STATISTICAL ANALYSIS PLAN for TBCOX2

Administrative information:

Sponsor name	Department of Infectious Diseases, Oslo University Hospital
Sponsor address	Oslo, Norway
EudraCT number / REC no	EudraCT number: 2014-004986-26
Trial title	Therapeutic vaccination and immune modulation – new treatment strategies for the multidrug-resistant tuberculosis pandemic. An open label phase I clinical trial of the therapeutic TB H56:IC31 Vaccine and cyclooxygenase-inhibitors.
Trial ID	TBCOX2
Trial registration number	ClinicalTrials.gov: NCT02503839

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ABBREVIATIONS

AE	Adverse Event
BCG	Bacille Calmette-Guérin
BMI	Body mass index
CI	Confidence Interval
COX-2i	COX-2 inhibitor
DMC	Data Monitoring Committee
eCRF	electronic Case Report Form
EAE	Expected Adverse Events (EAE)
EPTB	Extra-pulmonary Tuberculosis
ESR	Erythrocyte Sedimentation Rate
Hb	Haemoglobin
ICS	Intracellular Cytokine Staining
IQR	Interquartile range
MDR-TB	Multidrug-Resistant Tuberculosis
MedDRA	Medical Dictionary for Regulatory Activities
QFT	Quantiferon
SAE	Serious Adverse Event
SD	Standard Deviation
SUSAR	Suspected Unexpected Serious Adverse Reaction
TB	Tuberculosis
WB	Whole Blood
XDR-TB	Extensively Drug-resistant Tuberculosis

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1 Introduction

This statistical analysis plan (SAP) is based on the guidelines for the content of SAPs in clinical trials[1] The SAP describes in detail the analyses to be conducted and highlights any deviations from the analysis described in the protocol. Deviations from methods described in the SAP, if any, will be specified in the clinical trial report. Reporting of the trial will take place after the data base lock.

1.1 Background and rationale

The World Health Organization estimates that globally 10.0 million people fell ill with active tuberculosis (TB) in 2018[2]. Globally, 3.4% of new TB cases and 18% of previously treated cases had multi-drugresistant TB (MDR-TB) which may take years to treat with expensive and toxic second-line drugs with uncertain efficacy. Further, the shifting of the TB epidemic towards extensively drug resistant (XDR-TB) with very restricted treatment options could reverse many of the major gains made in TB control and increase spread, morbidity and mortality. This calls for new treatment modalities, including host-directed therapy (HDT), that could be affordable and simple alternatives for a substantial number of patients with MDR-TB and XDR-TB in resource-poor countries.

1.2 Trial Objectives

1.2.1 Primary Objective

To evaluate the safety and tolerability of etoricoxib (Arcoxia) and the therapeutic TB vaccine H56:IC31 alone and in combination in patients with active TB disease treated with conventional 26-week anti-TB therapy.

1.2.2 Secondary Objectives

a) To evaluate the effect of i) etoricoxib (Arcoxia) and ii) the therapeutic TB vaccine H56:IC31 on immunogenicity in patients with active TB disease treated with conventional 26-weeks anti-TB therapy. The immunogenicity endpoints are described and ranked according to importance in the sections 2.4.1 and 2.4.2 (Tables 1 and 2)

b) To evaluate whether Arcoxia will improve the H56:IC31 TB vaccine response compared to H56:IC31 alone in patients with active TB disease treated with conventional 26-weeks anti-TB therapy. The H56:IC31 response endpoints are described and ranked according to importance in the sections 2.4.3 (Table 3)

1.2.3 Hypotheses

1. Etoricoxib administered during the first 84 days of 26-weeks standard TB treatment improves *Mycobacterium tuberculosis* (Mtb) specific immunity in patients with active TB disease (Comparison 1, **Figure 1**).
2. H56:IC31 vaccine administered at 2 doses (day 84 and day 140) during 26-weeks standard TB treatment induces H56:IC31 vaccine responses in patients with active TB disease (Comparison 2, **Figure 1**).

3. Etoricoxib administered during the first 140 days improves H56:IC31 vaccine responses in patients with active TB disease who are given the H56:IC31 vaccine administered at 2 doses (day 84 and day 140) during 26-weeks standard TB treatment (Comparison 3, **Figure 1**).

