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A-055: Study to Evaluate H56:IC31 in Preventing Rate of TB Recurrence
NCT03512249

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Title: A Phase 2, Double-blind, Randomized, Placebo-Controlled Study to Evaluate the Safety and Efficacy of H56:IC31 in Reducing the rate of TB Disease Recurrence in HIV Negative Adults Successfully Treated for Drug-Susceptible Pulmonary Tuberculosis

Final Version 5.0 dated 19 May 2021

PROTOCOL

A Phase 2, Double-blind, Randomized, Placebo-Controlled Study to Evaluate the Safety and Efficacy of H56:IC31 in Reducing the rate of TB Disease Recurrence in HIV Negative Adults Successfully Treated for Drug-Susceptible Pulmonary Tuberculosis

Investigational Product: **H56:IC31**

Protocol Number: **A-055**

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Principal Investigator Agreement:

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

Signature [redacted] Date 21/May/2021

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

Signature [redacted] Date 31 May 2021

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Principal Investigator Agreement:

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Signature Digitally signed by [redacted] Date

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Principal Investigator Agreement:

I, the undersigned, have reviewed this protocol and agree to conduct this protocol in accordance with Good Clinical Practices (ICH-GCP), the ethical principles set forth in the Declaration of H[redacted]quirements.

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

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Clinical Protocol-Amended Protocol Approval Form

If electronically signed off in VeTMF, the signatory and date lines on this form will remain blank, and electronic signatures will be generated on a separate page; the names of the signatories to fulfil each role will still be entered on this page.

Product(s): H56:IC31

Protocol Number: A-055
Protocol Version: FV 5.0
Protocol Date: 19 May 2021

Signatory	**Date**
[redacted]	
(Senior Medical Director)	
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STATENS SERUM INSTITUT

Clinical Protocol-Amended Protocol Approval Form

Product(s): H56:IC31

Protocol Number: A-055
Protocol Version: v5.0
Protocol Date: 19/May/2021

Signatory	Date
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LIST OF ABBREVIATIONS

AE	adverse event
AFB	acid fast bacilli (on sputum smear microscopy - the Auramine technique)
ALP	alkaline phosphatase
ALT	alanine aminotransferase
anti-TNF	anti-tumor necrosis factor
AST	aspartate aminotransferase
[redacted]	[redacted]
ßHCG	beta human chorionic gonadotropin
BCG	bacille Calmette-Guérin
BMI	body mass index
BUN	blood urea nitrogen
CBC	complete blood count
CM	concomitant medication
CFR	Code of Federal Regulations
CPK	creatinine phosphokinase
CRO	contract research organisation
CT	computed tomography
DSMB	data safety monitoring board
ECG	electrocardiogram
eCRF	electronic case report form
EDC	electronic data capture
EDCTP	The European & Developing Countries Clinical Trial Partnership
ELISA	enzyme linked immunosorbent assay
ELISpot	enzyme linked immunospot
ESR	erythrocyte sedimentation rate
ET Visit	early termination visit
GCP	good clinical practice
GMP	good manufacturing practice
GGT	gamma glutamyl transferase
HIV	human immunodeficiency virus
HLA	human leukocyte antigen
IB	investigator's brochure
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
ICS	intracellular cytokine staining
IgG ELISA	immunoglobulin G enzyme linked immunosorbent assay
IMP	investigational medicinal product
IEC	Independent Ethics Committee
IP	investigational product
IRB	Institutional Review Board
ITT	intention to treat
IXRS	interactive voice/web response system
LTBI	latent tuberculosis infection
LLN	lower limit of normal

MDR	multi-drug-resistant
mITT	modified intention to treat
[redacted]	[redacted]
Mtb	*Mycobacterium tuberculosis*
NHP	non-human-primate
[redacted]	[redacted]
[redacted]	[redacted]
PBMC	peripheral blood mononuclear cell
PI	principal investigator
POI	prevention of infection
POR	prevention of recurrence
PP	per-protocol
PT	prothrombin time
PTT	partial thromboplastin time
QFT	QuantiFERON-TB
SAHPRA	South African Health Products Regulatory Authority
SAE	serious adverse event
[redacted]	[redacted]
SOP	standard operating procedure
SSA	Sub-Saharan Africa
SSI	Statens serum institute, Denmark
STB Visit	suspected TB visit
SUSAR	suspected unexpected serious adverse reaction
[redacted]	[redacted]
TB	tuberculosis
TST	tuberculin skin test
TZ	Tanzania
[redacted]	[redacted]
ULN	upper limit of normal
UN	United Nations
WB	whole blood
WB ICS	whole blood intracellular cytokine staining
WGS	whole genome sequencing
WHO	World Health Organization
Xpert MTB/RIF (Ultra)	Cartridge based nucleic acid amplification test (NAAT), automated diagnostic test that can identify *Mycobacterium tuberculosis* (MTB) DNA and resistance to rifampicin (RIF)
ZA	South Africa

STUDY ABSTRACT

Protocol Title	A Phase 2, Double-blind, Randomized, Placebo-Controlled Study to Evaluate the Safety and Efficacy of H56:IC31 in Reducing the rate of TB Disease Recurrence in HIV Negative Adults Successfully Treated for Drug-Susceptible Pulmonary Tuberculosis
Protocol Number	A-055
Primary Objective	To evaluate the following in HIV-negative participants who have completed at least 5 months (22 weeks) treatment for drug-susceptible pulmonary TB and who test negative for acid fast bacilli (AFB) on sputum smear microscopy prior to vaccination (participants unable to produce sputum, and considered asymptomatic by the investigator, may be considered *Mtb* negative): • Efficacy of H56:IC31 compared to placebo in reducing the rate of recurrent TB disease (relapse or reinfection)
Secondary Objective(s)	To evaluate the following in HIV-negative participants who have completed at least 5 months (22 weeks) treatment for drug-susceptible pulmonary TB and who test negative for AFB on sputum smear microscopy prior to vaccination (participants unable to produce sputum, and considered asymptomatic by the investigator, may be considered *Mtb* negative): Key secondary objective: • Safety of H56:IC31 compared to placebo Other secondary objectives: • Trends towards efficacy of H56:IC31 compared to placebo in reducing the rate of TB disease relapse • Trends towards efficacy of H56:IC31 compared to placebo in reducing the rate of TB disease reinfection • Antigen-specific cell-mediated immune responses to H56:IC31 • Humoral immune responses to H56:IC31
Design	This is a phase 2, double-blind, randomized (1:1), placebo-controlled study with 2 parallel groups. • H56:IC31 (investigational vaccine) • Placebo The 1st screening visit (V1) must be conducted ≤ 7 days after the start date of the TB treatment. The 2nd screening visit (V2) must be conducted ≥ 5 months (22 weeks) after the start date of TB treatment. The end of TB treatment is expected to be 6 months (24 weeks) after the start of TB treatment and may not be extended beyond 28 weeks. Upon written approval of the Sponsors and relevant approvals for the Protocol Amendment v5.0, participants may be screened at a combined V1 and V2. The combined visits must be conducted at ≥ 5 months (22 weeks) after the start date of TB treatment and TB treatment must be ongoing. The TB treatment must still be ongoing at V3= Day 0, and it must be verified that the participant has tested *Mtb* negative, i.e. both sputum samples from V2 must be negative by smear AFB microscopy or if unable to produce sputum is considered asymptomatic by the investigator. If all inclusion and exclusion criteria are verified the 1st vaccination is administered. The 2nd vaccination is administered on V5= Day 56. There will be follow-up visits after each vaccination on V4= Day 14 and V6= Day

<table>
<tr><td></td><td>70, with review of diary cards, AEs including injection site reactions and concomitant medications. For the safety cohort, see treatment groups below, there will also be safety laboratory testing on V4= Day 14 and V6= Day 70.
After this there is follow-up on V7= Day 238 and the last study visit V8= Day 421.

TB signs and symptoms screening will take place at all visits and telephone calls (TCs) after V3= Day 0 and onwards. The TC1 to TC5 will take place on the following days: Day 154, 196, 280, 322, and 364. If there is suspicion of TB at any of these occasions, based on the TB symptoms screening, 2 separate sputum samples will be obtained, and the case verification procedure will be initiated.

Immunogenicity / immunology laboratory samples: PBMCs and plasma for ICS and IgG ELISA will be collected from all participants on V3= Day 0, V6= Day 70 and at suspicion of TB recurrence. Whole blood for RNA analysis and blood subset counts will be collected from all participants at V3= Day 0 and at suspicion of TB recurrence.

For the immunogenicity cohort, see treatment groups below, at the SATVI, ZA and MMRC, TZ sites only, whole blood will be collected and stimulated for WB ICS on V3= Day 0 and V6= Day 70.</td></tr>
<tr><td>Number and Location of Clinical Study Site(s)</td><td>[redacted]</td></tr>
<tr><td>Study Population</td><td>900 HIV negative adults of 18 to 60 years of age diagnosed with drug-susceptible pulmonary tuberculosis are planned to be included after completion of at least 5 months (22 weeks) of treatment and tested AFB negative.</td></tr>
<tr><td>Treatment Groups</td><td>
<table>
<tr><th rowspan="2">Number of Doses</th><th colspan="2">Treatment Assignment N (planned)</th><th>Total N (planned)</th></tr>
<tr><th>H56:IC31
5 µg H56/
500 nmol IC31</th><th>Placebo</th><th></th></tr>
<tr><td>All randomized participants
2 x 0.5 mL (Days 0, 56)
intramuscularly in deltoid</td><td>450</td><td>450</td><td>900</td></tr>
<tr><td>First 150 randomized participants
across all sites
Safety cohort subgroup</td><td>75</td><td>75</td><td>150</td></tr>
<tr><td>First 100 randomized participants
[redacted] sites only
Immunogenicity cohort subgroup</td><td>50</td><td>50</td><td>100</td></tr>
</table>
</td></tr>
<tr><td>Length of follow-up</td><td>Each participant will be followed from inclusion, randomization and 1st vaccination on Study Day 0 through Study Day 421, which corresponds to 1 year after the 2nd vaccination.</td></tr>
<tr><td>Primary Endpoint</td><td>Primary efficacy endpoint:
• Rate of TB disease recurrence (relapse or reinfection), defined as TB diagnosed by confirmation of Mtb by culture of sputum, during the period starting 14 days after the 2nd vaccination (V6= Day 70) and ending 12 months after the 2nd vaccination (V8= Day 421)</td></tr>
</table>

1 INTRODUCTION

The World Health Organization (WHO) has set the goal of ending the tuberculosis (TB) epidemic by 2035 (WHO core team within global TB programme, 2015) and the United Nations (UN) has set a similar target within the Sustainable Development Goals of ending TB by 2030 (UN General Assembly, 2014). The WHO has noted that without new drugs, diagnostics, and vaccines these targets will not be met (WHO core team within global TB programme, 2015).

The European & Developing Countries Clinical Trial Partnership (EDCTP) has decided to fund this project where the primary objective is to unite African and European research institutions, clinical study sites and vaccine developers in a consortium to conduct an innovative and cost-effective TB vaccine phase 2 efficacy study. As TB vaccines target antigens are not implicated in the generation of resistance they are expected to be equally effective against both drug-resistant and drug-sensitive strains of TB. Therefore TB vaccines will be a critical tool for managing the spread of resistant strains, and also, through prevention of TB disease and hence transmission, will decrease the need for antibiotics. The prevention of recurrence (POR) Consortium consists of African and European partners. The African partners are; [redacted], [redacted], [redacted] (South Africa), [redacted], (South Africa), [redacted] South Africa), [redacted] South Africa) and [redacted] under the [redacted] (Tanzania). The European partners are; [redacted] (Denmark) and [redacted] (Italy).

1.1 Background

Over the last 2 centuries, TB is estimated to have killed one billion people, remains the world's most lethal infectious disease, and was South Africa's leading cause of death in 2015 (Statistics South Africa, 2017). The TB epidemic is responsible for economic devastation and a cycle of poverty and illness that entraps families, communities and even entire countries. Adults are the main transmitters of TB and a vaccine that is efficacious in preventing TB disease in adults will have the biggest and most immediate impact on the TB epidemic (Knight G et al, 2014). The burden of TB is largely borne by just 30 countries, 16 of which are in Sub-Saharan Africa (SSA). The highest incidences of TB infection and disease in the world are encountered in SSA. South Africa reports one of the highest incidences in the world with 781 incident cases per 100.000 in 2016 (WHO, Geneva, 2017).

There is growing bacterial resistance to available drugs, which means the disease is becoming more difficult to treat. There were an estimated 480,000 cases of multi-drug-resistant (MDR-) TB in 2015. The current tools for controlling TB are clearly insufficient, and without new efficacious TB vaccines, the 2035 World Health Organization's End TB strategic goals of a reduction of TB deaths and cases of TB disease will not be met, nor will the target within the UN Sustainable Development Goals of ending TB by 2030 (UN General Assembly, 2014).

Recurrence of TB disease following successful treatment is a significant issue with rates of recurrence varying between 2% and 8% (Gillespie SH et al, 2014; Friedrich SO et al, 2013; Merle CS et al, 2014; Imperial et al, 2018) as a result of either relapse, i.e. due to the same *Mtb* strain that the patient was initially treated for, or reinfection, i.e. due to a new infection with a different strain. The majority of recurrences occur within the first year following the end of treatment (Gillespie SH et al, 2014; Friedrich SO et al, 2013; Merle CS et al, 2014, Imperial et al, 2018). The availability of an efficacious TB vaccine preventing recurrence will be a significant advance in the control of TB disease.

1.2 Description of the H56:IC31 Vaccine

H56:IC31 is being developed by SSI and IAVI South Africa NPC and was the first multi-stage TB subunit vaccine candidate to enter clinical studies. H56:IC31 is specifically designed to mediate protection both as a preventive vaccine and as a therapeutic vaccine, making it the ideal investigational vaccine for the present POR clinical study (A-055).

The H56:IC31 vaccine candidate consists of a fusion protein of 3 antigens expressed at different stages of *Mycobacterium tuberculosis* (*Mtb*) infection (Wiker et al, 1992; Belisle et al, 1997; Horwitz et al, 2000; Ravn et al, 1999; Ulrichs et al, 1998; Pollock and Anderson, 1997; Brandt et al, 1996; Brandt et al, 2000; Rustad et al, 2009; Betts et al 2002; Muttucumaru et al, 2004; Govender et al, 2010) and a T helper type 1 cell (Th1)-stimulating adjuvant, IC31® developed by Valneva (Kochenderfer et al, 2006).

H56 fusion protein:

- Ag85B: An immunodominant antigen secreted in the acute phase of infection
- ESAT-6: The premier virulence-associated antigen highly expressed throughout all stages of infection
- Rv2660c: A stress-induced antigen, the expression of which is strongly associated with latent TB infection

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

The two components of IC31 may be combined in various ratios but a 25:1 molar ratio of KLK to ODN1a is the ratio used for H56:IC31. Since the ratio of ODN1a and KLK is constant in H56:IC31, adjuvant amounts will be expressed in this document only as nmol of KLK for ease of presentation.

H56:IC31 vaccine:

The H56 fusion protein is formulated with IC31 in a good manufacturing practice (GMP) compliant environment in a ready to use final formulated vaccine.

H56:IC31 is administered twice with a 56 days (+/-10) interval, as 5 μg H56 adjuvanted with IC31 consisting of 500 nmol KLK and 20 nmol ODN1a, in a total volume of 0.5mL by the intramuscular route in the deltoid area using standard aseptic technique.

Please refer to the H56:IC31 Investigator's Brochure (IB) for further information.

1.3 Non-clinical Experience with the H56:IC31 Vaccine

H56:IC31 was immunogenic and showed protective efficacy in mice, rats, guinea pigs (Aagaard et al, 2011) and non-human-primates (NHPs) (Lin et al, 2012, Billeskov et al, 2016), although the effect varied in both guinea pigs and NHPs.

In guinea pigs the efficacy was dependent on whether the vaccine was administered as a stand-alone vaccine (demonstrating protective efficacy) or as a BCG booster (non-protective). In mice, the H56 antigen also protected against TB when given post-exposure to *Mtb*, which was not the case in guinea pigs (one experiment).

In 3 independent NHP experiments, the H56 antigen and IC31 adjuvant were administered at the dose of 50 μg of H56 in 500 nmol IC31. There was no evidence of systemic toxicity and no apparent local reactions at the injection sites. Vaccination with H56:IC31 significantly delayed and reduced TB associated inflammation measured by erythrocyte sedimentation rate (ESR). Boosting with H56:IC31 after BCG vaccination also delayed and reduced clinical disease and pathology and increased survival, compared to no vaccination and vaccination with BCG alone. In one experiment, the animals with developed LTBI were treated with anti-TNF drug to reactivate TB disease, and the BCG/H56:IC31 vaccinated animals had minimal reactivation relative to the BCG vaccinated group. In a fourth NHP study, the H56 antigen and IC31 adjuvant were administered to animals at the dose of 15 μg of H56 in 500 nmol IC31. In this study, the BCG vaccinated group performed better than expected, and BCG/H56:IC31 did not perform significantly better than BCG.

