CLINICAL TRIAL REPORT

A Phase 2, Double-blind, Randomised, 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

SSI trial code: A-055

ClinicalTrials.gov number: NCT03512249

Trial phase: II

Version: Final 1.0

First participant's first visit: 31-Jan-2019

Last participant's last visit: 20-Mar-2023

Author:

Birgit Thierry-Carstensen

SSI contact person:

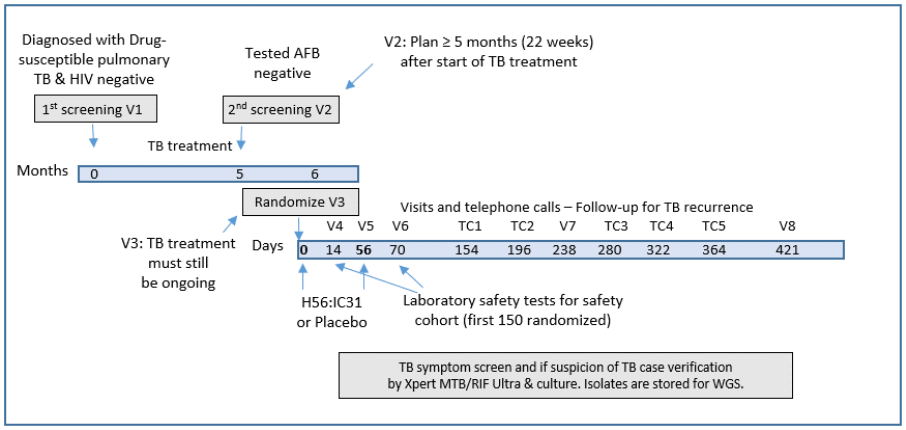
Per Skallerup
Section Manager, Vaccine Development
Statens Serum Institut
T: +45 3268 8554
Email: prsk@ssi.dk

1 Synopsis

Title	A Phase 2, Double-blind, Randomised, 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
Trial code	A-055
ClinicalTrials.gov ID	NCT03512249
Trial phase	II
Principal Investigators	Mark Hatherill (Coordinating PI) Justin Shenje (Site PI; A1) Rodney Dawson (Site PI, A2) Andreas Diacon (Site PI, A3) Raesibe Agnes Pearl Selepe (Site PI, A4) Issa Sabi (Site PI, A5) Craig Innes (Site PI, A6)
Trial sites	SATVI, Cape Town, ZA (A1) UCTLI, Cape Town, ZA (A2) Task, Cape Town, ZA (A3) Aurum Klerksdorp, ZA (A4) MMRC, Mbeya, TZ (A5) Aurum, Tembisa, ZA (A6)
Trial period	First participant's first visit: 31-Jan-2019 Last participant's last visit: 20-Mar-2023
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)
Key secondary (safety) 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

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®
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 • 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:IC31® in participants with TB recurrence diagnosis compared to the participants in the control cohort
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)
Key secondary (safety) endpoint	• 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 trial
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 (efficacy) • 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 (efficacy)

	• 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 (primary immunogenicity) • 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) (immunogenicity)
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 (efficacy) • 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 MTB/RIF Ultra or culture of sputum (efficacy) • 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 (efficacy) • Transcriptomic signatures (RNA analysis) and cellular composition (flow cytometry) of whole blood etc. at baseline (V3= Day 0) and at TB recurrence diagnosis (immunogenicity) • Blood subset counts of whole blood (supports the RNA analysis) at baseline (V3= Day 0) and at TB recurrence diagnosis (immunogenicity) • 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 (immunogenicity) • 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 (immunogenicity) This report only covers the results related to the 3 above-mentioned exploratory endpoints related to efficacy. The remaining exploratory endpoints related to immunogenicity will be reported separately.
Trial design	This was a phase 2, double-blind, randomised (1:1), placebo-controlled trial with 2 parallel groups: • H56:IC31® (test product) • Placebo (control product) 900 HIV negative adults with a diagnosis of drug-susceptible pulmonary TB were planned to be recruited and screened at 6 trial sites. If after at least 5 months (22 weeks) of TB treatment, participants were successfully treated, based on a negative AFB test result, they could be included, randomised and vaccinated. H56:IC31® or placebo was administered on Day 0 and Day 56 and the participants were followed for recurrent TB for 1 year after the 2nd dose; administered on Day 56.



The first 150 randomised participants were defined as the safety cohort, where the first 100 randomised participants at 2 specific sites (SATVI and MMRC) were defined as the immunogenicity cohort. Safety cohort participants had extra safety laboratory testing at V4= Day 14 and V6= Day 70. Immunogenicity cohort participants had extra immunogenicity samples taken.

From V3 and onwards, there was TB symptoms screening at all visits and at 5 telephone calls (TCs), and a STB visit was held, if there was suspicion of recurrent TB. If recurrent TB was not verified, the participant stayed in the trial.

There were short-term follow-up visits after each vaccination on V4= Day 14 and V6= Day 70, where diary cards were reviewed and AEs and concomitant medications (CMs) were assessed. In the diary cards, participants had recorded measurements of redness and swelling at the injection sites and answered solicited questions on AEs and CMs.

An internal data review committee (IDRC) assessed blinded safety data at 2 IDRC meetings held during the trial when:

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

The trial continued while the IDRC processes were ongoing. The DSMB that was established, was only to convene, if the IDRC recommended this, or a protocol defined pausing rule was triggered.

Trial population and main inclusion and exclusion criteria	900 HIV negative adults of 18 to 60 years of age diagnosed with drug-susceptible pulmonary tuberculosis were planned to be included after completion of at least 5 months (22 weeks) of treatment and negative AFB test. <u>Main inclusion criteria:</u> • HIV negative (self-reported) with a diagnosis of drug-susceptible pulmonary TB at the start of the TB treatment • Able to provide 2 separate sputum samples within ≤ 7 days of starting TB treatment. Participants were not expected to provide sputum samples prior to starting TB treatment if their 1st screening visit (V1) was performed on the same day as their 2nd screening visit (V2) • Tested *Mtb* negative by smear AFB microscopy of 2 separate sputum samples taken at V2 and was confirmed HIV negative at V2

	• 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 • Aged ≥ 18 years at V1 and ≤ 60 years at V3 <u>Main exclusion criteria:</u> • Diagnosis or co-diagnosis of extra pulmonary TB or hospitalised for the current episode of drug-susceptible pulmonary TB disease • History of receipt of treatment against active TB, prior to the current treatment episode, within the last 5 years • Insulin dependent diabetes • History of immunodeficiency, autoimmune disease, immunosuppression or chronic hepatitis • Severe anaemia, defined as haemoglobin less than 10 g/dL or a haematocrit less than 30% based on most recent haematology obtained before randomisation • 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
Planned/actual number of participants	900 HIV negative adults of 18 to 60 years of age diagnosed with drug-susceptible pulmonary tuberculosis were planned to be included after completion of at least 5 months (22 weeks) of treatment and negative AFB test. The randomisation was stopped, due to the expiry date of the IP, when 831 (of the planned 900) participants had been randomised.
Investigational products	The H56 fusion protein was formulated with IC31® in a GMP compliant environment in a ready to use final formulated vaccine. H56:IC31® was formulated with 5 mcg H56 adjuvanted with IC31® consisting of 500 nmol $KLKL_5KLK$ and 20 nmol ODN1a in 0.5 mL. Placebo was sterile saline (0.9%).
Dosages and route of administration	A total volume of 0.5 mL (H56:IC31® or placebo) was injected intramuscularly (i.m.) in the deltoid muscle using standard aseptic technique.
Statistical methods	The sample size for this trial was 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 trial Day 70. 3.Drop-out/loss to follow-up rate: 10%. 4.Vaccine efficacy (VE): 60%. 5.Type I error rate: 20% (2-sided). The trial was designed for the primary endpoint to be evaluated using a one-side α-level of 10%, therefore a one-sided log-rank p-value and 80% vaccine efficacy (VE) profile confidence limits were presented for the primary endpoint. Two-sided p-values and 95% VE profile confidence intervals were presented as well.

<table>
<tr><td></td><td>The primary analysis set for efficacy was the mITT analysis set. The primary efficacy endpoint was in addition analysed using the ITT and PP analysis sets. The analysis specified for the ITT and PP were regarded as supportive evidence.
Time to primary endpoint (TB recurrence) was estimated using the Kaplan-Meier method and compared between treatment groups stratified by trial site. The stratified log-rank statistic was used to test the null hypothesis of no difference in the rates of primary endpoint (TB recurrence) over the follow-up period in the H56:IC31® compared to the placebo group.</td></tr>
<tr><td>Efficacy results</td><td>In the primary efficacy analysis, the frequencies of participants with recurrent TB in the two groups; 5.8% (23/400) in the H56:IC31® group and 3.4% (14/406) in the placebo group, resulted in a one-sided log-rank p-value of 0.9558 (above 0.10) for a lower frequency of recurrent TB in the H56:IC31® group than in the placebo group.
<table>
<tr><th></th><th>H56:IC31</th><th>Placebo</th><th>P-value</th></tr>
<tr><td>mITT analysis set (N)</td><td>414</td><td>413</td><td></td></tr>
<tr><td>TB recurrence</td><td></td><td></td><td></td></tr>
<tr><td>Number of participants contributing to analysis</td><td>400 (100.0)</td><td>406 (100.0)</td><td></td></tr>
<tr><td>TB recurrence present</td><td>23 (5.8)</td><td>14 (3.4)</td><td></td></tr>
<tr><td>Censored without TB recurrence</td><td>377 (94.3)</td><td>392 (96.6)</td><td></td></tr>
<tr><td>Kaplan-Meier estimates</td><td></td><td></td><td></td></tr>
<tr><td>One-sided log-rank test*</td><td></td><td></td><td>0.9558</td></tr>
</table>
The overall conclusion of the primary efficacy analysis was thus that H56:IC31® did not lead to a statistically significant reduction in the rate of TB recurrence in comparison to placebo.
The supportive efficacy analysis (two-sided log-rank test) in the mITT analysis set from Day 70 supported the results of the primary efficacy analysis, as did the corresponding efficacy analysis from Day 70 in the PP analysis set.
Supplementary efficacy analyses were performed in different mITT subsets of participants to investigate the impact on the results of the primary efficacy analysis. The subgroups were: 1) participants who received 1 or 2 vaccinations, 2) exclusion of participants if 2nd injection outside ± 10 days window and 3) exclusion of participants with recurrent TB, if they were cultured in error. All performed supplementary efficacy analyses in these mITT subsets supported the results of the primary and supportive efficacy analysis in the mITT.
The overall conclusion of the secondary efficacy analyses of recurrent TB relapse and reinfection in the mITT analysis set from Day 70 was that H56:IC31® did not reduce the rate of TB relapse and TB reinfection in comparison to placebo. It was, moreover, noted, that the excess cases of recurrent TB in the H56:IC31® group seemed to be driven by TB relapse rather than by TB reinfection.
The results of the three exploratory efficacy analysis confirmed the results of the primary and secondary efficacy analysis.
From the forest plots on different subgroups an increased hazard for TB recurrence was observed in participants older than 35 years, without anaemia, and receiving both IP injections, but interaction tests were not significant.</td></tr>
<tr><td>Immunogenicity results</td><td>H56:IC31® vaccination induced significant increases in frequencies of antigen specific CD4+ T cells expressing any combination of IL-2, IFN-γ, TNF, IL-17, CD4+ T cells co-expressing IL-2 and TNF and polyfunctional IL-2+ IFN-γ+ TNF+ CD4+ T cells, measured at Day 70, relative to pre-vaccination. No changes in these responses were observed in placebo recipients.</td></tr>
</table>

	The fold change in H56-specific CD4+ T cell responses, computed between Day 70 and Day 0 was significantly higher in H56:IC31® recipients than in placebo recipients. Frequencies of antigen specific CD8+ T cells were not significantly modulated by vaccination. Participants receiving H56:IC31® mounted robust H56-specific humoral (serum IgG) responses.
Safety results	Overall, 72.3% of the participants in the H56:IC31® group experienced 1382 adverse events (AEs) and 64.2% in the placebo group reported 1033 AEs. Among the H56:IC31® vaccinees 3.6% had a severe AEs, 20.2% had a moderate AE and 67.0% had AEs of mild intensity only. 28 serious adverse events (SAEs), were reported, of these 16 SAEs were reported by 14 participants in the H56:IC31® group and 12 SAEs by 12 participants in the placebo group. No related SAEs and no SUSARs were reported. 2 adverse events of special interest (AESIs) were reported (by 2 participants in the placebo group). In the H56:IC31® group, the most frequently reported AEs were: injection site pain (34.0%), fatigue (27.0%), headache (24.1%), myalgia (21.9%), injection site erythema (18.6%), injection site swelling (17.6%), arthralgia (16.4%), nausea (12.8%) and pyrexia (6.3%). 39.8% of the H56:IC31® vaccinees reported an injection site reaction. The majority of the reported injection site reactions were of mild intensity (> 95%). 2.7% of the H56:IC31® vaccinees had a moderate injection site reaction. No injection site reactions of severe intensity were reported. There were no indications of higher or lower frequencies of injection site reactions after the 2nd injection than after the 1st injection. In the H56:IC31® group, the highest measured redness diameter was 30 mm and the highest measured swelling diameter was 40 mm. In the H56:IC31® group, 1 participant experienced related severe pyrexia for 4 days with onset 4 days after the 2nd vaccination (with no record of maximum temperature). 7 participants experienced 11 events of related moderate pyrexia for between 1 and 3 days with onset between 1 and 7 days after the 1st and/or 2nd vaccination. The recorded maximum temperatures were between 37.5 °C and 41.1 °C. The outcome of all pyrexia reactions was 'recovered / resolved'. No notable between group differences and no changes from V2 through V4 or V6 were noted for any of the biochemistry and haematology laboratory safety tests or vital signs measurements.
Overall conclusions	The overall conclusion of the primary efficacy analysis in the mITT analysis set from Day 70 was that H56:IC31® did not lead to a statistically significant reduction in the rate of TB recurrence in comparison to placebo. The supportive efficacy analysis (also in the mITT analysis set from Day 70) supported the results of the primary efficacy analysis, as did the corresponding efficacy analysis in the PP analysis set. H56:IC31® vaccination induced significant increases in frequencies of antigen specific CD4+ T cells expressing any of the tested cytokine combinations measured at Day 70, relative to pre-vaccination. The fold change in H56-specific CD4+ T cell responses was significantly higher in H56:IC31® recipients than in placebo recipients. Frequencies of antigen specific CD8+ T cells were not significantly modulated by vaccination. Participants receiving H56:IC31® mounted robust H56-specific humoral (serum IgG) responses. Overall, the H56:IC31® vaccine is considered well-tolerated. The majority of participants experienced only mild AEs. There was a low frequency of moderate injection site reactions and no severe injection site reactions. All SAEs were assessed as unrelated to the H56:IC31® vaccine.

2 Table of contents

3 List of in-text tables and figures

Tables:

Figures:

4 List of abbreviations and definition of terms

AE	adverse event
AESI	adverse event of special interest
Aeras	Aeras Global TB Vaccine Foundation NPC – a non-profit organisation dedicated to developing TB vaccines
AFB	acid fast bacilli on sputum smear microscopy (the Auramine technique) – a tuberculosis diagnostic test
ALP	alkaline phosphatase
ALT	alanine aminotransferase
AST	aspartate aminotransferase
ATC1 – ATC4	Anatomical Therapeutic Chemical Classification Codes 1- 4
Aurum	Aurum Institute, Johannesburg (Head office), South Africa
ßHCG	beta human chorionic gonadotropin
BARC	Bio Analytical Research Corporation
BCG	bacillus Calmette-Guérin
BMI	body mass index
CA	competent authority
CD4+ T cells	CD4-positive helper cells
CD8+ T cells	CD8-positive helper cells
CI	confidence interval
CM	concomitant medication
COVID-19	Coronavirus disease of 2019
CPK	creatinine phosphokinase
CRF	case report form
CRO	contract research organisation
CTM	clinical trial manager
CTR	clinical trial report
DBL	data base lock
DK	Denmark
DM	data management
DNA	deoxyribonucleic acid
DSMB	data safety monitoring board
eCRF	electronic case report form
EC	ethics committee
EDCTP	The European & Developing Countries Clinical Trial Partnership
ELISA	enzyme linked immunosorbent assay
EMB	ethambutol
EOT	end of trial
ESR	erythrocyte sedimentation rate
ET Visit	early termination visit
FV	final version

GA	general assembly
GCP	good clinical practice
GGT	gamma glutamyl transferase
GMP	good manufacturing practice
H56	H56 antigen which is a tuberculosis vaccine candidate consisting of a fusion protein of the 3 antigens; Ag85B, ESAT-6, and Rv2660c.
HIV	human immunodeficiency virus
HLGT	high level group term
HLT	high level term
IAVI	International AIDS Vaccine Initiative®, South Africa
IB	investigator's brochure
IC31®	2-component adjuvant comprised of the polypeptide $KLKL_5KLK$ and the oligodeoxynucleotide ODN1a, in a constant 25:1 molar ratio ($KLKL_5KLK$ to ODN1a). The dose when used with the H56 antigen is 500 nmol $KLKL_5KLK$ to 20 nmol ODN1a. For ease of presentation this is expressed as 500 nmol IC31® in this report.
ICF	informed consent form
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
ICS	intracellular cytokine staining
ID	identity
IDRC	internal data review committee
IgG	immunoglobulin G
IGRA	interferon gamma release assay
IEC	independent ethics committee
IFN-γ	interferon gamma
IL	interleukin
INH	isoniazid
I(M)P	investigational (medicinal) product
IT	Italy
ITT	intention to treat
KM	Kaplan-Meier
LLT	low level term
MDR-TB	multidrug (rifampicin and isoniazid) resistant tuberculosis
MDR/RR-TB	multidrug resistant or rifampicin resistant tuberculosis
MH	medical history
MAIT cells	mucosal associated invariant T cells
mITT	modified intention to treat
MMRC	Mbeya Medical Research Centre, Mbeya, Tanzania
Mtb	*Mycobacterium tuberculosis*
NA	not available or not applicable

NIMR	National Institute for Medical Research, Tanzania
NK	natural killer
NO	Norway
OSR	Emerging Bacterial Pathogens Unit, Ospedale San Raffaele, Italy
OOW	out of window
PBMC	peripheral blood mononuclear cell
PCL	profile confidence limit
PCIDA	preclinical and clinical immunoassay development and analysis
PD	protocol deviation
PI	principal investigator
POR	prevention of recurrence
PP	per protocol
PT	preferred term
PZA	pyrazinamide
QA	quality assurance
RIF	rifampicin
RNA	ribonucleic acid
RR-TB	rifampicin resistant tuberculosis
SAHPRA	South African Health Products Regulatory Authority
SAE	serious adverse event
SAP	statistical analysis plan
SAS	statistical analysis systems
SATVI	South African Tuberculosis Vaccine Initiative, University of Cape Town, South Africa
SD	standard deviation
SDTM	study data tabulation model
SDV	source data verification
SNP	single nucleotide polymorphisms
SOC	system organ class
SOP	standard operating procedure
SSI	Statens Serum Institut, Denmark
STB Visit	suspected TB visit
SUSAR	suspected unexpected serious adverse reaction
TASK	Task Applied Science, Cape Town, South Africa
TB	tuberculosis
TC	telephone calls
TEAE	treatment emergent adverse event
TMF	trial master file
TNF	tumour necrosis factor
TZ	Tanzania
UCTLI	University of Cape Town Lung Institute, South Africa

UK	United Kingdom
V1-V8	Visit 1–Visit 8
VE	vaccine efficacy
WB	whole blood
WB ICS	whole blood intracellular cytokine staining
WGS	whole genome sequencing
WP	work package
WHO	World Health Organisation
Xpert MTB/RIF Ultra	cartridge based nucleic acid amplification test, automated diagnostic test that can identify *Mycobacterium tuberculosis* (MTB) DNA and resistance to rifampicin (RIF)
ZA	South Africa

5 Ethics

5.1 Independent ethics committee and competent authority approvals

All trial related documents, including the protocol, protocol amendments and informed consent forms were reviewed and approved (as applicable) by the relevant independent ethics committee (IEC) of each participating clinical trial site and by the competent authorities (CAs) in South Africa (ZA) and Tanzania (TZ), prior to any protocol-specified procedures being conducted.

Please refer to Appendix 16.1.3 for a list of IEC and CA approvals.

5.2 Ethical conduct of the trial

The trial was conducted according to the ethical principles set forth in the Declaration of Helsinki, ICH-GCP, South African GCPs and local ethical and regulatory requirements, in ZA and TZ, as applicable.

5.3 Participant information and consent

The participants were recruited from tuberculosis (TB) clinics with established relationships to the trial sites. Informed consent was documented in writing on a version-controlled consent form approved by the IEC. The informed consent process was conducted in a private space to maintain confidentiality. The consent process was conducted in the participant's language of choice. All relevant information was provided in both oral and written form in a way that was understandable to the participant. Ample time and opportunity were given for the participant to inquire about details of the trial. The potential participant was encouraged to take the informed consent form home to discuss with family and friends before deciding whether or not to participate in the trial.

The investigator or the investigator's qualified designee explained the nature of the trial and informed the participant that participation was voluntary and that the participant could leave the trial at any time, without penalty or loss of benefits to which they were otherwise entitled. The participant was informed about the trial's purpose including why the participant was selected to participate, trial goals, expected benefits and risks, potential risks and that some potential risks were unforeseeable. The participant was provided with a description of the procedures and the estimated duration of time required for participation in the trial, as well as alternative interventions or courses of treatment, if applicable.

The participant received an explanation as to whether any compensation and any medical treatments were available if injury occurred and, if so, what they were, where further information might be obtained and who to contact in the event of a trial related injury. Participants were told who to contact for answers to any questions related to the trial. The extent of the confidentiality of participant's records was defined and the participant was informed that applicable data protection legislation applied.

The investigator or medically qualified personnel determined a participant's understanding of the key trial concepts (e.g., procedures, risks) prior to signing the consent form and conducted the final consenting.

In this trial, the participant information sheet and informed consent form (ICF) was written as one document and is referred to as the ICF. Modifications made by the investigator to an ICF template provided to the investigator by sponsor was reviewed and approved by the sponsor prior to being submitted to the IEC.

The signed original ICFs for each participant were maintained by the investigator as part of the participant's trial records. A copy of their signed ICFs was offered to each participant.

Two English master 'main ICF' documents were prepared, one master for ZA and one master for TZ. In addition, two English master 'sample storage ICFs', addressed the participant's consent to storage of samples for future TB research for ZA and TZ.

During the trial, the ICFs were updated, as a result of trial changes or updates that affected their contents. This occurred once for both types of ICFs, please see Table 1. All master ICFs are available in the trial master file (TMF).

Table 1: Master versions 'main' and 'sample storage' ICFs

Country	Master main ICF	Master sample storage ICF
ZA		
Initial	Version 1.0 25-Apr-2018	Version 1.0 25-Apr-2018
Updated	Version 2.0 28 May 2021	Version 2.0 09-Jul-2021
TZ		
Initial	Version 1.0 22 Jun 2018	Version 1.0 22-Jun-2018
Updated	Version 2.0 04-Jun-2021	Version 2.0 09-Jul-2021

Locally adapted ICFs, as per local IEC requirements, were prepared, based on the master versions. For each site, the ICFs were, furthermore, translated into local languages, as per local requirements.

For site A3 (TASK), an additional (site specific) HIV ICF (FV 1.0 29-May-2018), related to HIV testing was completed. All locally adapted ICF types, versions and translations are available in the TMF in English and in the relevant local languages.

6 Investigators and trial administrative structure

6.1 The prevention of recurrence of TB consortium

This clinical trial was one of the objectives of the prevention of recurrence (POR) of TB consortium, established and funded by The European & Developing Countries Clinical Trial Partnership (EDCTP).

The EDCTP decided in November 2017 to fund a project where the primary objective was to unite African and European research institutions, clinical trial sites and vaccine developers in a consortium to conduct an innovative and cost-effective TB vaccine trial. The EDCTP grant covered activities split into 5 work packages (WP) where the A-055 clinical trial is WP2.

The POR TB consortium comprised 8 partners; 6 partners from Sub-Saharan Africa and 2 from Europe.

The 6 African partners were:

- IAVI, Cape Town, ZA (IAVI, previously Aeras)
- South African Tuberculosis Vaccine Initiative, University of Cape Town, ZA (SATVI)
- University of Cape Town Lung Institute, ZA (UCTLI)
- Task Applied Science, Cape Town (head quarter), ZA (TASK)
- Aurum Institute, Johannesburg (head quarter), ZA (Aurum)
- Mbeya Medical Research Centre (MMRC) under the National Institute for Medical Research (NIMR), Mbeya, TZ

The 2 European partners were:

- Statens Serum Institut, Denmark (DK) (SSI)
- IRCCS San Raffaele Scientific Institute, Ospedale San Raffaele, Italy (IT) (OSR)

The above partners entered into a POR TB consortium agreement and each had one representative in the POR TB Consortium General Assembly (GA). SSI had a coordinating role and the SSI representative in the GA was the leader of the GA. The clinical trial was co-sponsored by IAVI and SSI.

6.2 Investigator's trial organisation

The co-sponsors selected the partner trial sites, SATVI, UCTLI, TASK, Aurum and MMRC for the A-055 trial. Pre-study visits were performed to ensure the feasibility of the sites for the trial, before they were finally selected.

6 trial sites were appointed, 5 sites in ZA and 1 site in TZ. For each site there was a responsible site principal investigator (site PI) who entered into an individual clinical trial agreement with the co-sponsors (IAVI and SSI). In ZA, there was, in addition to the 5 site PIs, also a national coordinating PI (who also served as the international coordinating PI). In TZ, there was only 1 site and the site PI also served as national coordinating PI. The 6 sites are shown in Table 2.

Before trial initiation at a site, a site initiation visit was performed. At the site initiation visit, it was ensured that trial products, ancillary supplies and trial procedures were in place, including relevant documents, approvals and agreements, as well as documentation of site staff training on the protocol and relevant trial manuals and subsequent inclusion of the site staff in the site delegation log with indication of delegated tasks.

Table 2: Overview of trial sites in A-055

Site number	Country	Site name (abbreviated), Town	Site PI Full name
Site A1	ZA	SATVI, Cape Town	Justin Shenje
Site A2	ZA	UCTLI, Cape Town	Rodney Dawson
Site A3	ZA	TASK, Cape Town	Elana van Brakel replaced by Ramonde Fiona Patientia (20-Jun-2019) replaced by Andreas Diacon (14-Jan-2021)
Site A4	ZA	Aurum, Klerksdorp	Raesibe Agnes Pearl Selepe
Site A5	TZ	MMRC, Mbeya	Issa Sabi
Site A6	ZA	Aurum, Tembisa	Modulakgotla Sebe replaced by Craig Innes (13-Sep-2023)

The international coordinating PI, Mark Hatherill, SATVI, ZA had a coordinating role and was a member of the internal data review committee (IDRC). According to the IDRC charter, he arranged two IDRC meetings during the trial to review and assess available blinded safety data, to ensure the continued safety of the participants in the trial.

The site PI from each trial site had 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 had the authority to terminate, suspend, or require changes to the clinical trial for safety concerns and could delay an individual's investigational product (IP) administration. The site PI was responsible for determining the intensity, seriousness and causality with respect to the IP for each adverse event. The site PI could delegate specific tasks to a designee who was a medically qualified team member who had to be a sub-investigator if assessing adverse events (AEs).

The site PI was responsible for all unused and used vials being accounted for during the trial and documented in the relevant trial documents/logs. The trained IP manager was delegated to maintain accurate IP accountability records. Upon receipt of the IP, the IP manager immediately inspected all vials for damage.

Furthermore, the site PI was responsible for the clinical assessment of laboratory tests, performed according to the protocol, at her/his site. These assessments could be delegated to other investigators, as well as the responsibility of keeping the sponsor's medically responsible informed about abnormal laboratory results that were vaccine-related AEs of medical importance.

The site PI delegated the laboratory procedures in the protocol to trained laboratory staff at the site. These staff members worked according to laboratory manuals provided by the sponsor.

For full addresses of the sites and the contact details of the site PIs and the international coordinating PI, please refer to Appendix 16.1.4. Curriculum vitae for all site PIs as well as sub-investigators are available in the TMF.

6.3 Sponsor's trial organisation

For this trial, 2 sponsors, IAVI and SSI, shared the sponsor responsibility as per a co-sponsor agreement. Each co-sponsor had a medically responsible person as well as one or more clinical trial managers (CTM). The medically responsible persons and the CTMs at IAVI and SSI were blinded during the trial.

IAVI was responsible for overall trial management, quality management, pharmacovigilance, IDRC and data safety monitoring board (DSMB) tasks, regulatory affairs, laboratory set-up and TMF, where SSI was responsible for the provision of the IP (H56:IC31®) including H56:IC31® manufacturing according to GMP and shipments of IP to the clinical trial sites in Africa, data management (DM), statistics, central monitoring and medical writing of the protocol and clinical trial report (CTR). SSI was also responsible for site monitoring (blinded as well as unblinded) until 31 May 2021, where this responsibility was transferred to IAVI.

The medically responsible persons were responsible for the sponsor's serious adverse event (SAE) causality assessments, as well as SAE expectedness assessments, to determine if a SAE was a SUSAR, being eligible for expedited reporting.

Moreover, the medically responsible persons chaired the 2 IDRC blinded safety data meetings held during the trial, including approval of the meeting conclusions and recommendations regarding the continuation of the trial, or pausing IP administration and enrolment and convening of the DSMB.

The medically responsible persons were to ensure oversight of the protocol defined rules that triggered the pausing of the trial and convening of the DSMB and based on DSMB recommendations, to finally decide whether IP administration and enrolment could be resumed or the trial should be terminated.

The DSMB was only to convene in case this was recommended by the IDRC, or in case a protocol defined pausing rule was triggered. No DSMB meetings were held during the trial. For further details on IDRC and DSMB procedures, please refer to the protocol, Appendix 16.1.1. The IDRC and DSMB charters are available in the TMF.

The CTMs from each co-sponsor divided their responsibility of managing and maintaining oversight of each of their well-defined areas and vendors in the trial. For further details on the sponsor responsibility split between IAVI and SSI, please refer to Appendix 16.1.4.

6.4 Contract research organisations and vendors in trial organisation

Certain functions and the associated tasks were outsourced to contract research organisations (CROs) or other vendors. For an overview of the CROs and vendors used in the trial, please see Table 3.

Table 3: Overview of CROs and vendors used in the trial

CRO or vendor name, Town and country	Activity / Role in trial	Period in trial where service was provided
Covance Inc., Centurion, ZA	Site monitoring (blinded and unblinded)	Until 31-May-2021
IQVIA Ltd., Centurion, ZA	Site monitoring (blinded and unblinded)	01-Jun-2021 until end of trial
Biostata Aps, Birkerød, DK	Data management Randomisation Statistics	Entire trial period
PPD, Cambridge, UK	Pharmacovigilance	Entire trial period
TRI, Cambridge, UK	Provider of IT system for risk management in the trial	Entire trial period
Veeva Vault, Pleasanton, USA	eTMF system	Entire trial period
Indigo language solutions, Stellenbosch, ZA	Translations into local languages	Entire trial period

For full addresses and the sponsor responsible for the oversight of the different CROs and vendors in the trial, please refer to Appendix 16.1.4.

6.5 Laboratory organisation

Laboratories included safety test and TB diagnostic laboratories as well as immunogenicity and immunology analytical laboratories. An overview of the laboratories used in the trial is shown in Table 4.

Table 4: Overview of laboratories used in the trial

Laboratory name (abbreviated)	Country	Activities / Role in trial	Sites using laboratory
BARC	ZA	1. Laboratory kit supplier for all sites 2. Safety tests and TB diagnostic laboratory for ZA sites 3. Immunology sample preparation for UCTLI and TASK 4. Sputum storage during the trial	1. All sites 2. All ZA sites 3. UCTLI (A2) and TASK (A3) 4. All ZA sites
NIMR, MMRC Main Laboratory	TZ	1. Safety tests and Rapid HIV tests for MMRC 2. PBMC extraction and initial cryopreservation of immunology specimens 3. WB ICS and cell subset count 4. Transfer of cryopreserved immunology specimens to the SATVI biobank	MMRC (A5)
NIMR, MMRC TB Laboratory	TZ	1. TB diagnostic tests for MMRC 2. Sputum storage for MMRC during the trial 3. Storage of all sputum samples	MMRC (A5)
Aurum, Klerksdorp Laboratory	ZA	1. Preparation of the collected screening, safety and TB samples and shipment to BARC 2. PBMC extraction and initial cryopreservation of immunology specimens 3. Transfer of cryopreserved immunology specimens to the SATVI biobank 4. Storage of V1 sputum samples	Aurum, Klerksdorp (A4)
UCTLI Laboratory	ZA	1. Preparation of the collected screening, safety and TB samples and shipment to BARC 2. Storage of V1 sputum samples	UCTLI (A2)
TASK Laboratory	ZA	1. Preparation of the collected screening, safety and TB samples and shipment to BARC 2. Storage of V1 sputum samples	TASK (A3)

Laboratory name (abbreviated)	Country	Activities / Role in trial	Sites using laboratory
SATVI Worcester Laboratory	ZA	1. Preparation of the collected screening, safety and TB samples and shipment to BARC 2. Storage of V1 sputum samples 3. PBMC extraction and initial cryopreservation of immunology specimens 4. WB ICS and cell subset count 5. Transfer of cryopreserved immunology specimens to the SATVI biobank	SATVI (A1)
Aurum, Tembisa Laboratory	ZA	1. Preparation of the collected screening, safety and TB samples and shipment to BARC 2. PBMC extraction and initial cryopreservation of immunology specimens 3. Transfer of cryopreserved immunology specimens to the SATVI biobank 4. Storage of V1 sputum samples	Aurum, Tembisa (A6)
SATVI, Cape Town Laboratory	ZA	1. WP4 and immunology leader 2. Biobanking of blood specimens 3. Immunology assays (WB ICS; PBMC Immune Correlates Analysis and leukocyte subset counts in WB)	All
Ospedale San Raffaele (OSR) Laboratory	IT	Whole genome sequencing (WGS) of sputum samples. Sputum samples and positive TB cultures were shipped to OSR for strain identification and phylogenetic analysis.	All
SSI PCIDA Laboratory	DK	Antigen specific antibody by IgG ELISA using plasma	All
Human Immunology Laboratory	UK	PBMC proficiency and external quality assessment management	All

For further details on laboratory accreditation certificates, as well as the normal ranges used in this trial (for the laboratory safety tests), please refer to Appendix 16.1.10.

7 Introduction

Tuberculosis (TB)

Mycobacterium tuberculosis (*Mtb*), the causative agent of TB kills more people than any other single pathogen and remains one of the biggest global health burdens. About a quarter of the global population is estimated to have been infected with TB (1) and is therefore at risk of developing active TB.

The Covid-19 pandemic, which occurred while this trial was ongoing, has negatively affected the global TB burden (2). The incidence rates of TB increased by 3.6% in 2021 relative to 2020, suggesting a reversal of the trend of nearly 2% decrease per year during the past two decades. The burden of drug-resistant TB also increased by 3% between 2020 and 2021 (2).

In 2022, 7.5 million people were newly diagnosed with active TB which caused an estimated 1.3 million deaths (1). 30 high TB burden countries accounted for 87% of all incident cases worldwide in 2022 (1).

In 2019, when this trial started, South Africa reported an incidence of 615 cases per 100,000 (the second highest reported incidence rate in a single country) and Tanzania 237 cases per 100,000 (3).

Recurrence of TB disease following successful treatment is a significant issue, with rates of recurrence varying between 2% and 8% (4-7), as a result of either relapse, due to the same *Mtb* strain that the patient was initially treated for, or reinfection due to a new infection with a different strain. The majority of recurrences occur within the first year following the end of treatment (4-7).

