—	—	—	—	X	X	X	X	—
Poststudy														
Unblind participant[k]	—	—	—	—	—	—	—	—	—	—	—	—		X

[a] Visit windows are detailed in Study Specific Procedures.

[b] Screening may occur over the course of several contacts/visits up to and including day 0 prior to vaccination.

[c] For specimen collection requirements, see Appendix L.

[d] Optional stool specimens may be collected at visit 2, 3, 4, or 5 (between enrollment and 7 days post vaccination 1), and at visit 9 or 10 (between 7 and 14 days post vaccination 2, for Groups 1, 2, and 4) from participants who agree to this procedure.

[e] Pregnancy prevention compliance occurs only with participants who were born female and are capable of becoming pregnant.

[f] Includes pre-test counseling. A subsequent follow-up contact is conducted to provide post-test counseling and to report results to participant.

[g] Items below are also listed in specimen table, Appendix L.

[h] For a participant who was born female, pregnancy test must be performed on the day of vaccination prior to vaccination. Pregnancy test to determine eligibility may be performed at screening or on day 0 prior to first vaccination. Persons who are NOT of reproductive potential due to having undergone total hysterectomy with bilateral oophorectomy (verified by medical records), are not required to undergo pregnancy testing. Serum pregnancy tests may be used to confirm the results of, or substitute for, a urine pregnancy test.

[i] Blood draws required at vaccination visits must be performed prior to administration of study product; however, it is not necessary to have results prior to administration. Lab tests may be drawn within the 3 days prior to vaccination.

[j] Reactogenicity assessments performed daily for at least 3 days postvaccination (see Section 8.7).

[k] Unblinding will take place after database lock

Group 3 Visit[a]:	01[b]	02	03	04	05	06	07	08	09	10	11	12	Early study discontinuation	Post
Day:		D0	D1	D3	D7	D14	D28	D56	D63	D70	D168	D224		
Month:		M0			M0.25	M0.5	M1	M2	M2.25	M2.5	M6	M8		
Procedure	Scr.	BCG												
Study procedures[c]														
Signed screening consent (if used)	X	—	—	—	—	—	—	—	—	—	—	—		—
Assessment of understanding	X	—	—	—	—	—	—	—	—	—	—	—		—
Signed protocol consent and extended safety surveillance consent	X	—	—	—	—	—	—	—	—	—	—	—		—
Medical history	X	—	—	—	—	—	—	—	—	—	—	—	X	—
Complete physical exam	X	—	—	—	—	—	—	—	—	—	—	X		—
Abbreviated physical exam	—	X	X	X	X	—	X	—	—	X	X	—	X	—
Stool sample collection - optional[d]	—	(X)	(X)	(X)	(X)	—	(X)	—	—	(X)	—	—		—
Risk reduction counseling	X	—	—	—	—	—	—	—	—	X	X	X		—
Pregnancy prevention assessment[e]	X	X	—	—	X	—	X	—	—	X	X	X		—
Confirm eligibility, obtain demographics, randomize	X	—	—	—	—	—	—	—	—	—	—	—		—
Social impact assessment	—	X	X	X	X	—	X	—	—	X	X	X		—
Concomitant medications	X	X	X	X	X	—	X	—	—	X	X	X		—
Intercurrent illness/adverse experience	—	X	X	X	X	—	X	—	—	X	X	X		—
HIV infection assessment[f]	X	—	—	—	—	—	—	—	—	X	X	X		—
Confirm HIV test results provided to participant	—	X	—	—	—	—	—	—	—	—	X	X		X
Local lab assessment[g]														
Urine dipstick	X	—	—	—	X	—	—	—	—	—	—	—	X	—
Pregnancy (urine or serum HCG)[h]	X	X	—	—	—	—	—	—	—	—	—	—		—
CBC, differential, platelet	X	—	—	—	X	—	—	—	—	—	X	—	X	—
Chemistry panel	X	—	—	—	X	—	—	—	—	—	X	—	X	—
Vaccination[i]	—	X	—	—	—	—	—	—	—	—	—	—		—
Reactogenicity assessments[j]	—	X	—	—	—	—	—	—	—	—	—	—		—
QFT-GIT	X	—	—	—	—	—	—	—	—	X	X	X	X	—
Poststudy														
Unblind participant[k]	—	—	—	—	—	—	—	—	—	—	—	—		X

[a] Visit windows are detailed in Study Specific Procedures.

[b] Screening may occur over the course of several contacts/visits up to and including day 0 prior to vaccination.

[c] For specimen collection requirements, see Appendix L.

[d] Optional stool specimens may be collected at visit 2, 3, 4, or 5 (between enrollment and 7 days post vaccination 1), and at visit 9 or 10 (between 7 and 14 days post vaccination 2, for Groups 1, 2, and 4) or visit 7 or 10 (for Group 3), from study participants who agree to this procedure.

[e] Pregnancy prevention compliance occurs only with participants who were born female and are capable of becoming pregnant.

[f] Includes pre-test counseling. A subsequent follow-up contact is conducted to provide post-test counseling and to report results to participant.

[g] Items below are also listed in specimen table, Appendix L.

[h] For a participant who was born female, pregnancy test must be performed on the day of vaccination prior to vaccination. Pregnancy test to determine eligibility may be performed at screening or on day 0 prior to first vaccination. Persons who are NOT of reproductive potential due to having undergone total hysterectomy with bilateral oophorectomy (verified by medical records), are not required to undergo pregnancy testing. Serum pregnancy tests may be used to confirm the results of, or substitute for, a urine pregnancy test.

[i] Blood draws required at vaccination visits must be performed prior to administration of study product; however, it is not necessary to have results prior to administration. Lab tests may be drawn within the 3 days prior to vaccination.

[j] Reactogenicity assessments performed daily for at least 3 days postvaccination (see Section 8.7).

[k] Unblinding will take place after database lock