No Koch-like reactions were seen in animals vaccinated up to 8 times with H56:IC31 (mice). There was no evidence of a broader toxic effect of H56:IC31 administered to rabbits in a more intensive regimen (4 doses; 100 μg H56 with or without 500 nmol IC31) than anticipated in the clinical program. The non-clinical information overall supports the suitability of H56:IC31 as a safe, immunogenic, and possibly effective vaccine to augment the immunity induced by a previous *Mtb* infection and/or BCG vaccination, and induce immunity in *Mtb*-uninfected individuals as well as in BCG-primed individuals.

Please refer to the H56:IC31 IB for further information.

1.4 Clinical Experience with the H56:IC31 Vaccine

Five H56:IC31 studies have been completed. In these studies a total of 248 participants have been vaccinated, 165 of whom have received H56:IC31, 24 of whom have received protocol-specified BCG, 35 of whom have received placebo, and 24 of whom have received H4:IC31

(a protein adjuvant candidate TB vaccine – see Section 1.5 - included in an arm of a phase 1b study (A-042) to evaluate immune responses to H56:IC31, H4:IC31 or BCG revaccination). The dose of H56:IC31 in these studies ranges from 5 to 50 μg H56 with 500 nmol IC31, administered 2 or 3 times 2 months apart.

Two of the studies, C-032-456 (Luabeya et al, 2015) and C-035-456 (Suliman et al, 2019), investigated the safety and immunogenicity of increasing intramuscular doses of H56:IC31 initially in humans with no evidence of *Mtb* infection, followed by individuals with latent tuberculosis infection (LTBI). A dose of (5 μg H56/500 nmol IC31) was selected for further clinical development based on data from these studies.

In preparation for the present POR study, the safety and immunogenicity of H56:IC31 were investigated in a third study (C-037-456) in 22 participants recently successfully treated for TB disease (16 H56:IC31 and 6 placebo) in South Africa. H56:IC31 was demonstrated to be safe and immunogenic in this population.

A fourth open label phase I study investigated H56:IC31 in participants undergoing TB treatment in Norway. In one of the study groups, 19 adults who had received TB treatment for 3 months at the time of the first dose, were vaccinated with 2 doses of H56:IC31 with 56 days interval. Preliminary clinical data indicates acceptable safety profile and immunogenicity was observed when H56:IC31 is administered during ongoing TB treatment.

H56:IC31 was associated with an acceptable safety profile in participants with and without LTBI, and in participants recently successfully treated for pulmonary TB. No vaccine-related SAEs were reported. Vaccination with H56:IC31 was associated most commonly (≥10% of participants with a related adverse event (AE) with mild to moderate injection site reactions of pain, warmth, and swelling; and mild to moderate systemic AEs of fatigue, myalgia, and nausea.

H56:IC31 induced a dominant CD4+ T cell response in both QFT (+) and QFT (-) participants, and in participants recently successfully treated for pulmonary TB. These responses were more pronounced in participants with prior TB exposure.

Please refer to the H56:IC31 IB for further information.

1.5 Clinical Experience with Vaccines Related to H56:IC31

Two other, closely related vaccine candidates consisting of fusion proteins of *Mtb* antigens combined with the IC31 adjuvant have been studied in humans: H1:IC31 and H4:IC31. H1:IC31 contains the H1 fusion protein, which includes antigens Ag85B and ESAT-6 and H4:IC31 contains the H4 fusion protein, which includes antigens Ag85B and TB10.4.

A total of 3 phase I clinical studies of H1:IC31 have been completed (THYB-01, THYB-02, and THYB-03), in which 95 healthy adult participants were vaccinated with H1 at a dose of 50 μg. The dose of IC31 in these studies ranged from 0 to 500 nmol. Clinical manifestations related to H1:IC31 include mild injection site reactions, constitutional symptoms (such as fatigue, malaise, and feeling cold) that were typically mild, and erythema and itching at previously administered tuberculin skin test (TST) site. Two SAEs possibly related to

vaccination have been reported (increased AST/ALT with fever, chills, generalized body weakness, and erythema at previous PPD injection site 12 hours post-vaccination in 1 participant; and elevated CPK 24 and 48 hours post-vaccination in 1 participant who is a heavy weight lifter), both of which resolved within 72 hours. Two participants were withdrawn after experiencing mild AEs that were considered to be possibly related to the vaccine: 1 participant had progressively increasing eosinophilia, and 1 participant had erythema and itching at his previous PPD injection site. Two phase II studies with H1:IC31 have also been completed: a dose finding study in 240 adolescents (THYB-04) and a study in 48 HIV-infected adults, 40% *Mtb* infected with no evidence of TB disease (THYB-05). There have been no major safety signals in either of these phase II studies, and no vaccine-related SAEs were reported.

A total of 4 phase I clinical studies of H4:IC31 have been completed and have unblinded data available, in which 234 healthy adult participants (previously vaccinated with BCG) were vaccinated (C-005-404, C-006-404, C-011-404, C-013-404). Of these participants, 198 received H4:IC31 and 36 received placebo. The doses of H4:IC31 in these studies range from 5 to 150 μg H4 with 0 to 500 nmol IC31. No related SAEs were reported in these studies. Clinical manifestations related to H4:IC31 include mild to moderate injection site reactions, and mild to severe hypersensitivity reactions at previously administered TST test site when study vaccine was administered even when the original TST was non-reactive. The most common AEs (occurring in ≥10% of participants) considered related to H4:IC31, other than injection site reactions, were mild to moderate fatigue, myalgia, headache, arthralgia, and mild to severe pyrexia. A phase II study (C-040-404) including 990 HIV-negative, healthy adolescents (12 to 17 years of age) who had been vaccinated as infants with BCG has been completed. All participants were randomized evenly into 3 study arms: placebo, H4:IC31 (15 μg), or BCG revaccination. The data showed that both H4:IC31 and BCG appeared to be safe and produced an immune response in the adolescents studied. No vaccine-related serious adverse events were reported in the study, and the most common vaccine-related adverse event was injection site swelling in BCG revaccinated participants, typical for BCG vaccination. Safety was assessed in all participants who received at least one injection. 550 participants experienced at least one AE. H4:IC31 and placebo had similar AE profiles (Nemes et al, 2018).

1.6 Rationale for Study

The clinical development of H56:IC31 is aimed towards the development of a vaccine to induce protective immunity in *Mtb*-uninfected individuals and to augment the immunity in already *Mtb* sensitized or infected individuals who have previously been vaccinated with BCG. Completed H56:IC31 phase I studies have evaluated the safety and immunogenicity of increasing intramuscular doses of H56:IC31 initially in uninfected humans followed by individuals with LTBI, previously vaccinated with BCG. A dose (5 μg H56/500 nmol IC31) has been selected for further development in QFT+ individuals. This dose was also determined to be safe and immunogenic in patients vaccinated as they approached the end of a 6-months course of TB treatment or who had very recently completed treatment for active pulmonary TB and who were, by the WHO definition, deemed to be cured (data on file).

As demonstration of protective efficacy against TB disease in the target population will require a large (many thousands of participants) and lengthy (3 or more years) field study,

shorter and smaller proof of concept phase II studies, investigating sub-populations at greater risk of TB infection and/or disease than those in the general population, have been proposed by leading experts in the TB vaccine field (Ellis RD et al, 2015). These studies will determine the impact of vaccination with investigational TB vaccines on POR TB disease in recently successfully treated TB patients (Imperial et al, 2018) and prevention of sustained infection (POI) in adolescents at high risk of infection with *Mtb*. Recurrent TB disease is ~4-fold more frequent than incident TB disease in the general population, and infection in high risk groups, such as adolescents living in areas where there is a high force of infection, can occur up to ~8-fold more frequently than incident TB disease (Nemes et al, 2018). Therefore, these studies can be completed more rapidly and more cost-effectively using smaller group sizes than could a proof of concept study with TB disease as an endpoint in the general population.. The outcomes of these proof of concept studies may then be used as Go/ No Go criteria to inform future investment and development, including large-scale manufacture and efficacy studies in a broader population.

Preclinical data suggest H56:IC31 may be more efficacious if administered while patients are still on treatment (Aagaard et al, 2011; Hoang T et al, 2013). Following the national guidelines for TB treatment in South Africa (ZA) and Tanzania (TZ) we will obtain sputum samples from patients after at least 5 months (22 weeks) of treatment, at the same time they are obtained within the national TB control programmes, and if the sputum is smear negative, the criterion for successful treatment within TB programmes, the individual will be eligible for randomization and vaccination if the duration of their treatment period is not expected to exceed 28 weeks.

As this is a proof of concept TB vaccine study, HIV positive individuals have been excluded as it is not yet known what effect HIV infection may have on the immune response to the vaccine. However, HIV positive individuals are an important population to include in future studies should efficacy be demonstrated.

2 STUDY OBJECTIVES AND DESIGN

2.1 Objectives

2.1.1 Primary Objective

To evaluate the following in HIV-negative participants who have completed at least 5 months (22 weeks) treatment for drug-susceptible pulmonary TB and who test negative for acid fast bacilli (AFB) on sputum smear microscopy prior to vaccination (participants unable to produce sputum, and considered asymptomatic by the investigator, may be considered *Mtb* negative):

- Efficacy of H56:IC31 compared to placebo in reducing the rate of recurrent TB disease (relapse or reinfection)

2.1.1.1 Primary Endpoint

- Rate of TB disease recurrence (relapse or reinfection), defined as TB diagnosed by confirmation of *Mtb* by culture of sputum, during the period starting 14 days after the 2nd vaccination (V6= Day 70) and ending 12 months after the 2nd vaccination (V8= Day 421)

2.1.2 Key Secondary Objective

To evaluate the following in HIV-negative participants who have completed at least 5 months (22 weeks) treatment for drug-susceptible pulmonary TB and who test negative for AFB on sputum smear microscopy prior to vaccination (participants unable to produce sputum, and considered asymptomatic by the investigator, may be considered *Mtb* negative):

- Safety of H56:IC31 compared to placebo

2.1.2.1 Key Secondary Endpoints

- Solicited adverse events and all adverse events occurring the first 14 days after each of the 1st and 2nd vaccinations
- Serious adverse events including medically important events occurring after the 1st vaccination through the end of the study

2.1.3 Other Secondary Objectives

To evaluate the following in HIV-negative participants who have completed at least 5 months (22 weeks) treatment for drug-susceptible pulmonary TB and who test negative for AFB on sputum smear microscopy prior to vaccination (participants unable to produce sputum, and considered asymptomatic by the investigator, may be considered *Mtb* negative):

- Trends towards efficacy of H56:IC31 compared to placebo in reducing the rate of TB disease relapse
- Trends towards efficacy of H56:IC31 compared to placebo in reducing the rate of TB disease reinfection
- Antigen-specific cell-mediated immune responses to H56:IC31
- Humoral immune responses to H56:IC31

2.1.3.1 Other Secondary Endpoints

- Rate of TB disease relapse, defined as participants meeting the primary endpoint of TB disease recurrence, AND determined by whole genome sequencing (WGS) of the *Mtb* isolate to be the same strain of *Mtb* as in the participant's original isolate from the time of diagnosis

- Rate of TB disease reinfection, defined as participants meeting the primary endpoint of TB disease recurrence, AND determined by WGS of the *Mtb* isolate to be a different strain than in the participant's original isolate from the time of diagnosis

- Antigen-specific cell-mediated immune responses by whole blood intracellular cytokine staining (WB ICS) at baseline (V3= Day 0), and 14 days after the 2nd vaccination (V6= Day 70) in the immunogenicity cohort

- Humoral immune responses by IgG ELISA of plasma samples taken from the immunogenicity cohort at baseline (V3= Day 0) and 14 days after the 2nd vaccination (V6= Day 70)

2.1.4 Exploratory Objectives

To evaluate the following in HIV-negative participants who have completed at least 5 months (22 weeks) treatment for drug-susceptible pulmonary TB and who test negative for AFB on sputum smear microscopy prior to vaccination (participants unable to produce sputum, and considered asymptomatic by the investigator, may be considered *Mtb* negative):

- Trends towards efficacy of H56:IC31 compared to placebo in reducing the rate of recurrent TB disease by exploratory efficacy endpoint definitions (Section 2.1.4.1)
- Transcriptomic signatures of inflammation or associated with TB disease recurrence
- Immunological correlates of risk and correlates of protection for TB disease recurrence
- Humoral immune responses to H56:IC31in participants with TB recurrence diagnosis compared to the participants in the control cohort

2.1.4.1 Exploratory Endpoints

- Rate of TB disease recurrence (relapse or reinfection), defined as participants meeting the primary endpoint of TB disease recurrence AND participants who started TB treatment without confirmation of *Mtb* by culture of sputum

  Rate of TB disease recurrence (relapse or reinfection), defined as participants meeting the primary endpoint of TB disease recurrence based on confirmation of *Mtb* by Xpert MTB/RIF Ultra or culture of sputum

- Rate of TB disease recurrence (relapse or reinfection), defined as participants meeting the primary endpoint of TB disease recurrence AND participants diagnosed between 30 days after the 1st vaccination and 14 days after the 2nd vaccination, based on confirmation of *Mtb* by culture of sputum

- Transcriptomic signatures (RNA analysis) and cellular composition (flow cytometry) of whole blood etc. at baseline (V3= Day 0) and at TB recurrence diagnosis

- Blood subset counts of whole blood (supports the RNA analysis) at baseline (V3= Day 0) and at TB recurrence diagnosis

- Antigen-specific cell-mediated immune responses by peripheral blood mononuclear cells (PBMC) ICS at baseline (V3= Day 0) and 14 days after the 2nd vaccination (V6= Day 70) and at TB recurrence diagnosis

- Humoral immune responses by IgG ELISA of plasma samples at baseline (V3= Day 0) and 14 days after the 2nd vaccination (V6= Day 70) and at TB recurrence diagnosis

2.2 Design

This is a phase 2, double-blind, randomized (1:1), placebo-controlled study with 2 parallel groups:

- H56:IC31 (investigational vaccine)
- Placebo

900 HIV-negative adults with a diagnosis of drug-susceptible pulmonary TB are planned to be included, recruited from TB clinics with established relationships to the study sites see Table 2-1.

5 study sites in South Africa: 2 sites from the [redacted] and three in Cape Town at [redacted] and [redacted].

1 study site in Tanzania (TZ): 1 site at [redacted].

Table 2-1 Treatment Groups and Number of Participants

Number of Doses	Treatment Assignment N (planned)		Total N (planned)
	H56:IC31 5 µg H56/ 500 nmol IC31	**Placebo**	
All randomized participants in study 2 x 0.5 mL (Days 0, 56) intramuscularly in deltoid	450	450	900
First 150 randomized participants across all sites Safety cohort subgroup	75	75	150
First 100 randomized participants [redacted] sites only Immunogenicity cohort subgroup and control cohort	50	50	100

For the summary schedule of participant evaluations, see Table 3.1. For a flowchart showing the study design, see Figure 2-1.

Screening Visits 1 and 2

The 1st screening visit (V1) must be conducted ≤ 7 days after the start date of the TB treatment. The 2nd screening visit (V2) must be conducted ≥ 5 months (22 weeks) after the start date of TB treatment. The end of TB treatment is expected to be 6 months (24 weeks) after the start of TB treatment and may not be extended beyond 28 weeks. At V1 informed consent will be obtained and inclusion and exclusion criteria (applicable for V1) will be assessed, by review of medical history. Demographics, height, weight, smoking history, Xpert MTB/RIF result or AFB smear positive result from the TB clinic (at MMRC Xpert MTB/RIF testing is performed at the site), and self-reported HIV status will be assessed. 2 separate sputum samples will be obtained and stored (frozen) for later comparison to

recurrent isolates by WGS at OSR, Italy. During the 5-month period between V1 and V2, the study staff will regularly liaise with screened participants, staff from the TB clinics and community health care workers to monitor TB treatment compliance and continued eligibility for study participation.

In- and exclusion criteria will be assessed at V2, by review of medical history, physical examination (complete), vital signs, pregnancy testing (all females) and HIV testing. For eligible participants, 2 separate sputum samples will be obtained, and safety laboratory tests (blood) and urinalysis (dipsticks) will be performed. One AFB smear for microscopy using the Auramine technique will be created from each of the sputum samples.

Upon written approval from the sponsors and relevant approvals for protocol amendment v5.0,the activities scheduled for the 1st screening visit (V1) will be performed on the same day as (or together with) the 2nd screening visit (V2). At a combined screening visit (V1 and V2) the participant must have completed at least 5 months (22 weeks) of TB treatment and while TB treatment must be ongoing.

Randomisation and follow-up
The TB treatment must still be ongoing at V3= Day 0, and it must be verified that the participant is tested *Mtb* negative, i.e. both sputum samples from V2 must be negative by smear AFB microscopy. Participants unable to produce sputum, but considered asymptomatic by the investigator, may be considered *Mtb* negative and eligible for inclusion. All inclusion and exclusion criteria will be assessed or re-assessed, by review of medical history, physical examination (symptom directed), weight, vital signs, pregnancy testing (all females) and confirmation of negative HIV status from V2.

Eligible participants, after verification of vaccination eligibility criteria, see Section 3.5, will be randomized (1:1) to receive either H56:IC31 or placebo, and the 1st vaccination will be administered.

Before administering the 2nd vaccination on V5= Day 56, physical examination (symptom directed), weight, vital signs, pregnancy testing (all females), TB symptoms screen, and vaccination eligibility criteria will be assessed, see Section 3.5 and 6.1.