Furthermore, there is growing bacterial resistance to available TB drugs, which means the disease is becoming more difficult to treat. In 2022, the estimated proportion of people with TB who had rifampicin and isoniazid resistant TB (MDR-TB) or rifampicin resistant TB (RR-TB), i.e. MDR/RR-TB, was 3.3% among new cases and 17% among those previously treated for TB (1). In absolute numbers, there were an estimated 410.000 incident cases of MDR/RR-TB in 2022 (1).

The World Health Organisation (WHO) has set the goal of ending the TB epidemic by 2035 (8). However, the current tools for controlling TB are clearly insufficient and as the WHO has noted; without new drugs, diagnostics and vaccines these targets will not be met (8).

TB vaccines

The availability of an efficacious TB vaccine for the prevention of recurrent TB will be a significant advance in the control of TB disease. Since adults are the main transmitters of TB, a vaccine that is efficacious in preventing TB disease in adults will have the biggest and most immediate impact on the current TB epidemic (9).

As TB vaccines target antigens, they are not implicated in the generation of resistance and thus 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 will in

addition potentially decrease the need for antibiotics through prevention of TB disease and hence transmission.

Clinical experience with H56:IC31® vaccine

The vaccine H56:IC31® had previously shown compelling safety and immunogenicity data (10-13). H56:IC31® was a particularly suitable candidate for testing in a POR trial because it induced robust CD4+ T cell responses for the antigens Ag85B and ESAT-6, with ESAT-6 being immunodominant, which persisted through 6 months after the last vaccination in adults recently treated for tuberculosis (14).

H56:IC31® has been developed by SSI and was the first multistage TB subunit vaccine candidate to enter clinical trials. H56:IC31® was specifically designed to mediate protection both as a preventive and therapeutic vaccine, making it the ideal investigational vaccine for the present POR TB clinical trial (A-055).

A total of 5 clinical trials with H56:IC31® have been completed to date, 4 in ZA and 1 in NO. In these trials; C-032 (10), C-035 (11), C-037 (14), A-042 (12), and TBCOX (13), a total of 168 participants received H56:IC31®. Antigen doses ranged from 5-50 mcg H56. In all trials, the adjuvant dose was kept constant at 500 nmol IC31®. The H56:IC31® vaccine was administered as intramuscular (i.m.) injections in a 2-dose regimen in all trials, except for C-035, where a 3-dose regimen was also investigated.

The first phase 1 clinical trial, C-032 (10), was a phase 1, first-in-human, open-label, dose-escalation trial of H56:IC31® in 25 healthy adults in ZA; 8 IGRA negative (50 mcg H56), followed by 8 IGRA positive (15 mcg H56) and 9 IGRA positive (50 mcg H56). Overall, H56:IC31® was associated with an acceptable safety profile and was immunogenic, in both IGRA negative and IGRA positive participants (10).

The second clinical trial, C-035 (11), was a phase 1/2a, double-blind, randomised, placebo-controlled, dose-finding trial in 98 healthy (IGRA negative or IGRA positive) adults in ZA, receiving H56:IC31® (81 participants) and placebo (17 participants). This trial had two phases: an initial dose-finding phase and a subsequent fixed-dose phase. H56 doses ranged from 5-50 mcg. The dose level of H56 was selected to be 5 mcg based on the results of this trial and in all subsequent trials only 5 mcg H56 in a 2-dose regimen with 2 months (56 days) interval has been investigated.

In the next phase 1, double-blind, randomised, placebo-controlled, trial, C-037 (14), H56:IC31® was for the first time investigated in HIV negative adults successfully treated for drug-susceptible pulmonary TB (16 received H56:IC31®; 6 received placebo) and H56:IC31® was found to be safe and immunogenic in this population.

Two more phase 1 trials investigated H56:IC31®. In the phase 1b, a partially blinded, randomised, placebo-controlled trial, A-042 (12), 84 healthy, BCG vaccinated adolescents in ZA received; H4:IC31® (24), H56:IC31® (24), BCG (24) or placebo (12). H56:IC31® was safe and immunogenic when administered in this population of BCG vaccinated adolescents. In the investigator-initiated, phase 1/2, open, randomised TBCOX trial in Norway (13), participants receiving ongoing TB treatment were vaccinated with H56:IC31®. Based on the safety results, including 22 H56:IC31® vaccinated participants, it was concluded that therapeutic vaccination with H56:IC31® was safe and immunogenic during TB treatment (13).

In the completed 5 clinical trials, H56:IC31® was associated with an acceptable safety profile in healthy adults (IGRA negative and IGRA positive) and adolescents, including adults successfully treated for drug-susceptible pulmonary TB. No vaccine-related SAEs have been reported to date. Vaccination with H56:IC31® in adults was most commonly (≥10% of participants with a related AE) associated with mild to moderate injection site reactions of pain, warmth and swelling and mild to moderate systemic AEs of fatigue, myalgia and nausea.

The present A-055 clinical trial was the first efficacy trial with H56:IC31®. As demonstration of protective efficacy against TB disease in the general population would require large (many thousands of participants) and lengthy field trials, shorter and smaller proof-of-concept phase 2 trials, investigating subpopulations 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 (4-6, 15, 16).

The A-055 trial investigated the efficacy (of vaccination) for prevention of recurrent (POR) TB disease in successfully treated TB patients. Recurrent TB disease is ~4-fold more frequent than incident TB disease in the general population (4-6, 15, 16). Therefore, it was expected that the trial could be completed rapidly and cost-effectively with a smaller sample size than a proof-of-concept trial investigating the efficacy of H56:IC31® against TB disease in the general population.

8 Trial objectives and endpoints

8.1 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)

8.2 Secondary objectives

8.2.1 Key secondary (safety) 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

8.2.2 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®

8.3 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
- 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:IC31® in participants with TB recurrence diagnosis compared to the participants in the control cohort

8.4 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).

8.5 Secondary endpoints

8.5.1 Key secondary (safety) 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 trial

8.5.2 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 (efficacy)

- 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 (efficacy)

- 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 (primary immunogenicity)

- 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) (immunogenicity)

8.6 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 (efficacy)

- 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 MTB/RIF Ultra or culture of sputum (efficacy)

- 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 (efficacy)

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

This report only covers the results related to the first 3 above-mentioned exploratory endpoints related to efficacy. The remaining exploratory endpoints related to immunogenicity will be reported separately.

9 Investigational plan

9.1 Overall trial design and plan

This was a phase 2, double-blind, randomised (1:1), placebo-controlled trial with 2 parallel groups:

- H56:IC31® (test product)
- Placebo (control product)

900 HIV negative adults with a diagnosis of drug-susceptible pulmonary TB were planned to be recruited and screened from TB clinics with established relationships to the 6 trial sites. If after at least 5 months (22 weeks) of TB treatment, participants were successfully treated, based on a negative AFB test result, they could be included, randomised and vaccinated. H56:IC31® or placebo was administered on Day 0 and Day 56 and the participants were followed for recurrent TB for 1 year after the 2nd dose. Additional details on TB case finding are provided in Section 9.5.1.1. For the trial flow diagram, please see Figure 1.

Figure 1: Trial flow diagram

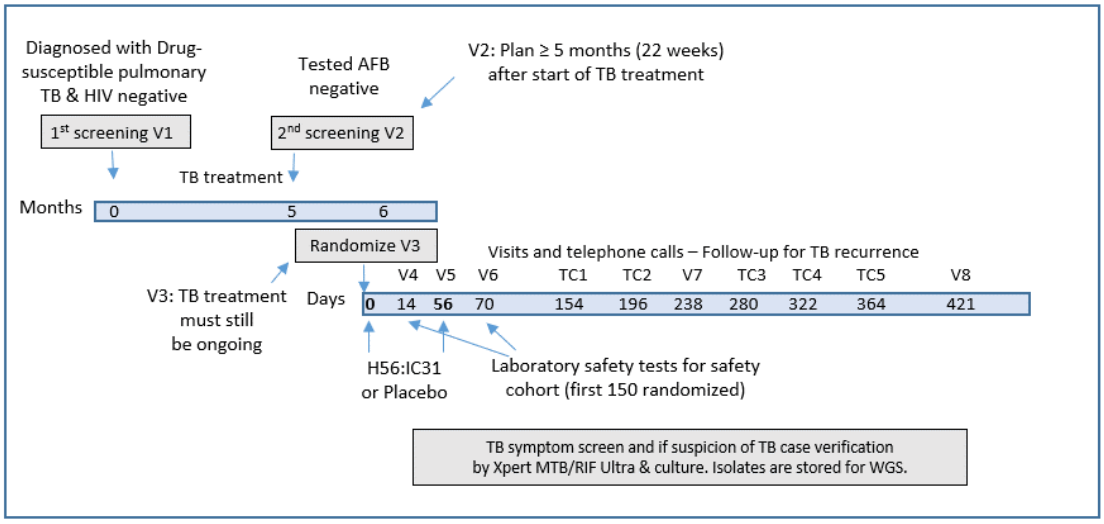


Figure 1 clarification note: From approval and implementation of protocol FV 5.0, participants could be screened at a combined V1 and V2 which was to be scheduled when the participant had completed 5 months (22 weeks) of TB treatment and the TB treatment was still ongoing.

The first 150 randomised participants were defined as the safety cohort, where the first 100 randomised participants at 2 specific sites (SATVI and MMRC) were defined as the immunogenicity cohort, please see Table 5.

Table 5: Overview of treatment groups with safety and immunogenicity cohort subgroups

Number of doses 2 x 0.5 mL (Days 0, 56) intramuscularly in deltoid muscle	**Treatment assignment N (planned)**		**Total N (planned)**
	H56:IC31® 5 mcg H56 500 nmol IC31®	**Placebo**	
All randomised participants in trial across all sites	450	450	900
First 150 randomised participants across all sites Safety cohort (subgroup)	75	75	150
First 100 randomised participants SATVI & MMRC sites only Immunogenicity cohort (subgroup and control cohort)	50	50	100

The summary schedule of investigational events is shown in Table 6.

After obtaining signed ICF at the 1st screening visit (V1), the TB diagnosis criteria and other in-/exclusion criteria (applicable for V1) were assessed. The V1 was to be conducted ≤ 7 days after the start date of the TB treatment and the 2nd screening visit (V2) ≥ 5 months (22 weeks) after the start date of the TB treatment. At V2, HIV, acid fast bacilli (AFB) and safety laboratory tests were performed. For further details, please see Section 9.3.

The day of randomisation and 1st vaccination for each participant was V3= Day 0, where TB treatment should still be ongoing. At V3 all in-/exclusion criteria were verified, including verification of negative HIV and AFB tests. If eligible for inclusion, participants were randomised to the trial based on a randomly-generated sequence of numbers (randomisation list) managed by a validated module in the eCRF. After verification of IP administration criteria, the 1st vaccination was administered and AEs were assessed.

From V3 and onwards, there was TB symptoms screening at all visits and at 5 telephone calls (TCs), and a STB visit was held, if there was suspicion of recurrent TB. If recurrent TB was not verified, the participant stayed in the trial. In this case, if triggered by a TB symptom screen later in the trial, another STB visit was conducted. For further details, please see Section 9.5.1.1.

The 2nd vaccination was administered at V5= Day 56. There were short-term follow-up visits after each vaccination on V4= Day 14 and V6= Day 70, where diary cards were reviewed and AEs and concomitant medications (CMs) were assessed. In the diary cards, participants recorded measurements of redness and swelling at the injection sites and answered solicited questions on AEs and CMs. For further details, please see Section 9.5.3.1.

At V7= Day 238 and V8= Day 421 participants were screened for TB symptoms and if the investigator suspected recurrent TB, the procedures for case verification were followed. SAEs and adverse events of special interest (AESIs) were furthermore assessed.

Immunogenicity cohort participants had extra immunogenicity samples taken for whole blood intracellular cytokine staining (WB ICS) at V3= Day 0 and V6= Day 70. The immunogenicity cohort was also a control cohort and for this purpose had extra immunogenicity samples taken at V8= Day 421. These samples were control samples for the corresponding immunogenicity samples taken from participants under diagnosis of recurrent TB during the trial. For further details, please see Section 9.5.2.

Safety cohort participants had extra safety laboratory testing at V4= Day 14 and V6= Day 70. For further details, please see Section 9.5.3.2.

An internal data review committee (IDRC) assessed blinded safety data at 2 IDRC meetings held during the trial when:

- The first 150 participants across all sites had received their 1st vaccination and the 14 days safety follow-up data was available

- The first 150 participants across all sites had received their 2nd vaccination and the 14 days safety follow-up data was available

The trial continued while the IDRC processes were ongoing.

The DSMB that was established was only to convene if the IDRC recommended this, or a protocol defined pausing rule was triggered. No DSMB meetings were held during the trial. For further details on IDRC and DSMB procedures, please refer to the protocol, Appendix 16.1.1. The IDRC and DSMB charters are available in the TMF.

The summary schedule of investigational events in the trial is shown in Table 6. For more detailed descriptions of the investigational events at the individual visits and on the case verification procedures for recurrent TB disease etc., please refer to the protocol, Appendix 16.1.1, and to the case report form (CRF), Appendix 16.1.2.

Table 6: Summary schedule of investigational events

	Trial 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 (*β*HCG) -<u>all</u> 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]										
Randomisation			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]

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 analysed 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 randomisation. If positive by AFB *smear microscopy* the participant is excluded from further participation in the trial. 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 trial. 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 counselling. 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 randomised across all sites)
7) If in the immunogenicity cohort (= first 50 participants randomised at the SATVI site and the first 50 randomised at the MMRC 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 IP administration
9) Only if not already done as part of another trial visit on the day of the STB Visit
10) At a participant's last trial 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) *S*erum chemistry: *AST, ALT, ALP, GGT, total bilirubin, creatinine*
13) Haematology: *haemoglobin, haematocrit, white cell count with differential, and platelet count*
14) Participants who are withdrawn from the trial 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 trial. In this case, if triggered by a TB symptom screen later in the trial, another STB Visit would be conducted.
16) If withdrawal is due to verified recurrent TB, the only trial 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 trial, 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 trial

9.2 Discussion of trial design and control group

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* sensitised or infected individuals who have previously been vaccinated with BCG. Completed H56:IC31® Phase I trials have evaluated the safety and immunogenicity of increasing intramuscular doses of H56:IC31® initially in uninfected humans followed by IGRA positive individuals, previously vaccinated with BCG. A dose (5mcg H56:500 nmol IC31®) was selected for further development in IGRA positive 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 recently completed treatment for active pulmonary TB and by the WHO definition, were deemed to be cured.

Demonstration of protective efficacy against TB disease in the target population would require a large (many thousands of participants) and lengthy (3 or more years) field trial. Therefore, shorter and smaller proof-of-concept phase II trials, investigating subpopulations 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 (15). These trials would determine the impact of vaccination with investigational TB vaccines on prevention of recurrent (POR) TB disease in recently successfully treated TB patients (4-6, 16, 17). Recurrent TB disease is ~4-fold more frequent than incident TB disease in the general population. Therefore, these trials can be completed rapidly and cost-effectively using smaller group sizes than a proof-of-concept study with TB disease in the general population as an endpoint.

In addition to the primary endpoint of TB recurrence, the exploratory endpoints of TB relapse and reinfection, are also of interest and investigated, through collection of sputum at screening and at the time of recurrent TB, for WGS analysis, to distinguish between TB relapse and reinfection.

9.3 Selection of trial population

900 HIV negative adults of 18 to 60 years of age diagnosed with drug-susceptible pulmonary tuberculosis were planned to be included after completion of at least 5 months (22 weeks) of treatment and negative AFB test.

Adults not known to be HIV positive with a diagnosis of drug-susceptible pulmonary TB per national guidelines (confirmed by Xpert MTB/RIF Ultra or AFB smear) and who started their TB treatment not more than 7 days before the 1st screening visit (V1) were, if eligible for inclusion, asked to produce 2 separate sputum samples at V1. The sputum samples were 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 did not provide V1 sputum samples.

Participants were also eligible for inclusion at V1, if the Xpert MTB/RIF Ultra test was unsuccessful at the local clinic, but the participant had started the drug-susceptible TB treatment regimen according to national guidelines. Such participants were screen failed prior to

randomisation (V3), if the site could not confirm drug-susceptible TB by a test or a clinical response to treatment.

The time period between V1 and the 2nd screening visit (V2) was approximately 5 months (22 weeks). Site staff remained in contact with participants, TB clinic staff and community health care workers during this period to monitor TB treatment compliance and continued eligibility for trial participation.

The completion of a standard TB treatment regimen is 6 months (24 weeks) in ZA and TZ which can be extended up to 28 weeks. Participants with a combined V1 and V2 were screened for the trial after at least 5 months (22 weeks) of TB treatment and TB treatment had to be ongoing.

At V2 samples for HIV tests and safety laboratory analysis were collected and evaluated. Furthermore, 2 sputum samples were collected, both samples needed to test negative for *Mtb* by smear AFB microscopy using the Auramine method. Participants unable to produce sputum and considered asymptomatic by the investigator, could be considered *Mtb* negative and eligible for inclusion.

9.3.1 Inclusion criteria

Participants had to meet all the following inclusion criteria at the time of randomisation and vaccination:

1. Completed the written informed consent process.

2. Agrees to give access to medical records for trial 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 trial site for the duration of the trial, provide updated contact information as necessary and has no current plans to move from the area for the duration of the trial.

9.3.2 Exclusion criteria

Participants had to meet none of the following exclusion criteria at the time of randomisation and vaccination:

1. Diagnosis or co-diagnosis of extra pulmonary TB
2. Hospitalised 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 IP
5. Insulin dependent diabetes
6. History of allergic disease or reactions likely to be exacerbated by any component of the IP
7. History or laboratory evidence of immunodeficiency, autoimmune disease or immunosuppression
8. History of chronic hepatitis
9. Severe anaemia, defined as haemoglobin less than 10 g/dL or a haematocrit less than 30% based on most recent haematology obtained before randomisation
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 programmes 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 sterilised, 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

9.3.3 Discontinuation of trial participants from vaccination or assessment

9.3.3.1 Discontinuation of individual participants from vaccination

Administration of additional investigational product (IP) was discontinued for individual participants if they experienced any of the following:

1. An objective clinical or laboratory parameter change which by the investigator was assessed as severe in intensity AND was judged to be related to the IP

2. AE thought to be an allergic reaction to the IP, including anaphylaxis or bronchospasm

3. Any extensive rash (>40% body surface) on the thorax, abdomen, or limbs, including but not limited to urticaria, generalised petechiae, or erythema multiforme judged to be related to the IP

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 IP received as part of this trial) between 1st and 2nd vaccination or any other vaccine as described in exclusion criterion #12

7. Any event that in the opinion of the site PI precludes administration of any further investigational product

8. Pregnancy

9.3.3.2 Deferral of individual participants from vaccination due to acute illness

At the time of the 1st dose at V3= Day 0, participants were not to be vaccinated if they had a fever ≥37.5°C (axillary temperature), evidence of acute illness, or took antipyretics; in such cases, randomisation and vaccination were deferred until the fever or acute illness had subsided.

At V5= Day 56, only randomised participants who had not met any of the criteria for discontinuation of IP administration, please see Section 9.3.3.1 and did not have a fever ≥37.5°C (axillary temperature), evidence of a new acute illness or took antipyretics, did receive the 2nd dose of the IP.

9.3.3.3 Discontinuation of individual participants from trial assessments

Randomised participants who received the 1st vaccination followed the schedule of investigational events, please see Table 6, unless informed consent was withdrawn. This was also the case for participants where IP administration had been discontinued.

Trial assessments were discontinued for an individual participant and the participant was withdrawn from the trial, after completion of the early termination (ET) visit, in case of the following:

- if the participant had a positive *Mtb* culture result
- if the participant had started new TB treatment

Referral for diagnosis and management of recurrent TB was triggered by the investigator based on clinical suspicion of recurrent TB. Participants were not withdrawn from the trial based on this referral, but were withdrawn if *Mtb* positive by culture in the trial, or if a referral clinic diagnosed recurrent TB and started TB treatment.

The trial recurrent TB case verification procedure initiated at a STB Visit. For further details, please refer to the protocol (p. 21), Appendix 16.1.1.

Participants who were withdrawn from the trial within 6 months after the last IP administration, were contacted at least 6 months after the last IP administration for recording of SAEs and AESIs. Discontinued participants were not replaced by new participants in this trial.

9.3.4 Rules for pausing the trial

The following rules triggered pausing by the sponsor's medically responsible person for enrolment and IP administration and a DSMB review:

1. One or more SUSARs occurred, OR
2. Anaphylaxis or bronchospasm within 4 hours of injection, indicative of an immediate hypersensitivity reaction to the trial injection, OR
3. An AE pattern of concern occur in a group of participants or for an individual

Furthermore, the outcome of the 2 IDRC meetings, where blinded safety data was assessed, could trigger the pausing of the trial and that the DSMB was convened.

The final decision to resume trial activities, amend the protocol, or terminate the trial was taken by the sponsor. Decisions regarding pausing and resumption of the trial activities were to be communicated to the EC by the site PI and to the applicable CA by the sponsor.

For further details, please refer to the protocol (p. 38 and p. 48), Appendix 16.1.1.

9.4 Treatments

H56:IC31® consists of a fusion protein of 3 antigens expressed at different stages of *Mtb* infection, developed by SSI and a T helper type 1 cell (Th1)-stimulating adjuvant, IC31® developed by Valneva.

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 $KLKL_5KLK$.

The first component, ODN1a, contains alternating sequences of the unusual base inosine and cytidine: oligo-d(IC)13. This motif is similar to CpG motifs that act as T cell adjuvants.

The second component, $KLKL_5KLK$, is a synthetic cationic antimicrobial peptide composed of lysine (K) and leucine (L) in the sequence KLKLLLLLKLK. $KLKL_5KLK$ 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 $KLKL_5KLK$ are forming a 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 $KLKL_5KLK$ to ODN1a is the ratio used for H56:IC31®. Since the ratio of ODN1a and $KLKL_5KLK$ is constant in H56:IC31®, adjuvant amounts are expressed as nmol of $KLKL_5KLK$ only, for ease of presentation in this document.

H56:IC31® vaccine

The H56 fusion protein is formulated with IC31® in a GMP compliant environment in a ready to use final formulated vaccine. H56:IC31® contains 5 mcg H56 adjuvanted with IC31® consisting of 500 nmol $KLKL_5KLK$ and 20 nmol ODN1a, in a total volume of 0.5 mL, for intramuscular (i.m.) injection in the deltoid muscle using standard aseptic technique.

9.4.1 Treatments administered

The investigational products (IPs), both H56:IC31® (test product) and placebo (control product), were sterile solutions for injection and both were administered i.m., in the deltoid muscle.
The dose volume was 0.5 mL. The dose regimen was 2 doses of 0.5 mL administered with 2 months (56 days) interval.

9.4.2 Identity of investigational (test and control) products

The identities of, procedures for and other details related to the IPs, covering both H56:IC31® and placebo, such as shipping, storage, randomisation, treatment assignment, preparation, blinding of syringes, administration, accountability, destruction, emergency unblinding, management of temperature excursions and product complaints procedures, were described in a vaccine management manual which is available in the TMF.

9.4.2.1 Investigational test product

The H56:IC31® vaccine, was manufactured, filled into vials and labelled at SSI according to GMP.

H56:IC31® was formulated in a buffer consisting of 4 mM Glycine, 8 mM Tris and 135 mM NaCl. The vaccine was formulated as follows; 1 part of H56 antigen (concentrated bulk formulated in 20 mM Glycine) to 4 parts IC31® adjuvant (1.25 x concentration formulated in 10 mM Tris and 168.75 mM NaCl).

Each vial contained one vaccine dose with H56 antigen (5 mcg / 0.5 mL) and IC31® adjuvant (500 nmol / 0.5 mL). The H56:IC31® vials contained 0.8 mL of greyish/colourless final formulated vaccine with an extractable volume > 0.5 mL.

The inner labels were trial specific while the labels for the outer packaging (i.e. the cardboard boxes) were trial and site specific. H56:IC31® was supplied as single dose vials for this trial.

H56:IC31® vials had to be stored at a temperature between +2°C and +8°C and protected from light.

Prior to withdrawing the 0.5 mL dose, the vial had to be gently swirled and inspected for particulate matter. Vials with visible particulates were to be quarantined and the sponsor notified. Vaccine doses for administration were to be drawn directly into a 1 to 3 mL polypropylene Luer-Lok™ syringes (similar to Becton Dickinson #300-962 or #309-585) via a 23 to 25-gauge needle using aseptic technique. It was important to use a Luer-Lok™ needle connection or an integral needle to avoid vaccine spray exposure from accidental dislodgement of the needle.

H56:IC31® had to be administered within 2 hours of being drawn into the syringe. The ambient temperature at which the syringe with the vaccine dose had to be kept should not exceed 25°C prior to administration.

The sites received an initial supply of H56:IC31® followed by additional shipments, according to needs. 2 different batches with 2 different 'use before dates' were used during the course of the clinical trial.

- Batch number 521202
- Batch number 711101

The unblinded site staff at the sites were continuously staying informed of the site recruitment rate and planned trial visits (V3 and V5) where the vaccinations were administered to ensure sufficient IP stock. For further details on supply logistics and the H56:IC31® batches, shipped from SSI to the individual sites during the trial, please refer to Appendix 16.1.6.

9.4.2.2 Investigational control product

The investigational control product, the placebo, consisted of 0.9% sterile saline (sodium chloride; NaCl) solution, purchased commercially by the local trial sites. A trial specific label was placed on the external carton stating the protocol number and that the saline was for clinical trial use only.

9.4.2.3 Investigational product temperature excursions

For both batches of test product (batch number 521202 and 711101), temperature deviations occurred at the sites. Each temperature deviation was evaluated by SSI QA. All reported incidences of temperature deviations resulted in SSI QA declaring the affected test product (H56:IC31® vials) "fit for use", except for one incidence which occurred at site A5, MMRC in TZ.

This incidence (date of incidence 01-Jan-2020) affected the test product (H56:IC31®), as 25 vials (from batch number 521202) were exposed to temperatures up to 14.9°C for up to 3 hours in the fridge at site A5 (MMRC). The affected 25 vials were later declared "to remain in quarantine and to not be permitted to use", by SSI QA.

3 participants (A50022, A50041 and A50042), who received their 2nd vaccination on 06-Jan-2020, by mistake received this test product (date of incident report 06-Feb-2020) and this way received test product (H56:IC31®) that had been exposed to the above-mentioned temperature deviation.

9.4.2.4 Investigational product complaints

1 participant (A30131) was affected by a product complaint (date of incident 09-Dec-2019):
The initial vial with H56:IC31® (batch number 521202) dispensed for administration of the V3= Day 0 dose was underfilled and it was not possible to draw up the dose of 0.5 mL. A new vial with H56:IC31® (batch number 521202) was therefore dispensed. In conclusion, this incident did not result in any deviation to the administered dose to the participant.

9.4.2.5 Investigational product used past expiry date

The H56:IC31® batches used in the trial had a shelf-life of 4 years. There were no incidences of use of IP past expiry date in this trial.

9.4.3 Treatment group assignment and randomisation methods

A participant screening number was automatically allocated in the eCRF to a participant when the participant's 1st screening visit (V1) was entered in the eCRF. The site numbers A1, A2, A3, A4, A5 and A6 were the first digits in the screening number. The system connected the participant to the specific site. The remaining 4 digits 0001-9999 indicated how many participants had been screened at the specific site and timepoint. All digits were allocated automatically by the system.

Participants were randomised to the trial based on a randomly-generated sequence of numbers (randomisation list) managed by a validated module in the eCRF. The randomisation list was prepared by a statistician who was not involved in the analysis of the trial to maintain the blind of the trial team. The day of randomisation for each participant was V3= Day 0. Once the participant had been randomised, the treatment assignment was only visible to unblinded site staff by a personal login to the eCRF system.

In addition to being automatically assigned to either H56:IC31® or placebo, a participant was also automatically assigned to one of the following cohorts, as part of the process, at the time of randomisation on V3= Day 0:

- Safety cohort (applicable for all sites)
- Immunogenicity cohort (applicable for sites A1 [SATVI] and A5 [MMRC])

For the randomisation list, please refer to Appendix 16.1.7.

9.4.4 Selection of dose level and dose regimen for the investigational product

The dose of H56:IC31® was selected based on the results of a phase 1/2a, double-blind, randomised, placebo-controlled, dose-finding trial in 98 healthy (IGRA negative or IGRA positive) adults in SA, receiving H56:IC31® (81 participants) and placebo, C-035 (17 participants). This trial had 2 phases; an initial dose-finding phase and a subsequent fixed-dose phase. H56 doses ranged from 5 to 50 mcg (11).

In the initial dose-ranging phase, only one 2-dose regimen of 3 dose levels of H56:IC31® in 50 IGRA negative participants were investigated to select a dose level for the second phase. These participants were randomised in the ratio 15:15:15:5 to receive 5 mcg, 15 mcg, or 50 mcg of H56 (all with 500 nmol IC31®), or placebo, all administered in the 2-dose regimen of Days 0 and 56.

The dose level of H56:IC31® for the second fixed-dose phase of the trial (i.e., 5 mcg:500 nmol) was selected by IAVI and SSI, in agreement with the recommendation of the Data Monitoring Committee in the trial, based on analysis of unblinded safety and immunogenicity data, through 28

days after the 2nd vaccination, from the first phase of the trial, in conjunction with safety and immunogenicity data from the C-032 trial (10).

In the second fixed-dose phase, the trial was expanded to evaluate both 2-dose (Days 0 and 56) and 3-dose (Days 0, 56 and 112) regimens, and to include IGRA positive participants. 48 participants were enrolled concurrently into 3 groups. One group of IGRA negatives received the 2 dose regimen (Days 0 and 56), and were randomised 12:4 to receive either H56:IC31® or placebo; and two groups of IGRA positives received either the 2 dose regimen or the 3-dose regimen, both randomised 12:4 to receive H56:IC31® or placebo.

The dose level of H56 was selected to be 5 mcg and the dose regimen to be 2 doses with 2 months (56 days) interval, based on the results of this trial (11). In all subsequent trials, this dose level and dose regimen has been employed.

As regards the adjuvant IC31®, the selected dose level has been 500 nmol IC31® in all clinical trials conducted with the vaccine antigen H56 to date.

9.4.5 Blinding

The unblinded persons in the trial were the IP manager (and designees) who managed the IP inventory log(s) at the trial sites, the unblinded site staff and the unblinded trial monitor(s) responsible for monitoring the IP at the trial site(s). All unblinded persons had to not reveal individual participant treatment assignments to any other trial team members. There was 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 trial.

The IP manager (and designees) were designated site team members, such as the pharmacist at the site(s). Unblinded site staff could not participate in the administration of vaccine and evaluation of AEs.

A delegation of responsibility log was maintained by the trial site and identified the individual(s) such as the IP manager (and designees) and other unblinded site staff, i.e., individuals with access to unblinded data.

The randomisation list was prepared by the unblinded statistician and was implemented as a module in the eCRF. Access (through the eCRF) to information which links a randomisation number to treatment assignment, was only be given to the IP manager (and designees) and to the unblinded trial monitor(s) responsible for monitoring the IP at the trial site(s).

All pharmacy source documents and dose preparation records that could link a randomisation number with a treatment assignment were kept confidential in a secure place (e.g., in the pharmacy with access limited to only unblinded trial team members) until notification from the sponsor that the trial has been unblinded.

The unblinded site staff were responsible for preparing the assigned treatment in the designated pharmacy. The syringes with the final prepared vaccine, ready for injection, were covered with a translucent coloured label supplied by the sponsor. This label obscured slight differences in colour and opacity between the H56:IC31® and placebo (to the blinded site staff injecting the vaccine), but still allowed sufficient visibility of the volume of the syringe content.

Labels accompanying the syringes of prepared IP doses did not indicate which investigational product (H56:IC31® or placebo) was in the syringe. Identical syringes, needles and labels were used to prepare and administer test and control products. For further details, please refer to the protocol, Appendix 16.1.1 and to the vaccine management manual in the TMF.

9.4.6 Unblinding

If there was an urgent clinical requirement to know a participant's treatment assignment, the site PI could 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 was to be notified within 24 hours of any clinically required break of the trial blind. The notification of the unblinding to the sponsor had to 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 had to be kept in the TMF.

It was recommended that the site PI consulted with the sponsor's medically responsible person prior to unblinding of a participant. However, in urgent circumstances, the trial site could unblind a participant at the site PI's discretion, without prior consultation with the sponsor's medically responsible person.

For further details, please refer to the protocol, Appendix 16.1.1 and to the vaccine management manual in the TMF.

9.4.7 Prior and concomitant therapy and vaccines in the trial

Specific therapies or vaccinations were reviewed on the day of inclusion and randomisation in the trial. The receipt of the following therapies or vaccines within the specified time periods excluded a participant from participation in the trial:

- History of receipt of treatment against active TB, prior to the current treatment episode, within the last 5 years (excl. crit. #3), or previous receipt of any investigational TB vaccine (excl. crit. #10).

- Receipt or planned receipt of any investigational drug or investigational vaccine from V1 through V8= Day 421 (excl. crit. #11), or receipt or planned receipt of any licensed vaccine from V1 through V6= Day 70 (excl. crit. #12)

- 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 were permitted (excl. crit. #13).

Implemented in protocol FV 5.0, Appendix 16.1.1, SARS-CoV-2 vaccines recommended by national vaccination programmes were allowed if administered > 28 days before or > 28 days after the administration of the 1st and 2nd doses of IP (excl. crit. #12). Before this protocol amendment such vaccines were not allowed between the 1st injection and 14 days post the 2nd injection of IP (Days 0 to 70), as other licensed vaccines.

The vaccinations administered in the trial were deferred in case of receipt of antipyretics due to acute illness. At the time of the 1st dose at V3= Day 0, participants could not be vaccinated if they were in treatment with antipyretics. In such cases, randomisation and vaccination was deferred until the fever or acute illness had subsided. At V5= Day 56, only randomised participants, eligible for vaccination and who did not take antipyretics received the 2nd dose of the IP. For further details, please see Section 9.3.3.2.

The collection of information on concomitant medications (CMs) used by participants following vaccination coincided with the collection period for AEs. In addition, information on all CMs associated with the treatment of AEs, SAEs and AESIs, were collected throughout the trial.

CMs included prescription and non-prescription drugs or other treatments and any vaccines other than the IP. The name of the medication, treatment start and stop dates (or 'ongoing') and indication were recorded on the CM page in the electronic case report form (eCRF).

9.4.8 Treatment compliance

As the IPs, H56:IC31® (test product) and placebo (control product), were administered by trial staff at the trial visits, assessment of participant treatment compliance is not applicable for this trial.

Accountability procedures at the trial sites ensured that the IP (test product as well as control product), was accounted for, as well as dispensed and administered according to the protocol.

9.5 Efficacy, immunogenicity and safety variables

Efficacy, immunogenicity and safety measurements to be performed at each visit are shown in Table 6.

9.5.1 Efficacy measurements assessed and schedule of assessments

9.5.1.1 TB recurrence case finding

After randomisation and vaccination at V3= Day 0, the participants were questioned about TB signs and symptoms at every trial visit and contact (e.g. telephone contacts). They were instructed to come to the trial site if TB signs or symptoms occurred between trial visits or contacts.

A participant was considered to have a clinical suspicion of TB when they presented 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, haemoptysis, or other TB signs or symptoms.

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

Referral for diagnosis and management of recurrent TB was triggered by the investigator based on clinical suspicion of recurrent TB. Participants were not withdrawn from the trial based on this referral but were to be withdrawn from the trial if they had a positive culture in the trial, or if the referral clinic diagnosed recurrent TB and started TB treatment.

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

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

If the participant tested *Mtb* negative by Xpert MTB/RIF Ultra and subsequently tested negative by culture, the participant continued in the trial.