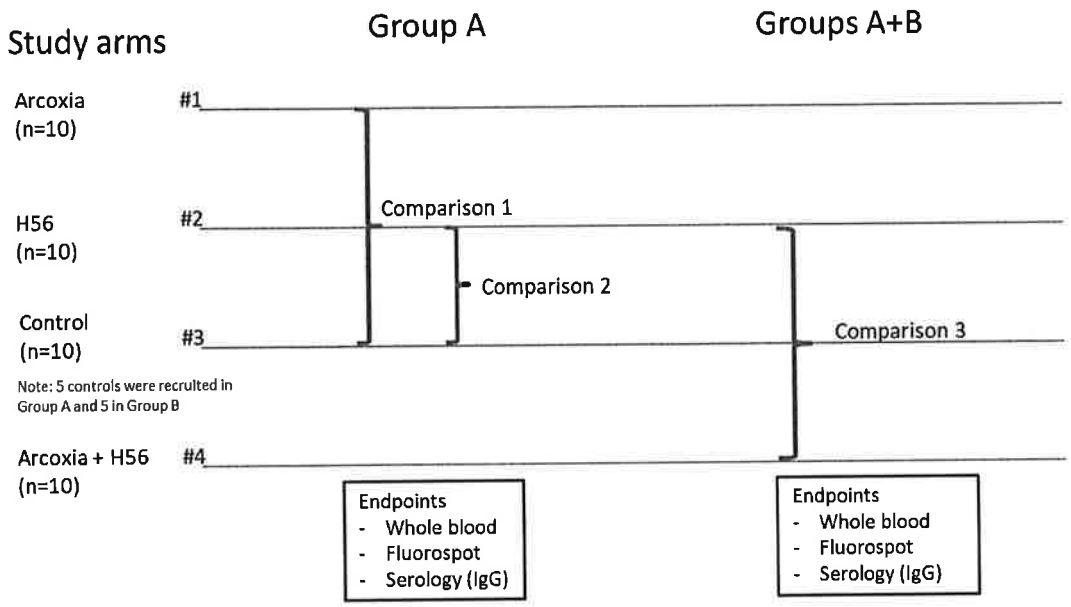


Figure 1: Illustration of the comparisons for each of the study hypotheses

2 Trial Methods

2.1 Trial Design

The TBCOX2 study is a randomized, open label, controlled, parallel group, multi-center, safety and explorative phase I study (**Figure 2**). The final treatment allocation to the four study arms is a 1:1:1:1 ratio with a randomization allocation ratio of first 2:2:1:0 for Group A and a subsequent randomization allocation ratio of 0:0:1:2 for Group B (see section 2.2 Randomization). Patients are randomized to either etoricoxib (COX-2 inhibitor (COX-2i); Arm 1 Arcoxia), TB vaccine (H56:IC31; Arm 2 H56:IC31), etoricoxib and TB vaccine (Arm 4 Arcoxia+H56:IC31), or no additional treatment (control arm; Arm 3 controls; **Figure 2**). In addition to these study-specific treatments, all study arms receive standard anti-TB therapy for 26 weeks (182 days), or longer in settings of single-drug resistance, drug intolerance or inadequate treatment response, as recommended by WHO treatment guidelines.

The study consists of two parts: The study starts with **Group A** that consists of full Arm 1, full Arm 2 and half of the control Arm 3. When safety data are satisfactory for all Group A participants at the safety interim analyses at study Day 98, **Group B** is enrolled and starting the study. Group B consists of half of the control Arm 3 and full Arm 4.