After each vaccination, it will be assessed if immediate AEs occurred within 60 minutes (for details see Section 3.6.3.1), TB signs and symptoms training will be performed, and diary cards will be handed out to the participants.

There will be short-term follow-up visits after each vaccination on V4= Day 14 and V6= Day 70, with weight, vital signs, TB symptoms screen and review of diary cards. Solicited AEs including injection site reactions and concomitant medications (CM), and spontaneously reported AEs, including serious AEs and AEs of special interest, will be assessed and recorded. If there is suspicion of TB disease, 2 separate sputum samples will be obtained, and the case verification procedure (see details below) for recurrent TB disease will be initiated.

For the safety cohort (= the first 150 randomized participants across all sites), see Table 2-1, there will also be safety laboratory testing on V4= Day 14 and V6= Day 70. For the remaining participants, there will only be laboratory safety testing at screening (V2), or at the ET Visit, if within 14 days after the investigational medicinal product (IMP) administration.

At the long-term follow-up visit on V7= Day 238, there will be a TB symptoms screen including weight and recording of serious adverse events (SAEs) and AEs of special interest. If there is suspicion of TB disease, 2 separate sputum samples will be obtained, and the case verification procedure (see details below) for recurrent TB disease will be initiated.

At the last study visit, V8= Day 421, whether or not there is suspicion of TB disease, 2 separate sputum samples will be obtained from all participants to identify missed cases, see Section 3.6.1. At this visit a negative Xpert MTB/RIF Ultra does not need to be verified by culture for participants with no clinical TB symptoms. Furthermore, SAEs and AEs of special interest will be assessed and recorded, if any.

Immunogenicity / immunology laboratory samples: PBMCs and plasma for ICS and IgG ELISA will be collected from all participants on V3= Day 0, V6= Day 70 and at suspicion of TB recurrence, as defined for the STB Visit, ET Visit or V8.Whole blood for RNA analysis and blood subset counts (to support the RNA analysis) will be collected from all participants at V3= Day 0 and at suspicion of TB recurrence, as defined for the STB Visit, ET Visit or V8, see Table 3-2.

For the immunogenicity cohort at the [redacted] TZ sites only (= first 50 randomized participants at [redacted] and first 50 randomized participants at [redacted] = in total 100 participants), see Table 2-1, whole blood will be collected and stimulated for WB ICS on V3= Day 0 and V6= Day 70, see Table 3-2. For the remaining participants and sites, there will be no collection of whole blood for WB ICS.

The immunogenicity cohort at the [redacted], TZ sites also serves as control cohort, see Section 7.4.5 and Table 2-1. Therefore there will be collection on V8= Day 421, of PBMCs and plasma for ICS and IgG ELISA and whole blood for RNA analysis and blood subset counts. These samples will be control samples for the corresponding immunology samples taken from participants under diagnosis of recurrent TB during the study, see Table 3-2.

TB signs and symptoms training will take place at all visits and telephone calls (TCs) from V3= Day 0 and onwards, and TB symptom screening at all visits and TCs from V4= Day 14 and onwards. The TC1 to TC5 will take place on the following days: Day 154, 196, 280, 322 and 364. If there is suspicion of TB at any of these occasions, based on the TB symptoms screening, 2 separate sputum samples will be obtained, and the case verification procedure (see details below) for recurrent TB disease will be initiated at a Suspected TB (STB) visit.

Participants who are withdrawn from the study within 6 months after the last product administration, will be contacted at least 6 months after the last product administration for recording of SAEs and AEs of special interest.

Referral for diagnosis and management of recurrent TB is triggered by the investigator based on clinical suspicion of recurrent TB. Participants will not be withdrawn from the study based on this referral but will be withdrawn if *Mtb* positive by culture in the study or if the referral clinic diagnoses recurrent TB and starts TB treatment. The case verification procedure is initiated at a STB Visit.

Case verification procedure for recurrent TB disease:

- 2 separate sputum samples are obtained:
  - 1 sputum sample is tested by Xpert MTB/RIF Ultra
  - The same sputum sample and the second sputum sample are both sent for culture
  - The remainder of the 2 separate sputum samples is stored (frozen) as 2 separate samples for later analysis by WGS if recurrent TB is confirmed

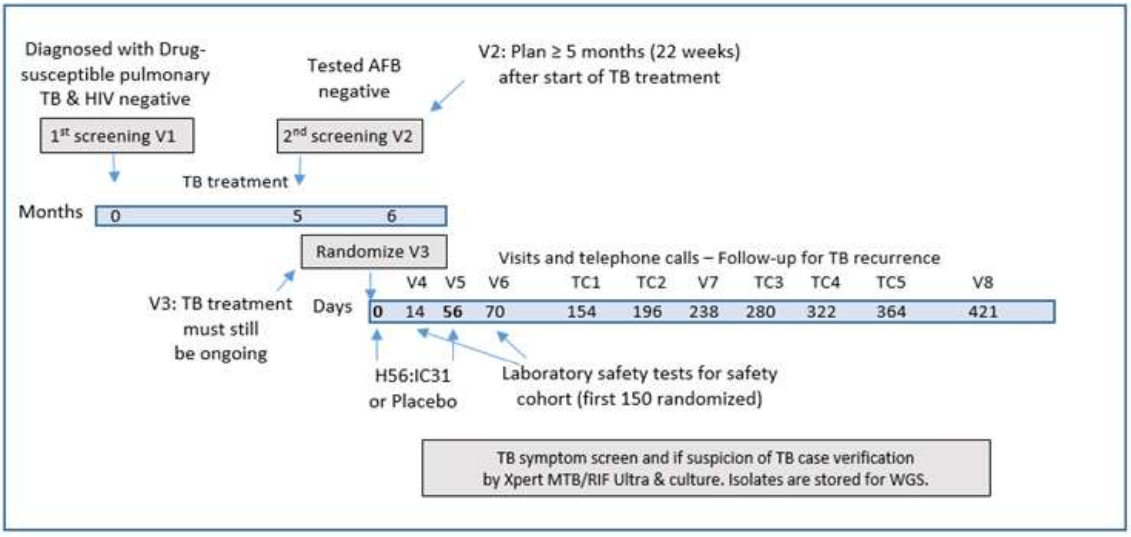
- The participant is HIV tested (including CD4 count for participants who test HIV positive) and immunology samples are taken, see Table 3-2.

- If *Mtb* positive by culture in the study, the participant is informed of TB test results, referred to TB treatment management as per national programme guidelines (if not previously done), and is withdrawn (at the ET Visit) due to verified recurrent TB.

- If *Mtb* negative by culture in the study the participant is informed of TB test results, continues in the study and no ET Visit is completed.

- If *Mtb* negative by culture or the study culture result is pending, but the referral clinic diagnosed recurrent TB and started TB treatment, 2 additional sputum samples must be collected as soon as possible for repeat *Mtb* testing by culture, and the participant is withdrawn from the study.

Figure 2-1 Study Flow Diagram



Upon written approval of the Sponsors and relevant approvals for the Protocol Amendment v5.0, participants may be screened at a combined V1 and V2. This visit will be scheduled when the participant has completed at least 5 months (22 weeks) of TB treatment and TB treatment must be ongoing.

An internal data review committee, consisting of the coordinating principal investigator (PI), the site PIs and the medically responsible person from each of the co-sponsors, will convene during the study to review the blinded reported SAEs, AEs including AEs of special interest, injection site reactions and laboratory safety test results to identify any possible safety issues when:

- The first 150 participants across all sites have received their 1st vaccination and the 14 days safety follow-up data is available
- The first 150 participants across all sites have received their 2nd vaccination and the 14 days safety follow-up data is available

An independent data safety monitoring board (DSMB) will be established, consisting of a panel of independent experts, who will operate according to a DSMB charter. The DSMB will be convened if:

- A stopping rule is triggered
- The internal data review committee requests a DSMB meeting based on their review of the safety data

For more details on the internal data review committee review of safety data and the DSMB procedures, please see sections 5.1.4 and 6.2.

3 STUDY PROCEDURES

3.1 Schedule of Participant Evaluations

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

A Summary Schedule of Evaluations depicting all visit-specific procedures is provided in Table 3-1.

Upon written approval from the sponsors and relevant approvals for protocol amendment v5.0, the activities scheduled for the 1st screening visit (V1) will be performed on the same day as (or together with) the 2nd screening visit (V2). At this combined screening visit (V1 and V2) the participant must have completed at least 5 months (22 weeks) of TB treatment and TB treatment must be ongoing. Activities scheduled for both V1 and V2 do not need to be repeated.

Table 3-1 Summary Schedule of Participant Evaluations

	Study Day / Suspected TB (STB Visit) / Early Termination (ET Visit) / Telephone Call (TC) / Visit Window ±days														
	Visit 1[1] Screening ≤ 7 days after Start of TB treatment	**Visit 2[1]** Screening ≥ 5 months (22 weeks) after start of TB treatment	**Visit 3[1]** **Day 0** Before end of TB treatment	**Visit 4** **Day 14**	**Visit 5** **Day 56**	**Visit 6** **Day 70**	**TC 1** **Day 154**	**TC 2** **Day 196**	**Visit 7** **Day 238**	**TC 3** **Day 280**	**TC 4** **Day 322**	**TC 5** **Day 364**	**Visit 8** **Day 421**	**STB[15] Visit** **NA**	**ET[16] Visit** **NA**
	-	-	-	±3 days	±10 days	±3 days	±7 days	±7 days	±7 days	±7 days	±7 days	±7 days	±7 days	NA	NA
Informed consent	x														
Demographics	x														
Medical history, medication & smoking history	x	x	x[5]												
In- / exclusion criteria	x	x	x[5]												
Weight	x (incl. height)	x	x	x	x	x			x				x	x[9]	x
Complete physical examination		x													
Symptom directed physical examination *- and if needed extra safety tests*			x[5]		x[5]										
Vital signs (Pre- and post-vaccination on vaccination days)		x	x	x	x	x									x[8]
Urine pregnancy test (βHCG) -all females		x	x[5]		x[5]										
HIV testing	x (self-reported)	x											x[10]	x	x[10]
Urine analysis[11]		x[3]		x[6]		x[6]									x[6,8]
Serum chemistry[12]		x[3]		x[6]		x[6]									x[6,8]
Hematology[13]		x[3]		x[6]		x[6]									x[6,8]
WB ICS – only immunogenicity cohort (whole blood)			x[5,7]			x[7]									
ICS & IgG ELISA (PBMCs and plasma)			x[5]			x							x[7,10]	x	x[10]
RNA analysis (whole blood)			x[5]										x[7,10]	x	x[10]
Blood subset count supporting RNA analysis (whole blood)			x[5]										x[7,10]	x	x[10]
Vaccination eligibility criteria verification			x[5]		x[5]										
Randomization			x[5]												
Vaccination			x		x										
TB signs and symptoms training			x	x	x	x	x	x	x	x	x	x			
TB symptom screen				x[4]	x[4,5]	x[4]	x[4]	x[4]	x[4]	x[4]	x[4]	x[4]	x	x[9]	x
Sputum collection	x[2]	x[3]											x[10]	x[4,17]	x[10,17]
Distribute diary cards			x		x										
Review diary cards				x		x									x[8]
Solicited AEs and injection site reactions *incl. CM*			x	x	x	x									x[8]
AEs (non-serious) *incl. CM*			x	x	x	x									x[8]
SAEs and AEs of special interest *incl. CM*			x	x	x	x	x	x	x	x	x	x	x	x[14]	x[14]

Table 3-1 Summary Schedule of Participant Evaluations

1) Visit 1 must at the latest be ≤ 7 days after the start of the TB treatment. HIV negative participants with a diagnosis of drug-susceptible pulmonary TB per national guidelines are recruited. At visit 2 the participant must have completed at least 5 months (22 weeks) of TB treatment, and at Visit 3 the TB treatment must be ongoing. Note: For participants with a combined screening visit (V1 and V2), V1 activities will be performed on the same day as V2. This combined screening visit should occur when the participant has completed at least 5 months (22 weeks) of TB treatment and TB treatment must be ongoing.
2) 2 separate sputum samples are collected preferably before the TB treatment is started but at the latest ≤ 7 days after starting the treatment. Visit 1 sputum samples will be frozen and stored. If TB disease recurrence is diagnosed the sputum samples will be analyzed to determine if it is a relapse or a reinfection of *Mtb*. Note: For participants with a combined screening visit (V1 and V2), V1 activities will be performed on the same day as V2. Therefore, sputum samples will be collected only at V2.
3) Only if a participant is still eligible for inclusion according to in- and exclusion criteria at visit 2 the safety tests & 2 separate sputum samples are collected. 2 sputum samples are sent for AFB *smear microscopy* testing. If negative by AFB *smear microscopy* the participant can proceed to visit 3 and randomization. If positive by AFB *smear microscopy* the participant is excluded from further participation in the study. If negative by AFB *smear microscopy* the remainder of both *Mtb* negative sputum samples is stored (frozen) for culture, if verified recurrent TB, and for exploratory use at the end of the study. Any left-over sputum samples is destroyed. Participants who cannot produce a sputum sample and are considered asymptomatic are considered *Mtb* negative and can proceed to visit 3
4) If the investigator based on the TB symptom screen, post-vaccination, decides to proceed with verification of recurrent TB, the STB Visit is completed. 2 separate sputum samples are collected for Xpert MTB/RIF Ultra and culture testing. The remainder of the 2 sputum samples is frozen for later analysis by WGS. The participant is HIV tested (including pre- and post-test counseling. A CD4 count is performed for participants who are HIV positive), and immunology samples are taken. The participant is referred to TB treatment management as per national programme guidelines and HIV management if HIV positive
5) Prior to the vaccination
6) If in the safety cohort (= first 150 participants randomized across all sites)
7) If in the immunogenicity cohort (= first 50 participants randomized at the [redacted] site and the first 50 randomized at the [redacted] site). 25 participants from each of the 2 sites receiving the H56:IC31 and 25 participants from each of the 2 sites receiving the placebo
8) If the ET Visit occurs within 14 days after investigational product administration
9) Only if not already done as part of another study visit on the day of the STB Visit
10) At a participant's last study visit (V8 or ET Visit), 2 separate sputum samples are collected, and 1 sputum sample is tested with Xpert MTB/RIF Ultra. If *Mtb* positive by Xpert MTB/RIF Ultra, the same sputum sample is sent for culture together with the 2nd sputum sample, and the participant returns to the site for HIV testing and immunology sample collection. The remainder of the 2 sputum samples is frozen for later analysis by WGS. The participant is referred to TB treatment management as per national programme guidelines and HIV management if HIV positive. If *Mtb* negative by Xpert MTB/RIF Ultra and no TB signs or symptoms are observed, the sputum samples are not culture confirmed and no HIV testing and no immunology sample collection will be performed. Note: If there is clinical suspicion of TB at V8, all V8 activities will be conducted and not a STB visit since at V8 two separate sputum samples for Mtb testing will be obtained from all participants even if there is no clinical suspicion of TB. This is to ensure that no cases of recurrent TB disease are missed during the trial. One sputum sample will be tested by Xpert MTB/RIF ultra. If Mtb negative by Xpert MTB/RIF ultra but there are clinical TB signs or symptoms, verification by culture testing will be requested from the laboratory and both of the sputum samples will then be processed for culture. In this instance HIV testing and immunology sample collection should also be performed at V8.
11) Urine analysis: *urine protein (dipstick), glucose (dipstick), leukocytes (dipstick) and blood (microscopy if positive by dipstick)*
12) Serum chemistry: *AST, ALT, ALP, GGT, total bilirubin, creatinine*
13) Hematology: *hemoglobin, hematocrit, white cell count with differential, and platelet count*
14) Participants who are withdrawn from the study within 6 months after the last product administration, are contacted at least 6 months after the last product administration for recording of SAEs and AEs of special interest
15) A STB Visit is conducted if the investigator based on the TB symptom screen, decides to proceed with verification of recurrent TB. If recurrent TB is not verified, the participant stays in the study. In this case, if triggered by a TB symptom screen later in the study, another STB Visit would be conducted.
16) If withdrawal is due to verified recurrent TB, the only study events at the ET Visit are SAEs and AEs of special interest and date of withdrawal
17) If *Mtb* negative by culture or the culture result is pending in the study, but the referral clinic diagnosed recurrent TB and started TB treatment, 2 additional sputum samples should be collected as soon as possible for repeat *Mtb* testing by culture, and the participant is withdrawn from the study

3.2 Study Participants

3.2.1 Recruitment and Informed Consent

Various methods of recruitment will be used such as advertising, referrals, or proposition. All recruitment materials must be approved by the sponsor and Institutional Review Board (IRB) and/or Independent Ethics Committee (IEC). Interested TB patients will be invited to participate in the informed consent process. Informed consent will be obtained by the use of a written consent form approved by the IRB and/or IEC and signed and dated by the TB patient at the time of consent.

Potential participants will be interviewed to ensure they meet all entry criteria relating to age, diagnosis, TB treatment status and history etc. The clinical investigator or medically qualified designee (e.g. nurse or physician's assistant) will conduct the consent discussion on an individual basis in an appropriate private space (which does not have to be at the study site) with each potential participant, and will allow adequate time for all questions to be addressed. The potential participant will be encouraged to take the informed consent form home to discuss with family and friends before deciding whether or not to participate in the study. Written informed consent will be obtained prior to conducting any screening procedures. A copy of the signed consent form shall be given to the participant. Further details regarding informed consent are presented in Section 9.2.