The case verification procedure for recurrent TB disease was initiated at a STB visit. For further details, please refer to the protocol (p. 21), Appendix 16.1.1.

At the final trial visit (V8= Day 421) or at the ET Visit, if discontinuation was not due to verified recurrent TB, 2 separate sputum samples for *Mtb* testing were obtained from all participants (capable of producing sputum), even if there was no clinical suspicion of TB disease (asymptomatic participants not capable of producing sputum were considered negative for TB disease recurrence).

These sputum samples were taken to ensure that no cases of recurrent TB disease were missed during the trial and to detect subclinical cases in this population at high risk of recurrence of TB disease.

1 sputum sample was tested by Xpert MTB/RIF Ultra. If *Mtb* positive by Xpert MTB/RIF Ultra (and if *Mtb* negative by Xpert MTB/RIF Ultra but there were clinical TB signs or symptoms), the same sputum sample was sent for culture together with the second sputum sample for verification. The remainder of each of the 2 separate sputum samples was stored (frozen) as 2 separate samples for later analysis by WGS if recurrent TB was verified. In addition, HIV testing and immunology sample collection were also performed.

Participants were contacted to be given their Xpert MTB/RIF Ultra and culture result once available (whether positive or negative) and symptomatic participants were referred as appropriate for TB management irrespective of the Xpert MTB/RIF Ultra and culture results.

9.5.1.2 Primary efficacy variable (TB recurrence)

Confirmation of *Mtb* by one positive sputum culture result was required for a participant to meet the primary efficacy endpoint of TB disease recurrence. The primary efficacy endpoint is described in Section 8.4.

9.5.1.3 Secondary efficacy variables (TB relapse and reinfection)

WGS was performed on *Mtb* isolates to distinguish relapse from reinfection.

Participants with a combined V1 and V2 had not provided sputum samples prior to starting TB treatment because their 1st screening visit (V1) was performed on the same day as V2. For these participants, it was therefore not possible to determine if their TB recurrence was due to relapse or reinfection. Of note this only affected 1 participant (A20243) with recurrent TB on Day 39.

The two secondary efficacy endpoints TB relapse and TB reinfection are described in Section 8.5.2.

9.5.1.4 Exploratory efficacy variables

Participants who started TB treatment without *Mtb* positive culture result, or recurrent TB was diagnosed based on a Xpert MTB/RIF Ultra positive result only, or the recurrent TB occurred between 30 days after the 1st vaccination and 14 days after the 2nd vaccination were assessed in exploratory efficacy analysis. The three exploratory efficacy endpoints are described in Section 8.6.

9.5.2 Immunogenicity measurements assessed and schedule of assessments

Table 7: Summary schedule of immunogenicity measurements

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]	SATVI, ZA
Plasma (obtained from PBMC)	IgG ELISA	Secondary endpoint[1]	Plasma (3 mL) obtained from PBMC	0[1], 70[1]	SSI, DK
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]	SSI, DK
Whole blood	RNA analysis	Exploratory endpoint[2]	2.5 mL	0[2], 421[1,5], STB[3], ET[4]	SATVI, ZA
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]	SATVI, ZA
PBMC	ICS	Exploratory endpoint[2]	50 mL	0[2], 70[2], 421[1,5], STB[3], ET[4]	SATVI, ZA

1) Participants in immunogenicity cohort (1st 50 randomised at SATVI and 1st 50 randomised at MMRC)
2) All participants in the trial
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 MTB/RIF Ultra positive at the ET Visit
5) Participants who are Xpert MTB/RIF Ultra positive at Visit 8 (Day 421)

9.5.2.1 Intracellular cytokine staining assay using whole blood (WB ICS)

Responses were measured by flow cytometry (up to 15 parameters) with the whole blood intracellular cytokine staining (WB ICS) assay. The variables of interest for assessment of antigen specific cell mediated immune response to vaccination were the percentage of CD4+ and CD8+ T cells that expressed selected cytokines and phenotypic markers alone or in combination in response to stimulation with H56 antigen.

This was a secondary endpoint in the trial and the primary immunogenicity endpoint, please see Section 8.5.2 and Table 7. This endpoint was measured at V3= Day 0 and V6= Day 70 in the immunogenicity cohort only, please see Section 9.1 and Table 5.

9.5.2.2 Antigen specific IgG ELISA assay using plasma (IgG ELISA)

Antigen specific antibody were assessed in plasma by enzyme linked immunosorbent assay (IgG ELISA). This was a secondary endpoint in the trial, please see Section 8.5.2 and Table 7. This endpoint was measured at V3= Day 0 and V6= Day 70 in the immunogenicity cohort, please see Section 9.1, Table 5.

For the remaining participants, this was an exploratory endpoint, please see Section 8.6 and Table 7, measured at V3= Day 0, V6= Day 70 and in participants with suspicion of a diagnosis of recurrent TB at the time of evaluation of TB recurrence.

9.5.2.3 RNA analysis and supporting blood subset counts using whole blood

Whole blood gene expression profiles were to 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 was characterised 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 were also investigated. The determination of absolute blood subset counts supported the RNA analysis.

These were exploratory endpoints in the trial, please see Section 8.6 and Table 7. These endpoints were 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. The results of these analyses will not be reported in this CTR.

9.5.2.4 Intracellular cytokine staining assay using PBMCs (ICS)

Analysis of cells stimulated with relevant antigens (ESAT-6, Ag85B, Rv2660c, as well as negative and positive controls) included 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 were measured by flow cytometry (up to 28 parameters) using a PBMC based ICS assay.

This was an exploratory endpoint in the trial, please see Section 8.6 and Table 7. This endpoint was measured in all participants at V3= Day 0, V6= Day 70 and in participants with suspicion of a diagnosis of recurrent TB at the time of evaluation of TB recurrence. The results of these analyses will not be reported in this CTR.

9.5.2.5 Exploratory immunology at TB recurrence – control samples

Exploratory immunology samples were, taken at the time of evaluation of TB recurrence, from participants with a suspected diagnosis of recurrent TB disease. These samples were PBMC and plasma for ICS and IgG ELISA and whole blood for RNA analysis and blood subset counts.

For the assessment of these samples, “control samples” from a “control cohort” were needed.

The immunology samples taken at V8= Day 421 from the participants in the immunogenicity cohort (excluding participants with recurrent TB, if any) served as these control samples, please see Table 7. The results of these analyses will not be reported in this CTR.

9.5.3 Safety measurements assessed and schedule of assessments

The key secondary (safety) endpoints in the trial are described in Section 8.5.1.

9.5.3.1 Adverse events

Allergic reactions were monitored for 60 minutes after the IP administrations, where appropriate medical staff, equipment and drugs for the treatment of acute anaphylactic reactions were immediately available.

The injection sites were examined before the participants left the clinic on the days when the IP was administered. In case of immediate injection site reactions (redness, swelling and tenderness/pain) or loco-regional reactions (such as axillary lymphadenopathy) these AEs were recorded in the eCRF.

Solicited AEs were defined in the protocol and the participants were specifically asked about the occurrence of these AEs in a diary. For this trial the solicited AEs were:

- injection site reactions of redness, swelling and tenderness/pain
- systemic AEs of fever, arthralgia, myalgia, fatigue, headache, rash, chills and nausea

Solicited injection site reactions were considered related to the IP.

All participants were provided with a diary, a thermometer to record axillary temperature, a ruler to measure injection site redness and swelling and instructions on using the diary for AE and CM recording, for the first 7 days after each vaccination. In the period from 8 days after the vaccinations until the diary review at 14 days after each vaccination, the participants were instructed to fill in the diary only in case of an AE or CM intake.

Adverse events of special interest (AESIs) represented a subset of AEs that include autoimmune diseases and other systemic disorders of interest that could potentially have an immune aetiology; AESIs were listed in Appendix A to the protocol, please refer to Appendix 16.1.1.

The PI should use clinical and scientific judgement in deciding whether other AEs (i.e., events not listed in Appendix A) could have an autoimmune origin. The investigator reported AESIs in the eCRF. The collection periods for AEs are shown in Table 8.

Table 8: Collection periods for adverse events

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

The investigator assessed the AEs for intensity (mild, moderate or severe), relatedness to IP (related or not related) and the outcome was recorded. For SAEs, related to the IP, the expectedness was, furthermore, assessed by the sponsors medically responsible person to determine if the related SAE was a SUSAR. For further details on general definitions, assessments and reporting of AEs in the trial, please refer to Sections 5.2-5.10 in the protocol, Appendix 16.1.1.

9.5.3.2 Clinical laboratory tests

Safety laboratory tests performed at each visit are summarised in Table 6. Results from laboratory tests in the trial were reviewed by the investigator. The clinical significance of any change in a vital laboratory parameter was determined by the investigator and was reported as an AE at the discretion of the investigator.

Additional laboratory tests were performed if the investigator deemed them necessary to fully evaluate an AE. In the event that the investigator elected to order non-protocol-specified laboratory tests, the investigator recorded the rationale for the tests and a determination of the clinical significance of the result in the source documents.

9.5.3.3 Vital sign measurements

Participants had vital signs taken prior to each injection of IP and remained in the clinic under close observation for at least 60 minutes after receiving the IP. Vital signs were repeated at 60±10 minutes post-vaccination before the participants left the clinic. Vital signs were, furthermore, measured and assessed once at the trial visits as summarised in Table 6. The results of vital signs were reviewed by the investigator. The clinical significance of any change in a vital sign was determined by the investigator and was reported as an AE at the discretion of the investigator.

9.5.3.4 Physical examinations

A complete physical examination was performed at screening (V2) and symptom directed physical examinations were, furthermore, performed prior to IP administration at V3= Day 0 and V5= Day 56. Results from these assessments were reviewed by the investigator. The clinical significance of any finding was determined by the investigator and was reported in the eCRF at the discretion of the investigator. The weight of the participant was recorded at all trial visits.

9.5.3.5 Pregnancies

If participants became pregnant during the trial, they did not receive any further doses of IP, but were encouraged to continue in the trial for safety, efficacy and immunogenicity follow-up.

Follow-up had to continue for pregnancy outcome including premature terminations and this data was included in the pregnancy reports. If the pregnancy resulted in a miscarriage or a planned termination, the event (spontaneous abortion or elective abortion) was reported as an AE or serious AE per the investigator's judgement, please refer to Section 5.14 in the protocol, Appendix 16.1.1, for further details on the data recorded and procedures for handling pregnancies in the trial.

9.5.4 Appropriateness of efficacy, immunogenicity and safety measurements

TB disease recurrence, the primary efficacy endpoint, please see Section 9.5.1.2, was detected by surveillance of the following TB signs and symptoms: unexplained cough for longer than 2 weeks duration, fever, night sweats, loss of weight, haemoptysis, or other TB signs or symptoms, during the protocol defined follow-up period. A participant was considered to have a clinical suspicion of TB if they presented with one or more of these signs or symptoms, and would in this case proceed with verification of the suspected TB through the measurements described in Section 9.5.1.1.

To catch up on possibly missed cases at the participant's last visits, all participants had, in addition to be checked for TB signs and symptoms, to give a sputum sample, which was tested by Xpert MTB/RIF Ultra, and if there was either TB signs and symptoms or a positive Xpert MTB/RIF Ultra test, the 2 sputum samples were sent for culture, for verification of TB disease recurrence.

Confirmation of *Mtb* by one positive sputum culture result was required for a participant to meet the primary efficacy endpoint of TB disease recurrence. This diagnostic method for confirmation of active TB is regarded as the gold standard.

The measurements from the whole blood intra cellular cytokine (WB ICS) assay were, as per the protocol, considered appropriate for assessment and statistical analysis as the primary immunogenicity endpoint (cell medicated immunogenicity) in the trial and those from the IgG ELISA assay for assessment and statistical analysis as the secondary immunogenicity endpoint (antibody mediated immunogenicity). These endpoints were selected to characterise both the expected antibody mediated and cell mediated protection mechanisms of H56:IC31®.

To ensure the quality of the PBMCs used for the exploratory immunogenicity analysis, PBMC proficiency and external quality assessment management was obtained from the external

independent party of Human Immunology Laboratory, UK. Further details are available in the laboratory manual in the TMF.

The safety measurements in this trial are regarded as standard safety measurements.

9.6 Data quality assurance

The study was monitored regularly, according to the protocol-specific monitoring plan. Source data consisted 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 trial sites, a document identifying all (expected) source documents was prepared and signed by the site PI before the initiation of the study. Data recorded on source documents and required to be captured were entered in the electronic case report form (eCRF) approved by the sponsors.

The data management protocol, the trial validation plan and the data handling report described the handling of the data in the trial, including specification of the eCRF used for capturing the data at the trial sites. During the trial the eCRF existed in 4 versions, please refer to Appendix 16.1.2.

Access to the eCRF was given depending on a person's role in the study, including if a person was in the blinded or unblinded study team. All persons with access to the eCRF had automatically access to an audit trail logging all activities. Queries for discrepant data were generated either automatically by the system upon entry or generated manually by the monitor or the data manager. All queries, whether generated by the system or by a user, were in an electronic format. The site PIs, or investigator(s) authorised by the site PI, electronically signed all eCRFs.

Coding of AEs and MH was performed using MedDRA version 21.1, listing MedDRA (LLT, PT, HLT, HLGT and SOC levels), where CM coding was performed by use of WHODrug (ATC4, ATC3, ATC2, and ATC1 levels).

A separate SAE reconciliation plan was used for reconciliation of records of SAEs in the pharmacovigilance database with the corresponding records in the clinical database (eCRF). Coding, SAE reconciliation and the statistical analysis plan were finalised before database lock.

The data from the eCRF was extracted to SAS datasets for mapping into SDTM data using SAS (release 9.4 or later), please refer to Appendix 16.1.9.

The protocol management plan prepared by the sponsors for the trial included quality management procedures, that were followed during the trial.

Site staff and sponsor staff were trained in the protocol and in the trial specific plans and manuals applicable for their role, before they could work with their specific tasks. In addition, they worked according to relevant SOPs implemented at their respective site or organisation. For further details on the trial specific plans, manuals and SOPs used during the trial, please refer to the TMF.

The sponsors implemented a quality management system to manage quality throughout the design, conduct, recording, evaluation, reporting and archiving phase of the trial. A risk-based approach was implemented for quality management which comprised:

- Critical process and data identification
- Risk identification
- Risk evaluation
- Risk control
- Risk communication
- Risk review
- Risk reporting

Central monitoring (in addition to unblinded and blinded site monitoring) was implemented for this trial. The central monitoring was performed by a cross-functional sponsor/CRO team, who also defined the central monitoring procedures in the risk-based monitoring and data evaluation plan, through identification and assessment of critical data and processes.

In the monitoring and data evaluation plan 12 site risk indicators (quality tolerance limits) were defined as well as a set of supporting data evaluation reports to be assessed regularly during the trial at risk-based monitoring meetings with participation of team members from the cross-functional sponsor/CRO team. The results of these meetings were completed site risk assessments matrices. If the outcome for a site was that > 4 quality tolerance limits had been exceeded, the site moved to the category ‘high risk site’ and the SDV level would be increased or other applicable action would be implemented as a mitigation action. The individual regular meetings were documented by central monitoring reports and risk-based monitoring reports including proposed actions to mitigate the exceeded quality tolerance limits.

Another cross-functional sponsor team identified risks and assessed, controlled and closed these risks as well as identified new risks, continuously during the trial. Routine risk review meetings were conducted and the risks were subsequently communicated and reported to relevant parties.

Quality oversight visits were, furthermore, conducted at all trial sites by the co-sponsors. At these visits the sponsor reviewed the clinical data and processes at the selected sites and suggested changes, improvements or corrections related to data and processes, as applicable.

A number of audits were conducted for this trial, both at CROs and at trial sites.

For the audit certificates, please refer to Appendix 16.1.8.

9.7 Statistical methods and sample size determinations

9.7.1 Statistical analysis plan

The statistical analysis plan (SAP), FV 2.0, dated 15 September 2023 was signed before the data base lock (DBL) and unblinding of the trial, please refer to Appendix 16.1.9.

9.7.1.1 General approaches

The exploratory immunogenicity measurements and endpoints were not within the scope of the SAP. For further detail, please see Section 9.5.2.

Before releasing data for final analysis, a data review and classification meeting was held to classify participants with respect to analysis populations. The product of the classification meeting was a detailed description of the analysis populations, including reason for exclusion from the analysis sets for the individual participants. The statistical analysis was based on:

- The clinical database, which included the electronic case report forms (eCRF) and laboratory data
- The analysis populations as documented at the classification meeting

9.7.1.2 Primary efficacy endpoint methodology

The primary efficacy endpoint was:

- Rate of TB disease recurrence

Time to event analyses had start- and end timepoint depending on the endpoint and analysis population, as described in Table 1 and Table 2 in the SAP. For further details, please refer to Appendix 16.1.9.

If the event was not within the period between the start timepoint and end timepoint, then the participant was censored as described in Table 2 in the SAP. Events on the day of the start time point were included in the analyses with a time to event duration of 1 day.

Time to event (or censoring) in days was calculated as:

$$Date_{end\text{-}timepoint} - Date_{start\text{-}timepoint} + 1$$

Censoring was only used if no events were available after the start timepoint. If the last visit was used as censoring and the last visit occurred later than the visit window of V8, then Day 428 was used as end timepoint. Participants censored later than Day 428 due to other censoring events were not transferred to Day 428. For details about procedures for censoring and the different types of censoring events, please refer to Table 2 in the SAP, Appendix 16.1.9.

The trial was designed for the primary endpoint to be evaluated using a one-side α-level of 10%, therefore a one-sided log-rank p-value and 80% vaccine efficacy (VE) profile confidence limits was planned to be presented for the primary endpoint. Two-sided p-values and 95% VE profile confidence intervals were planned to be presented as well.

The primary analysis set for efficacy was the mITT analysis set. The primary efficacy endpoint was in addition analysed using the ITT and PP analysis sets. The analysis specified for the ITT and PP were regarded as supportive evidence.

Time to primary endpoint (TB recurrence) was estimated using the Kaplan-Meier method and compared between treatment groups stratified by trial site. The stratified log-rank statistic was used to test the null hypothesis of no difference in the rates of primary endpoint (TB recurrence) over the follow-up period in the H56:IC31® compared to the placebo group.

The trial was designed for the primary endpoint to be evaluated using a one-side α-level of 10%, thus the lower one-sided p-value for the log-rank test was presented. If the p-value for the log-rank test was below 0.10, then the primary objective of the trial was considered met. The median time to TB and the p-value for a two-sided test were presented as well.

Time to primary endpoint (TB recurrence) was presented by treatment in cumulative plots, i.e., inverted KM estimates. One plot per trial site was made. Time to censoring was marked on the curves and the number of participants at risk per month from 14 days post 2nd vaccination (or Day 70 for those who did not receive a second vaccination) were presented. Month was calculated as days divided by 30.25 and rounded to the nearest integer.

Furthermore, time to primary endpoint (TB recurrence) was presented by treatment in cumulative plots without stratification by the trial site.

Two plots were presented, one with 80% confidence bands and one with 95% confidence bands.

The primary endpoint was, in addition, analysed using a Cox proportional hazard regression model. The Cox proportional hazard model relied on the assumption of proportional hazards over time. The proportionality of the hazards by treatment were evaluated using a graphical comparison of log-minus-log survival curves. If the proportional hazard assumption was found to be violated, the Cox regression model was extended appropriately, e.g., by introducing a time-dependent variable or include trial sites as strata. The Cox proportional hazard model included treatment and site as fixed effects. Treatment group interference was evaluated using a two-sided likelihood ratio test. VE was calculated as 1 minus the hazard ratio for H56:IC31® versus placebo and presented with 80% and 95% profile confidence limits.

As sensitivity analysis, the above was repeated:

- Excluding participants who received 2nd vaccination outside the +/- 10 days visit window i.e., before day 46 or after day 66

- Excluding positive results of samples sent for culture without TB symptoms or positive Xpert MTB/RIF Ultra

The aim was to clarify the specific impact (on the primary endpoint analysis) of each of the following events that occurred during the trial:

- The visit window for the 2nd vaccination was extended due to the Covid-19 pandemic
- A number of sputum samples were sent for culture, where this was not indicated

For more details on and the background of these two events, please see Section 9.8.

The cumulative incidence of primary endpoint (TB recurrence) per 100 person years with associated 95% confidence intervals during follow-up were summarised by treatment group and by treatment group and trial site. Person-days of observation was used to estimate the time at risk of primary endpoint (TB recurrence) and was calculated based on the participant's date of last trial contact or date of the primary endpoint (TB recurrence) - whichever was earlier - minus the date of the 2nd vaccination plus 14 days. Person years of observation was calculated as person-days of observation divided by 365.25. The incidence of the primary endpoint (TB recurrence) was calculated as the number of cases of primary endpoint (TB recurrences) diagnosed during trial follow-up, divided by the total person years of observation.

Comparison of the incidence of primary endpoint (TB recurrence) between treatment groups was performed using relative risk summaries and corresponding 95% confidence intervals.

Supportive robustness and consistency analysis were performed using the PP and ITT analysis sets.

For further details, please refer to the SAP, Appendix 16.1.9.

9.7.1.3 Secondary efficacy endpoint methodology

The secondary efficacy endpoints were:

- Rate of TB disease relapse
- Rate of TB disease reinfection

The relatedness of strains was analysed by WGS using two different methods providing the single nucleotide polymorphism (SNP) distance (according to whole genome SNP analysis) and the allele distance (according to core genome multi locus sequence typing analysis) and the results were categorised as follows:

The below thresholds applied for the paired genetic variation between the *Mtb* isolates sampled at the screening visit and the TB disease recurrence visit:

- A distance of ≤ 5 SNPs or alleles defined a recent transmission chain, i.e. the same strain (likely relapse)
- A distance between > 5 and ≤ 12 SNPs or alleles defined strains that are genetically related (interpretation case by case was required)
- With a distance above 12 SNPs or alleles the two strains were not regarded closely related (reinfection)

Inconsistency between SNP and allele distance was evaluated case by case before DBL.

The SNP and allele distances were summarised by recurrence and overall, including mean (SD), median, quartiles and minimum and maximum values. SNP and allele distances were categorised using the above thresholds and summarised. TB strain information including WGS distance and tNGS lineage was presented in listings.

TB recurrence was summarised by the subgroups of TB relapse and TB reinfection or undetermined.

9.7.1.4 Exploratory efficacy endpoint methodology

The three exploratory efficacy endpoints (defined in the protocol) are shown in Section 8.6.

These were all linked to the primary efficacy endpoint. For further details, please refer to the SAP, Appendix 16.1.9.

9.7.1.5 Immunogenicity endpoints methodology

The secondary immunogenicity endpoints were:

- Antigen specific cell mediated immune responses by WB ICS

Where, the percentages of T cell responses were presented in summary tables and box plots including median, quartiles and range by treatment and visit including change from V3= Day 0 to V6= Day 70. The immunogenicity analysis set was used.

- Humoral immune responses by IgG ELISA

Where summaries included immune response at V3= Day 0 and V6= Day 70, as well as fold increase from V3 to V6. Antibody titres were presented in a plot by treatment and time.
The immunogenicity analysis set was used.

For further details, please refer to the SAP, Appendix 16.1.9.

9.7.1.6 Safety endpoint methodology

AEs were regarded as treatment emergent AEs (TEAEs) if they occur after the 1st vaccination.

Solicited injection site reactions were considered related to the IP regardless of the causality assessment by the investigator. Therefore, related events consisted of both solicited events and investigator defined related events. Related AEs were defined as adverse reactions.

Presentations of SAEs included medically important AEs.

An overall summary table of TEAEs by treatment was presented, including number of events, number of participants and proportion of participants in the safety analysis set reporting TEAEs (including solicited AEs), solicited AEs, AESIs, treatment emergent SAEs, fatal SAEs, related AEs, related SAEs, suspected unexpected serious adverse reactions (SUSARs), AEs leading to discontinuation of IP, AEs leading to discontinuation of trial, TEAEs by severity and TEAEs by outcome. The same was repeated for AEs occurring within 14 days after vaccination, SAEs, AESIs, solicited AEs and solicited SAEs occurring within the 7 days after each vaccination and unsolicited AEs and SAEs occurring within the first 14 days after each vaccination.

Treatment emergent AEs, AEs within 14 days after vaccination, solicited AEs and solicited SAEs occurring within the 7 days after each vaccination and unsolicited AEs and SAEs occurring within the first 14 days after each vaccination were summarised by SOC and PT. The summaries included number of events, number of participants and proportion of participants reporting these events and were tabulated by treatment. Additional summaries by SOC and PT presented number of events, and number and proportion of participants with AEs within 14 days after vaccination by severity, by causality, by age group (18-35 years, 36 years ->) and by sex.

The number of participants with solicited injection site reactions were presented in a plot by type of reaction and maximum severity. The number of participants with solicited systemic events were presented in a plot by PT and maximum severity.

AESIs were defined as either an AESI marked by the investigator or as AESI as per the protocol, Appendix B. AESIs will be summarised by SOC and PT, including number of events, number of participants and proportion of participants reporting these events by treatment arm. Similar summaries were made for SAEs including medically important events.

For specific details on statistical methodology for injection site reactions, temperature reactions and laboratory safety data, please refer to the SAP, Appendix 16.1.9.

9.7.1.7 Handling of missing values

For the primary efficacy endpoint missing values were handled using censoring. The primary endpoint was analysed based on the mITT which included all randomised participants except those with TB disease recurrence before V6= Day 70 (or 14 days after 2nd dose for those who received both vaccinations). Participants without TB disease recurrence confirmed by of *Mtb* by culture of

sputum samples were censored. Asymptomatic participants not capable of producing sputum were censored as well. No imputation of missing values was performed. For further details on predefined censoring events, please refer to Table 2 in the SAP, Appendix 16.1.9.

Partial TB diagnosis and TB treatment start dates were handled as follows: If day was missing, the day was set to the 1st of the month. If the month was missing the month was set to January. If the year was missing the date was set to missing.

Partial TB treatment end dates were handled as follows: If day was missing, the day was set to the last day of the month. If the month was missing, the month was set to six months after the start month.

Partial AE start dates were handled as follows: If day is missing then the day was set to the first day of the month unless a vaccination occurred in the same month. In that case day was set to the day of vaccination. If month was missing the month was set to the first month of the year unless at least one vaccination occurred that year. In that case the month was set to the month of the first vaccination of the year. If the AE start date was missing the date was set to the date of the first vaccination.

Partial AE end dates were handled as follows: If the day was missing, the day was set to the last day of the month. If month was missing, the month was set to the last month of the year. If the AE end date was missing the AE was considered ongoing at end of trial and the date was not imputed.

Partial dates of general concomitant medication and medical history were not imputed.

9.7.1.8 Multiplicity adjustments

The primary analysis of the primary endpoint was based on the mITT analysis set. Analysis of the primary endpoint in other analysis sets were considered sensitivity analyses and adjustment for multiplicity was not necessary.

9.7.1.9 Multicentre trial adjustment for centre effects

As it was a multicentre trial, adjustment for centre effects for the statistical analyses was performed.

9.7.1.10 Interim analysis and data monitoring

There was no interim analysis of unblinded data, formal or informal, pre-planned or ad-hoc in this trial. An IDRC reviewed blinded safety data from the safety cohort at two occasions (as per the protocol); 14 days after the first 150 randomised participants in the safety cohort had received the 1st vaccination and 14 days after they had received the 2nd vaccination. For further details on IDRC members and procedures, please refer to the protocol, Appendix 16.1.1. The IDRC charter is available in the TMF.

9.7.2 Determination of sample size

The sample size for this trial was 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 trial 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) was 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 trial Day 70 follow-up period for each participant.

9.8 Changes in the conduct of the trial or planned analysis

The Covid-19 pandemic had implications on the trial conduct. The main impacts were delays in recruitment and visit windows being exceeded during the periods where the sites had to close for trial visits and activities. The two protocol amendments, FV 4.0 and FV 5.0, were implemented, as mitigation measures.

- FV 4.0, details on SARS-Cov-2 vaccines receipt were added to exclusion criterion #12 (allowing SARS-Cov-2 vaccines, if administered > 28 days prior to the day of the 1st injection of IP [V3; Day 0], or on this day and onwards),
- FV 5.0, the visit window for the 2nd injection of IP (V5; Day 56) was extended from +10 days to +2 months and the two screening visits (V1 & V2) were combined into one visit. This resulted in approximately 6 months extra recruiting time, which allowed for recruiting of more participants, before recruitment had to be stopped, due to the expiry date of the IP (H56:IC31®).

During the trial, a number of sputum samples were analysed by culture, where this was not indicated. For further details on this protocol deviation, please see Section 10.2.2.

To specifically address the possible individual effects (on the primary efficacy endpoint analysis) of 'the extended window of the 2nd injection of IP' and 'the sputum samples that were analysed by culture where this was not indicated', two extra sensitivity analyses were introduced in the SAP, Appendix 16.1.9. For further details, please see Section 9.7.1.2.

The following changes to the protocol (FV 5.0), Appendix 16.1.1, were included in the SAP, Appendix 16.1.9:

Section 7.1 in the protocol states that the PP analysis set will consist of all participants who received both doses of H56:IC31® or placebo within the specified dose intervals, and 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 SAP)'.

In the SAP, Appendix 16.1.9, it was defined for the PP analysis set as regards protocol deviations, that the PP analysis set would include participants; 'who had no major protocol deviations of clinical or statistical significance'.

Section 7.3.1 in the protocol states that supportive robustness and consistency analysis would be performed using the PP and ITT populations for the primary endpoint. Since

- the only difference between the mITT and the ITT population is that the mITT will include all randomised participants except those with TB disease recurrence before V6= Day 70
- the primary endpoint only includes TBs 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 mITT and ITT analyses would, therefore, be identical, which is the reason why, the ITT analyses were updated to be conducted with the primary endpoint, during the period starting 'at the time of the 1st vaccination (V3= Day 0)' and ending 12 months after the 2nd vaccination (V8= Day 421).

Section 7.3.1 in the protocol states that summaries of the median time to initial diagnosis of TB disease recurrence will be presented by treatment group and trial site.

Due to the expected low number of recurrent TB cases, it would not be possible to estimate the median time to initial diagnosis which was therefore not included in the SAP. The endpoint contains censored events and a non-parametric summary of time to TB disease recurrence is not appropriate.

The trial was designed for the primary endpoint to be evaluated using a one-side α-level of 10%, therefore a one-sided log-rank p-value and 80% vaccine efficacy (VE) profile confidence limits was planned to be presented for the primary endpoint. Two-sided p-values and 95% VE profile confidence intervals were planned to be presented as well.

As the trial ended up to have a result opposite of expected, the higher p-value was presented which is the p-value of H56:IC31® reducing events compared to placebo instead.

10 Trial participants

10.1 Disposition of participants

The disposition of participants is shown in Table 9 and Figure 2.

Table 9: Disposition of participants

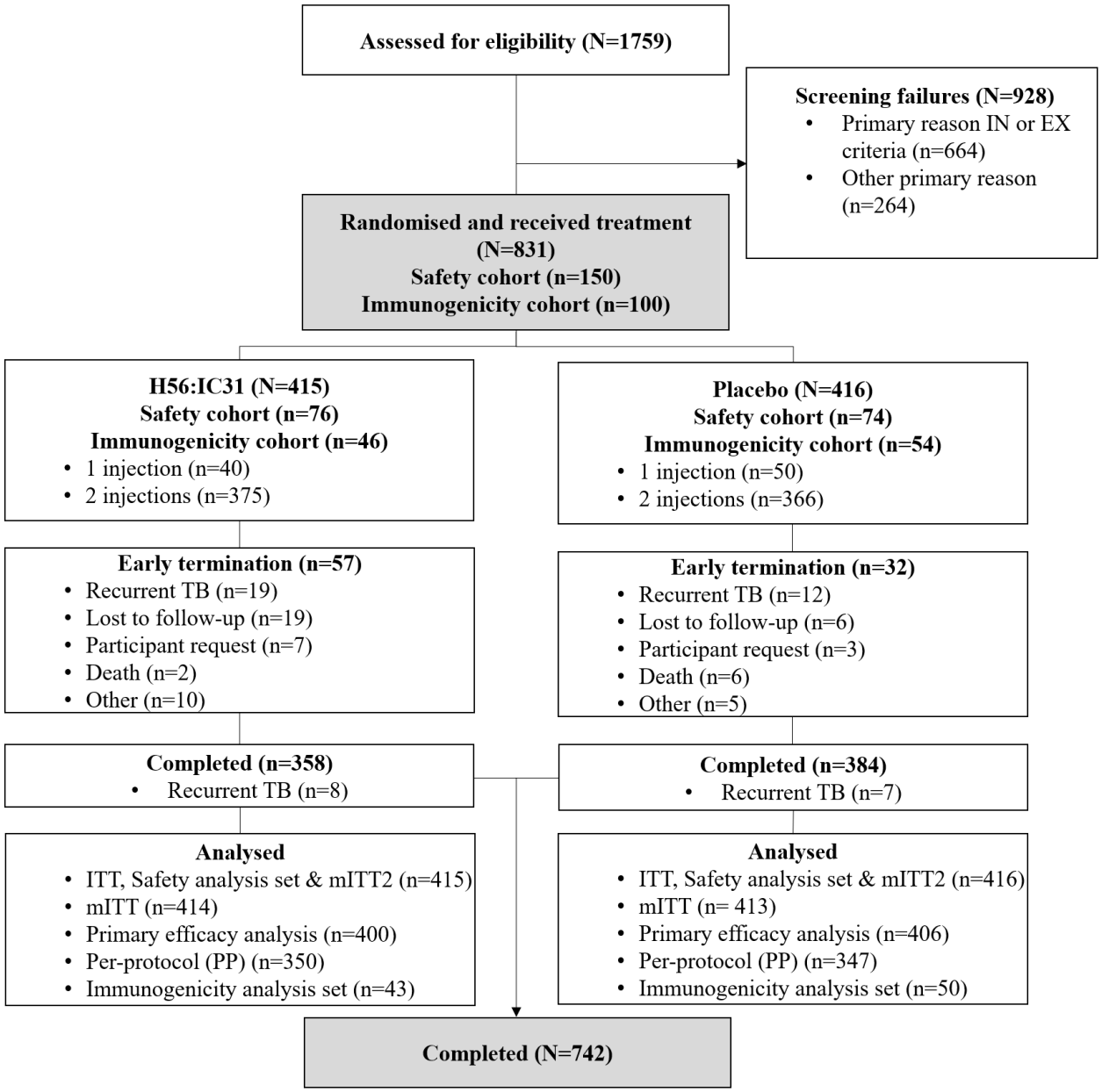
	H56:IC31® N (%)	Placebo N (%)	Total N (%)
Screened participants (N)			1759
Not assigned (N)			0
All randomised	415 (100.0)	416 (100.0)	831 (100.0)
ITT analysis set	415 (100.0)	416 (100.0)	831 (100.0)
Safety analysis set	415 (100.0)	416 (100.0)	831 (100.0)
Modified intention to treat (mITT)	414 (99.8)	413 (99.3)	827 (99.5)
Second mITT (mITT2)	415 (100.0)	416 (100.0)	831 (100.0)
Per protocol (PP)	350 (84.3)	347 (83.4)	697 (83.9)
Immunogenicity analysis set	43 (10.4)	50 (12.0)	93 (11.2)
Safety cohort	76 (18.3)	74 (17.8)	150 (18.1)
Immunogenicity cohort	46 (11.1)	54 (13.0)	100 (12.0)
End of trial			
Completed	358 (86.3)	384 (92.3)	742 (89.3)
With recurrent TB	8 (1.9)	7 (1.7)	15 (1.8)
Early termination	57 (13.7)	32 (7.7)	89 (10.7)
Total	415 (100.0)	416 (100.0)	831 (100.0)
Reason for early termination			
Recurrent TB	19 (33.3)	12 (37.5)	31 (34.8)
Death	2 (3.5)	6 (18.8)	8 (9.0)
Lost to follow-up	19 (33.3)	6 (18.8)	25 (28.1)
Withdrawal by participant	7 (12.3)	3 (9.4)	10 (11.2)
Other	10 (17.5)	5 (15.6)	15 (16.9)
Total	57 (100.0)	32 (100.0)	89 (100.0)

Page 1 of 1

N: Number of participants, %: Percentage of participants
Percentages are calculated relative to randomised treatment.
Trial: POR A-055
Program: t_ds.sas - output: t_ds.rtf - executed: 10NOV2023

Cross-reference: Table 14.1.1.1, Appendix 16.2.1

Figure 2: Disposition of participants



Sources: Tables 14.1.1.1 and 14.1.1.3, Appendix 16.2.1 and Listing 16.2.1.1, Appendix 16.2.3

This trial was initially planned with 2 screening visits approx. 5 months apart (V1 and V2). Due to delays in the screening of the participants, V1 and V2 were, however, after a protocol change, allowed to take place as one combined visit on the same date. This gave the sites 5 additional months for recruiting the participants. The randomisation was stopped, due to the expiry date of the IP, when 831 (of the planned 900) participants had been randomised.