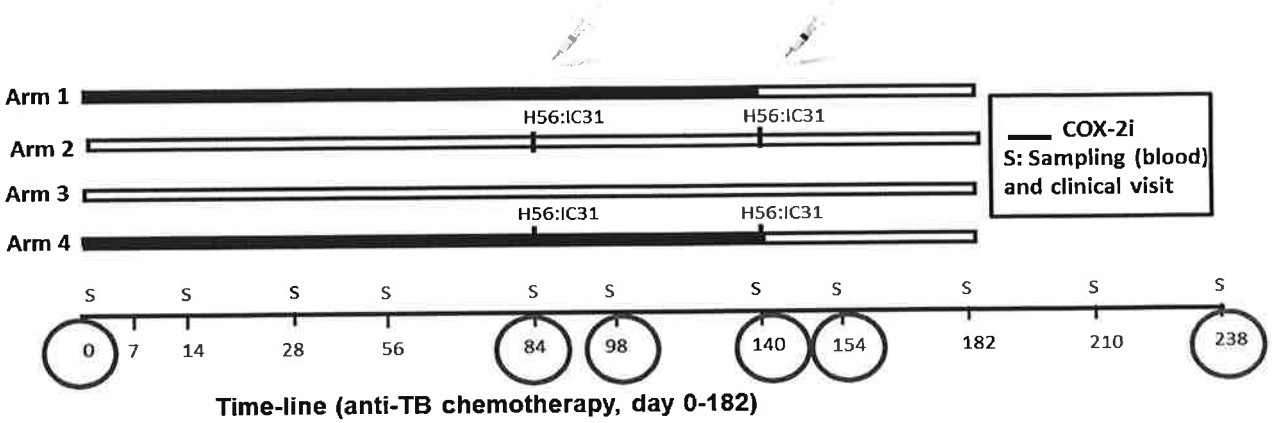


Figure 2: Study design of the TBCOX2 trial

Treatments/Intervention

All patients receive standard anti-TB therapy for 26 weeks (day 0-182).

Add-on treatment will be given according to allocation:

Arm 1 (Arcoxia, target n=10): etoricoxib started at day 0 and given for 20 weeks (day 0-140)

Arm 2 (H56:IC31, target n=10): H56:IC31 vaccine administrated at day 84 and day 140.

Arm 3 (controls, target n=10): No add-on treatment.

Arm 4 (Arcoxia + H56:IC31, target n=10): etoricoxib started at day 0 and given for 20 weeks (day 0-140), H56:IC31 TB vaccine administrated at day 84 and day 140.

Study groups

Group A: All patients allocated to Arm 1 (Arcoxia) and Arm 2 (H56:IC31). In addition, half of the patients allocated to Arm 3 (controls)

Group B: The other half of the patients allocated to Arm 3 (controls) and all patients allocated to Arm 4 (Arcoxia + H56:IC31)

2.2 Randomisation

By open label randomization TB patients meeting the inclusion criteria were included 2:2:1 in the Arms 1, 2 and 3 for Group A of the trial. When the last patient has reached study day 98 (2 weeks after the 1st H56:IC31 vaccine immunization) and safety of the treatments is found acceptable, participants for the Arms 4 and 3 will be recruited and randomized 2:1.

2.3 Sample size

No sample size calculation was performed as this is a Phase I trial. TBCOX2 is the first of its kind; exploratory, open label phase I study. Primary data on safety and efficacy of COX2-i (Arcoxia) as well as the H56:IC31 vaccine in patients with active TB are non-existing.

Based on the results in non-TB patients, this study aimed at including 40 patients, 10 in each arm (25 participants in Group A and 15 in Group B).

2.4 Statistical Framework

This is a phase 1 exploratory trial where different hypotheses with different outcome variables will be tested. The current SAP describes the general models/methods applied for the statistical analysis of the trial. These methods will be applied to a list of hypotheses and outcome variables as hierarchically defined in the Tables 1 to 3 and 6 to 9. We will describe deviations from the general methods for individual hypothesis and outcome variables, if applicable.

Tables 1 to 3 give an overview of all the outcome variables of primary and secondary priority as well as the respective hypotheses tests and their hierarchical order for the interpretation of the results. Tables 6 to 9 define all the outcome variables generated (calculated and coded) within this SAP. Outcome variables that are listed in Tables 6-9 but not in Tables 1-3 are of tertiary priority.

Explanation of outcomes described in Tables 1-3 and Tables 6-9:

- **Sin cytokine:** Cells expressing only one cytokine: sin_IFNγ or sin_IL2 or sin_TNFα*.
- **Duo cytokine:** Cells expressing two cytokines simultaneously: duo_IFNγ/IL2 or duo_IFNγ/TNFα*or duo_IL2/TNFα*.
- **Tri cytokines:** Cells expressing three cytokines simultaneously: tri_IFNγ/IL2/TNFα*.
- **Tot cytokines:** SUM of cytokine combinations_expressing cytokine of interest (sin_cytokines PLUS duo_cytokines PLUS tri_cytokines*): tot_IFNγ or tot_IL2 or tot_TNFα*
- **All cytokines (CYTOKINE$^+$) for Whole Blood ICS:** SUM of the following cytokine combinations: tot_IFNγ PLUS duo_IL2/TNFα PLUS sin_IL2 PLUS sin_TNFα.
- **All cytokines (CYTOKINE$^+$) for Fluorospot:** SUM of the following cytokine combinations: tot_IFNγ PLUS tot_IL2 MINUS duo_IFNγ/IL2.