3.2.2 Assignment of Participant Identification Numbers

The site PI is responsible for keeping the participant identification log(s). After informed consent is obtained, the participants will be screened to assess for eligibility for entry into the study.

For identification purposes, each participant for whom informed consent has been obtained will be assigned a unique participant identification number which will be listed in the participant identification log(s).

For screening failures, the reason for exclusion must be listed. Participants who are randomized in the study will also receive a unique randomization number.

The confidential participant identification log will identify the participants by full name, date of birth, participant screening number, address and contact number and will be stored in a secure location. The participant screening and enrollment log will identify participants by participant screening number, date of informed consent, date of randomization, randomization number, and date and reason for exclusion, if applicable.

Eligibility for entry into the study will be based on the inclusion and exclusion criteria defined in Sections 3.2.3 and 3.2.4. The investigator must document confirmation of eligibility prior to randomization.

The eCRF is used for allocating a randomization number to the participants that are eligible for inclusion. The unblinded site staff will, by use of the randomization number and their

exclusive access to the unblinded eCRF module, be able to identify if a given participant is to be vaccinated with H56:IC31 (investigational vaccine) or placebo.

In the eCRF, personal details, such as, the full name, address and contact number will not be recorded. In the eCRF, the participants may be identified by the use of: unique participant identification number(s), age on date of V3= Day 0 and sex. It is however, possible to trace back any eCRF to the participant identification log(s) in case needed.

At the end of the study, normally at the site close-out monitoring visit, the site PI must sign of the participant identification log(s) for correctness and completeness.

3.2.3 Inclusion Criteria

Participants must meet <u>all</u> inclusion criteria at the time of randomization and vaccination:

1. Completed the written informed consent process.
2. Agrees to give access to medical records for study-related purposes.
3. HIV-negative (self-reported) with a diagnosis of drug-susceptible pulmonary TB at the start of the TB treatment.
4. Able to provide 2 separate sputum samples within ≤ 7 days of starting TB treatment. Participants are not expected to provide sputum samples prior to starting TB treatment if their 1st screening visit (V1) is performed on the same day as their 2nd screening visit (V2).
5. Tested *Mtb* negative by smear AFB microscopy of 2 separate sputum samples taken on V2. Participants unable to produce sputum, but considered asymptomatic by the investigator, may be considered *Mtb* negative and eligible for inclusion.
6. Confirmed HIV negative on V2.
7. Completed ≥ 5 months (22 weeks) of TB treatment with treatment still ongoing at the time of the 1st vaccination on V3= Day 0, and total treatment time not extended beyond 28 weeks.
8. Aged ≥ 18 years on the date of V1 and ≤ 60 years on the date of V3= Day 0.
9. Agrees to stay in contact with the study site for the duration of the study, provide updated contact information as necessary, and has no current plans to move from the area for the duration of the study.

3.2.4 Exclusion Criteria

Participants must meet <u>none</u> of the exclusion criteria at the time of randomization and vaccination:

1. Diagnosis or co-diagnosis of extra pulmonary TB.
2. Hospitalized for the current episode of drug-susceptible pulmonary TB disease.
3. History of receipt of treatment against active TB, prior to the current treatment episode, within the last 5 years.
4. History of or ongoing severe disease that in the opinion of the investigator might affect the safety of the participant or the immunogenicity of the investigational product.
5. Insulin dependent diabetes.

6. History of allergic disease or reactions likely to be exacerbated by any component of the investigational product.
7. History or laboratory evidence of immunodeficiency, autoimmune disease or immunosuppression.
8. History of chronic hepatitis.
9. Severe anemia, defined as hemoglobin less than 10 g/dL or a hematocrit less than 30 % based on most recent hematology obtained before randomization.
10. Receipt of any investigational TB vaccine previously.
11. Receipt or planned receipt of any investigational drug or investigational vaccine from V1 through V8= Day 421.
12. Receipt or planned receipt of any licensed vaccine from V1 through V6= Day 70, except for SARS-Cov-2 vaccines recommended by national vaccination programs which will be allowed if given > 28 days before and from the time of administration of clinical trial product.
13. Receipt of treatment likely to modify the immune response (e.g. blood products, immunoglobulins, immunosuppressive treatment) within 42 days before V3= Day 0 through V6= Day 70. Inhaled and topical corticosteroids are permitted.
14. Has a body mass index (BMI) < 13 (weight, kg / height, m^2) on the date of V1.
15. Female participants of childbearing potential (not sterilized, menstruating or within 1 year of last menses, if post-menopausal): if not willing to use an acceptable method to avoid pregnancy (sterile sexual partner, sexual abstinence, hormonal contraceptives (oral, injection, transdermal patch, or implant) or intrauterine device from 28 days before V3= Day 0 until 2 months after the 2nd vaccination.
16. Female participants: if lactating / nursing, or pregnant as per positive pregnancy test on V2.
17. Not suitable for inclusion in the opinion of the investigator.

3.2.5 Screening Clinical Assessments and Laboratory Tests

Adults not known to be HIV positive with a diagnosis of drug-susceptible pulmonary TB per national guidelines (confirmed by Xpert MTB/RIF or AFB smear), and who started their TB treatment not more than 7 days before the 1st screening visit (V1) will, if eligible for inclusion, be asked to produce 2 separate sputum samples at V1, which will be stored (frozen) for later comparison to recurrent isolates by WGS, for differentiation between relapse (=identical strains) and reinfection (=different strains). Participants with a combined V1 and V2 will not provide V1 sputum samples.

Participants may also be eligible for inclusion at V1, if the Xpert rifampicin susceptibility test was unsuccessful at the local clinic, but the participant was started on a drug-susceptible treatment regimen according to national guidelines. The participant will then be screen failed prior to randomization (V3), if the site cannot confirm drug-susceptible TB by a test or a clinical response to treatment.

The time period between V1 and the 2nd screening visit (V2) is approximately 5 months (approximately 22 weeks). Site staff will remain in contact with participants, TB clinic staff and community health care workers during this period to monitor TB treatment compliance and continued eligibility for study participation. The completion of a standard TB treatment regimen is 6 months (24 weeks) which can be extended up to 28 weeks. Participants with a

combined V1 and V2 may only be screened for the trial after at least 22 weeks of TB treatment and TB treatment must be ongoing.

At V2 the samples for HIV screening and safety laboratory analysis are collected (for participants who are still eligible for inclusion) and subsequently evaluated. Furthermore, 2 sputum samples will be collected, both samples need to test negative for *Mtb* by smear AFB microscopy using the Auramine method. The remainder of both *Mtb* negative sputum samples will be stored (frozen) for culture, if verified recurrent TB, and for exploratory use at the end of the study. Any left-over sputum samples will be destroyed. The screening samples/evaluations may be taken/performed over more than one visit. If a combined V1 and V2 is performed, participants will only provide a sputum sample once at V2. These samples serve to verify that the participant is Mtb negative and would still be stored for exploratory analysis.

Participants unable to produce sputum, and considered asymptomatic by the investigator, may be considered *Mtb* negative and eligible for inclusion.

3.3 Study Randomization/Entry

Participants will be randomized to the study based on a randomly-generated sequence of numbers (randomization list) managed by a validated module in the eCRF. The randomization list will be prepared by a statistician who will not be involved in the analysis of the study in order to maintain the blind of the study team. The day of randomization for each participant will be Study Day 0.

Discontinued participants (for any reason) after randomization will not be replaced.

3.4 Blinding

The unblinded persons in the study are the study vaccine manager (and designee) who manages the participant inventory log(s) at the study site, the unblinded site staff, and the unblinded study monitor(s) responsible for monitoring the investigational product at the study site. All unblinded persons must take care to not reveal individual participant treatment assignments to any other member of the study team. There will be an unblinded contact person at the sponsor's site in order to manage queries from the unblinded site staff or the unblinded monitors in the study.

The study vaccine manager (and designee) should be a designated site team member, such as the study pharmacist. Unblinded site staff must not participate in the administration of vaccine and evaluation of adverse events.

A delegation of responsibility log will be maintained by the study site and will identify the individual(s) such as the study vaccine manager (and designee) and other unblinded site staff, i.e., individuals with access to unblinded data.

The randomization list will be prepared by the unblinded statistician and will be implemented as a module in the eCRF. Access (through the eCRF) to information which links a randomization number to treatment assignment, will only be given to the study vaccine

manager (and designee) and to the unblinded study monitor(s) responsible for monitoring the investigational product at the study site.

All pharmacy source documents and dose preparation records that can link a randomization number with a treatment assignment must remain secure (e.g., in the pharmacy with access limited to only unblinded persons) until notification from the sponsor that the study has been unblinded.

Labels accompanying the syringes of prepared investigational product doses will not indicate which investigational product is in the syringe. Identical syringes, needles and labels will be used for preparation and administration of investigational vaccine and placebo.

3.4.1 Unblinding for Clinical Emergencies

If there is an urgent clinical requirement to know a participant's treatment assignment, the site PI will request the urgent unblinding of a participant's treatment through use of the eCRF unblinding module, followed by immediate notification of the unblinding to the sponsor.

The sponsor must be notified within 24 hours of any clinically required break of the study blind. The notification of the unblinding to the sponsor must include the participant identification number, the date, a brief justification of the clinical requirement for unblinding, and the site PI's signature. The notification of the unblinding will be kept in the study file.

It is recommended that the site PI consults with the sponsor's medically responsible person prior to unblinding of a participant. However, in urgent circumstances, the study site can proceed with the unblinding of a participant at the site PI's discretion, without prior consultation with the sponsor's medically responsible person.

3.5 Investigational Product Administration

On Study Day 0, the participants must receive their first dose of investigational product as soon as possible after randomization. At the time of vaccination on Study Day 0, participants must not have a fever ≥37.5°C (axillary temperature), evidence of acute illness or take antipyretics; in such cases, randomization and vaccination will be deferred until the fever or acute illness has subsided.

If randomization and vaccination cannot be done on the same day, the participant must come back as soon as possible for a subsequent visit for randomization and vaccination. If randomization and vaccination do not occur on the same day, then the day of vaccination will be considered Study Day 0.

On Study Day 56, randomized participants who have not met any of the criteria for discontinuation of investigational product administration (see Section 6.1), and do not have a fever ≥37.5°C (axillary temperature), evidence of a new acute illness or take antipyretics, will receive a second dose of investigational product. In case of fever or acute illness, the second dose of investigational product should be deferred until the fever or acute illness has subsided. The allowed window period for deferral of the second dose is a maximum of 10 days.

The study vaccine manager will send the investigational product to the study site as a unit-dose syringe, which will be identified with a participant identification number, date dispensed, and time taken from fridge/shelf, and the volume prepared. The syringe will contain 0.5 mL of investigational vaccine or placebo. An investigator must be present at the study site at the time of all investigational product administrations.

Before administering the injection, the blinded study team member at the study site who will be administering the injection must inspect the syringe and investigational product volume, check that the syringe is identified with the correct patient identification number and verify the date dispensed and time taken from fridge / shelf. A vaccine placed outside the refrigerator can only be stored at room temperature (maximum 25 °C) for a maximum of 2 hours.

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

3.6 Study Evaluations

3.6.1 Efficacy Evaluations

After randomization and vaccination on Study Day 0, the participants will be questioned about TB signs and symptoms at every study visit and contact, and will be instructed to come to the study site if TB signs or symptoms occur between study visits or contacts.

When there is clinical suspicion of TB disease, 2 separate sputum samples for *Mtb* testing will be obtained. 1 sputum sample will be tested by Xpert MTB/RIF Ultra. The same sputum sample will be sent for culture together with the second sputum sample for verification. The remainder of the 2 separate sputum samples is stored (frozen) as 2 separate samples for later analysis by WGS if recurrent TB is verified.

Referral for diagnosis and management of recurrent TB is triggered by the investigator based on clinical suspicion of recurrent TB. Participants will not be withdrawn from the study based on this referral but will be withdrawn if *Mtb* positive by culture in the study or if the referral clinic diagnoses recurrent TB and starts TB treatment.

In cases where the *Mtb* culture result in the study was negative or is pending, but the referral clinic diagnosed recurrent TB and started TB treatment, 2 additional sputum samples should be collected as soon as possible for repeat *Mtb* testing by culture, and the participant is withdrawn from the study.

If *Mtb* negative by Xpert MTB/RIF Ultra and subsequent verification by culture, the participant continues in the study. For further details on the case verification procedure for recurrent TB disease, see Section 2.2.

At the final study visit (V8=Day 421) or at the ET Visit, if discontinuation is not due to verified recurrent TB, 2 separate sputum samples for *Mtb* testing will be obtained from all participants (capable of producing sputum), even if there is no clinical suspicion of TB disease, in order to ensure that no cases of recurrent TB disease are missed during the study and to detect subclinical cases in this population at high risk for recurrence of TB disease. If there is clinical suspicion of TB at V8, all V8 activities will be conducted and not a STB visit since at V8 two separate sputum samples for Mtb testing will be obtained from all participants even if there is no clinical suspicion of TB. This is to ensure that no cases of recurrent TB disease are missed during the trial. One sputum sample will be tested by Xpert MTB/RIF ultra. If Mtb negative by Xpert MTB/RIF ultra but there are clinical TB signs or symptoms, verification by culture testing will be requested from the laboratory and both of the sputum samples will then be processed for culture. In this instance HIV testing and immunology sample collection should also be performed at V8.

Participants will be contacted to be given their Xpert MTB/RIF Ultra and culture result once available (whether positive or negative) and symptomatic participants will be referred as appropriate for management irrespective of the Xpert MTB/RIF Ultra and culture result. Asymptomatic participants not capable of producing sputum will be considered negative for TB disease recurrence.

Confirmation of *Mtb* by one positive sputum culture result is required for a participant to meet the primary efficacy endpoint of TB disease recurrence. For the secondary efficacy endpoints of TB disease relapse and TB disease reinfection, WGS will be performed on *Mtb* isolates to distinguish relapse from reinfection. Participants with a combined V1 and V2 will not provide sputum samples prior to starting TB treatment because their 1st screening visit (V1) would be performed on the same day as V2. For these participants, it will therefore not be possible to determine if their TB recurrence was due to relapse or reinfection.

For the exploratory efficacy endpoints, participants who started TB treatment without confirmation of *Mtb* by culture or participants with TB disease recurrence between 30 days after the 1st vaccination and 14 days after the 2nd vaccination will also be included. All efficacy endpoint definitions are given in Section 2.1.

The HIV status (i.e., to assess if seroconversion occurred since HIV test at V2) will be determined at time of diagnosis of TB disease recurrence, and the CD4 count will be determined if the participant is HIV positive.

A participant is considered to have clinical suspicion of TB when he/she presents with one or more of the following TB signs and symptoms: unexplained cough for longer than 2 weeks duration, fever, night sweats, loss of weight, hemoptysis, or other TB signs or symptoms.

3.6.2 Immunogenicity and Immunology Laboratory Evaluations

A summary of immunogenicity and immunology assays to be performed on blood specimens is shown in Table 3-2.

For the immunogenicity cohort at the [redacted] sites only (= first 50 randomized participants at [redacted] and first 50 randomized participants at [redacted] = in total

100 participants), see Table 2-1, whole blood will be collected for WB ICS. For the remaining participants and sites, there will be no collection of whole blood for WB ICS.

For all participants in the study, the immunogenicity / immunology samples, assays, endpoints, blood volumes, study days of collecting samples and names of laboratories analyzing the samples are shown below in Table 3-2.

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

Table 3-2 Summary of Immunogenicity and Immunology Laboratory Evaluations

Sample Type	Assay	Immunogenicity/ Immunology Endpoint	Approximate Blood Volume (per Visit)	Study Days	Name and Location of Analysis Laboratory
Whole blood	WB ICS	Secondary endpoint[1] (Primary immunogenicity endpoint)	6 mL	0[1], 70[1]	[redacted]
Plasma (obtained from PBMC)	IgG ELISA	Secondary endpoint[1]	Plasma (3 mL) obtained from PBMC	0[1], 70[1]	[redacted]
Plasma (obtained from PBMC)	IgG ELISA	Exploratory endpoint[2]	Plasma (3 mL) obtained from PBMC	0[2], 70[2], 421[5], STB[3], ET[4]	[redacted]
Whole blood	RNA analysis	Exploratory endpoint[2]	2.5 mL	0[2], 421[1,5], STB[3], ET[4]	[redacted]
Whole blood (DLC iCE)	Blood subset count (support of RNA analysis)	Exploratory endpoint[2]	At least 0.5 mL collected in a 2.0 mL tube	0[2], 421[1,5], STB[3], ET[4]	[redacted]
PBMC	ICS	Exploratory endpoint[2]	50 mL	0[2], 70[2], 421[1,5], STB[3], ET[4]	[redacted]

1) Participants in immunogenicity cohort (1st 50 randomized a[redacted]nd 1st 50 randomized at[redacted]
2) All participants in study
3) Participants if STB Visit is conducted
4) Participants who have not already had this at a STB Visit which resulted in verification of recurrent TB and who are Xpert Ultra MTB/RIF positive at the ET Visit
5) Participants who are Xpert Ultra MTB/RIF positive at Visit 8 (Day 421)

For further details on the purpose of the assays and the immunogenicity/immunology endpoints, see Section 7.4.