V1 was completed by a total of 1759 participants, where V2 was completed by 1115 participants (including combined V1 and V2). 875 participants completed V3, where the eligibility for inclusion was finally assessed, and eligible participants were randomised and received their first injection.

For numbers and percentages of non-eligible participants for each individual in- and exclusion criterion, please refer to Table 14.1.1.3, Appendix 16.2.1.

The 5 most common inclusion criteria not met were: agreeing to stay in contact during the trial, inclusion criterion #9 (25.0%), being HIV negative, inclusion criterion #3 (4.3%), being able to provide 2 sputum samples, inclusion criterion #4 (4.1%), completing the informed consent process, inclusion criterion #1 (3.8%) and having complied to TB treatment requirements, inclusion criterion #7 (2.4%).

The 5 most common exclusion criteria met were: not being suitable for inclusion in the opinion of the investigator, exclusion criterion #17 (39.2%), not being willing to use acceptable method to avoid pregnancy, exclusion criterion #15 (5.4%), being immunodeficient, exclusion criterion #7 (4.7%), having a history of active TB within 5 years, exclusion criterion #3 (2.8%) and having severe disease, exclusion criterion #4 (2.6%).

A total of 928 participants were screening failures. For 664 the primary reason was given as non-eligibility according to in- or exclusion criteria, and for 264 the primary reason was given as ‘other’ (most commonly Covid-19 related impact on the trial conduct, participant withdrawal of informed consent or lost to follow-up), please refer to Listing 16.2.1.1, Appendix 16.2.3.

In total 831 participants were randomised to receive H56:IC31® (415) or placebo (416). All 831 participants received at least one injection, correctly as per the randomisation scheme, please refer to Listing 16.1.7.1, Appendix 16.2.3. As a result, the intention to treat (ITT) and the safety analysis sets both included all randomised participants.

There were 150 participants (from all sites, except for site A6, as site A6 was initiated after the inclusion of the first 150 participants) in the safety cohort; 76 received H56:IC31® and 74 received placebo, and 100 participants (from only sites A1 and A5) in the immunogenicity cohort; 46 received H56:IC31® and 54 received placebo. The participants were allocated to one, both or none of these two cohorts, at the time of randomisation, please see Table 9 and Figure 2.

In total 89.3% (742/831) of the randomised and vaccinated participants completed the trial; 86.3% (358/415) in the H56:IC31® group and 92.3% (384/416) in the placebo group, please see Table 9 and Figure 2.

For 10.7% (89/831) of the participants, trial participation was terminated early; 13.7% (57/415) in the H56:IC31® group and 7.7% (32/416) in the placebo group. Apart from recurrent TB, reasons for early termination were, lost to follow-up, participant request, death, or 'other', please see Table 9 and Figure 2. For details on early termination by visit, please refer to Table 14.1.1.2, Appendix 16.2.1, and for details on reasons for withdrawal from the trial for the individual participants, please refer to Listing 16.2.1.3, Appendix 16.2.3.

Overall, a total of 46 participants either discontinued the trial early due to recurrent TB (31 participants [19 in the H56:IC31® group and 12 in the placebo group]) or completed the trial with recurrent TB (15 participants [8 in the H56:IC31® group and 7 in the placebo group]), please see Table 9 and Figure 2. 41 of these participants had recurrent TB according to the primary efficacy endpoint definition i.e., verified by culture.

Note that if participants started new TB treatment after being diagnosed with recurrent TB at a local clinic or otherwise (without prior culture verification of recurrent TB), the reason for discontinuation could be 'Recurrent TB' or 'Other' in the end of trial form in the eCRF. Such participants were included in the 1st exploratory efficacy endpoint. For details on these participants, please see Section 11.1.6.1.

For details on the 6 analysis sets; intention to treat (ITT), modified ITT (mITT), mITT2, per protocol (PP), safety analysis set and immunogenicity analysis set, please see Section 10.3.

10.2 Protocol deviations

The number and frequencies of participants with protocol deviations (PDs), classified as minor or major, per predefined category are shown in Table 10. A major PD could lead to the participant being excluded from the PP analysis set, according to the SAP, please refer to Appendix 16.1.9.

Table 10: Protocol deviations by classification and category – All randomised participants

	H56:IC31® N (%)	Placebo N (%)	Total N (%)
All randomised (N)	415	416	831
Protocol deviation			
Major	132 (31.8)	123 (29.6)	255 (30.7)
Other	86 (20.7)	80 (19.2)	166 (20.0)
Missed procedure(s)	28 (6.7)	33 (7.9)	61 (7.3)
Visit window	14 (3.4)	12 (2.9)	26 (3.1)
Eligibility criteria	12 (2.9)	13 (3.1)	25 (3.0)
Informed consent	12 (2.9)	2 (0.5)	14 (1.7)
Dosing deviation	2 (0.5)		2 (0.2)
Safety reporting	2 (0.5)		2 (0.2)
Specimen deviation	1 (0.2)	1 (0.2)	2 (0.2)
Minor	321 (77.3)	349 (83.9)	670 (80.6)
Other	186 (44.8)	206 (49.5)	392 (47.2)
Visit window	151 (36.4)	153 (36.8)	304 (36.6)
Specimen deviation	130 (31.3)	145 (34.9)	275 (33.1)
Missed procedure(s)	9 (2.2)	15 (3.6)	24 (2.9)
Dosing deviation	8 (1.9)	10 (2.4)	18 (2.2)
Informed consent	2 (0.5)	4 (1.0)	6 (0.7)
Eligibility criteria		1 (0.2)	1 (0.1)
Total	349 (84.1)	355 (85.3)	704 (84.7)
Other	218 (52.5)	222 (53.4)	440 (52.9)
Visit window	162 (39.0)	161 (38.7)	323 (38.9)
Specimen deviation	131 (31.6)	146 (35.1)	277 (33.3)
Missed procedure(s)	36 (8.7)	46 (11.1)	82 (9.9)
Eligibility criteria	12 (2.9)	14 (3.4)	26 (3.1)
Dosing deviation	10 (2.4)	10 (2.4)	20 (2.4)
Informed consent	14 (3.4)	6 (1.4)	20 (2.4)
Safety reporting	2 (0.5)		2 (0.2)

N: Number of participants, %: Percentage of participants
Trial: POR A-055
Program: t_pd.sas - output: t_pd.rtf - executed: 10NOV2023

Cross-reference: Table 14.1.1.5, Appendix 16.2.1

10.2.1 Overview of protocol deviations

In total 84.7% (704/831) of the participants had at least one PD; with no differences between the groups. Among these, 80.6% (670/831) had minor PDs (77.3% [321/415] of the participants in the H56:IC31® group and 83.9% [349/416] in the placebo group), whereas 30.7% (255/831) had a major PD (31.8% [132/415] of the participants in the H56:IC31® group and 29.6% [123/416] in the placebo group). Overall, the PDs were reported at similar frequencies in the two treatment groups.

The PD category 'other' had the highest frequency of participants; 52.9% (440/831), with at least one PD (minor or major). The 'visit window' and 'specimen deviation' categories had the next highest percentages of participants with at least one PD (major or minor), please see Table 10. Many of these PDs could be explained by the impact of the Covid-19 pandemic on the trial conduct. Overall, 19.5% (162/831) of the participants were affected by Covid-19 related PDs. For further details, please refer to Table 14.1.1.6, Appendix 16.2.1.

3% (25/831) of the participants had a major PD related to one or more eligibility criteria. Some of these participants were excluded from the PP, please see Table 10. For details of PDs for the individual participants by trial site, and if the PD led to exclusion from the PP, please refer to Listing 16.2.2.1, Appendix 16.2.3.

10.2.2 Protocol deviation – Visit 8 – *Mtb* culture testing of Xpert MTB/RIF Ultra negative participants

The PD category 'other' includes a PD that occurred at the BARC laboratory during a period in the trial, and affected in total 154 participants from the ZA trial sites. It was related to the procedures for recurrent TB case finding at the last trial visit, V8= Day 421. The protocol specified that if a sputum sample tested by Xpert MTB/RIF Ultra was *Mtb* positive, this sputum sample was to be sent for culture verification together with a second sputum sample. For the 154 participants affected by this PD, two sputum samples were sent for culture verification, disregarding the Xpert MTB/RIF Ultra result. This increased the probability of detecting a missed recurrent TB case among these participants compared to the participants where the protocol was followed.

If recurrent TB was not diagnosed, this PD was categorised as minor. If recurrent TB was diagnosed, and there were no TB signs and symptoms and the Xpert MTB/RIF Ultra was *Mtb* negative, it was categorised as major, and the participants were excluded from the PP analysis set (primary reason: cultured in error). This was the case for 2 participants (A20070, A30158) who had received H56:IC31® and 2 participants (A10091, A20066) who had received placebo. The reason for excluding these cases from the PP is that they would not have been identified, if the protocol had been followed, please see Table 11. Of note, 1 participant (A30445) was categorised minor in error (as recurrent TB was diagnosed), and therefore this PD should have been major and should, seen in retrospective, have led to exclusion of this participant from the PP. For details of PDs for the the individual participants by trial site, please refer to Listing 16.2.2.1, Appendix 16.2.3.

10.2.3 Protocol deviation – Visit 8 by telephone – Xpert MTB/RIF Ultra testing not performed

Another PD in the 'other' category was also related to Xpert MTB/RIF Ultra testing of participants at V8. For 12 participants, the V8 was performed telephonically, and therefore the Xpert MTB/RIF Ultra test was not performed, and these participants could therefore, potentially, be missed recurrent TB cases.

Due to this, this PD was classified as major, and the participants were excluded from the PP analysis set (primary reason: 'missing Xpert at V8'). 5 participants had received H56:IC31® and 7 participants had received placebo, please see Table 11.

10.3 Data sets analysed

The protocol and the SAP defined 6 analysis sets:

- Intention to treat (ITT) analysis set
- Safety analysis set
- Modified ITT (mITT) analysis set
- Second modified (mITT2) analysis set
- Per protocol (PP) analysis set
- Immunogenicity analysis set

All randomised participants were included in the ITT analysis set.

The safety analysis set consists of all participants who received at least one injection of the IP, where the participants were evaluated according to the treatment actually received (H56:IC31® or placebo). As all participants received the treatment, all randomised participants were included in the safety analysis set.

The mITT analysis set includes all randomised participants except for those with TB disease recurrence before V6= Day 70 (or 14 days after the 2nd injection for those who received both injections). Participants were evaluated as randomised. The mITT was the primary analysis set for the primary and secondary efficacy endpoints.

The mITT2 analysis set includes all randomised participants except those with TB disease recurrence before Day 30 (or 30 days after the 1st injection). Participants were evaluated as randomised. The mITT2 was the analysis set for the 3rd exploratory efficacy endpoint.

4 participants were excluded from the mITT due to recurrent TB before V6= Day 70 (1 participant in the H56:IC31® group [A50184] and 3 participants in the placebo group [A20243, A40148, and

A50157]). These 4 participants were, however, all included in the mITT2 analysis set (for exploratory efficacy analysis), as the recurrent TB was diagnosed after Day 30. Thus, all randomised participants were included in the mITT2 analysis set, please refer to Listing 16.2.1.2, Appendix 16.2.3.

The PP analysis set consisted of all participants who received both doses of H56:IC31® or placebo within the specified time interval, who entered the evaluation period for efficacy 14 days after receipt of the 2nd dose of H56:IC31® or placebo with no HIV seroconversion, and who had no major PD of clinical or statistical significance, as defined in Section 3.1 in the SAP, Appendix 16.1.9. Participants were evaluated as treated.

The reasons for excluding 16.1% (134/831) of the participants from the PP analysis set; 15.7% (65/415) from the H56:IC31® group and 16.6% (69/416) from the placebo group, are shown in Table 11. The most frequent reason was that the 2nd injection had not been administered; 9.6% (40/415) in the H56:IC31® group and 12.0% (50/416) in the placebo group, followed by TB treatment error; 3.4% (14/415) in the H56:IC31® group and 1.7% (7/416) in the placebo group and 'missing Xpert at V8'; 1.2% (5/415) in the H56:IC31® group and 1.7% (7/416) in the placebo group.

Table 11: Exclusion from PP analysis set – All randomised participants

	H56:IC31® N (%)	Placebo N (%)	Total N (%)
All randomised (N)	415	416	831
PP analysis set	350 (84.3)	347 (83.4)	697 (83.9)
Reason for exclusion from PP analysis set*			
No 2nd IMP	40 (9.6)	50 (12.0)	90 (10.8)
TB treatment error	14 (3.4)	7 (1.7)	21 (2.5)
Missing Xpert at V8	5 (1.2)	7 (1.7)	12 (1.4)
Prohibited medication	1 (0.2)	4 (1.0)	5 (0.6)
Cultured in error	2 (0.5)	2 (0.5)	4 (0.5)
Withdrew before evaluation period for efficacy	3 (0.7)	1 (0.2)	4 (0.5)
2nd IMP OOW	2 (0.5)	1 (0.2)	3 (0.4)
HIV seroconversion	1 (0.2)		1 (0.1)
Unable to re-confirm TB		1 (0.2)	1 (0.1)
Total	65 (15.7)	69 (16.6)	134 (16.1)

Page 1 of 1

N: Number of participants, %: Percentage of participants
** One participant can be present with more than one reason*
Trial: POR A-055
Program: t_ppexcl.sas - output: t_ppexcl.rtf - executed: 10NOV2023
Cross-reference: Table 14.1.1.4, Appendix 16.2.1

The immunogenicity analysis set consisted of participants from the mITT population who received both doses of H56:IC31® or placebo and who were included in the immunogenicity cohort. Participants were evaluated as randomised.

For details on the disposition of participants in the 2 cohorts and 6 analysis sets by treatment group, please see Table 9 and Figure 2 and for the disposition of the individual participants, please refer to Listing 16.2.1.2, Appendix 16.2.3.

10.4 Demographics and other baseline characteristics

10.4.1 Demography

The key demographics and baseline characteristics of the 831 participants in the ITT analysis set are shown overall and by treatment group in Table 12. Overall, there were no notable demographic differences between the two treatment groups. The mean age of the participants was 34.7 years, distributed with 57.8% (480/831) in the younger age group (18-35 years) and 42.2% (351/831) in the older age group (36-60 years). There were fewer females than males in the trial; 27.6% (229/831) females and 72.4% (602/831) males. Most participants were black (66.1% [549/831]) followed by participants of mixed Cape ancestry (33.0% [274/831]). The majority, 76.2% (633/831) of the participants were residents in ZA, with the remaining 23.8% (198/831) being TZ residents.

Other demographics and baseline characteristics are shown in Table 13. Overall, there were no notable differences between the two treatment groups as regards these characteristics, including height, weight, body mass index (BMI) and smoking status at baseline. The baseline weight and BMI were low, as expected, in this population of successfully treated TB patients. The mean weight (incl. females and males) at baseline was 58.39 kg, and the mean BMI was 21.11 kg/m^2, distributed with 88.9% (739/831) in the low BMI group (13-25 kg/m^2) and 11.1% (92/831) in the high BMI group (≥25 kg/m^2). The lowest recorded BMI at baseline was 14.4 kg/m^2 where the highest was 42.3 kg/m^2 (according to exclusion criterion #14, participants with a BMI < 13 kg/m^2 on the date of V1 had to be excluded from the trial). For further details, please refer to Listing 16.2.4.1, Appendix 16.2.3.

The sites with the highest recruiting rates were A3 (TASK) in Cape Town, ZA and A5 (MMRC) in Mbeya, TZ. Together these two sites randomised and included approx. 50% of all participants; A3 included 229 (27.6%) and A5 included 198 (23.8%). The A1 (SATVI) site included 108 (13.0%) and A2 (UCTLI) included 139 (16.7%) of the participants, both in Cape Town, ZA, and A4 (Aurum, Klerksdorp) included 91 (11.0%) of the participants, please see Table 13. The A6 (Aurum, Tembisa) site included 66 (7.9%) of the participants, the lower number was partly due to the fact that this site was initiated later than the other 5 sites.

Table 12: Key demographics and baseline characteristics – ITT analysis set

	H56:IC31® N (%)	Placebo N (%)	Total N (%)
ITT analysis set (N)	415	416	831
Age (years)			
N	415	416	831
Mean (SD)	34.3 (11.2)	35.1 (11.0)	34.7 (11.1)
Median	33.0	34.0	33.0
q25 - q75	25.0 - 43.0	26.0 - 45.0	25.0 - 44.0
Min - Max	18 - 60	18 - 59	18 - 60
Age group (N,%)			
18-35 years	247 (59.5)	233 (56.0)	480 (57.8)
36-60 years	168 (40.5)	183 (44.0)	351 (42.2)
Total	415 (100.0)	416 (100.0)	831 (100.0)
Sex (N,%)			
Female	112 (27.0)	117 (28.1)	229 (27.6)
Male	303 (73.0)	299 (71.9)	602 (72.4)
Total	415 (100.0)	416 (100.0)	831 (100.0)
Race (N,%)			
Black or african american	276 (66.5)	273 (65.6)	549 (66.1)
Mixed cape ancestry	132 (31.8)	142 (34.1)	274 (33.0)
Other	7 (1.7)	1 (0.2)	8 (1.0)
Total	415 (100.0)	416 (100.0)	831 (100.0)
Country (N,%)			
South Africa	323 (77.8)	310 (74.5)	633 (76.2)
Tanzania	92 (22.2)	106 (25.5)	198 (23.8)
Total	415 (100.0)	416 (100.0)	831 (100.0)

N: Number of participants, %: Percentage of participants, SD: Standard deviation, q25: Lower quartile, q75: Upper quartile
Trial: POR A-055
Program: t_dm.sas - output: t_dm_itt.rtf - executed: 10NOV2023

Cross-reference: Table 14.1.2.1, Appendix 16.2.1

Table 13: Other demographics and baseline characteristics – ITT analysis set

	H56:IC31® N (%)	Placebo N (%)	Total N (%)
ITT analysis set (N)	415	416	831
Height (cm)			
N	415	416	831
Mean (SD)	166.52 (8.85)	166.22 (8.94)	166.37 (8.89)
Median	166.80	167.00	167.00
q25 - q75	159.50 - 173.00	160.00 - 172.50	159.80 - 173.00
Min - Max	144.5 - 191.0	142.0 - 193.2	142.0 - 193.2
Weight (kg)			
N	415	416	831
Mean (SD)	58.67 (10.01)	58.12 (10.92)	58.39 (10.47)
Median	57.70	56.95	57.30
q25 - q75	51.90 - 65.10	50.45 - 63.25	51.10 - 64.50
Min - Max	35.9 - 100.9	35.0 - 104.3	35.0 - 104.3
BMI (kg/m^2)			
N	415	416	831
Mean (SD)	21.17 (3.41)	21.04 (3.80)	21.11 (3.61)
Median	20.65	20.17	20.43
q25 - q75	18.89 - 22.57	18.60 - 22.48	18.74 - 22.56
Min - Max	14.4 - 37.3	14.7 - 42.3	14.4 - 42.3
BMI group (N,%)			
13 to 25 kg/m^2	374 (90.1)	365 (87.7)	739 (88.9)
25 kg/m^2 and above	41 (9.9)	51 (12.3)	92 (11.1)
Total	415 (100.0)	416 (100.0)	831 (100.0)
Smoking status (N,%)			
Non-smoker	149 (35.9)	175 (42.1)	324 (39.0)
Smoker	205 (49.4)	180 (43.3)	385 (46.3)
Ex-smoker	61 (14.7)	61 (14.7)	122 (14.7)
Total	415 (100.0)	416 (100.0)	831 (100.0)
Site (N,%)			
A1	47 (11.3)	61 (14.7)	108 (13.0)
A2	69 (16.6)	70 (16.8)	139 (16.7)
A3	128 (30.8)	101 (24.3)	229 (27.6)
A4	46 (11.1)	45 (10.8)	91 (11.0)
A5	92 (22.2)	106 (25.5)	198 (23.8)
A6	33 (8.0)	33 (7.9)	66 (7.9)
Total	415 (100.0)	416 (100.0)	831 (100.0)

	H56:IC31® N (%)	Placebo N (%)	Total N (%)

Page 2 of 2

N: Number of participants, %: Percentage of participants, SD: Standard deviation, q25: Lower quartile, q75: Upper quartile
BMI: Body mass index
Trial: POR A-055
Program: t_base.sas - output: t_base_itt.rtf - executed: 10NOV2023

Cross-reference: Table 14.1.2.2, Appendix 16.2.1

10.5 Baseline disease and comorbidity characteristics

The comorbidity terms (history of chronic or intermittent diseases, medical conditions or symptoms, that could, potentially, have an impact on the primary endpoint in the trial), as assessed by the sponsor's medically responsible, were predefined in Section 4.4 in the SAP, Appendix 16.1.9.

The frequencies of participants with anaemia and diabetes mellitus at baseline are shown in Table 14. Overall, there were no notable differences between the two treatment groups for these conditions or diseases. 9.4% (78/831) had anaemia at baseline, and 4.2% (35/831) had diabetes mellitus. The frequency of participants with any comorbidity at baseline was 28.2% (234/831), with no differences between the groups. For a tabular overview of comorbidities by SOC and PT, please refer to Table 14.1.3.2, Appendix 16.2.1 and for individual comorbidity data, please refer to Listing 16.2.4.2, Appendix 16.2.3.

Table 14: Additional baseline characteristics – ITT analysis set

	H56:IC31® N (%)	Placebo N (%)	Total N (%)
ITT analysis set (N)	415	416	831
Anaemia			
Yes	37 (8.9)	41 (9.9)	78 (9.4)
No	378 (91.1)	375 (90.1)	753 (90.6)
Total	415 (100.0)	416 (100.0)	831 (100.0)
Diabetes mellitus			
Yes	17 (4.1)	18 (4.3)	35 (4.2)
No	398 (95.9)	398 (95.7)	796 (95.8)
Total	415 (100.0)	416 (100.0)	831 (100.0)
Comorbidity			
Yes	120 (28.9)	114 (27.4)	234 (28.2)
No	295 (71.1)	302 (72.6)	597 (71.8)
Total	415 (100.0)	416 (100.0)	831 (100.0)

Page 1 of 1

N: Number of participants, %: Percentage of participants
Trial: POR A-055
Program: t_base_subgr.sas - output: t_base_subgr.rtf - executed: 10NOV2023

Cross-reference: Table 14.1.2.3, Appendix 16.2.1

10.6 Medical and surgical history

Overall, there were no notable differences between the groups as regards the medical and surgical history records, except for a few diseases/conditions, highlighted in the following.

The participants most frequently had a medical history within the system organ class (SOC) of infections and infestations; 10.6% (44/415) in the H56:IC31® group and 8.7% (36/416) in the placebo group, with the highest frequency in the preferred term (PT) of urinary tract infections; 2.2% (9/415) in the H56:IC31® group and 1.0% (4/416) in the placebo group.

Overall, the most frequently reported medical history was hypertension; 7.5% (31/415) in the H56:IC31® group and 8.7% (36/416) in the placebo group.

A medical history of arthralgia seemed to occur at a lower frequency in the H56:IC31® group (0.2% [1/415]) than in the placebo group (3.1% [13/416]), where the opposite was the case for neutropenia which occurred more frequently in H56:IC31® participants (3.4% [14/415]) than in placebo participants (1.2% [5/416]).

Increased gamma glutamyl transferase (GGT) was recorded as medical history for a similar proportion of participants in the in the two groups; 2.2% (9/415) in the H56:IC31® group and 2.9% (12/416) in the placebo group. This is not unexpected, as it is well-known that some of the anti-TB drugs, e.g., isoniazid and rifampicin, may be associated with hepatotoxicity and elevated GGT levels.

For a tabular overview of the medical and surgical history, by SOC and PT, please refer to Table 14.1.3.1, Appendix 16.2.1, and for details on the medical history for the individual participants, please refer to Listing 16.2.4.4, Appendix 16.2.3.

10.6.1 Prior and concomitant medication and new TB treatment

10.6.1.1 TB treatment prior to the first injection of IP

The trial population was participants successfully treated for drug-susceptible pulmonary TB, i.e., tested negative for acid fast bacilli (AFB) after at least 22 weeks of TB treatment, please see inclusion criterion #5, Section 9.3.1.

The participants were not eligible for inclusion in the trial, if the TB treatment was extended beyond 28 weeks, or if the TB treatment was no longer ongoing at the time of randomisation / first administration of IP, please see inclusion criterion #7, Section 9.3.1.

The TB treatment had to be according to the national recommendations in ZA or TZ. Overall, the anti-TB drugs in use, in both ZA and TZ, were isoniazid (INH) and rifampicin (RIF) for a standard treatment period of 24 weeks (up to 28 weeks), with addition of ethambutol (EMB) and pyrazinamide (PZA) during the first 8 weeks of treatment.

All participants, had received the 4 recommended anti-TB drugs as treatment for their current episode of drug-susceptible pulmonary TB. In addition, the majority of the participants also received pyridoxine, pyridoxine hydrochloride or Vitamin B complex; 72.5% (301/415) in the H56:IC31® group and 69.2% (288/416) in the placebo group, please refer to Table 14.1.3.4, Appendix 16.2.1. For the TB diagnosis dates and TB treatments administered to each participant, please refer to Listing 16.2.4.3, Appendix 16.2.3.

In Table 15 it is shown that the median time between the diagnosis of TB until the second screening visit (V2), where the AFB test (for successful TB treatment) was performed, was 23 weeks for both treatment groups, whereas the median duration of the TB treatment for the current episode was 24 weeks.

The median time between confirmation of successful TB treatment at V2 and the first IP administration at V3 was 10 days for both treatment groups, where the TB treatment stopped 7 days (H56:IC31® group) or 8 days (placebo group) later, please see Table 15.

10.6.1.2 General concomitant medication prior to and during the trial

Relevant prior CM was recorded as part of the medical history evaluations at baseline (V1, V2 and V3). After randomisation and the first administration of IP at V3, CMs were recorded in the participant diaries, reviewed by site staff at V4 and V6, and transferred to the eCRF. After V6, CMs were only recorded, if taken as treatment for AESIs or SAEs. For the summary schedule of investigational events, please see Table 6.

Overall, 49.6% (206/415) of the participants in the H56:IC31® group and 53.4% (222/416) in the placebo group received CM. There were no notable differences between the treatment groups.

The most frequent CMs administered were paracetamol; 16.6% (69/415) in the H56:IC31® group and 20.0% (83/416) in the placebo group, and acetylsalicylic acid, combined with caffeine and paracetamol; 5.1% (21/415) in the H56:IC31® group and 7.2% (30/416) in the placebo group.

1.0% (4/415) in the H56:IC31® group and 0.7% (3/416) in the placebo group received glucocorticoids, where ‘corticosteroids, weak (group I)’ was received by 1.0% (4/415) in the H56:IC31® group and 0.2% (1/416) in the placebo group. Moreover, 1 participant in the placebo group received ‘corticosteroids, potent (group III)’, 1 participant in the H56:IC31® group received ‘corticosteroids’ and, finally, 1 participant in the placebo group received ‘corticosteroids, plain’.

2 participants received prohibited medication. 1 participant (A20225) received a Covid-19 vaccination, and 1 participant (A40090) received glucocorticoids (prednisolone), both were excluded from the PP population, please refer to Listing 16.2.4.7, Appendix 16.2.3.

For a tabular presentation of all CMs coded by WHO Drug, please refer to Table 14.1.3.3, Appendix 16.2.1. For details of each participant, please refer to Listing 16.2.4.5, Appendix 16.2.3.

Table 15: TB diagnosis and treatment history – ITT analysis set

	H56:IC31® N (%)	Placebo N (%)
ITT analysis set (N)	415	416
Time from TB diagnosis to screening visit 2 (weeks)		
N	414	416
Mean (SD)	23.31 (1.32)	25.50 (46.03)
Median	23.00	23.00
q25 - q75	22.57 - 23.86	22.57 - 23.71
Min - Max	21.9 - 40.0	22.0 - 961.9
Duration of TB treatment for current episode (weeks)		
N	415	416
Mean (SD)	25.26 (3.70)	24.93 (1.88)
Median	24.14	24.29
q25 - q75	24.00 - 25.71	24.00 - 25.86
Min - Max	23.1 - 83.9	6.9 - 37.1
Time from completion of 22 weeks of TB treatment to 1st vaccination (days)		
N	415	413
Mean (SD)	11.00 (6.58)	10.60 (5.88)
Median	10.00	10.00
q25 - q75	7.00 - 14.00	7.00 - 13.00
Min - Max	-3.0 - 50.0	0.0 - 40.0
Time from 1st vaccination to TB treatment stop date (days)		
N	415	416
Mean (SD)	11.35 (25.62)	9.87 (17.93)
Median	7.00	8.00
q25 - q75	3.00 - 12.00	4.00 - 13.00
Min - Max	0.0 - 426.0	-112.0 - 261.0

Page 1 of 1

N: Number of participants, %: Percentage of participants, SD: Standard deviation, q25: Lower quartile, q75: Upper quartile
Trial: POR A-055
Program: t_th.sas - output: t_th.rtf - executed: 10NOV2023

Cross-reference: Modified from Table 14.1.3.4, Appendix 16.2.1

10.6.1.3 New TB treatment during the trial

New TB treatment was recorded as CM for 14 participants, during the trial, please refer to Listing 16.2.4.6, Appendix 16.2.3. Some of these participants had been prescribed the new TB treatment outside of the trial (at a local clinic) or within the trial due to recurrent TB, without verification of the recurrent TB as per the trial protocol, and therefore were only eligible for inclusion in the 1st exploratory efficacy analysis, please see Section 11.1.6.1.

2 participants had received the TB treatment with different diagnosis than recurrent TB; cor pulmonale (A10047) and non-TB mycobacterial infection (A20008) and 1 participant received only pyridoxine, vitamin B6 (A40071) with no indication.

3 participants who received the new TB treatment did have culture verified recurrent TB according to the protocol definition (A30102, A40069 and A60074) and were therefore included in the primary efficacy analysis, please refer to Listing 16.2.4.6, Appendix 16.2.3.

10.7 Measurements of treatment compliance

As this trial is a vaccine trial where the IP is injected by the site staff during the site visits, measurement of participant treatment compliance is not applicable.

10.8 Extent of exposure

The dose level of antigen and adjuvant per vaccine dose for the investigational test product was the same for all participants, namely H56 antigen (5 mcg / 0.5 mL) and IC31® adjuvant (500 nmol / 0.5 mL). For details on the antigen and adjuvant, please see Section 9.4.

For all participants the vaccination regimen was 2 injections with 56 days interval. However, 9.6% (40/415) of the participants in the H56:IC31® group and 12.0% (50/416) of the participants in the placebo group received only 1 dose (injection), please see Table 16.

The extent of exposure among the participants is shown in Table 16. For details on exposure of each participant, with indications of time between 1st and 2nd injection, injection dates, examinations of injection sites (Yes or No), immediate injection site AEs (Yes or No) and reason for vaccination not done, if applicable, please refer to Listing 16.2.5.1, Appendix 16.2.3.

Among the group of participants who received 2 injections, > 90% received the 2 doses 46 to 66 days apart (the window specified in the original protocol); 93.1% (349/375) in the H56:IC31® group and 94.0% (344/366) in the placebo group, please see Table 16.

Table 16: Exposure – Safety analysis set

	H56:IC31® N (%)	Placebo N (%)
Safety analysis set (N)	415	416
Number of vaccinations		
1	40 (9.6)	50 (12.0)
2	375 (90.4)	366 (88.0)
Total	415 (100.0)	416 (100.0)
Time from 1st to 2nd vaccination		
<46 days	2 (0.5)	1 (0.3)
46-66 days	349 (93.1)	344 (94.0)
>66 days	24 (6.4)	21 (5.7)
Total	375 (100.0)	366 (100.0)
Time from 2nd vaccination to end of trial		
28-91 days	6 (1.6)	1 (0.3)
92-182 days	9 (2.4)	2 (0.5)
183-273 days	9 (2.4)	4 (1.1)
>273 days	351 (93.6)	359 (98.1)
Total	375 (100.0)	366 (100.0)
Trial duration* (days)		
N	415	416
Mean (SD)	404.7 (88.9)	415.4 (76.0)
Median	422.0	422.0
q25 - q75	417.0 - 427.0	420.0 - 427.0
Min - Max	1 - 912	18 - 817

Page 1 of 1

N: Number of participants, %: Percentage of participants, SD: Standard deviation, q25: Lower quartile, q75: Upper quartile
** Time from 1st vaccination to end of trial.*
Trial: POR A-055
Program: t_ex.sas - output: t_ex.rtf - executed: 10NOV2023

Cross-reference: Table 14.1.3.5, Appendix 16.2.1

The visit window for the 2nd IP injection at V5 (± 10 days) was changed to (- 10 days; + 2 months), for a subgroup of participants affected by the Covid-19 lock-down, please see Section 9.8. This was approved by the relevant ECs, and described in the SAP, Appendix 16.1.9. Overall, 6.4% (24/375) in the H56:IC31® group and 5.7% (21/366) in the placebo group received their 2nd injection at V5 > 66 days after the 1st injection at V3. The impact of this visit window extension on the primary efficacy analysis was analysed in two sensitivity analysis, please see Sections 11.1.4.2 and 11.1.4.3.

The participants stayed in the trial (from V3= Day 0 until their last visit or contact) for a mean duration of 404.7 days in the H56:IC31® group and 415.4 days in the placebo group.

11 Efficacy and other evaluations

11.1 Efficacy results

A summary of TB recurrence (primary efficacy endpoint), from Day 70, overall and by site, is shown in Table 17. Out of the sputum samples sent for culture, there were overall 19.5% (23/118) recurrent TB cases in the H56:IC31® group and 12.7% (14/110) in the placebo group. Recurrent TB was thus (unexpectedly) more frequent in H56:IC31® vaccinated participants than in placebo recipients. At the 2 highest recruiting sites, TASK, Cape Town, ZA (A3) and MMRC, Mbeya, TZ (A5), recurrent TB was twice as frequent in the H56:IC31® group than in the placebo group. For details on participants in the mITT analysis set, from Day 70, please refer to Listing 16.2.6.1, Appendix 16.2.3.