* TNFα only for Whole Blood ICS.

Cytokine outcomes/responses as defined above are assessed by:

For the specific peptide stimulations: Ag85, ESAT6 (both 15aa, 10 overlapping peptide pools), CFP10 (25aa, 10, overlapping peptides), Rv2660 (20aa, 10 overlapping peptides). Purified Protein Derivate (PPD, 10μg/mL, SSI),

Fluorospot (FS):

- SUM (Ag85+ESAT6): sum of respective cytokine response.
- SUM (Ag85+ESAT6+CFP10): sum of respective cytokine response.

Wholeblood (WB):

- SUM (Ag85+ESAT6+Rv2660c) sum of respective cytokine response.

2.4.1 Outcome variables for Hypothesis 1

Hypothesis 1: Etoricoxib (**Arcoxia**) administered during the first 84 days of 26-weeks standard TB treatment improves Mtb specific immunity in patients with active TB disease

Table 1: Hierarchal ranking of primary and secondary priority outcomes and definition of hypotheses that will be tested comparing Arcoxia vs. controls. Formal testing will be performed on the primary priority outcomes only, tertiary priority outcomes are not listed here.

Method	Outcome	Time interval (days)	Comparison
Primary priority			
FLUOROSPOT (FS)	All_cytokine responses to SUM (Ag85+ESAT6+CFP10)	0-84	Arm (1+4) vs (2+3)
	Tot_IFNγ responses to SUM (Ag85+ESAT6+CFP10)	0-84	Arm (1+4) vs (2+3)
Whole blood (WB) intracellular cytokine analyses	All_cytokine CD4 responses to PPD	0-84	Arm (1+4) vs (2+3)
	All_cytokine CD4 responses to SUM (Ag85+ESAT6+Rv2660)	0-84	Arm (1+4) vs (2+3)
Secondary priority			
FLUOROSPOT (FS)	All_cytokine responses to ESAT6	0-84	Arm (1+4) vs (2+3)
	All_cytokine responses to Ag85	0-84	Arm (1+4) vs (2+3)
	All_cytokine responses to CFP10	0-84	Arm (1+4) vs (2+3)
	Duo_IL2/IFNγ responses to SUM (Ag85+ESAT6+CFP10)	0-84	Arm (1+4) vs (2+3)
Whole blood (WB) intracellular cytokine analyses	All_cytokine CD4/CD8 responses to ESAT6	0-84	Arm (1+4) vs (2+3)
	All_cytokine CD4/CD8 responses to Ag85	0-84	Arm (1+4) vs (2+3)
	All_cytokine CD4/CD8 responses to Rv2660	0-84	Arm (1+4) vs (2+3)
	Tri_IFNγ/IL2/TNFα CD4 responses to PPD	0-84	Arm (1+4) vs (2+3)
	Tri_IFNγ/IL2/TNFα CD4 responses to SUM (Ag85+ESAT6+Rv2660)	0-84	Arm (1+4) vs (2+3)
	Duo_IL2/IFNγ CD4 responses to SUM (Ag85+ESAT6+Rv2660)	0-84	Arm (1+4) vs (2+3)
	Duo_IL2/IFNγ CD4 responses to PPD	0-84	Arm (1+4) vs (2+3)
	All_cytokines CD8 responses to PPD	0-84	Arm (1+4) vs (2+3)
	All_ cytokines CD8 responses to SUM (Ag85+ESAT6+Rv2660)	0-84	Arm (1+4) vs (2+3)

2.4.2 Outcome variables for Hypothesis 2

Hypothesis 2: H56:IC31 vaccine administered at 2 doses (day 84 and day 140) during 26-weeks standard TB treatment induces H56:IC31 vaccine responses in patients with active TB disease.

Table 2: Hierarchal ranking of primary and secondary priority outcomes and definition of hypotheses that will be tested comparing H56:IC31 vs. controls. Formal testing will be performed on the primary priority outcomes only, tertiary priority outcomes are not listed.