3.6.3 Safety Evaluations

3.6.3.1 Pre-vaccination and Post-Vaccination Monitoring of Participants

Participants will have vital signs taken prior to each study vaccination. Participants will remain in the clinic under close observation for at least 60 minutes after receiving the investigational product. Vital signs will be repeated at 60±10 minutes post-vaccination before participants leave the clinic. The injection sites will be examined.

Allergic reactions to vaccination are possible, therefore, appropriate drugs and medical equipment to treat acute anaphylactic reactions must be immediately available and an investigator trained to recognize and treat anaphylaxis must be present in the clinic during the entire vaccination procedure and post-vaccination monitoring period.

3.6.3.2 Clinical Assessments and Laboratory Tests

Clinical assessments and laboratory tests to be performed at each visit are summarized in Table 3.1. Results from clinical assessments and laboratory tests obtained in the study must be reviewed by the investigator, and managed in accordance with the study site policies. The clinical significance of any change in a vital sign or laboratory parameter will be determined by the investigator, and may be reported as an AE at the discretion of the investigator. Additional laboratory tests may be performed if the investigator deems them to be necessary to fully evaluate an AE. In the event that the investigator elects to order non-protocol-specified laboratory tests, the investigator must record the rationale for the tests and a determination of clinical significance of the result in the source documents. The investigator must keep the sponsor's medically responsible person informed of vaccine-related AEs of medical importance.

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

3.6.3.3 Adverse Events

The collection periods for AEs are shown in Table 3-3.

Table 3-3 Collection Periods for Adverse Events

Type of Event	Collection Period
Unsolicited adverse events (non-serious)	First 14 days after each vaccination (spontaneous adverse events, if any) Diary review and interview at study visit 14 days after each vaccination.
Solicited adverse events	First 7 days after each vaccination (solicited adverse events recorded daily in diary card) Diary review and interview at study visit 14 days after each vaccination.
Adverse events of special interest	Entire post-vaccination study period (i.e., 421 days).
Serious adverse events including medically important adverse events	Entire post-vaccination study period (i.e.,421 days).

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

- Injection site reactions
  Redness, swelling, tenderness/pain

- Systemic adverse events
  Fever, Arthralgia, Myalgia, Fatigue, Headache, Rash, Chills, Nausea

Solicited injection site reactions will be considered causally related to the investigational product.

Adverse events of special interest represent a subset of AEs that include autoimmune diseases and other systemic disorders of interest that could potentially have an immune etiology. AEs of special interest are listed in Appendix A. The PI should use clinical and scientific judgment in deciding whether other AEs (i.e., events not listed in Appendix A) could have an autoimmune origin. The investigator will report AEs of special interest in the eCRF, hereby informing the sponsor's medically responsible persons.

All participants will be provided a diary card, a thermometer to record axillary temperature, a ruler to measure injection site redness and swelling, and instructions on how to use the diary for AE and CM recording, for the first 7 days after each vaccination.

In the period from 8 days after the vaccinations and until the diary card review at 14 days after each vaccination the participants are instructed to fill in the diary only in case they experience an AE or CM intake.

3.6.3.4 Site of Injection Examination

The injection sites will be examined immediately after the vaccinations, see section 3.6.3.1. After this, examinations for injection site reactions and loco-regional reactions such as axillary lymphadenopathy will be performed at the visits V4= Day 14 and V6= Day 70, see Table 3.1. The following will be assessed at these examinations:

- Redness, swelling, tenderness/pain and axillary lymphadenopathy

3.6.3.5 Concomitant Medications

The collection of information on CMs used by participants following vaccination will coincide with the collection period of AEs. In addition, information on all CMs associated with the treatment of AEs, SAEs and AEs of special interest, will be collected throughout the study.

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

3.6.4 Participant Follow-up and Contact

All participants who are assigned a randomization number and receive the first vaccination will be followed according to the protocol unless consent is withdrawn.

The participants will be instructed to contact a study team member to report new signs and symptoms or new or worsened AEs and will be referred for medical attention as applicable. For emergencies and other unscheduled visits to a medical facility other than the study site, medical records will, to the extent possible, be obtained by the investigator.

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

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

Participant-level deviations from protocol procedures, evaluations, and/or visits must be documented. When possible, missed visits and procedures must be rescheduled and performed at the nearest possible time point to the original schedule. Deviations will be reported to the IRB/IEC and applicable national regulatory authorities per their guidelines.

3.6.5 Lost to Follow-up

If the study site's study team members are unable to establish contact with a participant who misses a scheduled study visit, the study site must make every possible effort to re-establish contact and document such efforts. If contact is re-established, then the participant will resume participation in the study. If contact with the participant cannot be re-established by the participant's calculated V8= Day 421 visit date, then a determination of "lost to follow-up" should be made on the date of the last contact.

4 INVESTIGATIONAL PRODUCT

4.1 Description of Investigational Product

The administered volume is 0.5 mL for all doses of investigational product. Please refer to the most recent version of the Vaccine Management Manual and IB, for further details regarding the investigational product.

H56:IC31
The H56:IC31 vaccine is manufactured, filled into vials and labelled at SSI according to GMP. The inner labels are study specific while the labels for the outer packaging (i.e. the cardboard boxes) are study and site specific. Both are produced at SSI. H56:IC31 will be supplied in single dose vials by SSI at a concentration of 5 µg/0.5 mL with IC31 at a concentration of 500 nmol KLK and 20 nmol ODN1a/0.5 mL.

Placebo:
A 0.9 % saline placebo will be purchased commercially and used as a comparator in the study. A label should be placed on the external carton stating the protocol number and that

the saline is for clinical use only.

4.2 Receipt, Storage and Accountability

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

H56:IC3 must be stored between +2°C and +8°C in a secured location with no access for unauthorized and blinded personnel. The normal saline placebo will be stored according to manufacturer's requirements in the study pharmacy.

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

It is the site PI's responsibility that all unused and used vials are accounted for during the study and documented in the relevant study documents/logs. The study vaccine manager is delegated to maintain accurate investigational product accountability records. Instructions and forms to be completed and kept for accountability will be provided by the sponsor to the study vaccine manager. Upon completion of the study, all investigational product management records will be copied and the copies returned to the sponsor. The originals must be maintained at the study site with the rest of the study records. During the study an unblinded monitor will check that drug accountability is maintained.

4.3 Preparation

Refer to the most recent version of the Vaccine Management Manual for detailed instructions.

4.4 Disposal of Unused Investigational Product

After completion of the study the destruction of all used and unused vials is to be performed according to local regulatory and sponsor requirements. The destruction of investigational product may not be performed before the sponsor's acceptance has been given in written form. Any disposal of investigational product conducted at the clinical study site must be documented in the site study file.

5 SAFETY

5.1 Responsibilities for Ensuring the Safety of the Study Participants

The national regulatory authority, IRB/IEC, the study sponsor, the institution through which the research is performed and all members of the site PI's clinical team share the responsibility for ensuring that participants in this study are exposed to the least possible risk of adverse events that may result from participation in the study.

5.1.1 Principal Investigator

The site PI from each study site has a personal responsibility to closely monitor participants and an inherent authority to take whatever measures necessary to ensure the safety of the participants. The site PI has the authority to terminate, suspend or require changes to a clinical study for safety concerns and may delay an individual's investigational product administration. The site PI is responsible for determination of intensity, seriousness and causality with respect to the investigational product for each adverse event.

The site PI may delegate specific tasks to a designee who is a medically qualified team member (who must be a sub-investigator in the case of assessment of adverse events).

5.1.2 Study Sponsor

For this study SSI and IAVI are sharing sponsor responsibilities as co-sponsors. The sponsor has an institutional responsibility to ensure the safety of all participants in the study.

5.1.3 Medically Responsible Person

The medically responsible person in the study is a physician from the sponsor. The medically responsible person will review the safety of the product during the study and will assess the causality of the reported SAEs and provide the expectedness of related SAEs. The medically responsible person is like the site PI, blinded in this study.

Each co-sponsor has a medically responsible person who contributes to performing these tasks.

The site PI can, in case of an emergency, unblind a participant's treatment through a restricted access module in the eCRF (see Section 3.4.1).

5.1.4 Internal Data Review Committee and Independent Data Safety Monitoring Board

An internal data review committee, consisting of the coordinating PI, the site PIs and the medically responsible person from each of the co-sponsors, will convene during the study to review the blinded reported SAEs, AEs including AEs of special interest, injection site reactions and laboratory safety test results to identify any possible safety issues when:

- The first 150 participants across all sites have received their 1st vaccination and the 14 days safety follow-up data is available
- The first 150 participants across all sites have received their 2nd vaccination and the 14 days safety follow-up data is available

Randomization and vaccination will continue unless a stopping rule is triggered.

In case a stopping rule is triggered at any time by the site PIs and/or sponsor's medically responsible person, the study will be paused. Please see Section 6 for further details.

An independent DSMB will be established, consisting of a panel of independent experts, who will operate according to a DSMB charter. The DSMB will be convened if:

- A stopping rule is triggered
- The internal data review committee requests a DSMB meeting based on their review of the safety data

Based on the DSMB review, the DSMB will make recommendations to the sponsor regarding further conduct of the study and further administration of the investigational product.

The DSMB may require unblinding of any amount of safety information needed to conduct their assessment. All procedures associated with their review, including objectives, data handling, and elements to be included for review will be documented in the DSMB minutes.

The DSMB may recommend suspension or resumption of enrollment and investigational product administration after review of the safety data. However, the sponsor will make the final decision to suspend or resume study activities. The recommendations of the DSMB, along with the sponsor's decision, will be communicated to the site PIs, IRB/IEC, and the national regulatory authorities. The sponsor agrees to abide by any directives issued by the national regulatory authorities or the IRB/IEC.

5.1.5 Institutional Review Boards and Independent Ethics Committees

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

5.1.6 National Regulatory Authority

Each national regulatory authority receives all SUSARs and periodic safety reports summarizing the SAEs in the study and has the authority to terminate, suspend or require changes to the clinical study.

5.2 Definition of Adverse Event

Adverse event means any untoward medical occurrence associated with the use of a product in humans, whether or not considered product related, if deemed clinically significant by the investigator. An AE can be any unfavorable and unintended sign (e.g., an abnormal laboratory finding), symptom, or disease temporally associated with the use of a product, and does not imply any judgment about causality. An AE can arise with any use of the product (e.g., off-label use, use in combination with another product) and with any route of administration, formulation, or dose, including an overdose.

Medical conditions that exist prior to administration of the investigational product (pre-existing conditions) will be recorded in the eCRF on the participant's medical history page 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 AEs.

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

AEs will be reported using a recognized medical term or diagnosis that accurately reflects the event. AE evaluations will be reviewed by the site PI or sub-investigator. AE information is to be completed by members of the study team designated by the site PI. The onset and resolution dates of the event and action taken in response to the event will be documented.

5.3 Assessing Intensity of an Adverse Event

The safety concepts of "intensity" and "seriousness" are distinct concepts (see Section 5.7). Intensity refers to a degree of clinical manifestation. "Seriousness" refers to defined outcomes of an AE. A severe AE is not always serious and a serious AE is not always severe.

For all AEs, the investigator is responsible for assessing the intensity of the event, the seriousness and the causal relationship of the event to the investigational product.

The **intensity** of all AEs, will be classified as one of the following grades:

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

- "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 intensity of an AE will not be recorded as a new AE. Only the highest intensity level that occurs during the entire period of the AE will be recorded in the eCRF with the onset and resolution dates encompassing the entire duration of the event.

5.4 Assessing Causal Relationship "Relatedness" of an Adverse Event

For all AEs, the investigator and, for SAEs, also the sponsor's medically responsible person will determine if there is a **causal relationship** to the investigational product without knowledge of whether active vaccine or placebo was administered. A number of factors will be considered in making this assessment, including: 1) the temporal relationship of the event to the administration of the investigational product 2) whether an alternative etiology has been identified and 3) biological plausibility. 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 product?"

- **YES (related):** There is a reasonable possibility that the investigational product contributed to the AE. This includes AEs that are considered possibly, probably or certainly related.
- **NO (not related):** There is no reasonable possibility that the AE is causally related to the administration of the investigational product. There are other, more likely causes and administration of the investigational product is not suspected to have contributed to the AE.

The site PI and sponsor's medically responsible person will both determine the causality of a SAE and it is expected that communication and consultation may occur in this assessment.

Every effort should be made by the investigator to determine the existence of any pre-existing conditions (e.g., headaches on Study Day 0 with onset prior to study vaccination) which must be taken into consideration when assessing the causal relationship of an AE. Pre-existing conditions should be recorded in the eCRF as baseline history and substantiated by appropriate source documentation. Intermittent conditions such as headaches may not be present on Study Day 0 but may represent an AE if the intensity or duration of the event is worse than usual following administration of the investigational product.

5.5 Definition of Adverse Reaction

An adverse reaction is an AE assessed to be related to the investigational product.

5.6 Assessing "Outcome" of an Adverse Event

The outcome of an AE must be assessed by the investigator using the following terms:

- Fatal
- Not yet recovered
- Recovered with sequelae
- Recovered without sequelae
- Unknown

5.7 Assessing "Seriousness" of an Adverse Event

The “seriousness” of an AE must be assessed by the investigator by answering the following questions:

- Did the event result in **death** (i.e., the AE caused or led to the fatality)? Serious does not describe an event which hypothetically might have caused death if it were more severe.
- Has the event been or is the event **life-threatening** (i.e., the AE placed the participant at immediate risk of dying. It does not refer to an event which hypothetically may have led to death if it were more severe).
- Has the event required **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 intensity or frequency following receipt of investigational product, are **not** serious by this criterion.
- Has the event resulted in significant or persistent **disability/incapacity** (i.e., substantial reduction of the participant’s ability to carry out activities of daily living)?
- Has the event resulted in a **congenital anomaly or birth defect** (i.e., an adverse finding in a child or fetus of a participant exposed to the investigational product prior to conception or during pregnancy)?
- Was the event another **medically important condition** that may not have resulted in death, threatened life or required hospitalization but may have jeopardized the participant or required intervention to prevent one of the serious outcomes listed in these criteria? Based on medical and scientific judgment such events may also be considered serious. Example: 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 AE meeting the criteria for “seriousness” regardless of relationship to the administered product.

A serious adverse reaction is meeting the criteria for “seriousness” and was assessed to be related to the investigational product.

5.8 Assessing “Expectedness” of Serious Adverse Reactions

The expectedness of related SAEs i.e. serious adverse reactions, is assessed by the sponsor’s medically responsible person only. The expectedness is assessed against the IB. If the outcome of the assessment is that the reaction is unexpected, then the reaction may represent a suspected, unexpected, serious adverse reaction (SUSAR) and the blind should be broken for this specific participant by the [redacted] through the restricted access module in the eCRF.

Only, if the participant has received the investigational product (NOT placebo) AND the related SAE (i.e. serious adverse reaction) is unexpected (i.e. unlisted in the IB), the case represents a SUSAR.

Expected related SAEs (i.e. serious adverse reactions) are consistent with the IB for the investigational product.

5.9 Definition of Suspected Unexpected Serious Adverse Reaction (SUSAR)

When an AE is judged to be related to an investigational product, and judged to be serious and unexpected in a participant who received the active vaccine, it is a SUSAR and is subject to expedited reporting.

5.10 Reporting of Serious Adverse Events

All SAEs are reported by the clinical study site through the eCRF to the sponsor's medically responsible person and to the [redacted] for the entire study period. The clinical study site will be provided with the specific reporting procedures before study start.

All AEs will be assessed for seriousness, intensity and causal relationship to the investigational product by the investigator. If serious (SAEs), the sponsor's medically responsible person will give a second assessment on causal relationship. Both assessments will be documented, but the highest degree of causal relationship will determine the ultimate classification of the event as related or unrelated SAE.