Table 17: Summary of primary efficacy endpoint – TB recurrence – Overall and by site – mITT analysis set (from Day 70)

	H56:IC31® N (%)	Placebo N (%)
mITT analysis set (N)	414	413
TB recurrence from day 70		
All sites		
Sputum samples sent for culture*	118 (100.0)	110 (100.0)
Recurrence	23 (19.5)	14 (12.7)
No recurrence	95 (80.5)	96 (87.3)
Site A1		
Sputum samples sent for culture*	13 (100.0)	16 (100.0)
Recurrence	2 (15.4)	3 (18.8)
No recurrence	11 (84.6)	13 (81.3)
Site A2		
Sputum samples sent for culture*	19 (100.0)	26 (100.0)
Recurrence	1 (5.3)	2 (7.7)
No recurrence	18 (94.7)	24 (92.3)
Site A3		
Sputum samples sent for culture*	50 (100.0)	30 (100.0)
Recurrence	10 (20.0)	3 (10.0)
No recurrence	40 (80.0)	27 (90.0)
Site A4		
Sputum samples sent for culture*	12 (100.0)	9 (100.0)
Recurrence	2 (16.7)	2 (22.2)
No recurrence	10 (83.3)	7 (77.8)

	H56:IC31® N (%)	Placebo N (%)
Site A5		
Sputum samples sent for culture*	20 (100.0)	20 (100.0)
Recurrence	5 (25.0)	2 (10.0)
No recurrence	15 (75.0)	18 (90.0)
Site A6		
Sputum samples sent for culture*	4 (100.0)	9 (100.0)
Recurrence	3 (75.0)	2 (22.2)
No recurrence	1 (25.0)	7 (77.8)

Page 2 of 2

N: Number of participants, %: Percentage of participants
** Number of participants with one or more samples.*
Day 70 is interpreted as 2nd vaccination + 14 days.
Trial: POR A-055
Program: t_sum_tbrecur.sas - output: t_sum_tbrecur.rtf - executed: 10NOV2023
Cross-reference: Table 14.2.1.1, Appendix 16.2.1

11.1.1 Primary efficacy endpoint – TB recurrence – mITT analysis set (from Day 70)

The TB recurrence (primary efficacy endpoint) incidence rates among H56:IC31® vaccinees and placebo recipients, as well as the relative risk of recurrent TB among H56:IC31® vaccinees relative to placebo recipients, both with 95% confidence intervals (CIs), overall and by site, are shown in Table 18.

Table 18: Incidence rate and relative risk – Primary efficacy endpoint – TB recurrence – Overall and by site – mITT analysis set (from Day 70)

	H56:IC31®	Placebo	Relative risk (95% CI)
mITT analysis set (N)	414	413	
Overall TB recurrence			
Number of participants contributing to analysis	400 (100.0)	406 (100.0)	
Number of recurrent cases	23 (5.8)	14 (3.4)	1.3 (0.7; 1.6)
Person years of follow-up	366	379	
TB recurrence incidence rate* (95% CI)	6.3 (4.0; 9.4)	3.7 (2.0; 6.2)	
Site A1			
Number of participants contributing to analysis	45 (100.0)	61 (100.0)	
Number of recurrent cases	2 (4.4)	3 (4.9)	0.9 (0.1; 2.0)
Person years of follow-up	40	56	
TB recurrence incidence rate* (95% CI)	5.0 (0.6; 18.2)	5.3 (1.1; 15.6)	

Page 1 of 2

	H56:IC31®	Placebo	Relative risk (95% CI)
Site A2			
Number of participants contributing to analysis	68 (100.0)	68 (100.0)	
Number of recurrent cases	1 (1.5)	2 (2.9)	0.7 (0.0; 1.8)
Person years of follow-up	63	61	
TB recurrence incidence rate* (95% CI)	1.6 (0.0; 8.9)	3.3 (0.4; 11.9)	
Site A3			
Number of participants contributing to analysis	119 (100.0)	97 (100.0)	
Number of recurrent cases	10 (8.4)	3 (3.1)	1.4 (0.6; 1.8)
Person years of follow-up	107	92	
TB recurrence incidence rate* (95% CI)	9.3 (4.5; 17.2)	3.3 (0.7; 9.5)	
Site A4			
Number of participants contributing to analysis	46 (100.0)	44 (100.0)	
Number of recurrent cases	2 (4.3)	2 (4.5)	1.0 (0.1; 1.9)
Person years of follow-up	43	40	
TB recurrence incidence rate* (95% CI)	4.7 (0.6; 16.9)	4.9 (0.6; 17.9)	
Site A5			
Number of participants contributing to analysis	90 (100.0)	104 (100.0)	
Number of recurrent cases	5 (5.6)	2 (1.9)	1.6 (0.3; 2.2)
Person years of follow-up	86	99	
TB recurrence incidence rate* (95% CI)	5.8 (1.9; 13.6)	2.0 (0.2; 7.3)	
Site A6			
Number of participants contributing to analysis	32 (100.0)	32 (100.0)	
Number of recurrent cases	3 (9.4)	2 (6.3)	1.2 (0.2; 2.1)
Person years of follow-up	28	30	
TB recurrence incidence rate* (95% CI)	10.6 (2.2; 30.9)	6.6 (0.8; 24.0)	

Page 2 of 2

N: Number of participants, CI: Confidence intervals
** Rate per 100 person years of follow-up.95% confidence intervals are estimated using the poisson distribution.*
Day 70 is interpreted as 2nd vaccination + 14 days.
Trial: POR A-055
Program: t_ana_rate.sas - output: t_ana_rate.rtf - executed: 10NOV2023

Cross-reference: Table 14.2.1.4, Appendix 16.2.1

Overall, the TB recurrence incidence rate in the H56:IC31® group was 6.3% compared to 3.7% in the placebo group. The relative risk of recurrent TB was 1.3 among the H56:IC31® vaccinees relative to the placebo recipients.

11.1.1.1 Primary efficacy analysis – One-sided log-rank test – Primary efficacy endpoint –TB recurrence – mITT analysis set (from Day 70)

The Kaplan-Meier estimator test was used for the primary efficacy analysis of time to recurrent TB (primary efficacy endpoint) in the mITT, please refer to the protocol, Appendix 16.1.1 and the SAP, Appendix 16.1.9. The primary efficacy objective of the trial was considered met, if H56:IC31® reduced the rate of TB recurrence compared to placebo and the p-value of the **one**-sided log-rank test was **below 0.10**.

As the primary efficacy analysis was performed in the mITT, recurrent TB cases that occurred before Day 70 (defined as the 2nd administration of IP + 14 days), per the mITT definition, were excluded from the analysis. There were 4 such recurrent TB cases. In addition, overall, 21 mITT participants (14 from the H56:IC31® group and 7 from the placebo group) did not contribute to the primary efficacy analysis, as they left the trial before reaching Day 70 (defined as the 2nd administration of IP + 14 days) for other reasons than recurrent TB. As a result, 400 participants from the H56:IC31® group and 406 from the placebo group contributed to the Kaplan-Meier primary efficacy analysis, please see Table 19 and Figure 3.

The frequencies of participants with recurrent TB in the two groups; 5.8% (23/400) in the H56:IC31® group and 3.4% (14/406) in the placebo group, resulted in a **one**-sided log-rank p-value of 0.9558 (above 0.10) for a lower frequency of recurrent TB in the H56:IC31® group than in the placebo group.

The overall conclusion of the primary efficacy analysis was thus that H56:IC31® did not lead to a statistically significant reduction in the rate of TB recurrence in comparison to placebo, please see Table 19.

The cumulative frequencies of recurrent TB in the H56:IC31® group (blue line) and in the placebo group (red line), with 80% CIs (dotted lines), are plotted against number of months from Day 70, i.e., Day 70= Month 0 in Figure 3.

It is seen from the figure that a higher number of recurrent TB cases were identified in the H56:IC31® group (blue line) between Month 4 and 6 (after Day 70), than in the placebo group (red line). From Month 6 and onwards, the blue and red line follow each other more closely, indicating that the recurrent TB cases from Month 6 to Month 12 seem to occur more equally distributed in the two groups.

Just before the end of the follow-up period at Month 12 (after Day 0) where all participants were censored, identification of a recurrent TB case contributes more to the cumulative frequency increase (from Month 11 only 342/400 are left at risk in the H56:IC31® group and 368/406 in the placebo group), which partly explains the steeper blue and red curve increases around the Month 12 time point. Another possible explanation is that at Month 12 the last site visit (V8) took place, where all participants were tested with Xpert MTB/RIF Ultra, disregarding if they had TB signs or symptoms or not, aiming at catching up possibly missed recurrent TB cases, please see Figure 3. For a Kaplan-Meier plot with 95% CIs, please refer to Figure 14.2.1.8, Appendix 16.2.2.

Table 19: Primary efficacy analysis – One-sided log-rank test – Primary efficacy endpoint – TB recurrence – mITT analysis set (from Day 70)

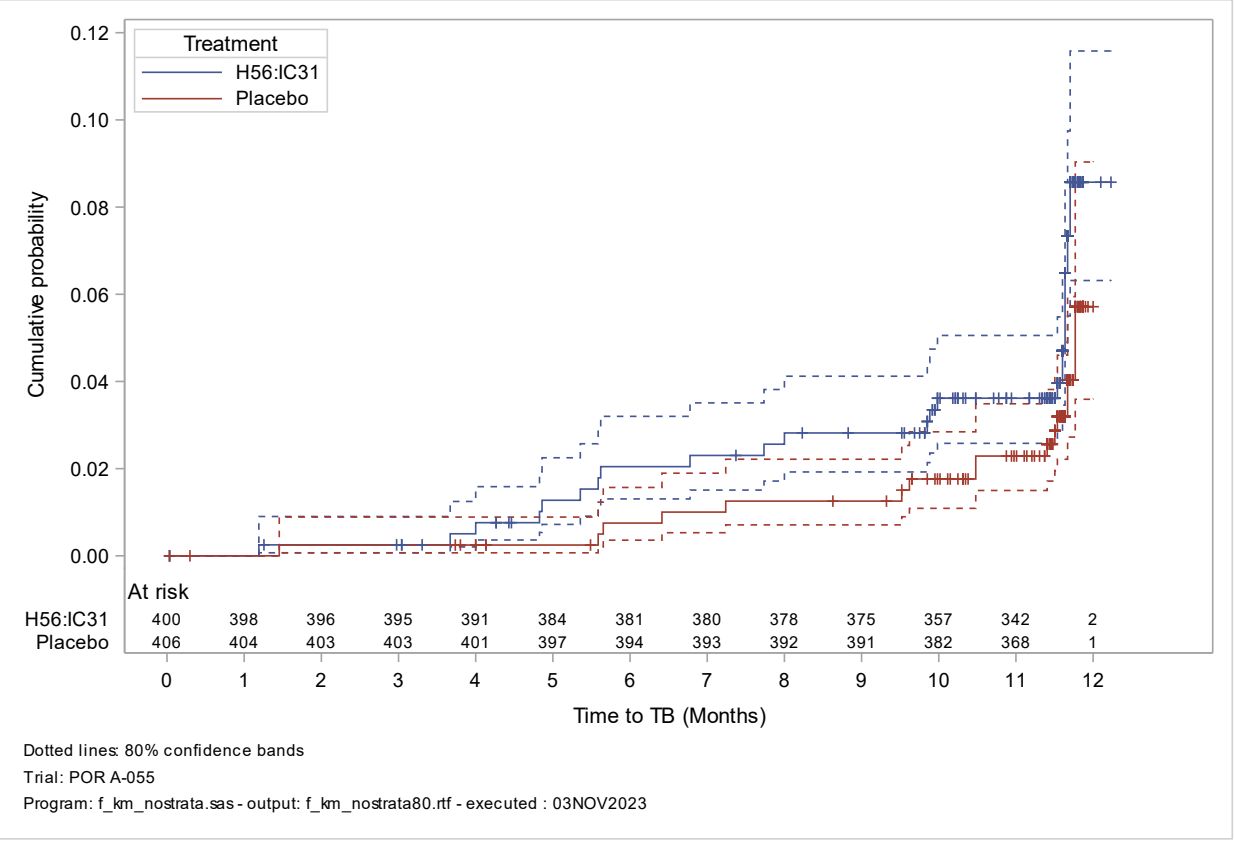
	H56:IC31®	Placebo	P-value
mITT analysis set (N)	414	413	
TB recurrence			
Number of participants contributing to analysis	400 (100.0)	406 (100.0)	
TB recurrence present	23 (5.8)	14 (3.4)	
Censored without TB recurrence	377 (94.3)	392 (96.6)	
Kaplan-Meier estimates			
One-sided log-rank test*			0.9558

Page 1 of 1

N: Number of participants
Day 70 is interpreted as 2nd vaccination + 14 days.
** Time to TB recurrence is estimated using the Kaplan-Meier method and compared between treatment groups stratified by trial site. The objective of the trial is considered met if H56:IC31 reduce the rate of TB compared to placebo and the p-value of the one-sided log-rank test is below 0.10.*
Trial: POR A-055
Program: t_ana_prim.sas - output: t_ana_prim.rtf - executed: 10NOV2023

Cross-reference: Table 14.2.1.2, Appendix 16.2.1

Figure 3: Time to primary efficacy endpoint – TB recurrence – With 80% confidence bands – mITT analysis set (from Day 70)



Cross-reference: Figure 14.2.1.7, Appendix 16.1.2

11.1.1.2 Supportive efficacy analysis – Two-sided log-rank test - Cox proportional hazard – Primary efficacy endpoint – TB recurrence – mITT analysis set (from Day 70)

The supportive efficacy analysis was the **two**-sided log-rank test in the mITT, but where the objective of the trial was considered met, if H56:IC31® reduced the rate of TB recurrence (primary efficacy endpoint) compared to placebo and the p-value was **below 0.05**, please see Table 20.

The participants and the frequencies of recurrent TB in the two groups are as for the primary efficacy analysis. The p-value for the **two**-sided log-rank test was 0.0885. This p-value (above 0.05) confirms the result of the primary efficacy analysis of no statistically significant reduction in the rate of TB recurrence in participants vaccinated with H56:IC31® compared to placebo.

From the Cox proportional hazard analysis, the estimated vaccine efficacy was -73.8 with an estimated 95% profile confidence limit (PCL) of (-246.9; 9.8). The calculated p-value for the two-sided likelihood ratio test was 0.0989 (above 0.05) and not statistically significant.

The overall conclusion of the supportive efficacy analysis was that H56:IC31® did not reduce the rate of TB recurrence in comparison to placebo, and thus confirmed the result of the primary efficacy analysis, please see Table 20.

Table 20: Supportive efficacy analysis – Two-sided log-rank test – Cox proportional hazard – Primary efficacy endpoint – TB recurrence – mITT analysis set (from Day 70)

	H56:IC31®	Placebo	P-value
mITT analysis set (N)	414	413	
TB recurrence			
Number of participants contributing to analysis	400 (100.0)	406 (100.0)	
TB recurrence present	23 (5.8)	14 (3.4)	
Censored without TB recurrence	377 (94.3)	392 (96.6)	
Kaplan-Meier estimates			
Two-sided log-rank test*			0.0885
Cox proportional hazard			
VE(%)	-73.8		
80% PCL	(-171.3; -13.0)		
95% PCL	(-246.9; 9.8)		
Two-sided likelihood ratio test			0.0989
Type 3 test for trial site			0.2521

Page 1 of 1

N: Number of participants, Day 70 is interpreted as 2nd vaccination + 14 days.
** Time to TB recurrence is estimated using the Kaplan-Meier method and compared between treatment groups stratified by trial site. The objective is considered met if H56:IC31 reduce the rate of TB compared to placebo and the p-value of the two-sided log-rank test is below 0.05.*
Trial: POR A-055
Program: t_ana_supp.sas - output: t_ana_supp.rtf - executed: 10NOV2023

Cross-reference: Table 14.2.1.3, Appendix 16.2.1

The type 3 test for trial sites, please see Table 20, confirmed that there were no statistically significant differences in TB recurrence between the trial sites.

11.1.2 Primary efficacy endpoint – TB recurrence – PP analysis set (from Day 70)

The results of the one-sided log-rank test (primary efficacy analysis), for analysing the TB recurrence (primary efficacy endpoint) in the PP analysis set are shown in Table 21 and Figure 4.

350 participants from the H56:IC31® group and 347 from the placebo group contributed to the statistical analysis in the PP analysis set. The frequencies of participants with recurrent TB were 5.4% (19/350) in the H56:IC31® group and 2.9% (10/347) in the placebo group. These frequencies resulted in a **one**-sided log-rank p-value of 0.9746 (above 0.05). This p-value is similar to the **one**-sided log-rank p-value of 0.9558 for the primary efficacy analysis in the mITT.

The overall conclusion of this efficacy analysis in the PP analysis set was that H56:IC31® did not reduce the rate of TB recurrence in comparison to placebo, and thus confirmed the result of the primary efficacy analysis (with a higher p-value), please see Table 21 and Figure 4.

For details on the results of the supportive efficacy analysis (two-sided log-rank test and Cox proportional hazard) of TB recurrence (primary efficacy endpoint), as well as incidence rates and relative risks, overall and by trial site, for the PP analysis set, please refer to Tables 14.2.1.9 and 14.2.1.10, Appendix 16.2.1.

Table 21: Efficacy analysis – One-sided log-rank test – Primary efficacy endpoint – TB recurrence – PP analysis set (from day 70)

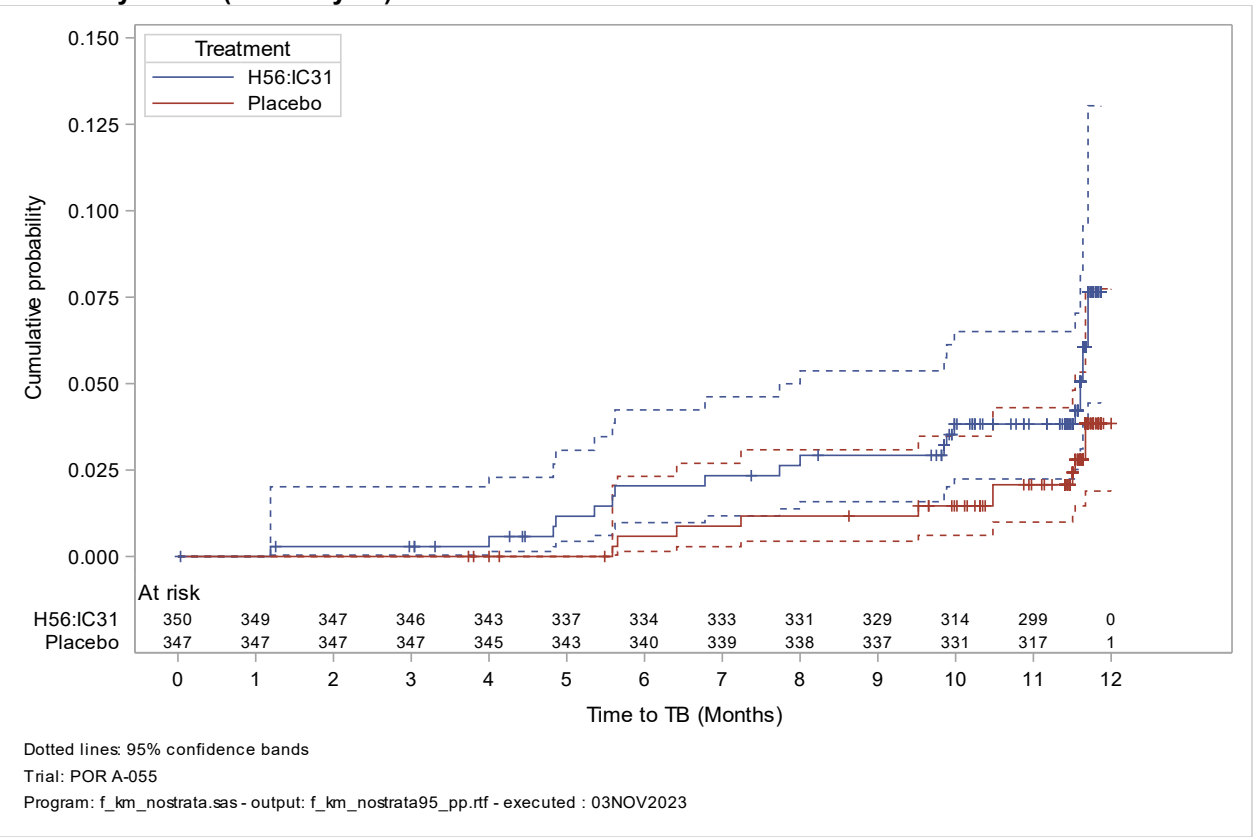
	H56:IC31®	Placebo	P-value
PP analysis set (N)	350	347	
TB recurrence			
Number of participants contributing to analysis	350 (100.0)	347 (100.0)	
TB recurrence present	19 (5.4)	10 (2.9)	
Censored without TB recurrence	331 (94.6)	337 (97.1)	
Kaplan-Meier estimates			
One-sided log-rank test*			0.9746

Page 1 of 1

N: Number of participants
Day 70 is interpreted as 2nd vaccination + 14 days.
** Time to TB recurrence is estimated using the Kaplan-Meier method and compared between treatment groups stratified by trial site. The supportive objective of the trial is considered met if H56:IC31 reduce the rate of TB compared to placebo and the p-value of the one-sided log-rank test is below 0.10.*
Trial: POR A-055
Program: t_ana_prim.sas - output: t_ana_prim_pp.rtf - executed: 10NOV2023

Cross-reference:Table 14.2.1.8, Appendix 16.2.1

Figure 4: Time to primary efficacy endpoint – TB recurrence – With 95% confidence bands – PP analysis set (from Day 70)



Cross-reference: Figure 14.2.1.9, Appendix 16.1.2

11.1.3 Primary efficacy endpoint – TB recurrence – ITT analysis set (from Day 0)

A total of 4 participants with recurrent TB with onset before Day 70 were included in the ITT analysis set: 1 in the H56:IC31® group (A50184) and 3 in the placebo group (A20243, A40148, A50157). These participants with recurrent TB were not included in the primary and supportive efficacy analyses performed in the mITT and PP analysis sets.

The results of the primary and supportive efficacy analysis of recurrent TB in the ITT analysis set from Day 0, as well as incidence rates and relative risks, overall and by site, confirmed the results of the primary efficacy analysis in the mITT and PP analysis sets, please refer to Tables 14.2.1.5 to 14.2.1.7 and 14.2.1.11, Appendix 16.2.1 and Figure 14.2.1.10, Appendix 16.2.2. For details on participants in the ITT analysis set from Day 0, please refer to Listing 16.2.6.2, Appendix 16.2.3.

11.1.4 Primary efficacy endpoint – TB recurrence – Supplementary efficacy analysis – mITT analysis set (from Day 70)

The supplementary efficacy analyses presented in this section were based on efficacy subsets of participants, as pre-specified in the SAP, please refer to Appendix 16.1.9.

The purpose was to investigate if exclusion of participants affected by different types of protocol changes or non-compliance would have an impact on the result of the primary or supportive efficacy analyses in the mITT analysis set from Day 70. The three efficacy subsets analyses were: receipt of 1 and 2 injections of IP, exclusion of participants who received the 2nd injection of IP outside the ± 10 days window and exclusion of participants with recurrent TB discovered by procedural error (i.e., if their sample was cultured in error).

11.1.4.1 Summary – Primary efficacy endpoint – TB recurrence – By receipt of 1 and 2 vaccinations – mITT analysis set (from Day 70)

The rates of participants with TB recurrence from Day 70, overall and by participants in the mITT who received 1 and 2 vaccinations, were calculated as numbers of recurrent TB cases out of total numbers of sputum samples sent for culture (i.e., overall 118 samples in the H56:IC31® group and 110 samples in the placebo group), please refer to Table 14.2.1.12, Appendix 16.2.1.

Overall, 19.5% (23/118) of the participants in the H56:IC31® group and 12.7% (14/110) of the participants in the placebo group had recurrent TB (primary efficacy endpoint) after Day 70, calculated this way. When dividing participants into groups by number of vaccinations received (1 or 2) the rates of participants with TB recurrence were as follows:

- 7.1% (1/14) - H56:IC31® group and 28.6% (4/14) - placebo group (1 vaccination)
- 21.2% (22/104) - H56:IC31® group and 10.4% (10/96) - placebo group (2 vaccinations)

From these calculated rates there were thus, no trends towards 2 vaccinations of H56:IC31® performing better in reducing recurrent TB than 1 vaccination of H56:IC31® and actually, the opposite seemed to be the case. However, this analysis of TB recurrence rates by number of vaccinations was exploratory and the numbers were low, especially in the groups receiving 1 vaccination, please refer to Table 14.2.1.27, Appendix 16.2.1.

11.1.4.2 Sensitivity – Primary efficacy analysis – TB recurrence – Exclusion if 2nd vaccination outside ± 10 days window for V5 (Day 56)

This sensitivity analysis shows the results of the **one**-sided log-rank test (primary efficacy analysis), analysing TB recurrence (primary efficacy endpoint) in the mITT analysis set from Day 70, but excluding participants who received the 2nd vaccination outside of the protocol defined visit window (Day 46 to 66), please refer to Table 14.2.1.13, Appendix 16.2.1.

Overall, 6 participants with recurrent TB (primary efficacy endpoint) received the 2nd vaccination outside of the protocol window; 1 participant in the H56:IC31® group and 5 participants in the placebo group. These 6 participants were excluded from this sensitivity analysis.

The overall conclusion of this sensitivity analysis was that H56:IC31® did not reduce the rate of TB recurrence in comparison to placebo, and thus confirmed the result of the primary efficacy analysis.

11.1.4.3 Sensitivity – Supportive efficacy analysis – TB recurrence – Exclusion if 2nd vaccination outside ± 10 days window for V5 (Day 56)

This sensitivity analysis shows the results of the **two**-sided log-rank test (supportive efficacy analysis), analysing TB recurrence (primary efficacy endpoint) in the mITT analysis set from Day 70, but excluding participants who received the 2nd vaccination outside of the protocol defined visit window (Day 46 to 66), please refer to Table 14.2.1.14, Appendix 16.2.1.

Overall, 6 participants with recurrent TB (primary efficacy endpoint) received the 2nd vaccination outside of the protocol window; 1 participant in the H56:IC31® group and 5 participants in the placebo group. These 6 participants were excluded from this sensitivity analysis.

The overall conclusion of this sensitivity analysis was that H56:IC31® did not reduce the rate of TB recurrence in comparison to placebo, and thus confirmed the result of the supportive efficacy analysis.

11.1.4.4 Sensitivity – Primary efficacy analysis – TB recurrence – Exclusion if cultured in error (no TB symptoms & Xpert MTB/RIF Ultra negative at V8)

This sensitivity analysis shows the results of the **one**-sided log-rank test (primary efficacy analysis), analysing TB recurrence (primary efficacy endpoint) in the mITT analysis set from Day 70, but excluding participants who were cultured in error (who had no TB symptoms and were Xpert MTB/RIF Ultra negative at V8). For further details, please see Section 10.2.

Overall, 5 participants with recurrent TB (primary efficacy endpoint) were cultured in error; 3 participants in the H56:IC31® group (A20070, A30158 and A30445) and 2 participants in the placebo group (A10091 and A20066). These 5 participants were excluded from this sensitivity analysis. For the results of the primary efficacy analysis in the mITT, where the 5 participants with recurrent TB that were cultured in error were excluded, please refer to Table 14.2.1.15, Appendix 16.2.1.

The overall conclusion of this sensitivity analysis was that H56:IC31® did not reduce the rate of TB recurrence in comparison to placebo, and thus confirmed the result of the primary efficacy analysis.

11.1.4.5 Sensitivity – Supportive efficacy analysis – TB recurrence – Exclusion if cultured in error (no TB symptoms & Xpert MTB/RIF Ultra negative at V8)

This sensitivity analysis shows the results of the **two**-sided log-rank test (supportive efficacy analysis), analysing TB recurrence (primary efficacy endpoint) in the mITT analysis set from Day 70, but excluding participants who were cultured in error (who had no TB symptoms and were Xpert MTB/RIF Ultra negative at V8). For further details, please see Section 10.2.

Overall, 5 participants with recurrent TB (primary efficacy endpoint) were cultured in error; 3 participants in the H56:IC31® group (A20070, A30158 and A30445) and 2 participants in the placebo group (A10091 and A20066). These 5 participants were excluded from this sensitivity analysis. For the results of the supportive efficacy analysis in the mITT, where the 5 participants

with recurrent TB that were cultured in error were excluded, please refer to Table 14.2.1.16, Appendix 16.2.1.

The overall conclusion of this sensitivity analysis was that H56:IC31® did not reduce the rate of TB recurrence in comparison to placebo, and thus confirmed the result of the supportive efficacy analysis.

11.1.5 Secondary efficacy endpoints – TB relapse and reinfection – mITT analysis set (from Day 70)

For a summary of SNP (single nucleotide polymorphisms) and allele distances by whole genome sequencing (WGS) for recurrent TB cases in the mITT analysis set from Day 70, please refer to Table 14.2.2.1, Appendix 16.2.1. Relapses and reinfections are clearly separated, with very small SNP and allele distances for relapses and large SNP and allele distances for reinfections. The summaries and analysis of recurrent TB, separated into recurrent TB relapse and TB reinfection, in the following sections, are a result of these WGS measurements and analysis.

For an overview of the total numbers of cases of TB recurrence determined to be TB relapses and TB reinfections and the cases that were undetermined, please see Table 22.

Table 22: Summary of recurrent TB type – TB relapse – TB reinfection – Undetermined – mITT analysis set from Day 70

	H56:IC31® N (%)	Placebo N (%)
mITT analysis set (N)	414	413
TB recurrence from day 70		
Overall		
Sputum samples sent for culture*	118 (100.0)	110 (100.0)
Recurrence	23 (19.5)	14 (12.7)
Relapse	12 (52.2)	6 (42.9)
Reinfection	8 (34.8)	7 (50.0)
Undetermined	3 (13.0)	1 (7.1)
No recurrence	95 (80.5)	96 (87.3)

Page 1 of 1

N: Number of participants, %: Percentage of participants, WGS: Whole genome sequencing
** Number of participants with one or more samples.*
Day 70 is interpreted as 2nd vaccination + 14 days.
Trial: POR A-055
Program: t_sum_tbrecur_wgs.sas - output: t_sum_tbrecur_wgs_site.rtf - executed: 10NOV2023

Cross-reference: Modified from Table 14.2.4.1, Appendix 16.2.1

For details on the corresponding WGS results for the ITT analysis set from Day 0, please refer to Table 14.2.2.4, Appendix 16.2.1. For details at participant level, please refer to Listing 16.2.6.5, Appendix 16.2.3.

11.1.5.1 Recurrent TB – Relapse – Cox proportional hazard – mITT analysis set (from Day 70)

Overall, 18 participants with recurrent TB (primary efficacy endpoint) were determined by WGS to be recurrent TB relapse; 12 participants in the H56:IC31® group and 6 participants in the placebo group. These 18 participants were included in this secondary efficacy analysis of recurrent TB relapse.

The results of the Cox proportional hazard estimate statistical analysis, for analysing TB relapse in the mITT analysis set from Day 70 are shown in Table 23. For a figure of time to relapse with 95% CIs, please refer to Figure 14.2.2, Appendix 16.2.2.

Table 23: Analysis of TB relapse – WGS – Cox proportional hazard – mITT analysis set (from Day 70)

	H56:IC31®	Placebo	P-value
mITT analysis set (N)	414	413	
TB relapse			
Number of participants contributing to analysis	400 (100.0)	406 (100.0)	
TB relapse present	12 (3.0)	6 (1.5)	
Censored without TB relapse	388 (97.0)	400 (98.5)	
Cox proportional hazard			
VE(%)	-116.1		
80% PCL	(-322.8; -15.6)		
95% PCL	(-522.2; 16.3)		
Two-sided likelihood ratio test			0.1128
Type 3 test for trial site			0.2697

Page 1 of 1

N: Number of participants, VE: Vaccine efficacy, PCL: Profile confidence limits
Day 70 is interpreted as 2nd vaccination + 14 days.
The estimates are from a Cox proportional hazard model with treatment and trial site as fixed effects.
Trial: POR A-055
Program: t_ana_sec.sas - output: t_ana_sec_relap.rtf - executed: 10NOV2023

Cross-reference: Table 14.2.2.2, Appendix 16.2.1

400 participants from the H56:IC31® group and 406 from the placebo group contributed to the Cox proportional hazard estimate statistical analysis, i.e., the same participants as in the primary and supportive efficacy analysis. The frequencies of participants with TB relapse were 3.0% (12/400) in the H56:IC31® group and 1.5% (6/406) in the placebo group. These frequencies resulted in a not statistically significant p-value (two-sided likelihood ratio test) of 0.1128 (above 0.05).

The overall conclusion of this secondary efficacy analysis of TB relapse in the mITT analysis set from Day 70 was that H56:IC31® did not reduce the rate of TB relapse in comparison to placebo. Of note, this trial was not powered to show a difference in TB relapses/reinfections.

For the incidence of TB relapse rates and relative risks, overall and by trial site, for the mITT analysis set from Day 70, please refer to Table 14.2.2.3, Appendix 16.2.1. For details on the corresponding results for the ITT analysis set, please refer to Tables 14.2.2.5 and 14.2.2.6, Appendix 16.2.1.

11.1.5.2 Recurrent TB – Reinfection – Cox proportional hazard – mITT analysis set (from Day 70)

Overall, 15 participants with recurrent TB (primary efficacy endpoint) were determined by WGS to be recurrent TB reinfection; 8 participants in the H56:IC31® group and 7 participants in the placebo group. These 15 participants were included in this secondary efficacy analysis of recurrent TB reinfection.

The results of the Cox proportional hazard estimate statistical analysis, for analysing TB reinfection in the mITT analysis set from Day 70 are shown in Table 24. For a figure of time to reinfection with 95% CIs, please refer to Figure 14.2.3, Appendix 16.2.2.

Table 24: Analysis of TB reinfection – WGS – Cox proportional hazard – mITT analysis set (from Day 70)

	H56:IC31®	Placebo	P-value
mITT analysis set (N)	414	413	
TB reinfection			
Number of participants contributing to analysis	400 (100.0)	406 (100.0)	
TB reinfection present	8 (2.0)	7 (1.7)	
Censored without TB reinfection	392 (98.0)	399 (98.3)	
Cox proportional hazard			
VE(%)	-21.1		
80% PCL	(-137.6; 37.7)		
95% PCL	(-245.3; 56.5)		
Two-sided likelihood ratio test			0.7113

N: Number of participants, VE: Vaccine efficacy
Day 70 is interpreted as 2nd vaccination + 14 days.
The estimates are from a Cox proportional hazard model with treatment as fixed effect.
Trial: POR A-055
Program: t_ana_sec.sas - output: t_ana_sec_reinf.rtf - executed: 10NOV2023

Cross-reference: Table 14.2.3.1, Appendix 16.2.1

400 participants from the H56:IC31® group and 406 from the placebo group contributed to the Cox proportional hazard estimate statistical analysis, i.e., the same participants as in the primary and supportive efficacy analyses. The frequencies of participants with TB reinfection were 2.0% (8/400) in the H56:IC31® group and 1.7% (7/406) in the placebo group. These frequencies resulted in a not statistically significant p-value (two-sided likelihood ratio test) of 0.7113 (above 0.05).

The overall conclusion of this secondary efficacy analysis of TB reinfection in the mITT analysis set from Day 70 was that H56:IC31® did not reduce the rate of TB reinfection in comparison to placebo. Of note, this trial was not powered to show a difference in TB relapses/reinfections.

For the incidence of TB reinfection rates and relative risks, overall and by trial site, for the mITT analysis set from Day 70, please refer to Table 14.2.3.2, Appendix 16.2.1. For details on the corresponding results for the ITT analysis set, please refer to Tables 14.2.3.3 and 14.2.3.4, Appendix 16.2.1.

11.1.5.3 Recurrent TB (relapse and reinfection) – Subgroup analysis – mITT analysis set (from Day 70)

For summary tables of TB recurrence type (TB relapse or reinfection) for the mITT analysis set from Day 70 overall and by; trial sites, country, BMI group, age group, sex, smoking status, anaemia, diabetes mellitus, comorbidities at baseline, use of steroids and anti-inflammatory drugs, number of vaccinations received, Covid-19 related PD, or Covid-19 infection, please refer to Tables 14.2.4.1 to 14.2.4.13, Appendix 16.2.1.

For summary tables of TB recurrence type (TB relapse or reinfection) from the ITT analysis set from Day 0 overall and by; trial sites, country, BMI group, age group, sex, smoking status, anaemia, diabetes mellitus, comorbidities at baseline, use of steroids and anti-inflammatory drugs, number of vaccinations received, Covid-19 related PD, or Covid-19 infection, please refer to Tables 14.2.4.14 to 14.2.4.26, Appendix 16.2.1.

The overall conclusion from all supplementary subgroup efficacy analysis was that H56:IC31® did not reduce the rate of recurrent TB (relapse or reinfection) in comparison to placebo. Of note, the trial was not powered to show subgroup differences.

11.1.6 Other efficacy endpoints

11.1.6.1 1st exploratory efficacy endpoint – TB recurrence (culture verified and/or start new TB treatment) – Cox proportional hazard – mITT analysis set (from Day 70)

Overall, 48 participants with recurrent TB (culture verified and/or start new TB treatment) were included in this 1st exploratory efficacy analysis; 27 in the H56:IC31® group and 21 in the placebo group, please see Table 25. This means that overall 11 additional participants with assumed recurrent TB (who started new TB treatment without verification of recurrent TB) were included, if compared to the primary efficacy analysis, where the corresponding number was a total of 37 recurrent TB cases (23 in the H56:IC31® group and 14 in the placebo group), please see Table 19.

The results of the Cox proportional hazard analysis, for analysing TB recurrence (culture verified and start new TB treatment) in the mITT analysis set from Day 70 are shown in Table 25.

400 participants from the H56:IC31® group and 406 from the placebo group contributed to the Cox proportional hazard analysis, i.e., the same participants as in the primary and supportive efficacy analysis. The frequencies of participants with recurrent TB (culture verified and/or start new TB treatment) were 6.8% (27/400) in the H56:IC31® group and 5.2% (21/406) in the placebo group. These frequencies resulted in a p-value (two-sided likelihood ratio test) of 0.3058 (above 0.05).

The overall conclusion of this 1st exploratory efficacy analysis of recurrent TB (culture verified and/or start new TB treatment) in the mITT analysis set from Day 70 was that H56:IC31® did not reduce the rate of TB recurrence in comparison to placebo.

This 1st exploratory efficacy endpoint was defined to include the 37 culture verified recurrent TB cases (primary efficacy endpoint) as well as 11 additional cases where new TB treatment was started without prior culture verification of recurrent TB. The 11 additional cases were a mixture of participants who had been prescribed the new TB treatment at a local clinic or within the trial due to suspicion of recurrent TB, but without prior culture verification (A10063, A10184, A20083, A20170, A20236, A30134, A30462, A40010), but also included 3 cases (A10047, A20008, A40071), all placebo treated, where the new TB treatment was prescribed for other diagnosis than suspicion of recurrent TB; cor pulmonale (A10047), non-TB mycobacterial infection (A20008), or where the new TB treatment was pyridoxine, vitamin B6, (A40071) in a participant with no suspicion of recurrent TB and who completed the trial with a negative Xpert MTB/RIF Ultra test result at V8.

Seen in retrospective, this 1st exploratory efficacy endpoint should have been defined more precisely, to not include cases where new TB treatment was started, if there was no suspicion of recurrent TB, please refer to Listing 16.2.4.6, Appendix 16.2.3.

Overall, 46 participants either discontinued (31) or completed (15) the trial with primary reason given as recurrent TB, please see Table 9, including the 41 participants with culture verified recurrent TB in the ITT population and 5 participants who discontinued the trial due to start of new TB treatment for suspected, but not culture verified, recurrent TB (A10063, A10184, A20083, A20170, A20236).

3 of the 8 participants that did start new TB treatment for suspected, but not culture verified, recurrent TB were not included among the 46 above-mentioned participants. The H56:IC31® vaccinee (A30462) discontinued the trial with primary reason given as ‘other’, but also had started new TB treatment for suspected, but not culture verified, recurrent TB. The 2 placebo recipients (A30134 and A40010) completed the trial, but had started new TB treatment for suspected, but not culture verified, recurrent TB at V8. As the end of trial form only captured culture verified recurrent TB for trial completers, these 2 participants were, however, not included.

Including the above-mentioned 3 participants, overall 49 participants either discontinued (32) or completed (17) the trial with recurrent TB, either culture verified or started new TB treatment for suspected, but not culture verified, recurrent TB.

Table 25: Analysis – 1st exploratory efficacy endpoint – TB recurrence (culture verified and/or start new TB treatment) – Cox proportional hazard – mITT analysis set (from Day 70)

	H56:IC31®	Placebo	P-value
mITT analysis set (N)	414	413	
TB recurrence			
Number of participants contributing to analysis	400 (100.0)	406 (100.0)	
TB recurrence present	27 (6.8)	21 (5.2)	
Censored without TB recurrence	373 (93.3)	385 (94.8)	
Cox proportional hazard			
VE(%)	-34.7		
80% PCL	(-96.6; 7.2)		
95% PCL	(-141.3; 23.8)		
Two-sided likelihood ratio test			0.3058
Type 3 test for trial site			0.4082

Page 1 of 1

N: Number of participants, VE: Vaccine efficacy, PCL: Profile confidence limits
Day 70 is interpreted as 2nd vaccination + 14 days.
The estimates are from a Cox proportional hazard model with treatment and trial site as fixed effects.
Trial: POR A-055
Program: t_ana_sec.sas - output: t_ana_sec_new.rtf - executed: 10NOV2023

Cross-reference: Table 14.2.7.2, Appendix 16.2.1

For the numbers of TB recurrences (culture verified and start new treatment) for the mITT analysis set from Day 70, please refer to Table 14.2.7.1, Appendix 16.2.1 and for details on the results of a statistical sensitivity analysis (with treatment and country as fixed effects), please refer to Table 14.2.7.3, Appendix 16.2.1.

For incidence rates of TB recurrences (culture verified and start new treatment) as well as relative risks, overall and by site, for the mITT analysis set, please refer to Table 14.2.7.4, Appendix 16.2.1. For the corresponding tables for the ITT population from Day 0, please refer to Tables 14.2.7.5 to 14.2.7.7, Appendix 16.2.1.

11.1.6.2 2nd exploratory efficacy endpoint – TB recurrence (culture verified and positive Xpert MTB/RIF Ultra only) – Cox proportional hazard – mITT analysis set (from Day 70)

Overall, 77 participants with recurrent TB (culture verified and positive Xpert MTB/RIF Ultra only) were included as recurrent TB cases in this 2nd exploratory efficacy analysis; 49 participants in the H56:IC31® group and 28 participants in the placebo group.

The results of the Cox proportional hazard analysis, for analysing TB recurrence (culture verified and positive Xpert MTB/RIF Ultra only) in the mITT analysis set from Day 70 are shown in Table 26.

Table 26: Analysis – 2nd exploratory efficacy endpoint – TB recurrence (culture verified and positive Xpert MTB/RIF Ultra only) – Cox proportional hazard – mITT analysis set (from Day 70)

	H56:IC31®	Placebo	P-value
mITT analysis set (N)	414	413	
TB recurrence			
Number of participants contributing to analysis	395 (100.0)	404 (100.0)	
TB recurrence present	49 (12.4)	28 (6.9)	
Censored without TB recurrence	346 (87.6)	376 (93.1)	
Cox proportional hazard			
VE(%)	-100.3		
80% PCL	(-175.2; -47.2)		
95% PCL	(-227.3; -25.4)		
Two-sided likelihood ratio test			0.0034
Type 3 test for trial site			0.1202

Page 1 of 1

N: Number of participants, VE: Vaccine efficacy, PCL: Profile confidence limits
Day 70 is interpreted as 2nd vaccination + 14 days.
The estimates are from a Cox proportional hazard model with treatment and trial site as fixed effects.
Trial: POR A-055
Program: t_ana_sec.sas - output: t_ana_sec_xpert.rtf - executed: 10NOV2023

Cross-reference: Table 14.2.8.2, Appendix 16.2.1

395 participants from the H56:IC31® group and 404 from the placebo group contributed to the Cox proportional hazard analysis. The frequencies of participants with recurrent TB (culture verified and positive Xpert MTB/RIF Ultra only) were 12.4% (49/395) in the H56:IC31® group and 6.9% (28/404) in the placebo group. These frequencies resulted in a statistically significant p-value (two-sided likelihood ratio test) of 0.0034 in favour of placebo.

The overall conclusion of this 2nd exploratory efficacy analysis of recurrent TB (culture verified and positive Xpert MTB/RIF Ultra only) in the mITT analysis set from Day 70 was that H56:IC31® increased the rate of TB recurrence in comparison to placebo.

For the numbers of TB recurrences (culture verified only, positive Xpert MTB/RIF Ultra only, culture verified and positive Xpert MTB/RIF Ultra) for the mITT analysis set from Day 70, please refer to Table 14.2.8.1, Appendix 16.2.1 and for details on the results of a statistical sensitivity analysis (with treatment and country as fixed effects), please refer to Table 14.2.8.3, Appendix 16.2.1.

For incidence rates of TB recurrences (culture verified and positive Xpert MTB/RIF Ultra only) as well as relative risks, overall and by site, for the mITT analysis set, please refer to Table 14.2.8.4, Appendix 16.2.1. For the corresponding tables for the ITT population from Day 0, please refer to Tables 14.2.8.5 to 14.2.8.7, Appendix 16.2.1.

11.1.6.3 3rd exploratory efficacy endpoint – TB recurrence (culture verified) – Cox proportional hazard – mITT2 (from Day 30)

The 4 participants with recurrent TB; 1 in the H56:IC31® group (A50184) and 3 in the placebo group (A20243, A40148, A50157) that occurred before Day 70 and therefore were excluded from the mITT and PP analysis sets and all primary and secondary efficacy analysis, were all included in the mITT2 analysis set and in this 3rd exploratory efficacy analysis. This was the case, as all 4 recurrent TB cases occurred after Day 30, the requirement for inclusion in the mITT2 analysis set used for the for the 3rd exploratory efficacy analysis, please see Table 27 .

Table 27: Analysis – 3rd exploratory efficacy endpoint – TB recurrence (culture verified) – Cox proportional hazard – mITT2 (from Day 30)

	H56:IC31®	Placebo	P-value
mITT2 analysis set (N)	415	416	
TB recurrence			
Number of participants contributing to analysis	403 (100.0)	410 (100.0)	
TB recurrence present	24 (6.0)	17 (4.1)	
Censored without TB recurrence	379 (94.0)	393 (95.9)	
Cox proportional hazard			
VE(%)	-58.5		
80% PCL	(-141.8; -5.1)		
95% PCL	(-204.7; 15.3)		
Two-sided likelihood ratio test			0.1502
Type 3 test for trial site			0.5987

Page 1 of 1

N: Number of participants, VE: Vaccine efficacy, PCL: Profile confidence limits
The estimates are from a Cox proportional hazard model with treatment and trial site as fixed effects.
Trial: POR A-055
Program: t_ana_sec.sas - output: t_ana_sec_mitt2.rtf - executed: 10NOV2023

Cross-reference: Table 14.2.9.2, Appendix 16.2.1

403 participants from the H56:IC31® group and 410 from the placebo group contributed to the Cox proportional hazard analysis. The frequencies of participants with recurrent TB (from Day 30) were 6.0% (24/403) in the H56:IC31® group and 4.1% (17/410) in the placebo group. These frequencies resulted in a p-value (two-sided likelihood ratio test) of 0.1502.

The overall conclusion of this 3rd exploratory efficacy analysis of recurrent TB (from Day 30) in the mITT2 analysis set was that H56:IC31® did not reduce the rate of TB recurrence (from Day 30) in comparison to placebo.

For the numbers of TB recurrences (from Day 30), please refer to Table 14.2.9.1, Appendix 16.2.1 and for details on the results of a statistical sensitivity analysis (with treatment and country as fixed effects), please refer to Table 14.2.9.3, Appendix 16.2.1. For incidence rates of TB recurrences (from Day 30) as well as relative risks, overall and by site, for the mITT2 analysis set, please refer

to Table 14.2.9.4, Appendix 16.2.1. For the corresponding tables for the ITT population from Day 0, please refer to Tables 14.2.8.5 to 14.2.8.7, Appendix 16.2.1. For details on participants in the ITT analysis set from Day 30, please refer to Listing 16.2.6.2, Appendix 16.2.3.

11.1.7 Results of statistical issues encountered during the analysis

The lack of efficacy of H56:IC31® was unexpected, and not covered by the considerations in relation to the sample size calculations and choice of one-side statistically testing, as defined in the protocol and in the SAP, Appendix 16.1.9.

11.1.8 Adjustment for covariates

No adjustments of covariates differed from those planned in the SAP, Appendix 16.1.9.

11.1.9 Handling of withdrawals, discontinuations or missing data

For the primary efficacy endpoint missing values were handled using censoring. The primary endpoint was analysed based on the mITT which included all randomised participants except those with TB disease recurrence before V6= Day 70 (or 14 days after 2nd dose for those who received both vaccinations). Participants without TB disease recurrence (confirmed by of *Mtb* by culture of sputum samples) were censored. Asymptomatic participants not capable of producing sputum were censored as well. No imputation of missing values was performed.

Partial TB diagnosis and TB treatment start dates were handled as follows: If day was missing, the day was set to the 1st of the month. If the month was missing the month was set to January. If the year was missing the date was set to missing.

Partial TB treatment end dates were handled as follows: If day was missing, the day was set to the last day of the month. If the month was missing, the month was set to six months after the start month. Partial AE start dates were handled as follows: If day is missing then the day was set to the first day of the month unless a vaccination occurred in the same month. In that case day was set to the day of vaccination. If month was missing the month was set to the first month of the year unless at least one vaccination occurred that year. In that case the month was set to the month of the first vaccination of the year. If the AE start date was missing the date was set to the date of the first vaccination. Partial AE end dates were handled as follows: If the day was missing, the day was set to the last day of the month. If month was missing, the month was set to the last month of the year. If the AE end date was missing the AE was considered ongoing at end of trial and the date was not imputed. Partial dates of general concomitant medication and medical history were not imputed.

11.1.10 Interim analysis and data monitoring

There was no interim analysis in this trial.

11.1.11 Multicentre trials

The Type 3 test for trial sites was performed and shown in tables displaying the results of the statistical analysis when relevant. Overall, the conclusions of the Type 3 test results for trial sites

were that the fact that the analysed data was collected at 6 individual sites, did in general not introduce statistically significant trial site effects which again could have had an impact on the overall statistical conclusions.

For the primary statistical efficacy analysis in the mITT analysis set, the Type 3 test result for trial sites resulted in a p-value of 0.2521 (above 0.05), please see Table 20. This non-significant p-value confirmed that site effects did not have an impact on the overall results of the primary statistical efficacy analysis.

For figures of time to TB recurrence (primary efficacy endpoint) by sites A1 to A6, please refer to Figures 14.2.1.1 to 14.2.1.6, Appendix 16.2.2. For a figure of time to TB recurrence for all sites, please refer to Figure 14.2.1.11, Appendix 16.2.2 and for all countries Table 14.2.1.12, Appendix 16.1.2.

For a table of analysis of TB recurrence (primary efficacy endpoint) in the mITT from Day 70, by sites A1 to A6, please refer to Table 14.2.1.17, Appendix 16.2.1 and for a table of analysis of TB recurrence in the mITT from Day 70 by country, please refer to Table 14.2.1.18, Appendix 16.2.1.

11.1.12 Multiple comparison/multiplicity

The primary analysis of the primary endpoint was based on the mITT analysis set. Analysis of the primary endpoint in other analysis sets were considered sensitivity analysis and adjustment for multiplicity was not necessary.

11.1.13 Examination of subgroups

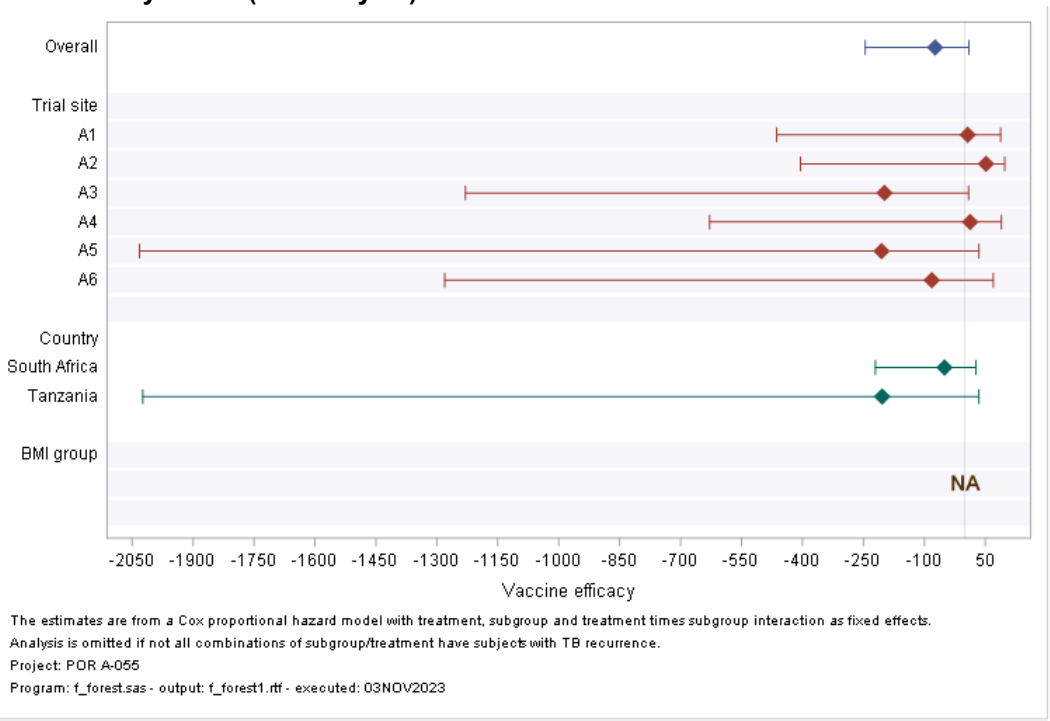
The estimated vaccine efficacy overall and by subgroups are shown as forest plots in Figure 5 (trial sites, countries), Figure 6 (age group, sex, smoking status, anaemia) and Figure 7 (diabetes mellitus, comorbidities, steroids and anti-inflammatory drugs and number of vaccinations).

There seemed to be an age group effect on the vaccine efficacy where the vaccine efficacy was lower (negative) in the older age group (36 to 60 years) than in the younger age group (18 to 35 years), please see Figure 6. The difference was not statistically significant as the p-value for the joint test for interaction was 0.0697, please refer to Table 14.2.1.20, Appendix 16.2.1.

Also, increased hazard for TB recurrence was observed, but not statistically significant, in participants without anaemia (with a p-value for joint test for interaction of 0.1104, please refer to Table 14.2.1.23, Appendix 16.2.1) and in participants who received 2 injections of IP (with a p-value for joint test for interaction of 0.1277, please refer to Table 14.2.1.27, Appendix 16.2.1). Overall, none of the interaction tests were significant. No other notable group effects on vaccine efficacy were identified from the forest plots.

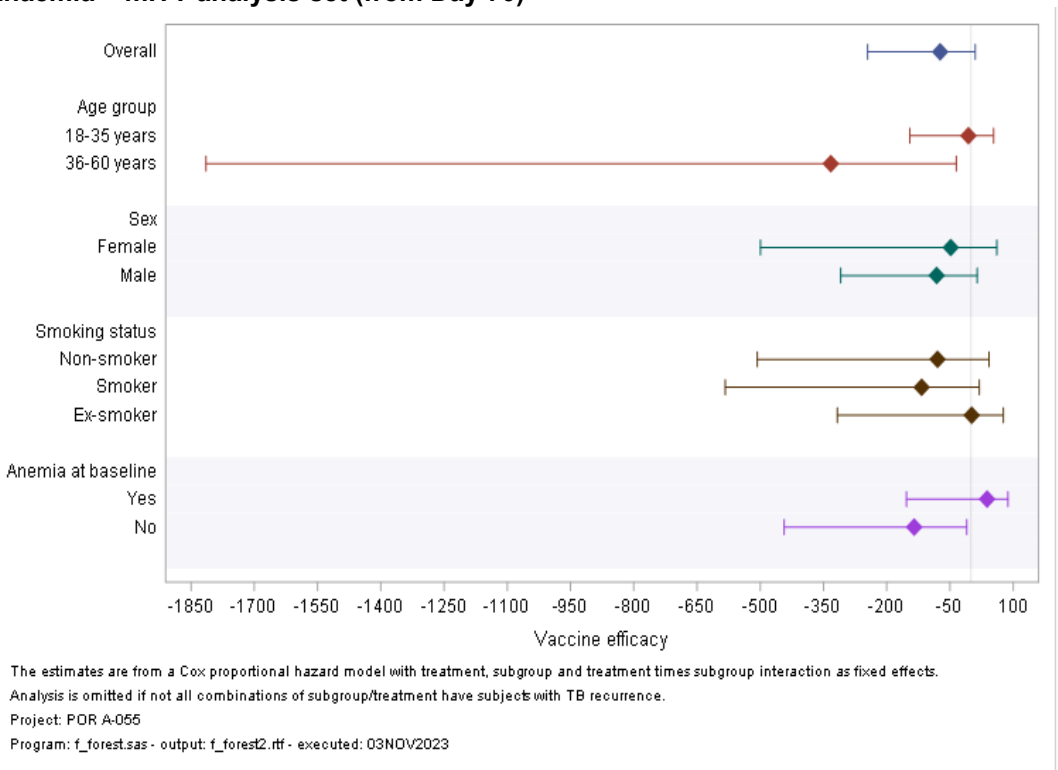
For p-values for joint tests for interaction for all the mentioned subgroups, please refer to Tables 14.2.1.17 to 14.2.1.29, Appendix 16.2.1.

Figure 5: Forest plot of estimated vaccine efficacy overall and by trial sites and country – mITT analysis set (from Day 70)



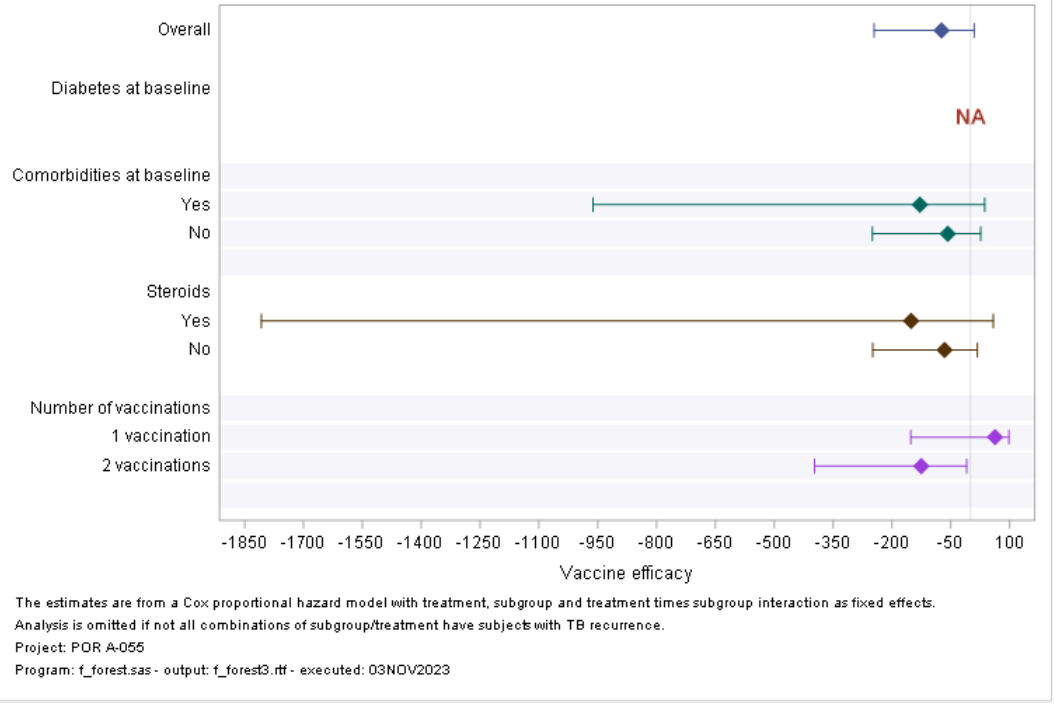
Cross-reference: Figure 14.2.1.22, Appendix 16.2.2

Figure 6: Forest plot of estimated vaccine efficacy overall and by age group, sex, smoking status and anaemia – mITT analysis set (from Day 70)



Cross-reference: Figure 14.2.1.23, Appendix 16.2.2

Figure 7: Forest plot of estimated vaccine efficacy overall and by diabetes mellitus, comorbidities, steroids and anti-inflammatory drugs, number of vaccinations – mITT analysis set (from Day 70)



Cross-reference: Figure 14.2.1.24, Appendix 16.2.2

For tables of TB recurrence (primary efficacy endpoint) in the mITT from Day 70, by trial site, country, age group, sex, smoking status, anaemia, diabetes mellitus, comorbidity, steroids and anti-inflammatory drugs, number of injections of IP, Covid-19 relevant PDs and Covid-19 infection, including p-values for joint tests for interaction, please refer to Tables 14.2.1.17 to 14.2.1.29, Appendix 16.2.1.

For figures of time to TB recurrence (primary efficacy endpoint) in the mITT from Day 70, by trial site, country, age group, sex, smoking status, anaemia, diabetes mellitus, comorbidity, steroids and anti-inflammatory drugs, number of injections of IP, please refer to Figures 14.2.1.11 to 14.2.1.21, Appendix 16.2.2.

11.1.14 Supplementary listings – Efficacy – Sputum collection

For further details of participants on sputum collection, please refer to Listing 16.2.6.3 (participants with TB recurrence) and Listing 16.2.6.4 (participants without TB recurrence), both in Appendix 16.2.3.

11.2 Efficacy results summary

Overall, in the mITT analysis set, the TB recurrence incidence rate in the H56:IC31® group was 6.3% (95% CI: 4.0; 9.4) compared to 3.7% (95% CI: 2.0; 6.2) in the placebo group. The relative risk of recurrent TB was 1.3 (95% CI: 0.7; 1.6) among the H56:IC31® vaccinees relative to the placebo recipients, please see Table 18.

The frequencies of participants with recurrent TB in the two groups; 5.8% (23/400) in the H56:IC31® group and 3.4% (14/406) in the placebo group, resulted in a **one**-sided log-rank p-value of 0.9558 (above 0.10) for a lower frequency of recurrent TB in the H56:IC31® group than in the placebo group.

The overall conclusion of the primary efficacy analysis (**one**-sided log-rank test) in the mITT analysis set from Day 70 was thus that H56:IC31® did not lead to a statistically significant reduction in the rate of TB recurrence in comparison to placebo, please see Table 19 and Figure 3.

The supportive efficacy analysis (**two**-sided log-rank test) in the mITT analysis set from Day 70 supported the results of the primary efficacy analysis, please see Table 20, as did the corresponding efficacy analysis from Day 70 in the PP analysis set, please see Table 21 and Figure 4.

Supplementary efficacy analyses were performed in different mITT subsets of participants to investigate the impact on the results of the primary efficacy analysis. The subgroups were: 1) participants who received 1 or 2 vaccinations, 2) exclusion of participants if 2nd injection outside ± 10 days window and 3) exclusion of participants with recurrent TB, if they were cultured in error. All performed supplementary efficacy analyses in these mITT subsets supported the results of the primary and supportive efficacy analysis in the mITT.

The overall conclusion of the secondary efficacy analysis of recurrent TB relapse and reinfection in the mITT analysis set from Day 70 was that H56:IC31® did not reduce the rate of TB relapse and TB reinfection in comparison to placebo. It was, moreover, noted, that the excess cases of recurrent TB in the H56:IC31® group seemed to be driven by TB relapse rather than by TB reinfection, please see Table 23 and Table 24.

The results of the three exploratory efficacy analyses confirmed the results of the primary and secondary efficacy analyses, please see Table 25, Table 26 and Table 27.

From the forest plots on different subgroups an increased hazard for TB recurrence was observed in participants older than 35 years, without anaemia, and receiving both IP injections, but interaction tests were not statistically significant, please see Figure 5, Figure 6 and Figure 7.

11.3 Immunogenicity evaluation

The trial had two immunogenicity endpoints which were both classified as ‘other secondary endpoints’ in Section 8.5.2 in the protocol, Appendix 16.1.1:

- 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)

11.4 Immunogenicity results

11.4.1 Primary immunogenicity endpoint

In the protocol the primary immunogenicity endpoint, please see Table 7, was defined as antigen specific cell mediated immune responses by WB ICS at baseline (V3= Day 0) and 14 days after the 2nd vaccination (V6= Day 70) in the immunogenicity cohort, please see Section 11.3.

The analysis set used for assessing this endpoint was changed in Section 6.4 in the SAP, Appendix 16.1.9, where it was specified that the immunogenicity analysis set was to be used and that the immunogenicity cohort was to be used as an exploratory analysis. The immunogenicity analysis set is defined in Section 10.3.

The SAP specified that the variables of interest for assessment of antigen specific cell mediated immune response to vaccination were the percentage of CD4+ and CD8+ T cells that express IL-2, IFN-γ, TNF, and IL-17 in the following combinations:

- H56 protein-specific CD4+ T cells expressing the total cytokine response, i.e., any combination of IL-2, IFN-γ, TNF, and/or IL-17
- H56 protein-specific CD4+ T cells co-expressing IL-2 and TNF
- H56 protein-specific CD4+ T cells co-expressing IL-2, IFN-γ and TNF
- H56 protein-specific CD8+ T cells expressing any combination of IL-2, IFN-γ, TNF, and/or IL-17 (total response)

Due to a few outliers in the dataset, the medians rather than the means will be used in the following.

11.4.1.1 CD4+ T cell responses

Overall, CD4+ T cell responses that were specific for H56 protein were low in both groups before vaccination and the administration of H56:IC31® induced significant increases in these responses.

The median fold increase from Day 0 to Day 70 in CD4+ T cells expressing the total cytokine response, i.e., any combination of IL-2, IFN-γ, TNF and/or IL-17, was 3.8 in the H56:IC31® group versus 1.0 in the placebo group, please see Table 28 and please refer to Figure 14.2.4.1, Appendix 16.2.2. Similar results were observed in the immunogenicity cohort, please refer to Table 14.2.5.2, Appendix 16.2.1 and Figure 14.2.4.2, Appendix 16.2.2.

Table 28: H56 protein-specific CD4+ T cells expressing any combination of IL-2, IFN-γ, TNF, or IL-17 (%) – WB ICS – Immunogenicity analysis set

	H56:IC31®	Placebo
Immunogenicity analysis set (N)	43	50
CD4⁺ T cells expressing any combination of IL-2, IFN- γ, TNF- α, or IL-17 (%)		
Visit 3 (Day 0) - Baseline		
N	42	48
Mean (SD)	0.141 (0.233)	0.127 (0.114)
Median	0.074	0.093
q25 - q75	0.048 - 0.139	0.044 - 0.180
Min - Max	0.02 - 1.39	0.01 - 0.47
Visit 6 (Day 70)		
N	40	49
Mean (SD)	0.568 (1.416)	0.345 (1.472)
Median	0.328	0.088
q25 - q75	0.165 - 0.506	0.061 - 0.171
Min - Max	0.07 - 9.19	0.02 - 10.40
Fold increase from Baseline to Visit 6 (Day 70)		
N	39	47
Mean (SD)	5.8 (5.5)	8.6 (49.7)
Median	3.8	1.0
q25 - q75	2.4 - 7.8	0.7 - 1.5
Min - Max	0 - 30	0 - 342

Page 1 of 1

N: Number of participants, SD: Standard deviation, q25: Lower quartile, q75: Upper quartile
WB ICS: Intracellular Cytokine Staining Using Whole Blood, IL-2: Interleukin-2, IFN- γ: Interferon gamma
TNF- α: Tumor Necrosis Factor alpha, IL-17: Interleukin-17
Antigen specific CD4⁺ T cells expressing any of the cytokines IL-2, IFN- γ, TNF- α, or IL-17 after stimulation with H56.
Trial: POR A-055
Program: t_tcell.sas - output: t_tcell_cd4tot.rtf - executed: 10NOV2023

Cross-reference: Table 14.2.5.1, Appendix 16.2.1

For CD4+ T cells co-expressing IL-2 and TNF the median fold increase from Day 0 to Day 70 was higher in the H56:IC31® group than in the placebo group (4.6 versus 0.8), please refer to Table 14.2.5.5, Appendix 16.2.1 and Figure 14.2.4.5, Appendix 16.2.2. Similar results were observed in the immunogenicity cohort, please refer to Table 14.2.5.6, Appendix 16.2.1 and Figure 14.2.4.6, Appendix 16.2.2.

For CD4+ T cells co-expressing IL-2, IFN-γ and TNF, the median fold increase from Day 0 to Day 70 was higher in the H56:IC31® group than in the placebo group (2.7 versus 0.9), please refer to Table 14.2.5.3, Appendix 16.2.1 and Figure 14.2.4.3, Appendix 16.2.2. Similar results were observed in the immunogenicity cohort, please refer to Table 14.2.5.4, Appendix 16.2.1 and Figure 14.2.4.4, Appendix 16.2.2.

11.4.1.2 CD8+ T cell responses

CD8+ T cell responses specific for H56 protein were low in both groups before vaccination and no strong response was observed following administration of H56:IC31®. The median fold increase from Day 0 to Day 70 in CD8+ T cells expressing the total cytokine response (i.e., any combination of IL-2, IFN-γ, TNF, and/or IL-17) was 1.2 in the H56:IC31® group versus 1.5 in the placebo group, please see Table 29, and please refer to Figure 14.2.4.7, Appendix 16.2.2.