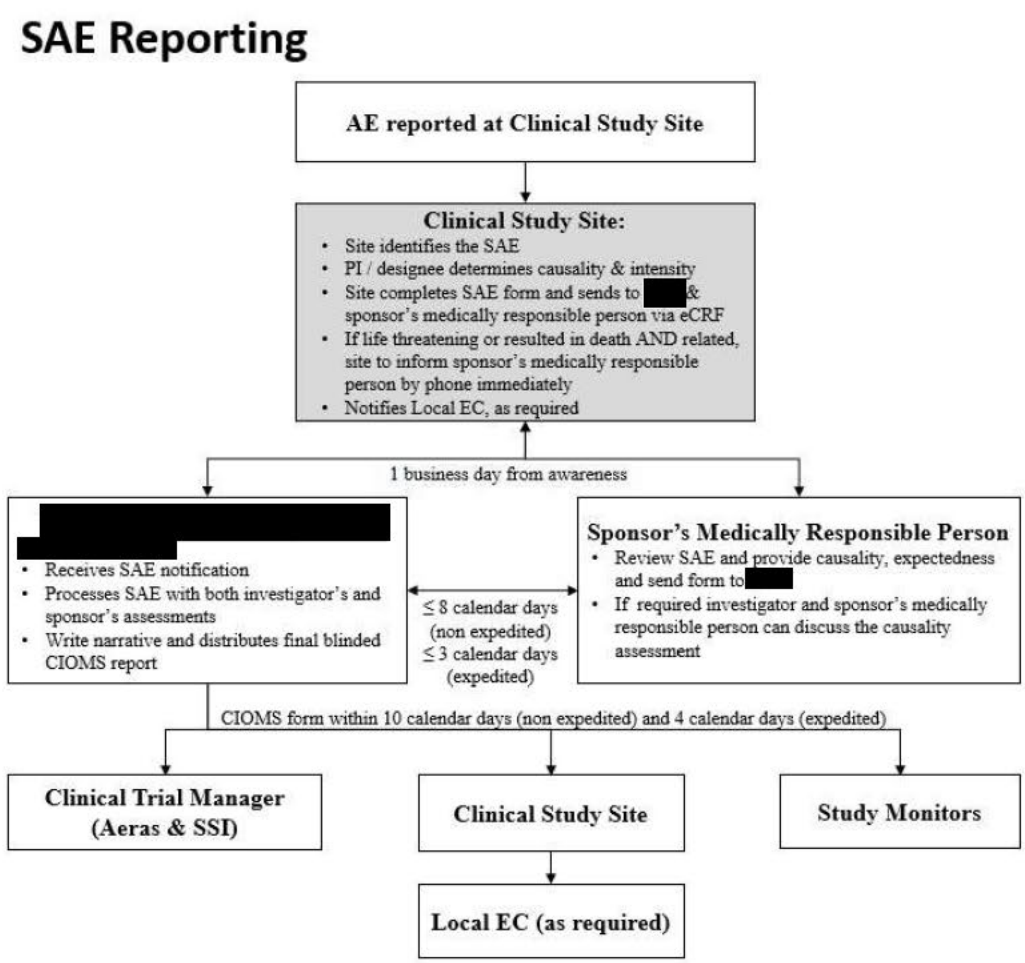
For related SAEs i.e. serious adverse reactions, the sponsor's medically responsible person will subsequently assess the expectedness and, if unexpected, report to the [redacted] within one business day.

The SAE form for the event must be completed by the investigator within one business day of the clinical study site becoming aware of the event. Investigators must not wait to collect additional information to fully document the SAE before notifying the sponsor's medically responsible person and the [redacted]

Fatal or life-threatening SAEs that the investigator suspects are related to the investigational product should be telephoned to sponsor's medically responsible person immediately upon the investigator's awareness of the event. If the investigator or sponsors medically responsible person is required by the protocol or chooses to suspend enrollment s/he shall immediately create a written memorandum for record to the study file.

Fatal or life-threatening SAEs or SUSARs that occur in Tanzania must be telephoned, faxed or e-mailed to TFDA by the investigator within 24 hours of awareness.

Please refer to below flowchart for the reporting of SAEs:

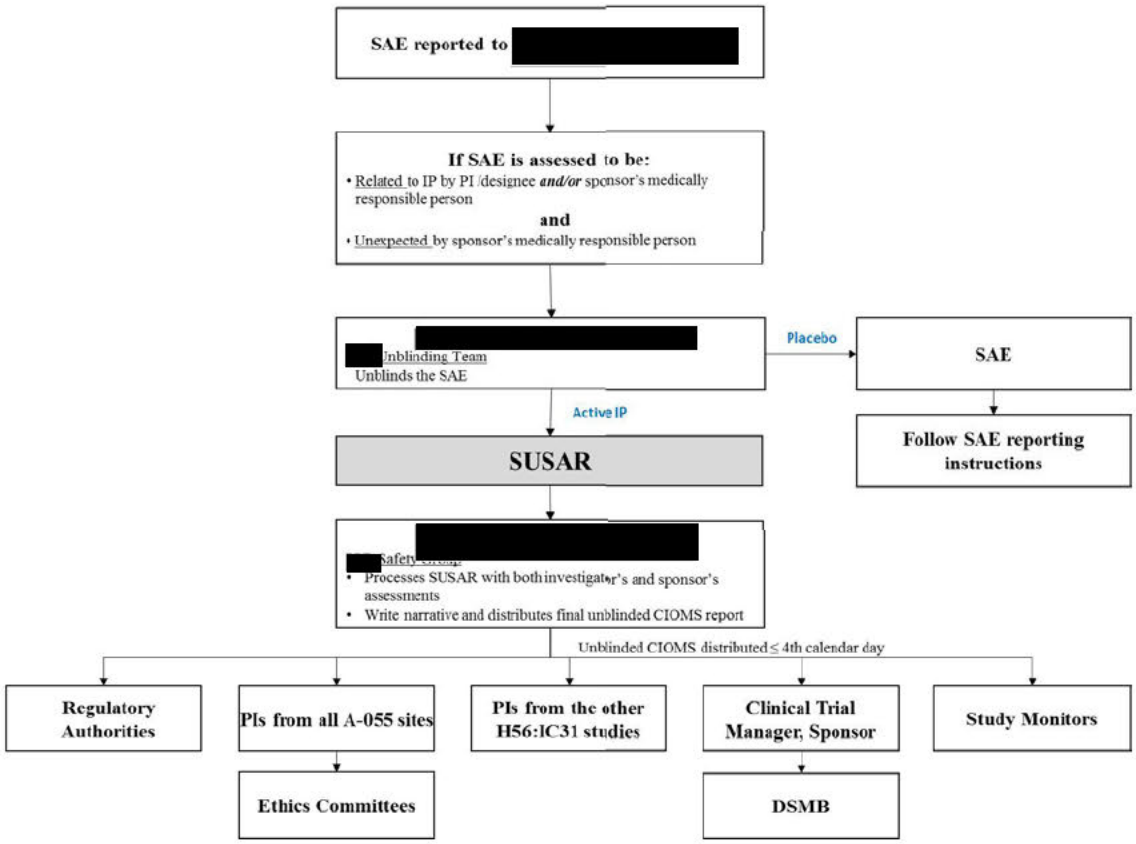


Expedited Reporting of SUSARs

Cases of SAEs determined to be both related to investigational product and unexpected should be unblinded. Such SAEs 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.

The sponsor has authorized the [redacted] to execute its responsibilities for expedited safety report submission to the appropriate competent authorities within specific time periods of being notified of the event (within 7 or 15 calendar days depending the character of the SUSAR); therefore, it is important that the investigator submits additional information requested as soon as it becomes available. SUSARs are reported even after the study is over, if the site PI becomes aware of them. Please see below flowchart:

SUSAR Reporting



5.11 Other Events Requiring Immediate Reporting

The investigator must report the following events to the sponsor's medically responsible person within 24 hours of becoming aware of the event. The clinical study site will be provided with the specific reporting procedures before study start:

- Withdrawal of consent during the study for safety reasons.
- Emergency and accidental unblinding.
- Protocol violation affecting the safety of a participant or involving the vaccination process.
- AE thought to be an allergic reaction to the investigational product.
- Any event that, in the opinion of the investigator, precludes further administration of the investigational product.
- Pregnancy.

5.12 Adverse Event Treatment and Follow-up

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

The investigator will continue follow-up on AEs, including laboratory abnormalities until the event has resolved. AEs 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 resolution date will be recorded in the eCRF as the last date on which the participant experienced the AE. If an AE resolution date is uncertain the investigator should estimate the completion date based on medical judgment and interview of the participant. Approximate dates of resolution from interviews may be taken as AE resolution dates.

AEs that are still present at the end of the study (last visit) should be recorded as ongoing in the eCRF. Information recorded in the eCRF must be substantiated in the source documents and followed up until resolution or stabilization. If an AE evolves into a condition that becomes "serious," it will be designated as serious on the AE form in the eCRF and a supplemental SAE form will be completed as specified in Section 5.10.

Follow-up on SAEs must continue until resolution or stabilized, even if this extends beyond the SAE reporting period (i.e., after the final study visit). For analysis purposes, the outcome for SAEs will be determined on the final study visit.

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

5.13 Participant Diary

Day 0-7 after each study vaccination:
All participants will be asked to fill in a diary daily for 7 days after each vaccination. In the diary the participants are asked to measure and report on the day of vaccination and the following 7 days details of the following AEs:

- Solicited:
  - Injection site reactions (Redness, Swelling, Tenderness/Pain)
  - Systemic AEs (Fever, Arthralgia, Myalgia, Fatigue, Headache, Rash, Chills, Nausea)

- Any other symptoms

Solicited injection site reactions will be considered causally related to the investigational product.

All participants will receive and be instructed in completing the diary. A ruler (for measurement of injection site reactions) and a thermometer (for axillary use) to be used

during the specified post-vaccination diary period after each study vaccination will be provided. The diary is a tool to aid the investigator to engage in a conversation with the participant about any AEs and/or CM intake that may have occurred between visits.

If a diary is lost, the investigator will discuss the occurrence of any AEs and/or CM with the participant and document the discussion. The diary will be considered source documentation and information assessed to be an AE by the investigator will be recorded in the eCRF. Source should document the clinical significance assessment.

Any change to an observation or event recorded by the participant in the diary based on the investigator's evaluation of the event, must be explained by notation in source documentation by the investigator.

Day 8-14 after each study vaccination:
From day 8-14 after each study vaccination the participants are instructed to fill in the diary only in case they experience an adverse event(s) (or concomitant medication intake). The participant will on day 14 after each vaccination be interviewed by the investigator, if any signs or symptoms have been observed by the participant.

5.14 Follow-up of Participants Who Become Pregnant

If a participant becomes pregnant during the study, she will not receive any further doses of investigational product, but she should be encouraged to continue in the study for safety, efficacy and immunogenicity follow-up. Follow-up should continue for pregnancy outcome including premature terminations, and data are to be included in the safety reports.

A pregnancy eCRF page must be completed by the investigator, within one business day of the clinical study site becoming aware of the event. Following entry into the eCRF, sponsor's medically responsible person and the [redacted] must be notified of the pregnancy. At a minimum, the estimated date of conception, the estimated due date, and the date the participant received the investigational product should be provided. The clinical study site will be provided with specific reporting procedures before study start.

The health status of the mother and child, the date of delivery, and the child's sex, birth weight and multiparity should be reported by completing the applicable pregnancy eCRF page. 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. The outcome will also be reported to the sponsor's medically responsible person and to the [redacted].

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

A congenital anomaly or birth defect (i.e., an adverse finding in a child or fetus of a participant exposed to the investigational product before conception or during pregnancy) must be reported as a SAE.

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 sponsor's medically responsible person and to the [redacted]

6 PAUSING AND STOPPING RULES

These rules govern the pausing and stopping of investigational product administration at any time during the study.

6.1 Rules for Discontinuing Investigational Product in an Individual Participant

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

1. An objective clinical or laboratory parameter change which by the investigator is assessed as severe in intensity AND is judged to be related to the investigational product.
2. AE thought to be an allergic reaction to the investigational product, including anaphylaxis or bronchospasm.
3. Any extensive rash (>40% body surface) on the thorax, abdomen, or limbs, including but not limited to urticaria, generalized petechiae, or erythema multiforme judged to be related to the investigational product.
4. Development of active TB.
5. Development of severe disease, immunodeficiency, autoimmune disease, immunosuppression or reported HIV seroconversion.
6. Receipt of investigational drug therapy or investigational vaccine (other than investigational product received as part of this study) between 1st and 2nd vaccination or any other vaccine as described in Exclusion Criteria #12.
7. Any event that in the opinion of the site PI precludes administration of any further investigational product.
8. Pregnancy.

Participants who are withdrawn from the study within 6 months after the last product administration, will be contacted at least 6 months after the last product administration for recording of SAEs and AEs of special interest.

6.2 Rules for Pausing the Study

The following rules will trigger pausing by the sponsor's medically responsible person for enrollment, and investigational product administration:

1. One or more SUSARs occur.

OR

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

OR

3. An AE pattern of concern occur in a group of participants or for an individual.

If the study is paused, the decision will be recorded in a memorandum to the study file and will trigger a DSMB review. If a recommendation to resume study enrollment and investigational product administration is made, the DSMB will record their judgment in a memorandum to the study file and notify the sponsor. The DSMB memorandum will be forwarded to the sponsor's medically responsible person and site PIs. The DSMB may recommend resumption of enrollment with changes to the protocol if it judges that such changes will reduce safety risks. However, the final decision to resume study activities, amend the protocol, or terminate the study will be made by sponsor.

The clinical study site will be allowed to resume activities only upon receipt of written notification from sponsor. Decisions regarding pausing and resumption of the study will be communicated to the IRB/IEC by the site PI and to the applicable national regulatory authorities by the sponsor.

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

7 STATISTICAL CONSIDERATIONS

The planned statistical analyses for this study are outlined below. A detailed statistical analysis plan will be created and finalized prior to database lock.

7.1 Analysis Sets

The safety analysis set will consist of all participants who received at least one dose of investigational product. The primary analysis set for efficacy (modified intent-to-treat [mITT] analysis set) will include all randomized participants except those with TB disease recurrence before V6=Day 70 (or 14 days after dose 2 for those who received both vaccinations). The second mITT [mITT2] analysis set will include all randomized participants except those with TB disease recurrence prior to 30 days post 1st dose. The per-protocol [PP] analysis set will consist of all participants who received both doses of H56:IC31 or placebo within the specified dose intervals, who entered the evaluation period for efficacy 14 days after receipt of the second dose of H56:IC31 or placebo with no HIV seroconversion, and who had no major protocol deviations (to be defined in the statistical analysis plan).
The immunogenicity analysis set will consist of participants from the mITT population who received both doses of H56:IC31 or placebo and who are included in the immunogenicity cohort (refer to Section 2.2). The ITT analysis set will consist of all randomized participants.

7.2 Demographics and Protocol Compliance

Demographic parameters (age, sex, and race/ethnicity) and other baseline characteristics will be summarized by treatment group for all participants in the safety analysis set, and may be summarized for participants in the mITT and PP analysis sets depending upon the numbers of participants in these analysis sets.

Listings of randomized participants who missed any dose of investigational product and of participants with protocol deviations will be provided.

7.3 Efficacy Analyses

7.3.1 Analysis of Primary Efficacy Endpoint

The primary efficacy endpoint in this study will be the detection of TB disease recurrence at any time greater than or equal to 14 days after receipt of the second dose of H56:IC31 or placebo through Study Day 421 (i.e. Study Day 70 through Study Day 421) (mITT analysis):

- Rate of TB disease recurrence (relapse or reinfection), defined as TB diagnosed by confirmation of *Mtb* by culture of sputum, during the period starting 14 days after the 2nd vaccination (V6= Day 70) and ending 12 months after the 2nd vaccination (V8= Day 421)

The number (percentage) of participants meeting this endpoint will be summarized by treatment group and study site/country. The primary analysis set for efficacy will be the mITT analysis set (defined in Section 7.1).

Summaries of median time to initial diagnosis of TB disease recurrence, person-time at risk, and associated 95% confidence intervals will be presented by treatment group and study site, for participants in the mITT analysis set. The log-rank statistic will be used to test the null hypothesis of no difference in the rates of TB Disease Recurrence over the follow-up period in the H56:IC31 compared to the placebo group. Additional analyses of the primary efficacy endpoint will include a Cox proportional hazards regression model to examine additional covariates such as study site, body mass index, age, and smoking.

The cumulative person-years incidence of TB disease recurrence and associated 95% confidence intervals during study follow-up will be summarized by treatment group and study site. Person-time observation will be used to estimate the time at risk of TB disease recurrence and will be calculated based on participant's date of last contact with the study or date of TB disease recurrence (whichever is earlier) minus the date at 14 days after receipt of the second dose of H56:IC31 or placebo. The incidence of TB disease recurrence will be calculated as the number of cases of TB disease recurrence diagnosed during study follow-up, divided by the total person-time of observation.
Comparison of the incidence of TB disease recurrence in each treatment group will be performed using relative risk summaries and corresponding 95% confidence intervals, as appropriate.

Supportive robustness and consistency analysis will be performed using the PP and ITT populations (defined in Section 7.1).

7.3.2 Analyses of Secondary Efficacy Endpoints

The following secondary efficacy endpoints will be analyzed using the same methods as described for the primary efficacy endpoint of TB disease recurrence (see Section 6.3.1).

Methods for comparison of WGS of paired *Mtb* isolates in participants with TB disease recurrence will be described in the statistical analysis plan.

- Rate of TB disease relapse, defined as participants meeting the primary endpoint of TB disease recurrence, AND determined by WGS of the *Mtb* isolate to be the same strain of *Mtb* as in the participant's original isolate from the time of diagnosis

- Rate of TB disease reinfection, defined as participants meeting the primary endpoint of TB disease recurrence, AND determined by WGS of the *Mtb* isolate to be a different strain than in the participant's original isolate from the time of diagnosis

.Analyses of Exploratory Efficacy Endpoints
The following exploratory efficacy endpoints will be analyzed using the same methods as described for the primary efficacy endpoint of TB disease recurrence (see Section 7.3.1):

- Rate of TB disease recurrence (relapse or reinfection), defined as participants meeting the primary endpoint of TB disease recurrence AND participants who started TB treatment without confirmation of *Mtb* by culture of sputum

- Rate of TB disease recurrence (relapse or reinfection), defined as participants meeting the primary endpoint of TB disease recurrence based on confirmation of *Mtb* by Xpert MTB/RIF Ultra or culture of sputum

- Rate of TB disease recurrence (relapse or reinfection), defined as participants meeting the primary endpoint of TB disease recurrence AND participants diagnosed between 30 days after the 1st vaccination and 14 days after the 2nd vaccination, based on confirmation of *Mtb* by culture of sputum

7.4 Immunogenicity and Immunology Analyses

Immunogenicity and immunology analysis will be summarized for all time points as collected and as available. The immunogenicity endpoint analysis will be based on participants in the immunogenicity analysis set (defined in Section 7.1). No imputation for missing data will be performed. Data will be transformed as appropriate prior to analysis.