Table 29: H56 protein-specific CD8+ T cells expressing any combination of IL-2, IFN-γ, TNF, or IL-17 (%) – WB ICS – Immunogenicity analysis set

	H56:IC31®	Placebo
Immunogenicity analysis set (N)	43	50
CD8⁺ T cells expressing any combination of IL-2, IFN- γ, TNF- α, or IL-17 (%)		
Visit 3 (Day 0) - Baseline		
N	42	48
Mean (SD)	0.308 (0.891)	0.138 (0.196)
Median	0.088	0.058
q25 - q75	0.039 - 0.184	0.030 - 0.154
Min - Max	0.00 - 5.73	0.00 - 1.12
Visit 6 (Day 70)		
N	40	49
Mean (SD)	0.307 (0.542)	0.398 (1.379)
Median	0.157	0.072
q25 - q75	0.058 - 0.345	0.027 - 0.288
Min - Max	0.01 - 3.29	0.00 - 9.52
Fold increase from Baseline to Visit 6 (Day 70)		
N	39	47
Mean (SD)	2.4 (2.9)	6.7 (24.4)
Median	1.2	1.5
q25 - q75	0.8 - 2.4	0.6 - 3.2
Min - Max	0 - 12	0 - 167

Page 1 of 1

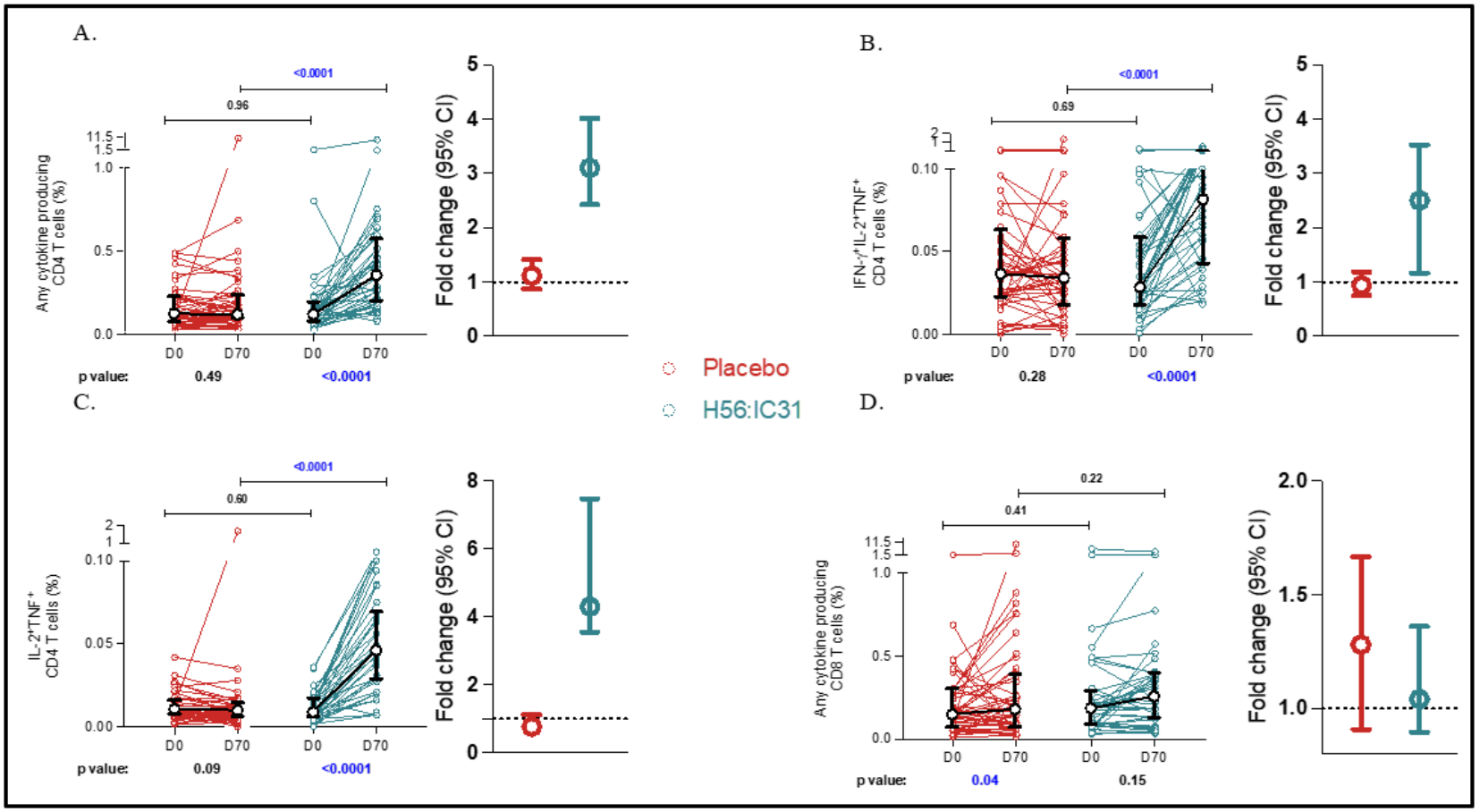
N: Number of participants, SD: Standard deviation, q25: Lower quartile, q75: Upper quartile
WB ICS: Intracellular Cytokine Staining Using Whole Blood, IL-2: Interleukin-2, IFN- γ: Interferon gamma
TNF- α: Tumor Necrosis Factor alpha, IL-17: Interleukin-17
Antigen specific CD8⁺ T cells expressing any of the cytokines IL-2, IFN- γ, TNF- α, or IL-17 after stimulation with H56.
Trial: POR A-055
Program: t_tcell.sas - output: t_tcell_cd8tot.rtf - executed: 10NOV2023

Cross-reference: Table 14.2.5.7, Appendix 16.2.1

11.4.1.3 Statistical analysis CD4+ and CD8+ cell responses

The statistical analysis on the immunogenicity endpoints of CD4+ and CD8+ T cells was reported separately in the immunogenicity report, please refer to Appendix 16.2.5. The results of the CD4+ and CD8+ T cell responses are shown in Figure 8.

Figure 8: H56 specific CD4+ and CD8+ T cell responses in H56:IC31® vaccinees and placebo recipients



Cross-reference: Figure 1, Appendix 16.2.5

H56:IC31® vaccination induced significant increases in frequencies of antigen specific CD4+ T cells expressing any combination of IL-2, IFN-γ, TNF and/or IL-17 (Figure 8 A), CD4+ T cells co-expressing IL-2 and TNF (Figure 8 B), and polyfunctional IL-2+ IFN-γ+ TNF+ CD4+ T cells (Figure 8 C), measured at Day 70, relative to pre-vaccination. No changes in these responses were observed in placebo recipients. The fold change in H56-specific CD4+ T cell responses, computed between Day 70 and Day 0 was significantly higher in H56:IC31® recipients than in placebo recipients. Frequencies of antigen specific CD8+ T cells were not significantly modulated by vaccination (Figure 8 D).

For further details on the immunogenicity analysis, including exploratory outcomes, please refer to the immunogenicity report, Appendix 16.2.5.

11.4.2 Secondary immunogenicity endpoints

In the protocol the secondary immunogenicity endpoint was defined as 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).

The analysis set used for assessing this endpoint was changed as described in Section 6.4 in the SAP, Appendix 16.1.9, where it was specified that the immunogenicity analysis set was to be used and that the immunogenicity cohort was to be used as an exploratory analysis. The immunogenicity analysis set is defined in Section 10.3.

The median fold increase in anti-H56 IgG titres from Day 0 to Day 70 was higher in the H56:IC31® group than in the placebo group (1.8 versus 0.6), please see Table 30. The geometric mean anti-H56 IgG titres increased following administration of the vaccine in the H56:IC31® group but not in the placebo group, please refer to Figure 14.2.5.1, Appendix 16.2.2. Similar results were observed in the immunogenicity cohort, please refer to Table 14.2.6.2, Appendix 16.2.1 and Figure 14.2.5.2, Appendix 16.2.2. Individual anti-H56 IgG participant data are provided in Listing 16.2.6.7, Appendix 16.2.3.

Table 30: Humoral immune responses – IgG ELISA – Immunogenicity analysis set

	H56:IC31®	Placebo
Immunogenicity analysis set (N)	43	50
Anti-H56 IgG (EU/mL)		
Visit 3 (Day 0) - Baseline		
N	43	49
Mean (SD)	110.2 (694.2)	5.9 (10.7)
Median	4.2	2.9
q25 - q75	1.6 - 6.1	1.4 - 5.8
Min - Max	0.3 - 4556.2	0.3 - 55.4
Visit 6 (Day 70)		
N	42	50
Mean (SD)	139.6 (708.3)	3.1 (3.2)
Median	6.7	2.2
q25 - q75	1.6 - 30.6	1.1 - 3.9
Min - Max	0.5 - 4576.0	0.2 - 17.7
Fold increase from Baseline to Visit 6 (Day 70)		
N	42	49
Mean (SD)	7.2 (20.4)	0.8 (0.6)
Median	1.8	0.6
q25 - q75	1.0 - 6.1	0.4 - 1.0
Min - Max	0 - 131	0 - 2

Page 1 of 1

N: Number of participants, SD: Standard deviation, q25: Lower quartile, q75: Upper quartile
Trial: POR A-055
Program: t_antiH56.sas - output: t_antiH56.rtf - executed: 10NOV2023

Cross-reference: Table 14.2.6.1, Appendix 16.2.1

11.4.3 Other immunogenicity endpoints

The exploratory immunogenicity endpoints in this trial will be reported elsewhere.

11.5 Immunogenicity results summary

H56:IC31® vaccination induced significant increases in frequencies of antigen specific CD4+ T cells expressing any combination of IL-2, IFN-γ, TNF, IL-17 (Figure 8 A), CD4+ T cells co-expressing IL-2 and TNF (Figure 8 B), and polyfunctional IL-2+ IFN-γ+ TNF+ CD4+ T cells (Figure 8 C), measured at Day 70, relative to pre-vaccination. No changes in these responses were observed in placebo recipients. The fold change in H56-specific CD4+ T cell responses, computed between Day 70 and Day 0 was significantly higher in H56:IC31® recipients than in placebo recipients. Frequencies of antigen specific CD8+ T cells were not significantly modulated by vaccination (Figure 8 D).

Participants receiving H56:IC31® mounted robust H56-specific humoral (serum IgG) responses.

12 Safety evaluation

12.1 Adverse events

12.1.1 Brief summary of adverse events

Overall, 72.3% of the participants in the H56:IC31® group experienced 1382 adverse events (AEs) and 64.2% in the placebo group reported 1033 AEs. Among the H56:IC31® vaccinees 3.6% had a severe AEs, 20.2% had a moderate AE and 67.0% had AEs of mild intensity only.

28 serious adverse events (SAEs), were reported, of these 16 SAEs were reported by 14 participants in the H56:IC31® group and 12 SAEs by 12 participants in the placebo group. No related SAEs and no SUSARs were reported. 2 adverse events of special interest (AESIs) were reported (by 2 participants in the placebo group).

In the H56:IC31® group, the most frequently reported AEs were: injection site pain (34.0%), fatigue (27.0%), headache (24.1%), myalgia (21.9%), injection site erythema (18.6%), injection site swelling (17.6%), arthralgia (16.4%), nausea (12.8%) and pyrexia (6.3%).

39.8% of the H56:IC31® vaccinees reported an injection site reaction. The majority of the reported injection site reactions were of mild intensity (> 95%). 2.7% of the H56:IC31® vaccinees had a moderate injection site reaction. No injection site reactions of severe intensity were reported. There were no indications of higher or lower frequencies of injection site reactions after the 2nd injection than after the 1st injection. In the H56:IC31® group, the highest measured redness diameter was 30 mm and the highest measured swelling diameter was 40 mm.

In the H56:IC31® group, 1 participant experienced related severe pyrexia for 4 days with onset 4 days after the 2nd vaccination (with no record of maximum temperature). 7 participants experienced 11 events of related moderate pyrexia for between 1 and 3 days with onset between 1 and 7 days after the 1st and/or 2nd vaccination. The recorded maximum temperatures were between 37.5 °C and 41.1 °C. The outcome of all pyrexia reactions was ‘recovered / resolved’.

No notable between group differences and no changes from V2 through V4 or V6 were noted for any of the biochemistry and haematology laboratory safety tests or vital signs measurements.

Overall, the H56:IC31® vaccine is considered well-tolerated. The majority of participants experienced only mild AEs. There was a low frequency of moderate injection site reactions and no severe injection site reactions and all SAEs were assessed as unrelated to the H56:IC31® vaccine.

12.1.2 Overall analysis of all adverse events

Overall, 72.3% (300/415) of the participants in the H56:IC31® group experienced 1382 AEs and 64.2% (267/416) in the placebo group reported 1033 AEs, please see Table 31 and Table 32. Among these were the solicited AEs reported by 57.6% (239/415) of the participants in the H56:IC31® group and 46.9% (195/416) in the placebo group. For a separate summary of solicited AEs, please refer to Table 14.3.2.5, Appendix 16.2.1.

Table 31: Summary of all adverse events – Solicited and unsolicited – Safety analysis set

	H56:IC31® N (%) E	Placebo N (%) E
Safety analysis set (N)	415	416
Any adverse events	300 (72.3) 1382	267 (64.2) 1033
Solicited AE	239 (57.6) 1034	195 (46.9) 706
AESI		2 (0.5) 2
SAE	14 (3.4) 16	12 (2.9) 12
Fatal SAE	2 (0.5) 2	6 (1.4) 6
Related AE	242 (58.3) 1074	190 (45.7) 741
Related SAE		
SUSAR		
AE leading to discontinuation of IMP	1 (0.2) 1	4 (1.0) 5
AE leading to discontinuation of trial	3 (0.7) 3	6 (1.4) 6
Intensity		
Severe	15 (3.6) 18	10 (2.4) 10
Moderate	84 (20.2) 165	93 (22.4) 163
Mild	278 (67.0) 1199	234 (56.3) 860
Outcome		
Fatal	2 (0.5) 2	6 (1.4) 6
Not recovered/not resolved	30 (7.2) 36	29 (7.0) 33
Recovered/resolved with sequelae	4 (1.0) 4	3 (0.7) 3
Recovered/resolved	293 (70.6) 1340	253 (60.8) 987
Unknown		4 (1.0) 4

N: Number of participants, %: Percentage of participants, E: Number of events, AE: Adverse event
AESI: Adverse event of special interest, SAE: Serious adverse event, SUSAR: Suspected unexpected serious adverse reaction
IMP: Investigational medicinal product
Only treatment emergent AEs are presented
Solicited AEs: Events occurring within 7 days after a vaccination and of the following types: the injection site reactions redness, swelling, and tenderness/pain, and the systemic adverse events fever, arthralgia, myalgia, fatigue, headache, rash, chills, and nausea.
Trial: POR A-055
Program: t_ae.sas - output: t_ae.rtf - executed: 10NOV2023

Cross-reference: Table 14.3.1.1, Appendix 16.2.1

3.6% (15/415) in the H56:IC31® group and 2.4% (10/416) in the placebo group had an AE of severe intensity, where 20.2% (84/415) in the H56:IC31® group and 22.4% (93/416) in the placebo group had a moderate AE. For the remaining 67.0% (278/415) participants in the H56:IC31® group and 56.3% (234/416) in the placebo group, all experienced AEs were of mild intensity, please see Table 31.

The outcome of the AEs was most frequently 'recovered/resolved', noted for 70.6% (293/415) of the participants in the H56:IC31® group and 60.8% (253/416) in the placebo group. Less frequently the outcome was 'not recovered / not resolved' (7.2% (30/415) of the participants in the H56:IC31® group and 7.0% (29/416) in placebo group. 4 participants in the H56:IC31® group and 3 participants in the placebo group had an AE where the outcome was 'recovered with sequelae' and 4 participants in the placebo group had an AE where the outcome was 'unknown', please see Table 31.

Overall, 28 SAEs, were reported, of these 16 SAEs were reported by 14 participants in the H56:IC31® group and 12 SAEs by 12 participants in the placebo group.
8 SAEs in 8 participants had fatal outcome, 2 in the H56:IC31® group and 6 in the placebo group.

No related SAEs and no SUSARS were reported. 2 adverse events of special interest (AESIs) were reported (by 2 participants in the placebo group), please refer to Table 14.3.1.1, Appendix 16.2.1.

For details on all AEs for each participant, please refer to Listing 16.2.7.1, Appendix 16.2.3 and for details on all adverse reactions, please refer to Listing 16.2.7.5, Appendix 16.2.3.

12.1.3 Most frequently reported adverse events

The most frequently (group frequencies ≥ 2.0%) reported AEs are shown by system organ class (SOC) and preferred term (PT) in Table 32. For a corresponding table including all reported AEs, please refer to Table 14.3.1.2, Appendix 16.2.1.

The most frequent AE (i.e., highest frequency in one of the groups) was injection site pain reported by 34.0% (141/415) in the H56:IC31® group and 13.7% (57/416) in the placebo group, followed by AE frequencies for fatigue (27.0%; 112/415), headache (24.1%; 100/415) in the H56:IC31® group with similar frequencies in the placebo group. For myalgia the frequency was 21.9% (91/415) in the H56:IC31® group which was higher than in the placebo group (13.7%; 57/416).

Injection site erythema (18.6%; 77/415) and injection site swelling (17.6%; 73/415) occurred, as expected, at higher frequencies in the H56:IC31® group, than in the placebo group (8.7% and 5.5%, respectively).

Other notable AEs reported at frequencies ≥ 2.0% in the H56:IC31® group were: arthralgia (16.4%; 68/415), nausea (12.8%; 53/415), pyrexia (6.3%; 26/415), chills (4.8%; 20/415) and rash (3.6%; 15/415) with similar frequencies in the placebo group. For remaining AEs and placebo group frequencies, please see Table 32, Figure 9, Figure 10 and Figure 11.

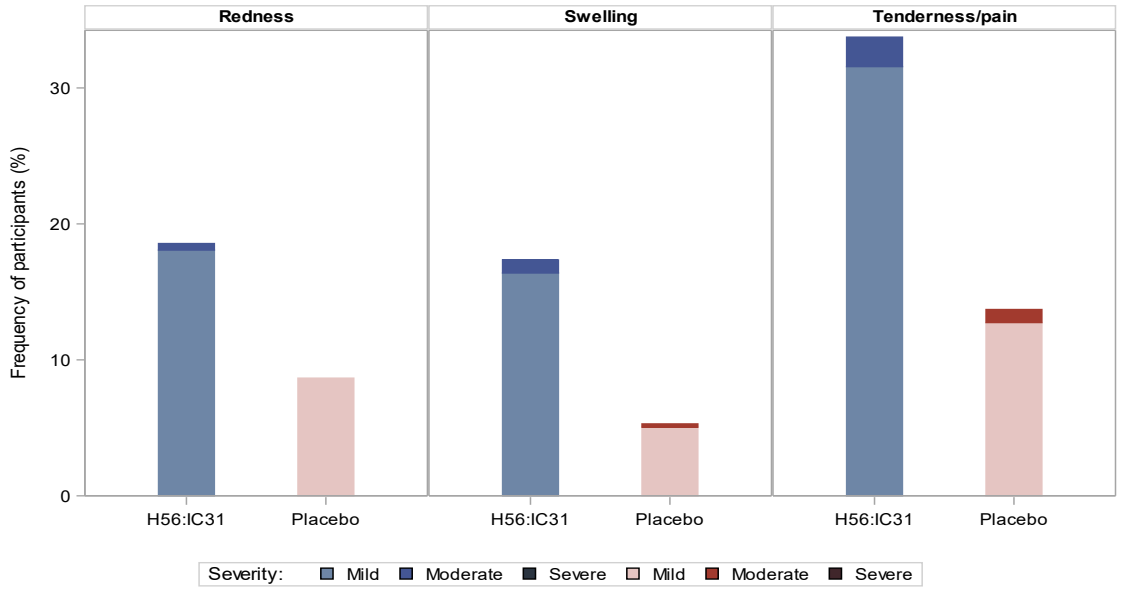
Table 32: Adverse events by system organ class (SOC) and preferred term (PT) – Solicited and unsolicited – AEs with PT group frequency ≥ 2.0% – Safety analysis set

	H56:IC31® N (%) E	Placebo N (%) E
Safety analysis set (N)	415	416
Any adverse events	300 (72.3) 1382	267 (64.2) 1033
General disorders and administration site conditions	216 (52.0) 664	168 (40.4) 389
Injection site pain	141 (34.0) 212	57 (13.7) 72
Fatigue	112 (27.0) 160	104 (25.0) 155
Injection site erythema	77 (18.6) 98	36 (8.7) 45
Injection site swelling	73 (17.6) 101	23 (5.5) 27
Pyrexia	26 (6.3) 31	23 (5.5) 28
Chills	20 (4.8) 30	18 (4.3) 25
Musculoskeletal and connective tissue disorders	118 (28.4) 245	97 (23.3) 188
Myalgia	91 (21.9) 123	57 (13.7) 79
Arthralgia	68 (16.4) 92	62 (14.9) 83
Back pain	12 (2.9) 12	10 (2.4) 11
Nervous system disorders	104 (25.1) 150	109 (26.2) 167
Headache	100 (24.1) 136	105 (25.2) 158
Gastrointestinal disorders	76 (18.3) 99	77 (18.5) 102
Nausea	53 (12.8) 63	47 (11.3) 59
Abdominal pain	10 (2.4) 12	8 (1.9) 8
Toothache	6 (1.4) 6	9 (2.2) 9
Diarrhoea	4 (1.0) 4	9 (2.2) 9
Infections and infestations	63 (15.2) 77	60 (14.4) 76
Upper respiratory tract infection	18 (4.3) 18	25 (6.0) 27
Skin and subcutaneous tissue disorders	28 (6.7) 31	27 (6.5) 34
Rash	15 (3.6) 17	14 (3.4) 18

N: Number of participants, %: Percentage of participants, E: Number of events, SOC: System organ class
Only treatment emergent AEs are presented
Sorted by total frequency of participants, total frequency of events, SOC, and preferred term.
Trial: POR A-055
Program: t_ae_soc.sas - output: t_aesoc.rtf - executed: 10NOV2023

Cross-reference: Modified from Table 14.3.1.2, Appendix 16.2.1

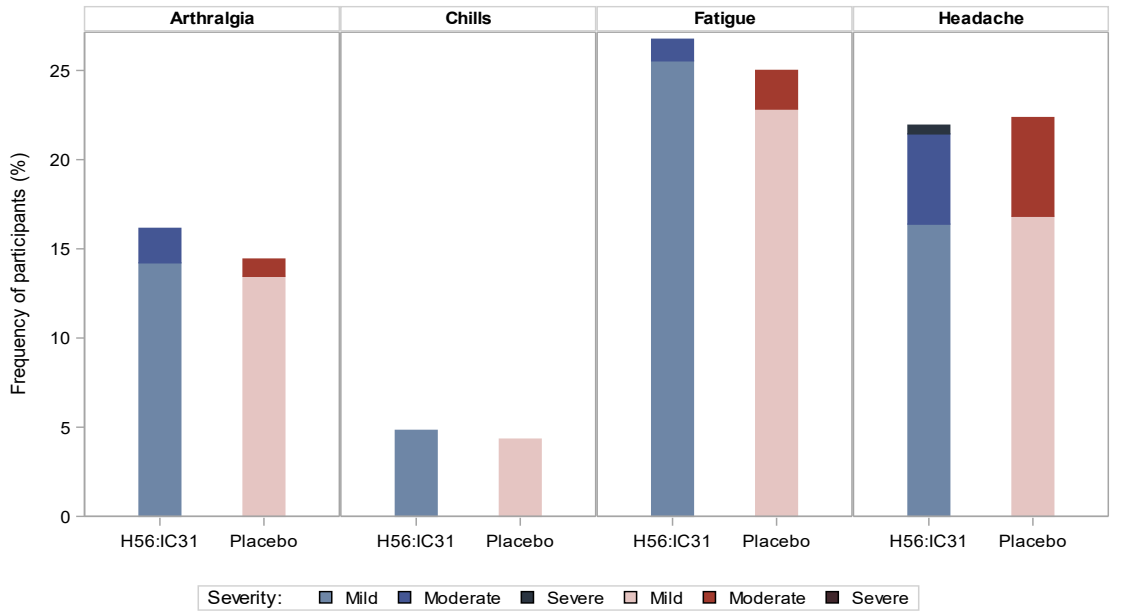
Figure 9: Frequency of participants with injection site redness, swelling and tenderness/pain by severity – Safety analysis set



For participants having the same injection site reation with different severities only the reaction with the maximum severity is used for the figure.
Project: POR A-055
Program: f_bar_injsr_freq.sas - output: f_bar_injsr_freq.rtf - executed: 03NOV2023

Cross-reference: Figure 14.3.2.1, Appendix 16.2.2

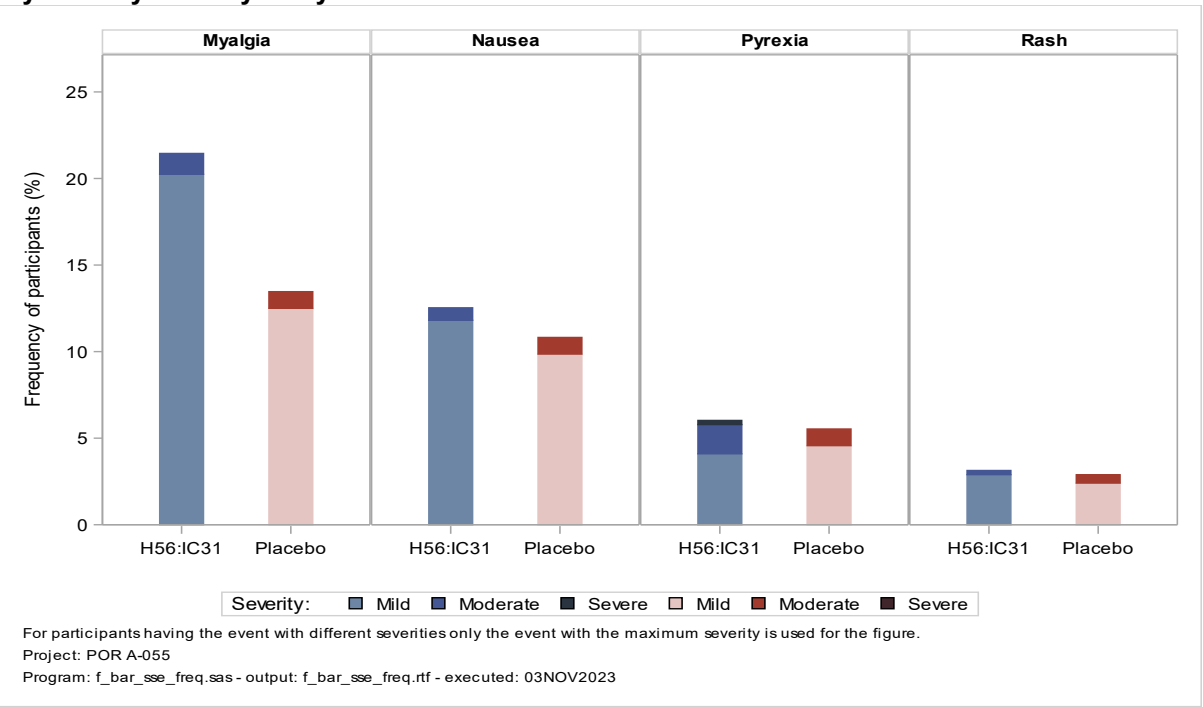
Figure 10: Frequency of participants with arthralgia, chills, fatigue and headache by severity – Safety analysis set



For participants having the event with different severities only the event with the maximum severity is used for the figure.
Project: POR A-055
Program: f_bar_sse_freq.sas - output: f_bar_sse_freq.rtf - executed: 03NOV2023

Cross-reference: Figure 14.3.2.2, Appendix 16.2.2

Figure 11: Frequency of participants with myalgia, nausea, pyrexia and rash by severity – Safety analysis set



Cross-reference: Figure 14.3.2.2, Appendix 16.2.2

In the period within 14 days after the vaccinations, 3 participants, all in the H56:IC31® group, reported 2 severe headaches and 1 severe pyrexia, please refer to Table 14.3.1.19, Appendix 16.2.1 and Listing 16.2.7.5, Appendix 16.2.3. One of the severe headaches had onset on the day of the 2nd vaccination and a duration of 12 days (A50002) where the other severe headache had onset 7 days after the 2nd vaccination with a duration of 1 day (A50026).

For further details on the severe pyrexia, please see Section 12.1.5. These 3 severe AEs were assessed as related to the vaccination and the outcome was 'recovered / resolved'. In the same time period, overall 13.0% (54/415) of the H56:IC31® vaccinees reported an AE of moderate intensity. The most frequently reported moderate AE was headache (5.1%; 21/415). For details on frequencies and PTs of the remaining AEs of moderate intensity in this time period, please refer to Table 14.3.1.7, Appendix 16.2.1.

For details on solicited AEs within 7 days and unsolicited AEs within 14 days after the 2 injections, please refer to Tables 14.3.1.3 to 14.3.1.6, Appendix 16.2.1. For details on all AEs within 14 days after the 2 injections, overall, by severity, causality, age group and sex, please refer to Tables 14.3.1.19 and 14.3.1.20 and Tables 14.3.1.7 to 14.3.1.10, Appendix 16.2.1.

For details on each participant, please refer to Listing 16.2.7.3 (solicited systemic reactions within 7 days after the vaccinations) and Listing 16.2.7.4 (unsolicited AEs within 14 days after the vaccinations), both in Appendix 16.2.3.

12.1.4 Analysis of injection site reactions

39.8% (165/415) of the participants in the H56:IC31® group and 18.8% (78/416) in the placebo group had at least one solicited injection site reaction. All injection site reactions were assessed as related to the IP administration.

The majority of the reported injection site reactions were of mild intensity (> 95%). 2.7% (11/415) of the H56:IC31® vaccinees and 1.2% (5/416) of the placebo recipients had a moderate injection site reaction. No severe injection site reactions were reported. The outcome of all injection site reactions was noted as 'recovered / resolved', please refer to Table 14.3.2.2, Appendix 16.2.1.

In general, the frequencies were higher in the H56:IC31® group, indicating that injection of vaccine, as expected, did lead to higher frequencies of injection site reactions than injection of placebo (saline). In the H56:IC31® group injection site reaction frequencies were: 34.0% (141/415) for pain, 18.6% (77/415) erythema and 17.6% (73/415) for swelling. For placebo group frequencies, please see Table 32 and Figure 9. For details on all injection site reactions for each participant, please refer to Listing 16.2.7.2, Appendix 16.2.3.

The frequencies of injection site tenderness/pain, redness and swelling, comparing the 14 days periods after the 1st and 2nd injections, were similar within the H56:IC31® and placebo groups. There were thus no indications of higher or lower frequencies of injection site reactions after the 2nd injection than after the 1st injection, please see Table 33.

The majority of the injection site reactions occurred through Day 0 to Day 7 in both groups, and most had resolved on Day 8, please see Table 33. In general, the frequencies were higher in the H56:IC31® group at all time points, similarly to the overall frequencies in Table 32.

The maximum diameters of redness and swelling, measured by the participants as part of the diary procedures in the 7 days after the 2 vaccinations are shown in Table 34 (redness) and Table 35 (swelling).

In the H56:IC31® group, the lowest redness maximum diameter was 1 mm and the highest was 30 mm, after both the 1st and 2nd vaccination. The mean maximum measurements on the vaccination days; 5.2 mm (1st vaccination) and 5.0 mm (2nd vaccination), increased in following period (Day 1 to Day 7) to 7.6 mm (1st vaccination) and 9.6 mm (2nd vaccination).

In the H56:IC31® group, the lowest swelling maximum diameters was 1 mm (1st vaccination) and 2 mm (2nd vaccination) and the highest was 30 mm (1st vaccination) and 40 mm (2nd vaccination). The mean maximum measurements on the vaccination days; 6.3 mm (1st vaccination) and 3.9 mm (2nd vaccination), increased in following period (Day 1 to Day 7) to; 8.9 mm (1st vaccination) and 8.8 mm (2nd vaccination).

Overall, there were no injection site redness or swelling measurements ≥ 50 mm, and no injection site redness or swelling reactions of severe intensity. In the H56:IC31® group, there were 2 moderate injection site redness reactions (in 2 participants) and 4 moderate injection site swelling reactions (in 4 participants), please refer to Table 14.3.1.7, Appendix 16.2.1. None of the measured moderate redness reactions had a diameter > 30 mm, and none of the measured moderate swelling reactions had a diameter > 40 mm, please see Table 34 and Table 35.

In addition to the injection site reactions covered by the injection site reaction analysis in the present section; namely injection site tenderness/pain (PT: injection site pain), injection site redness (PT: injection site erythema) and injection site swelling (PT: injection site swelling), the following injection site reactions (PTs) occurred among the H56:IC31® vaccinees: 3 injection site pruritis reactions (in 2 participants), 2 injection site rash reactions (in 2 participants) and 1 injection site discoloration reaction (in 1 participant), please refer to Table 14.3.1.2, Appendix 16.2.1.

Table 33: Injection site reactions (redness, swelling tenderness/pain) within 14 days after vaccination – Safety analysis set

	H56:IC31® N (%) E	Placebo N (%) E
Safety analysis set (N)	415	416
Day 0: 1st vaccination		
Redness	19 (4.6) 19	14 (3.4) 14
Swelling	16 (3.9) 16	7 (1.7) 7
Tenderness/pain	32 (7.7) 32	18 (4.3) 18
Day 1 to 7 after 1st vaccination		
Redness	34 (8.2) 35	11 (2.6) 11
Swelling	34 (8.2) 36	10 (2.4) 10
Tenderness/pain	78 (18.8) 83	23 (5.5) 25
Other	4 (1.0) 4	
Day 8 to 14 after 1st vaccination		
Swelling	1 (0.2) 1	
Tenderness/pain	2 (0.5) 2	
Other	1 (0.2) 1	
Day 56: 2nd vaccination		
Redness	14 (3.4) 14	9 (2.2) 9
Swelling	12 (2.9) 12	4 (1.0) 4
Tenderness/pain	29 (7.0) 29	11 (2.6) 11
Day 1 to 7 after 2nd vaccination		
Redness	30 (7.2) 30	10 (2.4) 11
Swelling	35 (8.4) 35	5 (1.2) 5
Tenderness/pain	64 (15.4) 66	15 (3.6) 17
Other	1 (0.2) 1	
Day 8 to 14 after 2nd vaccination		
Swelling		1 (0.2) 1
Tenderness/pain		1 (0.2) 1

Page 1 of 1

N: Number of participants, %: Percentage of participants, E: Number of events
Trial: POR A-055
Program: t_ae_isrlym.sas - output: t_ae_isrlym.rtf - executed: 10NOV2023

Cross-reference: Table 14.3.2.1, Appendix 16.2.1

Table 34: Maximum diameter of redness (mm) within 7 days after vaccination – Safety analysis set

	H56:IC31®	Placebo
Safety analysis set (N)	415	416
Max diameter of redness (mm)		
Day 0: 1st vaccination		
E	17	14
Mean (SD)	5.2 (3.6)	4.6 (2.9)
Median	4.0	3.0
q25 - q75	3.0 - 8.0	2.0 - 7.0
Min - Max	1 - 14	2 - 11
Day 1 to 7 after 1st vaccination		
E	32	11
Mean (SD)	7.6 (6.7)	6.5 (4.0)
Median	5.0	4.0
q25 - q75	3.5 - 10.5	4.0 - 10.0
Min - Max	1 - 30	3 - 16
Day 56: 2nd vaccination		
E	13	9
Mean (SD)	5.0 (3.4)	7.7 (10.1)
Median	5.0	3.0
q25 - q75	2.0 - 7.0	1.0 - 10.0
Min - Max	1 - 11	1 - 30
Day 1 to 7 after 2nd vaccination		
E	28	8
Mean (SD)	9.6 (7.9)	5.3 (4.4)
Median	8.0	3.5
q25 - q75	4.0 - 11.5	2.0 - 7.5
Min - Max	2 - 30	2 - 14

E: Number of reactions with non-missing measurement of size, SD: Standard deviation, q25: Lower quartile, q75: Upper quartile
Trial: POR A-055
Program: t_ae_maxdia.sas - output: t_ae_maxdiared.rtf - executed: 10NOV2023

Cross-reference: Table 14.3.2.3, Appendix 16.2.1

Table 35: Maximum diameter of swelling (mm) within 7 days after vaccination – Safety analysis set

	H56:IC31®	Placebo
Safety analysis set (N)	415	416
Max diameter of size of swelling (mm)		
Day 0: 1st vaccination		
E	14	6
Mean (SD)	6.3 (4.9)	2.5 (1.0)
Median	6.0	2.5
q25 - q75	3.0 - 7.0	2.0 - 3.0
Min - Max	1 - 20	1 - 4
Day 1 to 7 after 1st vaccination		
E	34	7
Mean (SD)	8.9 (8.0)	17.7 (34.2)
Median	6.0	4.0
q25 - q75	3.0 - 12.0	3.0 - 10.0
Min - Max	1 - 30	1 - 95
Day 56: 2nd vaccination		
E	11	4
Mean (SD)	3.9 (2.8)	13.8 (16.3)
Median	3.0	9.5
q25 - q75	3.0 - 4.0	1.0 - 26.5
Min - Max	2 - 12	1 - 35
Day 1 to 7 after 2nd vaccination		
E	30	3
Mean (SD)	8.8 (9.4)	11.7 (8.5)
Median	5.0	12.0
q25 - q75	2.0 - 10.0	3.0 - 20.0
Min - Max	1 - 40	3 - 20

Page 1 of 1

E: Number of reactions with non-missing measurement of size, SD: Standard deviation, q25: Lower quartile, q75: Upper quartile
Trial: POR A-055
Program: t_ae_maxdia.sas - output: t_ae_maxdiaswell.rtf - executed: 10NOV2023

Cross-reference: Table 14.3.2.4, Appendix 16.2.1

12.1.5 Analysis of pyrexia and weight loss

For maximum temperature measured by the participants as part of the diary procedures in the 7 days after the 2 vaccinations please refer to Table 14.3.2.6, Appendix 16.2.1. In the H56:IC31® group, there were 15 maximum temperature measurements after the 1st vaccination and 8 after the 2nd vaccination. These measurements ranged between 37.5 °C and 41.1 °C with a mean value of 38.3 °C on Days 1 to 7, both after the 1st and 2nd vaccination, please refer to Table 14.3.2.6, Appendix 16.2.1.