7.4.1 Intracellular Cytokine Staining (WB ICS) Using Whole Blood

This is a secondary endpoint in the study, see Section 2.1.3.1 and Table 3-2, and it is the primary immunogenicity endpoint. This endpoint will be measured in the immunogenicity cohort (see Table 2-1) at V3= Day 0 and V6= Day 70.

Responses will be measured by flow cytometry (up to 15 parameters) with the whole blood based ICS assay. The variables of interest for assessment of antigen-specific cell-medicated immune response to vaccination will be the percentage of CD4+ and CD8+ T cells that express selected cytokines and phenotypic markers alone or in combination in response to stimulation with peptide pools representing the entire amino acid sequence of the vaccine antigens.

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

Analytic approaches for these endpoints will be informed by best practice and the most recent advances in vaccinology and systems biology.

7.4.2 Antigen-specific IgG ELISA Assay Using Plasma

This is a secondary endpoint in the study, see Section 2.1.3.1 and Table 3-2, and is the secondary immunogenicity endpoint. This endpoint will be measured in the immunogenicity cohort (see Table 2-1) at V3= Day 0 and V6= Day 70. For remaining participants, it is an exploratory endpoint at V3= Day 0, V6= Day 70 or at suspicion of a diagnosis of recurrent TB at the time of evaluation of TB recurrence. Antigen-specific antibody will be assessed in plasma by enzyme linked immunosorbent assay (ELISA). Summaries will include immune response at all available pre- and post-vaccination immunology time points, and change from pre-vaccination to post-vaccination time points.

7.4.3 RNA Analysis and Blood Subset Counts Supporting RNA Analysis Using Whole Blood

These are exploratory endpoints in the study, see Section 2.1.4.1 and Table 3-2. These endpoints will be measured in all participants at V3= Day 0 and in participants with suspicion of a diagnosis of recurrent TB at the time of evaluation of TB recurrence. Whole blood gene expression profiles will be measured by RNA sequencing and microfluidic qRT-PCR on PAXgene samples in a subset of participants: those who developed TB recurrence and a selected group of controls.

Cellular composition of whole blood leukocytes will be characterized by flow cytometry in order to deconvolve transcriptomic patterns to account for changes in peripheral blood cell numbers.

Transcriptomic signatures of inflammation and gene expression pathways as well as transcriptomic signatures associated with risk of TB recurrence and prediction of adaptive responses will also be investigated.

The determination of absolute blood subset counts will support the RNA analysis.

Analytic approaches for these endpoints will be informed by best practice and the most recent advances in vaccinology and systems biology.

7.4.4 Intracellular Cytokine Staining (ICS) Using PBMCs

This is an exploratory endpoint in the study, see Section 2.1.4.1 and Table 3-2. This endpoint will be measured in all participants at V3= Day 0 and V6= Day 70 and in participants with suspicion of a diagnosis of recurrent TB at the time of evaluation of TB recurrence.

Analysis of cells stimulated with relevant antigens (ESAT-6, Ag85B, Rv2660c, as well as negative and positive controls) will include T, B and other lymphocyte lineages (such as NK and MAIT cells), expression of differentiation and activation markers as well as cell functions (such as cytokine production and cytotoxic potential). Responses will be measured by flow cytometry (up to 28 parameters) using a PBMC based ICS assay.

7.4.5 Exploratory Immunology at TB Recurrence - Control Samples

The exploratory immunology samples to be taken at the time of evaluation of TB recurrence, from participants with a suspected diagnosis of recurrent TB disease will be: PBMC and plasma for ICS and IgG ELISA and whole blood for RNA analysis and Blood subset counts (that support the RNA analysis), see Table 3-2.

For the analysis of these samples, “control samples” from a “control cohort” are needed.

The immunology samples taken at V8= Day 421 from the participants in the immunogenicity cohort (excluding participants with recurrent TB, if any), see Table 2-1, will be these “control samples”. See Table 3.1.

7.4.6 Adverse Events

The primary variable for evaluation of the safety profile will be the number and percentage of adverse events recorded after the first administration of vaccine on Study Day 0. The number of events and number (percentage) of participants with unsolicited adverse events and solicited adverse events will be summarized by MedDRA system organ class and preferred term. Additional summaries will present the number of events and number (percentage) of participants with adverse events by severity and by relationship to investigational product; Listings will be provided for participants with serious adverse events.

7.4.7 Injection Site Reactions

The number (percentage) of participants recording injection site reactions (redness, swelling and tenderness/pain) and axillary lymphadenopathy will be summarized by treatment group and post-vaccination time point. In addition, measurements (recorded in mm) of redness and swelling will be summarized as continuous parameters by treatment group.

7.4.8 Clinical Laboratory and Vital Sign Parameters

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

vaccination assessments and for change from pre-vaccination to post-vaccination assessments.

The number (percentage) of participants with post-vaccination clinical laboratory values or vital sign values recorded as newly abnormal following study vaccination will be tabulated at each post-vaccination time point and overall. Clinical laboratory and vital sign abnormalities will also be reported as adverse events and included in the summaries of adverse events.

7.5 Sample Size Considerations

The sample size for this study is based on the following assumptions:

1. Estimated TB Disease Recurrence rate in placebo group: 4%/year.
2. Follow-up period for each participant: 12 Months post Study Day 70.
3. Drop-out/loss to follow-up rate: 10%.
4. Vaccine Efficacy (VE): 60%.
5. Type I error rate: 20% (2-sided).

Based on the aforementioned assumptions, a sample size of 900 participants (N=450 in each treatment arm [1:1] per active versus placebo comparison) is expected to provide the 23 TB Disease Recurrence endpoints per active versus placebo comparison required to detect with 80% power a VE of 60%, assuming a 10% drop-out rate and 12-month post Study Day 70 follow-up period for each participant.

7.6 Plan for Statistical Summaries and Analyses

7.6.1 Preliminary Data Reviews

An internal data review committee will review the unblinded safety data when 150 participants have received the 1st vaccination and when 150 participants have received the 2nd vaccination.

An independent DSMB with independent experts will be established. The DSMB will only be convened if, a stopping rule is triggered, or if the internal data review committee's safety review triggers a DSMB meeting. Please refer to Section 5.1.4 for details of the procedures of the internal data review committee and the DSMB.

7.6.2 Exploratory Analysis of Immunogenicity Cohort

Whole blood ICS assays are to be performed and analyzed by [redacted] (Table 3-2) prior to unblinding of the trial. The blinded biostatistician will provide a list of unique, randomly assigned dummy participant identification (ID) numbers to a designated technologist who will not be involved in the conduct or analyses of the data. Two designated scientists who are not involved in any operational activities of the trial will analyze the data. They will receive a list linking each dummy ID with the participant's treatment (H56:IC31 or placebo) to compare responses in participants receiving active vs. placebo; they will not be able to link the treatment with the real participant ID. A SOP will detail the transfer of samples to the

processing technologists and the transfer of data for analysis. Only after database lock will the staff be unblinded to the real participant IDs to enable linking of clinical outcomes to immunological responses.

7.6.3 Final Study Report

The final study report will include all available data related to participant disposition; demography and baseline characteristics; vaccine administration; protocol deviations; efficacy, immunogenicity (excluding exploratory analyses), and safety analyses; clinical assessments; and concomitant medications through the final study visit. The database will be locked prior to preparation of the final study report when all of the above data have been entered, reviewed, and all queries related to the data have been addressed.

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

7.7 Computer Methods

Statistical analyses will be performed using SAS®.

8 DATA COLLECTION, MONITORING, AND RECORD RETENTION

For the purpose of monitoring and auditing the study, source data will consist of original records and certified copies of original records contained in source documents (e.g. existing medical records and/or study records developed and maintained by the investigator).

At the investigational site, a document identifying all (expected) source documents should be prepared and signed by the site PI before the initiation of the study.

Data recorded on source documents and required to be captured in the database will be entered in electronic case report forms (eCRFs) using an electronic data capture (EDC) system provided and approved by sponsor.

The study will be monitored regularly, according to the protocol-specific monitoring plan. On site monitoring will be performed by a clinical CRO, sub-contracted by the sponsor, throughout the study period.

Data management and statistical analysis will be sub-contracted by sponsor to a CRO. Before releasing the database for statistical analysis and clinical study reporting, the data manager will ensure that all quality control procedures in connection with data capture, cleaning and reconciliation of data have been finalized and documented.

All study records (source documents, signed informed consent forms, IRB/IEC correspondence and approval letters, investigational product management records, and other essential documents) will be kept secured for a minimum of 15 years after the last participant’s last visit. Additional storage will be according to the guidance of the applicable national regulatory authority. The investigator will ensure that study records are not disposed

or removed from the clinical site without prior written notification and approval from sponsor.

9 ETHICS AND REGULATORY

9.1 Ethical and Regulatory Considerations

The study will be conducted according to the ethical principles set forth in the Declaration of Helsinki, ICH-GCP, South African GCPs and local regulatory requirements, as applicable. The clinical study site(s) should have written operating procedures and recruitment and retention guidelines appropriate for the specific age group and health status (e.g., HIV-positive, active TB disease) of the study population.

The protocol (and protocol amendments if applicable) and informed consent forms will be reviewed and approved (as applicable) by the IRB/IEC of each participating clinical study site and the regulatory authorities in South Africa and Tanzania, prior to any protocol-specified procedures being conducted.

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

Modifications to the protocol may not be implemented without prior written IRB/IEC and regulatory authority 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 to the regulatory authorities by the sponsor. A copy of the correspondence to verify the submission will be maintained.

The investigator must inform the IRB/IEC of modifications to the informed consent form or any other documents previously submitted for review/approval, of any new information that may adversely affect the safety of the 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. The sponsor will inform the regulatory authorities of such information as required.

Any site-generated documents or forms to be provided to the participant (e.g., information cards, form letters from the investigator), and all forms of study advertising (flyers, brochures, print advertisements, radio or television scripts, etc.) must be approved by sponsor 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.

Written informed consent will be obtained from each participant prior to any protocol-specified procedures being conducted. HIV testing will be performed with appropriate pre- and post-test counseling.

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

After the study has been unblinded, the participant should be informed by the study staff on which treatment (H56:IC31 or placebo) the participant received and the results of the study. Participants who receive placebo will not be offered H56:IC31 at the end of the study even if prevention of recurrence is demonstrated.

9.2 Informed Consent

The principles of informed consent in the current edition of the Declaration of Helsinki and ICH-GCP, and any other applicable GCPs, should be implemented prior to any protocol-specified procedures being conducted. The written consent document will embody the elements of informed consent as described in the Declaration of Helsinki and will also comply with local regulations. Informed consent will be documented in writing on a version-controlled consent form approved by the IRB/IEC.

The informed consent process will be conducted in a private space to maintain confidentiality. The consent process will be conducted in the participant's language of choice. All relevant information should be provided in both oral and written form in a way that is understandable to the participant. Ample time and opportunity must be given for the participant to inquire about details of the study. The potential participant should be encouraged to take the informed consent form home to discuss with family and friends before deciding whether or not to participate in the study.

The investigator or the investigator's qualified designee will explain the nature of the study and inform the participant 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 must 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 must be provided with a description of the procedures and the estimated duration of time required for participation in the study, as well as alternative interventions or courses of treatment, if applicable.

The participant must receive an explanation as to whether any compensation and any medical treatments are available if injury occurs and, if so, what they are, where further information may be obtained, and who to contact in the event of a study-related injury. IVIA carries an insurance programme including cover for clinical studies. The policy covers claims arising from injury/injuries caused by the investigational products used in this clinical study, if used in accordance with the instructions given in the protocol. Participants must be told who to contact for answers to any questions related to the study. The extent of the confidentiality of participant's records must be defined and the participant must be informed that applicable data protection legislation applies.

The participant must 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 must be informed that his/her signature on the informed consent form indicates that he/she has decided to participate in the study, having read and discussed the information presented.

The assessment of a participant's understanding of the key study concepts (e.g., procedures, risks) prior to signing the consent form and the final consenting should be conducted by the investigator or medically qualified personnel. The investigator or medically qualified personnel should be aware of situations in which partners, family, or community members may be exerting undue influence on an individual to participate in the study.
Modifications made by the investigator to an informed consent form template provided to the investigator by sponsor will be reviewed and approved by sponsor prior to being submitted to the IRB/IEC.

The original, signed informed consent form for each participant will be maintained by the investigator as part of the participant's study records. A copy of the signed informed consent form will be provided to each participant.

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

10 STUDY COMPLETION

The site PI or designated study site staff will notify the IRB/IEC when the study has been completed. Sponsor will notify the regulatory authorities in both South Africa and Tanzania.

11 PUBLICATIONS

The final clinical study report will be made available to the site PIs for purposes of publications. The site PIs and study staff must send all manuscripts, abstracts, and presentations using data from this study to the sponsor for review prior to their submission. The sponsor reserves the right to delete any part or parts of such materials deemed to be confidential or proprietary.

12 CHANGES IN THE PROTOCOL

The protocol may not be modified without written approval from the sponsor except where necessary to eliminate an immediate hazard(s) to study participants or when the change(s) involves only logistical or administrative aspects of the study. All substantial changes to the protocol must be submitted and approved by the IRB/IEC and applicable national regulatory authorities prior to their implementation.

12.1 Amendment 1 (Protocol v2.0)

What's new in Protocol Final Version 2.0, excludes formatting, organizational, typographical and minor editorial changes.

Section No., Paragraph No., Page No. in Final version 1.0	Text in Final Version 1.0	Reason for change	Text in Final Version 2.0
1.1, 3rd, p. 10	N/A	New reference X 2	(Imperial et al, 2018)
1.4, 1st, p. 12	In these studies a total of **239** participants have been vaccinated, **156** of whom….	IB annual update FV8.0 to FV9.0	In these studies a total of **248** participants have been vaccinated, **165** of whom….
1.4, 1st, p. 13	(Suliman et al in prep.)	New reference	(Suliman et al, 2019)
1.4, 3rd, p. 13	…, **10** adults who had received TB treatment for 3 months…….	IB annual update FV8.0 to FV9.0	…, **19** adults who had received TB treatment for 3 months…….
1.5; 3rd, p. 19	(data on file)	IB annual update FV8.0 to FV9.0	(Nemes et al, 2018)
1.6, 1st & 2nd, p. 15	See text in Protocol Final 1.0	IB annual update FV8.0 to FV9.0. Description of the general investigational plan-minor corrections and updating with new references	See text in Protocol Final 2.0
2.2, 2nd, p. 19	….Xpert MTB/RIF Ultra result from the TB clinic…..	Specification in more detail	….Xpert MTB/RIF Ultra result **or AFB smear result** from the TB clinic…..
2.2, 2nd, p. 19 3.2.5, 2nd, p. 28	N/A	IRC commitment to add language in the protocol about contacting participants during the screening period to reduce the screening failure rate	During the 5-month period between V1 and V2, the study staff will regularly liaise with screened participants, staff from the TB clinics and community health care workers to monitor TB treatment compliance and continued eligibility for study participation
2.2, 3rd, p. 19 Table 3-1 foot note 3) 3.2.5, 3rd, p. 28	The remainder of the 2 sputum samples will be stored (frozen) for exploratory use at the end of the study.	Specification in more detail	The remainder of the 2 sputum samples will be stored (frozen) **for culture, if verified recurrent TB, and** exploratory use at the end of the study
2.2, 3rd, p. 19 2.2, 1st and 2nd, p. 20	…, pregnancy testing (females of childbearing potential)….	All females will be pregnancy tested	…, pregnancy testing (all females)
2.2, 4th, p. 20	….and medically important AEs…..	Specification in more detail (throughout protocol)	….and AEs of special interest…..

Section No., Paragraph No., Page No. in Final version 1.0	Text in Final Version 1.0	Reason for change	Text in Final Version 2.0
2.2, 5th, p. 20	For the safety cohort (= the first 150 randomized participants across all sites), see Table 2-1, there will also be safety laboratory testing on V4= Day 14 and V6= Day 70 **or at the early termination (ET) visit,** as defined. For the remaining participants, there will only be laboratory safety testing at screening (V2).	ET visit procedures of safety testing (i.e. if IMP is administered within 14 days of early termination) applies for all participants (and not only for participants in the safety cohort), as explained in Table 3.1	For the safety cohort (= the first 150 randomized participants across all sites), see Table 2 1, there will also be safety laboratory testing on V4= Day 14 and V6= Day 70. For the remaining participants, there will only be laboratory safety testing at screening (V2), **or at the early termination (ET) visit**, if within 14 days after IMP administration.
2.2, 6 months safety follow-up, p. 21 Table 3-1, foot note 14 6.1, 6 months safety follow-up p. 49	Participants who are withdrawn from the study will be followed for safety tests for a minimum of 6 months after the last product administration.	Specification to make procedures clear.	Participants who are withdrawn from the study within 6 months after the last product administration, will be contacted at least 6 months after last product administration for recording of SAEs and AEs of special interest
2.2, Case verification procedure, p. 21 3.1, Table 3-1, p. 25 3.6.1, 2nd, p. 31 Table 3-2, p. 33	See texts in Protocol Final 1.0	Revisions throughout the protocol, to specify the case verification procedure, incl. sampling at STB Visit and withdrawal at ET Visit and that case verification is defined as a Mtb culture positive result, where the XPert Ultra MTB/RIF results are used to rule out recurrent TB at V8 or ET Visit.	See texts in Protocol Final 2.0
2.2, p. 22 5.1.3, 1st, p.38 5.1.4, 1st, p. 38	…...An internal data review committee consisting of the site principal investigators (PIs) and sponsor's medically responsible person will convene during the study to review…..	Specification to make more clear	An internal data review committee, consisting of the **coordinating principal investigator (PI)**, the site PIs and **the medically responsible person from each of the sponsors**, will convene during the study to review…….

Section No., Paragraph No., Page No. in Final version 1.0	Text in Final Version 1.0	Reason for change	Text in Final Version 2.0
3.1, Table 3-1 Summary Schedule of Participant Evaluations, p. 24-25 3.6.2, Table 3-2 Summary of Immunogenicity and Immunology Laboratory Evaluations, p. 33	See Table 3-1 in Protocol Final 1.0 See Table 3-2 in Protocol Final 1.0	Table 3-1 incl. notes updated to separate out study procedures at the STB Visit and at the ET Visit, to make procedures for sputum samples, case verification and referral to TB treatment more precise. Table 3-2 had to be updated accordingly. ET Visit references updated accordingly throughout the protocol.	See revised Table 3-1 in Protocol Final 2.0. See revised Table 3-2 in Protocol Final 2.0
3.2.2, 4th, p. 26	The participant identification log(s) will identify the participants by full name, sex, address, contact number, date of birth, date of consent, participant identification number(s) and randomization date (for included participants) and will be stored in a secure location.	Participant identification log(s) described in more detail	The confidential participant identification log will identify the participants by full name, date of birth, participant screening number, address and contact number and will be stored in a secure location. The participant screening and enrollment log will identify participants by participant screening number, date of informed consent, date of randomization, randomization number, and date and reason for exclusion, if applicable.
3.2.5, 1st, p. 28	….confirmed by Xpert MTB/RIF Ultra…..	Specification in more detail	….confirmed by Xpert MTB/RIF Ultra **or AFB smear**……
3.2.5, 1st, p. 28	N/A	Addition of language to explain that confirmation of rifampicin susceptibility may be pending until randomization at V3	Participants may also be eligible for inclusion at V1, if the Xpert rifampicin susceptibility test was unsuccessful at the local clinic, but the participant was started on a drug-susceptible treatment regimen according to national guidelines. The participant will then be screen failed prior to randomization (V3), if the site cannot confirm drug-susceptible TB by a test or a clinical response to treatment

Section No., Paragraph No., Page No. in Final version 1.0	Text in Final Version 1.0	Reason for change	Text in Final Version 2.0
3.6.1, 3rd, p. 32	….unexplained cough for longer than 2 weeks duration, fever, night sweats, loss of weight, or hemoptysis	Adjustment to match the text in the eCRF	….unexplained cough for longer than 2 weeks duration, fever, night sweats, loss of weight, hemoptysis, **or other TB signs or symptoms**
3.6.2, Table 3-2, p. 33	Column 1, row 4: **Whole blood**.	Specification in more detail and editorial changes in foot notes	Column 1, row 4: **Whole blood (DLC iCE)**
3.6.3.3, 2nd, p. 35	N/A	Specification of reporting of AEs of special interest from investigator to sponsor's medically responsible person	The investigator will report AEs of special interest in the eCRF, hereby notifying the sponsors medically responsible persons
5., p. 37	N/A	Specification of SAE reporting and unblinding procedures by use of eCRF.	It has been added in various subsections in section 5, that reporting and unblinding of SAEs is conducted by use of the eCRF.
5.10, SAE Reporting Flowchart, p. 45	Site completes SAE form and sends to PPD	Adjustment of Flowchart to match instructions in text	Site completes SAE form and sends to PPD **& sponsor's medically responsible person via eCRF**
5.10, SAE Reporting Flowchart, p. 45	If life-threatening or resulted in death, site to inform sponsor's medically responsible person by phone	Adjustment of Flowchart to match instructions in text	If life-threatening or resulted in death **and related**, site to inform sponsor's medically responsible person by phone **immediately**
5.10, Fatal or life-threatening SAEs in TZ, p. 44	N/A	Procedures for fatal or life-threatening SAEs or SUSARs that occur at site in TZ to be detailed in protocol	Fatal or life-threatening SAEs or SUSARs that occur in Tanzania must be telephoned, faxed or e-mailed to TFDA by the investigator within 24 hours of awareness.
5.11, 2nd bullet, p. 46	Emergency unblinding	Specification in more detail	Emergency **and accidental** unblinding

Section No., Paragraph No., Page No. in Final version 1.0	Text in Final Version 1.0	Reason for change	Text in Final Version 2.0
7.1, 1st, p. 50	N/A	Specification of analysis populations in more detail	The second mITT [mITT2] analysis set will include all randomized participants except those with TB disease recurrence prior to 30 days post 1st dose. The immunogenicity analysis set will consist of participants from the mITT population who received both doses of H56:IC31 or placebo **and who are included in the immunogenicity cohort (refer to section 2.2).**
7.4.2, 1st, p. 53	This is a secondary endpoint in the study, see Section 2.1.3.1 and Table 3 2. This endpoint will be measured in all participants at V3= Day 0 and V6= Day 70 and in participants with suspicion of a diagnosis of recurrent TB at the time of evaluation of TB recurrence.	Specification of analysis procedures in more detail	This is a secondary endpoint in the study, see Section 2.1.3.1 and Table 3-2, and is the secondary immunogenicity endpoint. This endpoint will be measured in the immunogenicity cohort (see Table 2-1) at V3= Day 0 and V6= Day 70. For remaining participants, it is an exploratory endpoint at V3= Day 0, V6= Day 70 or at suspicion of a diagnosis of recurrent TB at the time of evaluation of TB recurrence.
7.4.6, Adverse events, p. 55	1) …The number (percentage) of participants with... X 2 2) ...each participant will be counted once per preferred term at the greatest severity or most related state recorded for that term…	Specification to make the adverse events analysis more clear	1) ...The number of **events and number** (percentage) of participants with... X 2 2) Deleted
12. Changes in the protocol	This is the **1st** final version of the protocol	Update to new version	This is the **2nd** final version of the protocol. Addition of Table with changes from Final version 1.0 to Final version 2.0
13. References	Addition of new references to list	Addition of new references to list	Addition of new references to list

12.2 Amendment 2 (Protocol v3.0)

What's new in Protocol Final Version 3.0, excludes formatting, organizational, typographical and minor editorial changes.

Section No., Paragraph No., Page No. in Final version 2.0	Text in Final Version 2.0	Reason for change	Text in Final Version 3.0
2.1.3.1, 3rd bullet, page 17	Antigen-specific cell-mediated immune responses by whole blood intracellular cytokine staining (WB ICS) at baseline (V3= Day 0), and 14 days after the 2nd vaccination (V6= Day 70)	Clarify specific population to be analyzed	Antigen-specific cell-mediated immune responses by whole blood intracellular cytokine staining (WB ICS) at baseline (V3= Day 0), and 14 days after the 2nd vaccination (V6= Day 70) in the immunogenicity cohort
2.1.3.1, 4th bullet, page 17	Humoral immune responses by IgG ELISA of plasma samples taken at baseline (V3= Day 0) and 14 days after the 2nd vaccination (V6= Day 70) and at TB recurrence diagnosis	Clarify specific population to be analyzed and data time point to exclude TB recurrence diagnosis. This change reconciles the discrepancy between Section 2.1.3 and Section 7.4.2 of version 2.0	Humoral immune responses by IgG ELISA of plasma samples taken from the immunogenicity cohort at baseline (V3= Day 0) and 14 days after the 2nd vaccination (V6= Day 70)

12.3 Amendment 3 (Protocol v4.0)

What's new in Protocol Final Version 4.0, excludes formatting, organizational, typographical and minor editorial changes.

Section No., Paragraph No., Page No. in Final version 3.0	Text in Final Version 3.0	Reason for change	Text in Final Version 4.0
3.2.4, page 27	12. Receipt or planned receipt of any licensed vaccine from V1 through V6= Day 70	In anticipation of the implementation of national SARS-Cov-2 vaccines programs.	12. Receipt or planned receipt of any licensed vaccine from V1 through V6= Day 70, except for SARS-Cov-2 vaccines recommended by national vaccination programs which will be allowed if given > 28 days before and from the time of administration of clinical trial product.
6.1, page 48	6. Receipt of investigational drug therapy, investigational vaccine (other than	In anticipation of the implementation of national SARS-Cov-2 vaccines programs.	6. Receipt of investigational drug therapy or investigational vaccine (other than investigational

Section No., Paragraph No., Page No. in Final version 3.0	Text in Final Version 3.0	Reason for change	Text in Final Version 4.0
	investigational product received as part of this study) and/or any other vaccine between 1st and 2nd vaccination.		product received as part of this study) between 1st and 2nd vaccination or any other vaccine as described in Exclusion Criteria #12.
None	None	For operational reasons study team and PIs decided to allow partial unblinding of the immunogenicity cohort in order to conduct exploratory whole blood ICS while the trial is pending completion.	7.6.2 Exploratory Analysis of Immunogenicity Cohort Whole blood ICS assays are to be performed and analyzed by [redacted] (Table 3-2) prior to unblinding of the trial. The blinded biostatistician will provide a list of unique, randomly assigned dummy participant identification (ID) numbers to a designated technologist who will not be involved in the conduct or analyses of the data. Two designated scientists who are not involved in any operational activities of the trial will analyze the data. They will receive a list linking each dummy ID with the participant's treatment (H56:IC31 or placebo) to compare responses in participants receiving active vs. placebo; they will not be able to link the treatment with the real participant ID. A SOP will detail the transfer of samples to the processing technologists and the transfer of data for analysis. Only after database lock will the staff be unblinded to the real participant IDs to enable linking of clinical outcomes to immunological responses.

12.4 Amendment 4 (Protocol v5.0)

What's new in Protocol Final Version 5.0, excludes formatting, organizational, typographical and minor editorial changes.

Section No., Page No. in Final version 4.0	Text in Final Version 4.0	Reason for change	Text in Final Version 5.0
1.4, page 13	A fourth open label phase I study is presently investigating H56:IC31 in participants currently undergoing TB treatment in Norway. In one of the study groups, 19 adults who had received TB treatment for 3 months at the time of the first dose, were vaccinated with 2 doses of H56:IC31 with 56 days interval.	The trial has been completed since v4.0 and preliminary data has been released.	A fourth open label phase I study investigated H56:IC31 in participants undergoing TB treatment in Norway. In one of the study groups, 19 adults who had received TB treatment for 3 months at the time of the first dose, were vaccinated with 2 doses of H56:IC31 with 56 days interval. Preliminary clinical data indicates acceptable safety profile and immunogenicity was observed when H56:IC31 is administered during ongoing TB treatment
2.2, page 19	None	The COVID-19 pandemic has significantly affected enrollment and randomization and its ability to recruit and fully enroll participants in time before the current batch of IP expires. To allow us to fully complete enrollment this amendment allows the sites to recruit for an additional 5 months by combining V1 and V2 screening visits. Without combining these visits enrollment will have to be stopped in August 2021 to accommodate the 5-month period between visits 1 and 2. Continuing enrollment until Jan 2022 will allow us to fully recruit the study to accrue sufficient endpoints as per the sample size calculations to support the primary analysis.	Upon written approval from the sponsors and relevant approvals for protocol amendment v5.0,the activities scheduled for the 1st screening visit (V1) will be performed on the same day as (or together with) the 2nd screening visit (V2). At a combined screening visit (V1 and V2) the participant must have completed at least 5 months (22 weeks) of TB treatment and while TB treatment must be ongoing.
2.2, page 22 (footnote to Figure 2-1)	None	Same as reason for changes to section 2.2, page 19	Upon written approval of the Sponsors and relevant approvals for the Protocol Amendment v5.0, participants may be

Section No., Page No. in Final version 4.0	Text in Final Version 4.0	Reason for change	Text in Final Version 5.0
			screened at a combined V1 and V2. This visit will be scheduled when the participant has completed at least 5 months (22 weeks) of TB treatment and TB treatment must be ongoing.
3.1, page 23	None	Same as reason for changes to section 2.2, page 19	Upon written approval from the sponsors and relevant approvals for protocol amendment v5.0, the activities scheduled for the 1st screening visit (V1) will be performed on the same day as (or together with) the 2nd screening visit (V2). At this combined screening visit (V1 and V2) the participant must have completed at least 5 months (22 weeks) of TB treatment and TB treatment must be ongoing. Activities scheduled for both V1 and V2 do not need to be repeated.
3.1, Table 3-1, page 25	None	Same as reason for changes to section 2.2, page 19	Bullet 1: Note: For participants with a combined screening visit (V1 and V2), V1 activities will be performed on the same day as V2. This combined screening visit should occur when the participant has completed at least 5 months (22 weeks) of TB treatment and TB treatment must be ongoing. Bullet 2: Note: For participants with a combined screening visit (V1 and V2), V1 activities will be performed on the same day as V2. Therefore, sputum samples will be collected only at V2.
3.1, Table 3-1, page 25	None	New text added to further clarify the trial's intention to perform culture on sputum taken at V8 if the participants are MTB negative by Xpert MTB/RIF ultra but have clinical TB signs or symptom.	Bullet 10: Note: If there is clinical suspicion of TB at V8, all V8 activities will be conducted and not a STB visit since at V8 two separate sputum samples for Mtb testing will be obtained from all participants even if there is no clinical suspicion of TB. This is to ensure that no cases of recurrent TB disease are missed during the trial. One sputum sample will be tested by Xpert MTB/RIF ultra. If Mtb negative by Xpert MTB/RIF ultra but there are clinical TB signs or symptoms, verification by culture testing will be requested from the laboratory and both of the sputum samples will then be processed for culture. In this instance HIV testing and immunology sample collection should also be performed at V8.

Section No., Page No. in Final version 4.0	Text in Final Version 4.0	Reason for change	Text in Final Version 5.0
3.2.3, page 27	None	Same as reason for changes to section 2.2, page 19	Participants are not expected to provide sputum samples prior to starting TB treatment if their 1st screening visit (V1) is performed on the same day as their 2nd screening visit (V2).
3.2.5, page 28	None	Same as reason for changes to section 2.2, page 19	Participants with a combined V1 and V2 will not provide V1 sputum samples.
3.2.5, page 28	None	Same as reason for changes to section 2.2, page 19	Participants with a combined V1 and V2 may only be screened for the trial after at least 22 weeks of TB treatment and TB treatment must be ongoing.
3.2.5, page 29	None	Same as reason for changes to section 2.2, page 19	If a combined V1 and V2 is performed, participants will only provide a sputum sample once at V2. These samples serve to verify that the participant is Mtb negative and would still be stored for exploratory analysis.
3.6.1, page 32	At the final study visit (V8=Day 421) or at the ET Visit, if discontinuation is not due to verified recurrent TB, 2 separate sputum samples for *Mtb* testing will be obtained from all participants (capable of producing sputum), even if there is no clinical suspicion of TB disease, in order to ensure that no cases of recurrent TB disease are missed during the study and to detect subclinical cases in this population at high risk for recurrence of TB disease. The same case verification procedure as stipulated above in case of clinical suspicion of TB applies, except that, if *Mtb* negative by Xpert MTB/RIF Ultra and no clinical TB signs or symptoms, no further verification	Same as reason for changes to section 3.1, Table 3-1, page 25	At the final study visit (V8=Day 421) or at the ET Visit, if discontinuation is not due to verified recurrent TB, 2 separate sputum samples for *Mtb* testing will be obtained from all participants (capable of producing sputum), even if there is no clinical suspicion of TB disease, in order to ensure that no cases of recurrent TB disease are missed during the study and to detect subclinical cases in this population at high risk for recurrence of TB disease. If there is clinical suspicion of TB at V8, all V8 activities will be conducted and not a STB visit since at V8 two separate sputum samples for Mtb testing will be obtained from all participants even if there is no clinical suspicion of TB. This is to ensure that no cases of recurrent TB disease are missed during the trial. One sputum sample will be tested by Xpert MTB/RIF ultra. If Mtb negative by Xpert MTB/RIF ultra but there are clinical TB signs or symptoms, verification by culture testing will be requested from the laboratory and both of the sputum samples will then be processed for culture. In this instance HIV testing and immunology sample collection should also be performed at V8.

Section No., Page No. in Final version 4.0	Text in Final Version 4.0	Reason for change	Text in Final Version 5.0
	by culture testing is required.		
3.6.1, page 32	None	Same as reason for changes to section 2.2, page 19	Participants with a combined V1 and V2 will not provide sputum samples prior to starting TB treatment because their 1st screening visit (V1) would be performed on the same day as V2. For these participants, it will therefore not be possible to determine if their TB recurrence was due to relapse or reinfection.

13 REFERENCES

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14 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 PI should use clinical and scientific judgment in deciding whether other adverse events (i.e., events not listed here) could have an autoimmune origin.

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