1 H56:IC31® vaccinated participant (A10044) experienced severe pyrexia for 4 days with onset 4 days after the 2nd vaccination. The maximum temperature was not measured. This pyrexia reaction was assessed as related to the vaccination. After 4 days, the pyrexia resolved and the participant recovered, please refer to Table 14.3.1.7, Appendix 16.2.1 and Listing 16.2.7.3, Appendix 16.2.3.

7 H56:IC31® vaccinated participants (A10044, A20015, A20070, A30058, A30072, A40046 and A60076) experienced 11 events of related moderate pyrexia for between 1 and 3 days, with onset between 1 and 7 days after the 1st and/or 2nd vaccination, please refer to Table 14.3.1.7, Appendix 16.2.1 and Listing 16.2.7.3, Appendix 16.2.3. Not all had recordings of temperature measurements. The recorded measurements were between 37.5 °C and 41.1 °C. 1 participant (A50184) had 1 event of unrelated moderate pyrexia with a duration of 24 days. For all participants the outcome was noted as 'recovered / resolved', please refer to Listing 16.2.7.3, Appendix 16.2.3.

For the weight of the participants measured at the 2 screening visits (V1 and V2), at baseline (V3) and at all subsequent trial visits (V4 to V8), please refer to Table 14.3.3.5, Appendix 16.2.1.

There was a clear increase in weight from the first screening visit (V1) to the time of the V3 (baseline) where the participants had successfully completed the TB treatment for the TB episode just diagnosed at the time of V1. The mean weight at the time of V1 was 54.05 kg (H56:IC31® group) and 53.51 kg (placebo group) compared to mean weights of 58.67 kg (H56:IC31® group) and 58.12 kg (placebo group) at V3 where the participants had successfully completed the TB treatment. From V3 and onwards no differences in weight were noted. Also, no differences in weights between the two groups were noted, please refer to Table 14.3.3.5 to 14.3.3.6, Appendix 16.2.1 and Figure 14.3.3, Appendix 16.2.2.

Weight loss was one of the TB signs and symptoms, that could lead to suspicion of recurrent TB and trigger the case verification procedure for recurrent TB specified in the protocol. Weight changes were also monitored (blindly) during the trial by the sponsor's medically responsible(s), and in case of an identified weight loss, a query to initiate a suspected TB visit was sent to the concerned site PI. There seemed to be a trend towards a lower proportion of loss of weight out of all TB signs and symptoms, at the telephone calls (TC 1 to 5), compared to the site visits (V4 to V8), which could indicate that weight loss is more likely to be detected at trial visits where the weight is measured by site staff, than through telephone contacts, please refer to Table 14.3.4, Appendix 16.2.2.

12.1.6 Supplementary listings – Safety – AEs > 14 days after the vaccination

For further details of participants with injection site reactions with onset > 14 days after the vaccinations, please refer to Listing 16.2.7.10 and with non-serious systemic AEs with onset > 14 days after the vaccinations, please refer to Listing 16.2.7.11, both in Appendix 16.2.3.

12.2 Analysis of deaths, other serious and medically important adverse events

Overall, 28 SAEs were reported, 16 SAEs by 14 participants in the H56:IC31® group and 12 SAEs by 12 participants in the placebo group. 8 SAEs in 8 participants had fatal outcome, 2 in the H56:IC31® group and 6 in the placebo group. No related SAEs and no SUSARS were reported, please see Table 36 and Table 37. Overall, 4 participants in the H56:IC31® group and 7 participants in the placebo group discontinued the trial and/or the IP due to an AE, please see Section 12.2.1.3.

Table 36: Summary of serious adverse events – Safety analysis set

	H56:IC31® N (%) E	Placebo N (%) E
Safety analysis set (N)	415	416
Any serious adverse events	14 (3.4) 16	12 (2.9) 12
SAE	14 (3.4) 16	12 (2.9) 12
Fatal SAE	2 (0.5) 2	6 (1.4) 6
Related SAE		
SUSAR		
SAE leading to discontinuation of IMP		3 (0.7) 3
SAE leading to discontinuation of trial	3 (0.7) 3	6 (1.4) 6
Intensity		
Severe	13 (3.1) 15	10 (2.4) 10
Moderate	1 (0.2) 1	2 (0.5) 2
Outcome		
Fatal	2 (0.5) 2	6 (1.4) 6
Recovered/resolved with sequelae	3 (0.7) 3	1 (0.2) 1
Recovered/resolved	9 (2.2) 11	5 (1.2) 5

Page 1 of 1

N: Number of participants, %: Percentage of participants, E: Number of events, AE: Adverse event
AESI: Adverse event of special interest, SAE: Serious adverse event, SUSAR: Suspected unexpected serious adverse reaction
IMP: Investigational medicinal product
Only treatment emergent AEs are presented
Solicited AEs: Events occurring within 7 days after a vaccination and of the following types: the injection site reactions redness, swelling, and tenderness/pain, and the systemic adverse events fever, arthralgia, myalgia, fatigue, headache, rash, chills, and nausea.
Trial: POR A-055
Program: t_ae.sas - output: t_sae.rtf - executed: 10NOV2023

Cross-reference: Modified from Table 14.3.1.13, Appendix 16.2.1

Table 37: Serious adverse events by SOC and preferred term – Safety analysis set

	H56:IC31® N (%) E	Placebo N (%) E
Safety analysis set (N)	415	416
Any SAE	14 (3.4) 16	12 (2.9) 12
Injury, poisoning and procedural complications	3 (0.7) 3	6 (1.4) 6
Gun shot wound	1 (0.2) 1	2 (0.5) 2
Chest injury		1 (0.2) 1
Craniocerebral injury		1 (0.2) 1
Road traffic accident		1 (0.2) 1
Stab wound	1 (0.2) 1	
Toxicity to various agents	1 (0.2) 1	
Traumatic haemothorax		1 (0.2) 1
Infections and infestations	3 (0.7) 3	1 (0.2) 1
Pneumonia	2 (0.5) 2	
Abscess neck		1 (0.2) 1
Lower respiratory tract infection	1 (0.2) 1	
Pregnancy, puerperium and perinatal conditions	2 (0.5) 3	1 (0.2) 1
Abortion incomplete		1 (0.2) 1
Ectopic pregnancy	1 (0.2) 1	
Polyhydramnios	1 (0.2) 1	
Stillbirth	1 (0.2) 1	
Cardiac disorders	1 (0.2) 2	1 (0.2) 1
Cor pulmonale		1 (0.2) 1
Supraventricular tachycardia	1 (0.2) 1	
Tachycardia	1 (0.2) 1	
Nervous system disorders	2 (0.5) 2	
Cerebrovascular accident	1 (0.2) 1	
Epilepsy	1 (0.2) 1	

N: Number of participants, %: Percentage of participants, E: Number of events, SOC: System organ class
SAE: Serious adverse event
Only treatment emergent AEs are presented
Sorted by total frequency of participants, total frequency of events, SOC, and preferred term.
Trial: POR A-055
Program: t_ae_soc.sas - output: t_sae_soc.rtf - executed: 10NOV2023

Cross-reference: Table 14.3.1.14, Appendix 16.2.1

12.2.1 Deaths, other serious adverse events, discontinuations due to adverse events and adverse events of special interest

12.2.1.1 Deaths

2 of the participants with fatal SAEs had received the H56:IC31® vaccine, where the remaining 6 fatal SAEs occurred in 6 participants who had received placebo, please see Table 36 and please refer to Listing 16.2.7.9, Appendix 16.2.3. The 2 fatal SAEs in the H56:IC31® group are summarised below:

- Participant A30096 had severe pneumonia with onset 52 days after the 2nd vaccination with H56:IC31® and with a duration of 2 days, after which the participant died. This event of severe pneumonia was assessed as not related to the IP administration. For further details, please refer to Listing 16.2.7.9, Appendix 16.2.3 and for further reference to the narrative, please see Section 14

- Participant A30278 had a severe gunshot wound 125 days after the 2nd vaccination with H56:IC31® and died after 1 day. This event was assessed as not related to the IP administration. For further details, please see Listing 16.2.7.9, Appendix 16.2.3 and for further reference to the narrative, please see Section 14

The preferred terms of the 6 fatal SAEs that occurred in 6 participants in the placebo group are shown in Table 38.

Table 38: Preferred terms – Fatal SAEs – Participants treated with placebo

Participant treated with placebo	Preferred term (PT) of fatal SAEs
A10047	Cor pulmonale
A10162	Death
A20173	Road traffic accident
A30207	Gunshot wound
A30516	Gunshot wound
A50003	Craniocerebral injury

For further details, please refer to Listing 16.2.7.9, Appendix 16.2.3 and for further reference to the narratives of fatal SAEs, please see Section 14.

12.2.1.2 Serious adverse events (other than deaths)

There were 14 non-fatal SAEs in 12 participants in the H56:IC31® group. The PTs of these non-fatal SAEs are shown in Table 39.

Of note, 1 H56:IC31® vaccinated participant (A60074) had the SAE of hospitalisation (for recurrent TB) with onset 304 days after the 2nd vaccination due to which he discontinued the trial. This SAE was of moderate intensity, and was assessed as not related to the IP and the participant recovered with sequelae. As the recurrent TB was culture verified, the participant was included in the primary efficacy analysis. None of the remaining H56:IC31® vaccinated participants with SAEs had recurrent TB during the trial, please refer to Listing 16.2.7.7, Appendix 16.2.3 and for further reference to the narrative, please see Section 14.

Table 39: Preferred terms – Non-fatal SAEs – Participants treated with H56:IC31®

Participant treated with H56:IC31®	Preferred term (PT) of non-fatal SAEs
A10044	Toxicity to various agents
A20018	Substance-induced psychotic disorder
A30212	Pneumonia
A30517	Cerebrovascular accident
A40004	Lower respiratory tract infection
A40070	Stab wound
A40189	Epilepsy
A50054	Haemangioma of skin
A50254	Ectopic pregnancy
A50262	Supraventricular tachycardia & Tachycardia – 2 SAEs
A60020	Polyhydramnios & Still birth – 2 SAEs
A60074	Hospitalisation

6 placebo recipients experienced the following 6 non-fatal SAEs: chest injury (A10111), traumatic haemothorax (A30026), abscess neck (A40010), abortion incomplete (A40177), pneumothorax (A50065) and skin ulcer (A50252).

Of note, the placebo recipient (A50065) with the non-fatal SAE of pneumothorax, also had culture verified recurrent TB and was included in the primary efficacy analysis. None of the remaining placebo recipients with SAEs had recurrent TB. For further details of placebo recipients, please refer to Listing 16.2.7.7, Appendix 16.2.3 and for further reference to the narratives of non-fatal SAEs, please see Section 14.

12.2.1.3 Discontinuations due to adverse events

In the H56:IC31® group, overall 4 participants discontinued the trial and/or the IP due to an AE, please see Table 31 and please refer to Listing 16.2.7.8, Appendix 16.2.3. 2 participants left the trial due to a fatal SAE (A30096, A30278), please see Section 12.2.1.1, and 1 participant due to hospitalisation (A60074), please see Section 12.2.1.2. For 1 participant (A40189) the IP was discontinued due to the non-serious AE of seizure, please refer to Listing 16.2.7.8, Appendix 16.2.3.

In the placebo group, overall 7 participants discontinued the trial and/or the IP due to an AE which was a higher number than in the H56:IC31® group, please see Table 31 and please refer to Listing 16.2.7.8, Appendix 16.2.3. 3 participants discontinued the trial and the IP due to a fatal SAE (A30207, A30516, and A50003) and 3 participants discontinued the trial (after having received both doses of IP) due to a fatal SAE (A10047, A10162, A20173), please see Section 12.2.1.1. For 1 participant (A60115), the IP was discontinued due to the non-serious AEs of pruritus and generalised angioedema, please refer to Listing 16.2.7.8, Appendix 16.2.3.

12.2.1.4 Adverse events of special interest

2 adverse events of special interest (AESIs) were reported by 2 participants in the placebo group. The reported PTs were facial paralysis (A30155) and erythema multiforme (A50104). Both events were non-serious, moderate and resolved and the participants recovered, please refer to Table 14.3.1.11 and 14.3.1.12, Appendix 16.2.1. For further details on these 2 AESIs, please refer to Listing 16.2.7.6, Appendix 16.2.3.

12.2.2 Narratives of deaths, other serious adverse events and pregnancies

For safety narratives of deaths, SAEs other than deaths and pregnancies, please refer to Appendix 16.2.4.

12.3 Clinical laboratory evaluation

The biochemistry and haematology safety tests were performed for all participants at V2 (screening). Subsequently, the safety tests were only performed at V4 and V6, and only for safety cohort participants (i.e., the first 150 randomised participants in the trial across the sites; 76 participants in the H56:IC31® group and 74 in the placebo group). A safety test could also be performed at the discretion of the investigator at any visit, or at an early termination (ET) visit, if applicable. The results of the safety laboratory tests are shown for the safety analysis set in the tables and listings.

12.3.1 Individual laboratory measurements by participant and abnormal values

The individual biochemistry laboratory safety test results are available in Listing 16.2.9.1, Appendix 16.2.3.

The individual haematology laboratory safety test results are available in Listing 16.2.9.2, Appendix 16.2.3.

The individual urinalysis safety test results are available in Listing 16.2.9.3, Appendix 16.2.3.

12.3.2 Evaluation of laboratory values

The boxplots of the results of all biochemistry and haematology laboratory safety tests were evaluated to detect between group differences and changes over time from V2 (screening) to V6 (Day 70).

No notable between group differences and no changes from V2 through V4 or V6 were noted for any of the box plots of the biochemistry and haematology laboratory safety tests, please refer to Figures 14.3.5.1 to 14.3.5.15, Appendix 16.2.2.

12.3.2.1 Aspartate aminotransferase (AST)

At baseline (V2), the aspartate aminotransferase (AST) mean value was 0.449 ukat/L (SD: 0.297) in the H56:IC31® group and 0.487 ukat/L (SD: 0.629) in the placebo group. There were no notable changes when comparing these values to the mean values at V4 and V6, please refer to Table 14.3.5.1, Appendix 16.2.1 and Figure 14.3.5.1, Appendix 16.2.2.

12.3.2.2 Alanine aminotransferase (ALT)

At baseline (V2), the alanine aminotransferase (ALT) mean value was 0.336 ukat/L (SD: 0.227) in the H56:IC31® group and 0.340 ukat/L (SD: 0.306) in the placebo group. There were no notable changes when comparing these values to the mean values at V4 and V6, please refer to Table 14.3.5.2, Appendix 16.2.1 and Figure 14.3.5.2, Appendix 16.2.2.

12.3.2.3 Alkaline phosphatase (ALP)

At baseline (V2), the alanine aminotransferase (ALT) mean value was 1.626 ukat/L (SD: 0.533) in the H56:IC31® group and 1.664 ukat/L (SD: 0.658) in the placebo group. There were no notable changes when comparing these values to the mean values at V4 and V6, please refer to Table 14.3.5.3, Appendix 16.2.1 and Figure 14.3.5.3, Appendix 16.2.2.

12.3.2.4 Gamma glutamyl transferase (GGT)

At baseline (V2), the gamma glutamyl transferase (GGT) mean value was 0.938 ukat/L (SD: 1.137) in the H56:IC31® group and 0.982 ukat/L (SD: 1.270) in the placebo group. There were no notable changes when comparing these values to the mean values at V4 and V6, please refer to Table 14.3.5.4, Appendix 16.2.1 and Figure 14.3.5.4, Appendix 16.2.2.

12.3.2.5 Total bilirubin

At baseline (V2), the total bilirubin mean value was 7.423 µmol/L (SD: 4.762) in the H56:IC31® group and 7.680 µmol/L (SD: 4.326) in the placebo group. There were no notable changes when comparing these values to the mean values at V4 and V6, please refer to Table 14.3.5.5, Appendix 16.2.1 and Figure 14.3.5.5, Appendix 16.2.2.

12.3.2.6 Creatinine

At baseline (V2), the creatinine mean value was 60.98 µmol/L (SD: 12.09) in the H56:IC31® group and 60.82 µmol/L (SD: 14.37) in the placebo group. There were no notable changes when comparing these values to the mean values at V4 and V6, please refer to Table 14.3.5.6, Appendix 16.2.1 and Figure 14.3.5.6, Appendix 16.2.2.

12.3.2.7 Haemoglobin

At baseline (V2), the haemoglobin mean value was 144.22 g/L (SD: 14.95) in the H56:IC31® group and 144.88 g/L (SD: 15.74) in the placebo group. There were no notable changes when comparing these values to the mean values at V4 and V6, please refer to Table 14.3.5.7, Appendix 16.2.1 and Figure 14.3.5.7, Appendix 16.2.2.

12.3.2.8 Haematocrit

At baseline (V2), the haematocrit mean value was 0.4343 L/L (SD: 0.0418) in the H56:IC31® group and 0.4367 L/L (SD: 0.0424) in the placebo group. There were no notable changes when comparing these values to the mean values at V4 and V6, please refer to Table 14.3.5.8, Appendix 16.2.1 and Figure 14.3.5.8, Appendix 16.2.2.

12.3.2.9 Leukocytes

At baseline (V2), the leukocytes mean value was 6.002x10^9/L (SD: 2.166) in the H56:IC31® group and 5.867x10^9/L (SD: 1.826) in the placebo group. There were no notable changes when comparing these values to the mean values at V4 and V6, please refer to Table 14.3.5.9, Appendix 16.2.1 and Figure 14.3.5.9, Appendix 16.2.2.

12.3.2.10 Lymphocytes

At baseline (V2), the lymphocytes mean value was 6.002x10^9/L (SD: 0.658) in the H56:IC31® group and 1.980x10^9/L (SD: 0.647) in the placebo group. There were no notable changes when comparing these values to the mean values at V4 and V6, please refer to Table 14.3.5.10, Appendix 16.2.1 and Figure 14.3.5.10, Appendix 16.2.2.

12.3.2.11 Monocytes

At baseline (V2), the monocytes mean value was 0.505x10^9/L (SD: 0.193) in the H56:IC31® group and 0.508x10^9/L (SD: 0.178) in the placebo group. There were no notable changes when comparing these values to the mean values at V4 and V6, please refer to Table 14.3.5.11, Appendix 16.2.1 and Figure 14.3.5.11, Appendix 16.2.2.

12.3.2.12 Neutrophils

At baseline (V2), the neutrophils mean value was 3.256x10^9/L (SD: 1.705) in the H56:IC31® group and 3.171x10^9/L (SD: 1.498) in the placebo group. There were no notable changes when comparing these values to the mean values at V4 and V6, please refer to Table 14.3.5.12, Appendix 16.2.1 and Figure 14.3.5.12, Appendix 16.2.2.

12.3.2.13 Basophils

At baseline (V2), the basophils mean value was 0.030x10^9/L (SD: 0.020) in the H56:IC31® group and 0.028x10^9/L (SD: 0.019) in the placebo group. There were no notable changes when comparing these values to the mean values at V4 and V6, please refer to Table 14.3.5.13, Appendix 16.2.1 and Figure 14.3.5.13, Appendix 16.2.2.

12.3.2.14 Eosinophils

At baseline (V2), the eosinophils mean value was 0.196x10^9/L (SD: 0.183) in the H56:IC31® group and 0.186x10^9/L (SD: 0.184) in the placebo group. There were no notable changes when comparing these values to the mean values at V4 and V6, please refer to Table 14.3.5.14, Appendix 16.2.1 and Figure 14.3.5.14, Appendix 16.2.2.

12.3.2.15 Platelets

At baseline (V2), the platelets mean value was 282.4x10^9/L (SD: 73.8) in the H56:IC31® group and 281.1x10^9/L (SD: 78.6) in the placebo group. There were no notable changes when comparing these values to the mean values at V4 and V6, please refer to Table 14.3.5.15, Appendix 16.2.1 and Figure 14.3.5.15, Appendix 16.2.2.

12.3.3 Laboratory values over time

This section is not applicable.

12.3.3.1 Individual participant changes in laboratory values

This section is not applicable.

12.3.3.2 Individual clinically significantly abnormal laboratory values

Individual clinically significantly abnormal laboratory safety test results are listed by the related participants in Listing 16.2.9.4, Appendix 16.2.3.

12.4 Vital signs, physical examinations, and pregnancies

12.4.1 Vital signs

For individual vital sign measurements for the safety analysis set, please refer to Listing 16.2.8.1, Appendix 16.2.3.

12.4.1.1 Blood pressure

The systolic blood pressure was measured at V2 (screening), at V3 (baseline; pre- and post-vaccination), at V5 (pre- and post-vaccination) and at V4 and V6 (14 days after the 1st and 2nd vaccination).

The mean systolic blood pressure was 119.8 mmHg (SD: 14.2) in the H56:IC31® group and 121.3 mmHg (SD: 14.8) in the placebo group at V3 (baseline).

There were no notable changes from pre-vaccination to post-vaccination and no notable changes between the treatment groups or between the visits during the trial, please refer to Table 14.3.3.1, Appendix 16.2.1, for the results of the measurements.

The diastolic blood pressure was measured at the same time points as the systolic blood pressure. The mean diastolic blood pressure was 76.2 mmHg (SD: 10.1) in the H56:IC31® group and 77.1 mmHg (SD: 11.0) in the placebo group at V3 (baseline).

There were no notable changes from pre-vaccination to post-vaccination and no notable changes between the treatment groups or between the visits during the trial, please refer to Table 14.3.3.2, Appendix 16.2.1, for the results of the measurements.

12.4.1.2 Pulse rate

The pulse rate was measured at V2 (screening), at V3 (baseline; pre- and post-vaccination), at V5 (pre- and post-vaccination) and at V4 and V6 (14 days after the 1st and 2nd vaccination).

The mean pulse rate was 73.3 beats/min (SD: 12.3) in the H56:IC31® group and 73.7 beats/min (SD: 12.9) in the placebo group at V3 (baseline).

There were no notable changes from pre-vaccination to post-vaccination and no notable changes between the treatment groups or between the visits during the trial, please refer to Table 14.3.3.3, Appendix 16.2.1, for the results of the measurements.

12.4.1.3 Axillary temperature

The axillary temperature was measured at V2 (screening), at V3 (baseline; pre- and post-vaccination), at V5 (pre- and post-vaccination) and at V4 and V6 (14 days after the 1st and 2nd vaccination).

The mean axillary temperature was 36.06 °C (SD: 0.43) in the H56:IC31® group and 36.02 °C (SD: 0.41) in the placebo group at V3 (baseline).

There were no notable changes from pre-vaccination to post-vaccination and no notable changes between the treatment groups or between the visits during the trial. For the results of the measurements, please refer to Table 14.3.3.4, Appendix 16.2.1.

12.4.2 Physical examination findings

All clinically significant abnormal physical examination findings are listed by participant in Listing 16.2.8.2, Appendix 16.2.3.

All weight measurements recorded at all visits are listed by participant in Listing 16.2.8.3, Appendix 16.2.3.

All TB signs and symptoms recorded at all visits are listed by participant in Listing 16.2.8.4, Appendix 16.2.3.

12.4.3 Pregnancies

There were 9 pregnancies in 9 participants in the trial (4 in the H56:IC31® group and 5 in the placebo group). In 6 of the pregnancies, there were no pregnancy related SAE, whereas 3 pregnant participants experienced 4 pregnancy related SAEs, please see Table 40.

Table 40: Pregnant participants – Pregnancy outcomes – Preferred terms pregnancy related SAEs

Pregnant participant	Treatment	Pregnancy outcome	Preferred term (PT) of pregnancy related SAEs
A10057	H56:IC31®	Lost to follow-up	-
A50254	H56:IC31®	Foetus fatal	Ectopic pregnancy
A60007	H56:IC31®	Baby normal	-
A60020	H56:IC31®	Foetus fatal	Polyhydramnios & Still birth – 2 SAEs
A10095	Placebo	Baby normal	-
A20088	Placebo	Baby normal	-
A40177	Placebo	Foetus fatal	Abortion incomplete
A50069	Placebo	Baby normal	-
A50238	Placebo	Baby normal	-

For further details on pregnancy outcomes, please refer to Listing 16.2.9.6 and 16.2.9.7, Appendix 16.2.3 and for further reference to the narratives of pregnancies, please see Section 14.

For details on individual pregnancy test results for the safety analysis set, please refer to Listing 16.2.9.5, Appendix 16.2.3.

12.5 Safety results summary

No notable between group differences and no changes from V2 through V4 or V6 were noted for any of the biochemistry and haematology laboratory safety tests or vital signs measurements.

Overall, the H56:IC31® vaccine is considered well-tolerated. The majority of participants experienced only mild AEs. There was a low frequency of moderate injection site reactions and no severe injection site reactions and all SAEs were assessed as unrelated to the H56:IC31® vaccine.

13 Discussion and overall conclusions

13.1 Discussion

Mycobacterium tuberculosis (*Mtb*), the causative agent of TB kills more people than any other single pathogen and remains one of the biggest global health burdens. About a quarter of the global population is estimated to have been infected with TB (1) and is therefore at risk of developing active TB.

H56:IC31® has been developed by SSI and was the first multistage TB subunit vaccine candidate to enter clinical trials. H56:IC31® was specifically designed to mediate protection both as a preventive and therapeutic vaccine, making it the ideal investigational vaccine for the present POR TB clinical trial (A-055).

The present A-055 clinical trial was the first efficacy trial with H56:IC31®. As demonstration of protective efficacy against TB disease in the general population would require large (many thousands of participants) and lengthy field trials, shorter and smaller proof-of-concept phase 2 trials, investigating subpopulations 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 (4-6, 15, 16).

Overall, the TB recurrence incidence rate in the H56:IC31® group was 6.3% (95% CI: 4.0; 9.4) compared to 3.7% (95% CI: 2.0; 6.2) in the placebo group with a relative risk of recurrent TB of 1.3 (95% CI: 0.7; 1.6) after H56:IC31® vaccination (relative to placebo). The rates of participants who had recurrent TB were 5.8% (23/400) in the H56:IC31® group and 3.4% (14/406) in the placebo group (primary efficacy analysis) which led to the conclusion that the H56:IC31® vaccine does not reduce the rate of recurrent TB.

The supportive-, secondary-, supplementary- and the three exploratory- efficacy analyses all confirmed this result, so it was a very clear result. However, the underlying mechanisms for this efficacy result are not yet understood and further analysis is ongoing to obtain better understanding. From the forest plots on different subgroups an increased hazard for TB recurrence was observed in participants older than 35 years, without anaemia, and who received both IP injections, but interaction tests were not significant.

Interestingly, the lack of efficacy of H56:IC31® seemed to be driven by TB relapse rather than by TB reinfection and the recurrent TB cases, recorded from Month 0 (Day 70) and onwards, seemed more frequent (in the H56:IC31® group) in the period from Month 4 to 6, than during the remaining periods in the follow-up period (of in total 12 months).

The efficacy results of this trial will be used to inform vaccine developers and to learn from the experience gained, both the scientific aspects related to the efficacy of the investigated TB vaccine and the clinical trial design aspects related to how the efficacy of TB vaccines is best investigated in clinical trials.

H56:IC31® vaccination induced significant increases in frequencies of antigen specific CD4+ T cells expressing any combination of IL-2, IFN-γ, TNF, IL-17, CD4+ T cells co-expressing IL-2 and TNF, and polyfunctional IL-2+ IFN-γ+ TNF+ CD4+ T cells, measured at Day 70, relative to pre-vaccination. No changes in these responses were observed in placebo recipients. Frequencies of antigen specific CD8+ T cells were not significantly modulated by vaccination. The median fold increase in anti-H56 IgG titres from Day 0 to Day 70 was higher in the H56:IC31® group than in the placebo group.

AEs were experienced more frequently by H56:IC31® vaccinated participants than by placebo recipients. 72.3% of the participants in the H56:IC31® group experienced 1382 AEs and 64.2% in the placebo group reported 1033 AEs. 28 SAEs, were reported, there were no related SAEs and no SUSARS. Among the H56:IC31® vaccinees 3.6% had a severe AEs, 20.2% had a moderate AE and 67.0% had AEs of mild intensity only.

In the H56:IC31® group, the most frequently reported AEs were; injection site pain (34.0%), fatigue (27.0%), headache (24.1%), myalgia (21.9%), injection site erythema (18.6%), injection site swelling (17.6%), arthralgia (16.4%), nausea (12.8%) and pyrexia (6.3%).

39.8% of the H56:IC31® vaccinees reported an injection site reaction. The majority of the reported injection site reactions were of mild intensity (> 95%). 2.7% of the H56:IC31® vaccinees had a moderate injection site reaction. There were no severe injection site reactions. In the H56:IC31® group, the highest measured redness diameter was 30 mm and the highest measured swelling diameter was 40 mm.

No notable between group differences and no changes from V2 through V4 or V6 were noted for any of the biochemistry and haematology laboratory safety tests or vital signs measurements.

13.2 Conclusions

The overall conclusion of the primary efficacy analysis in the mITT analysis set from Day 70 was that H56:IC31® did not lead to a statistically significant reduction in the rate of TB recurrence in comparison to placebo. The supportive efficacy analysis (in the mITT analysis set from Day 70) supported the results of the primary efficacy analysis, as did the efficacy analysis in the PP analysis set.

H56:IC31® vaccination induced significant increases in frequencies of antigen specific CD4+ T cells expressing any of the tested cytokine combinations measured at Day 70, relative to pre-vaccination. The fold change in H56-specific CD4+ T cell responses was significantly higher in H56:IC31® recipients than in placebo recipients. Frequencies of antigen specific CD8+ T cells were not significantly modulated by vaccination. Participants receiving H56:IC31® mounted robust H56-specific humoral (serum IgG) responses.

Overall, the H56:IC31® vaccine is considered well-tolerated. The majority of participants experienced only mild AEs. There was a low frequency of moderate injection site reactions and no severe injection site reactions. All SAEs were assessed as unrelated to the H56:IC31® vaccine.

14 Tables, figures and narratives of deaths, SAEs and pregnancies

The following EOT tables are available in Appendix 16.2.1.

The following EOT figures are available in Appendix 16.2.2.

The Listings are available in Appendix 16.2.3.

The Narratives are available in Appendix 16.2.4:

- Deaths, please refer to Section 16.2.4.1
- SAEs (other than deaths), please refer to Section 16.2.4.2
- Pregnancies, please refer to Section 16.2.4.3

15 References

1. WHOGlobalTB. Tuberculosis report 2023. Geneva, Switzerland: World health organisation; https://iris.who.int/bitstream/handle/10665/373828/9789240083851-eng.pdf?sequence=1. 2023.

2. Bagcchi S. WHO's Global Tuberculosis Report 2022. Lancet Microbe. 2023;4(1):e20.

3. WHOGlobalTB. Tuberculosis report 2020. Geneva, Switzerland: World health organisation; https://www.who.who.int/health-topics/tuberculosis. 2020.

4. Gillespie SH, Crook AM, McHugh TD, Mendel CM, Meredith SK, Murray SR, et al. Four-month moxifloxacin-based regimens for drug-sensitive tuberculosis. N Engl J Med. 2014;371(17):1577-87.

5. Friedrich SO, Rachow A, Saathoff E, Singh K, Mangu CD, Dawson R, et al. Assessment of the sensitivity and specificity of Xpert MTB/RIF assay as an early sputum biomarker of response to tuberculosis treatment. Lancet Respir Med. 2013;1(6):462-70.

6. Merle CS, Fielding K, Sow OB, Gninafon M, Lo MB, Mthiyane T, et al. A four-month gatifloxacin-containing regimen for treating tuberculosis. N Engl J Med. 2014;371(17):1588-98.

7. Imperial MZ, Nahid P, Phillips PPJ, Davies GR, Fielding K, Hanna D, et al. A patient-level pooled analysis of treatment-shortening regimens for drug-susceptible pulmonary tuberculosis. Nat Med. 2018;24(11):1708-15.

8. WHOEndTB. Global strategy and targets for tuberculosis prevention, care and control. Geneva, Switzerland World health organisation; https://www.who.int/health-topics/tuberculosis. 2015.

9. Knight GM, Griffiths UK, Sumner T, Laurence YV, Gheorghe A, Vassall A, et al. Impact and cost-effectiveness of new tuberculosis vaccines in low- and middle-income countries. Proc Natl Acad Sci U S A. 2014;111(43):15520-5.

10. Luabeya AK, Kagina BM, Tameris MD, Geldenhuys H, Hoff ST, Shi Z, et al. First-in-human trial of the post-exposure tuberculosis vaccine H56:IC31 in Mycobacterium tuberculosis infected and non-infected healthy adults. Vaccine. 2015;33(33):4130-40.

11. Suliman S, Luabeya AKK, Geldenhuys H, Tameris M, Hoff ST, Shi Z, et al. Dose Optimization of H56:IC31 Vaccine for Tuberculosis-Endemic Populations. A Double-Blind, Placebo-controlled, Dose-Selection Trial. Am J Respir Crit Care Med. 2019;199(2):220-31.

12. Bekker LG, Dintwe O, Fiore-Gartland A, Middelkoop K, Hutter J, Williams A, et al. A phase 1b randomized study of the safety and immunological responses to vaccination with H4:IC31, H56:IC31, and BCG revaccination in Mycobacterium tuberculosis-uninfected adolescents in Cape Town, South Africa. EClinicalMedicine. 2020;21:100313.

13. Jenum S, Tonby K, Rueegg CS, Ruhwald M, Kristiansen MP, Bang P, et al. A Phase I/II randomized trial of H56:IC31 vaccination and adjunctive cyclooxygenase-2-inhibitor treatment in tuberculosis patients. Nat Commun. 2021;12(1):6774.

14. Tait D. Safety and immunogenicity of the H56:IC31 tuberculosis vaccine candidate in adults successfully treated for drug-susceptible pulmonary TB: a phase 1 randomised trial. Submitted to The journal of Infectious Diseases. 2023.

15. Ellis RD, Hatherill M, Tait D, Snowden M, Churchyard G, Hanekom W, et al. Innovative clinical trial designs to rationalize TB vaccine development. Tuberculosis (Edinb). 2015;95(3):352-7.

16. Jindani A, Harrison TS, Nunn AJ, Phillips PP, Churchyard GJ, Charalambous S, et al. High-dose rifapentine with moxifloxacin for pulmonary tuberculosis. N Engl J Med. 2014;371(17):1599-608.

17. Garcia-Basteiro AL, White RG, Tait D, Schmidt AC, Rangaka MX, Quaife M, et al. End-point definition and trial design to advance tuberculosis vaccine development. Eur Respir Rev. 2022;31(164).

16 Appendices

16.1 Trial information

16.1.1 Protocol and protocol amendments

16.1.2 Sample case report form

16.1.3 List independent ethics committee and competent authority approvals

16.1.4 List investigators and important trial team members at the investigator's, sponsor's, CRO's and vendor's sites

16.1.5 Signature page

16.1.6 Batch information test product (H56:IC31)

16.1.7 Randomisation list

16.1.8 Audit certificates

16.1.9 Documentation of statistical methods

16.1.10 Laboratory analysis and normal ranges

16.2 Participant data, results and analysis (Tables, Figures, Listings)

16.2.1 Tables

16.2.2 Figures

16.2.3 Listings

16.2.4 Narratives of deaths, other serious adverse events and pregnancies

16.2.5 Immunogenicity report

16.2.6 Statistical report