Method	Outcome	Time interval (days)	Comparison
Primary priority			
FLUOROSPOT (FS)	All_cytokine responses to H56	84-154	Arm 2 vs 3
	Tot_IFNγ responses to H56	84-154	Arm 2 vs 3
	All_cytokine responses to SUM (Ag85+ESAT6)	84-154	Arm 2 vs 3
Whole blood (WB) intracellular cytokine analyses	All_cytokine CD4 responses to SUM (Ag85+ESAT6+Rv2660)	84-154	Arm 2 vs 3
Serology/antibody measurements	IgG antibody responses to H56: IC31	84-154	Arm 2 vs 3
Secondary priority			
FLUOROSPOT (FS)	All_cytokine responses to ESAT6	84-154	Arm 2 vs 3
	All_cytokine responses to Ag85	84-154	Arm 2 vs 3
	All_cytokine responses to CFP10	84-154	Arm 2 vs 3
	Duo_IL2/IFNγ responses to H56	84-154	Arm 2 vs 3
	Tot_IFNγ responses to SUM (Ag85+ESAT6)	84-154	Arm 2 vs 3
	Duo_IL2/IFNγ responses to SUM (Ag85+ESAT6)	84-154	Arm 2 vs 3
Whole blood (WB) intracellular cytokine analyses	All_cytokine CD4/CD8 responses to ESAT6	84-154	Arm 2 vs 3
	All_cytokine CD4/CD8 responses to Ag85	84-154	Arm 2 vs 3
	All_cytokine CD4/CD8 responses to Rv2660	84-154	Arm 2 vs 3
	All_cytokine CD4/CD8 responses to PPD	84-154	Arm 2 vs 3
	Tri_IFNγ/IL2/TNFα CD4 responses to SUM (Ag85+ESAT6+Rv2660)	84-154	Arm 2 vs 3
	Duo_IL2/IFNγ CD4 responses to SUM (Ag85+ESAT6+Rv2660)	84-154	Arm 2 vs 3
	All_cytokine CD8 responses to SUM (Ag85+ESAT6+Rv2660)	84-154	Arm 2 vs 3

2.4.3 Outcome variables for Hypothesis 3

Hypthesis 3: Etoricoxib (Arcoxia) administered during the first 140 days of 26-weeks standard TB treatment improves H56:IC31 vaccine responses given at 2 doses (day 84 and day 140) in patients with active TB disease.

Table 3: Hierarchal ranking of primary and secondary priority outcomes and definition of hypotheses that will be tested comparing Arcoxia + H56:IC31 vs. H56:IC31 alone. Formal testing will be performed on the primary priority outcomes only, tertiary priority outcomes are not listed.

Method	Outcome	Time interval (days)	Comparison
Primary priority			
FLUOROSPOT (FS)	All_cytokine responses to H56	84-154	Arm 4 vs 2
	Tot_IFNγ responses to H56	84-154	Arm 4 vs 2
	All_cytokine responses to SUM (Ag85+ESAT6)	84-154	Arm 4 vs 2
Whole blood (WB) intracellular cytokine analyses	All_cytokine CD4 responses to SUM (Ag85+ESAT6+Rv2660)	84-154	Arm 4 vs 2
Serology/antibody measurements	IgG antibody responses to H56:IC31	84-154	Arm 4 vs 2
Secondary priority			
FLUOROSPOT (FS)	All_cytokine responses to ESAT6	84-154	Arm 4 vs 2
	All_cytokine responses to Ag85	84-154	Arm 4 vs 2
	All_cytokine responses to CFP10	84-154	Arm 4 vs 2
	Duo_IL2/IFNγ responses to H56	84-154	Arm 4 vs 2
	Tot_IFNγ FS responses to SUM (Ag85+ESAT6)	84-154	Arm 4 vs 2
	Duo_IL2/IFNγ FS responses to SUM (Ag85+ESAT6)	84-154	Arm 4 vs 2
Whole blood (WB) intracellular cytokine analyses	All_cytokine CD4/CD8 responses to ESAT6	84-154	Arm 4 vs 2
	All_cytokine CD4/CD8 responses to Ag85	84-154	Arm 4 vs 2
	All_cytokine CD4/CD8 responses to Rv2660	84-154	Arm 4 vs 2
	All_cytokine CD4/CD8 responses to PPD	84-154	Arm 4 vs 2
	Tri_IFNγ/IL2/TNFα CD4 responses to SUM (Ag85+ESAT6+Rv2660)	84-154	Arm 4 vs 2
	Duo_IL2/IFNγ CD4 responses to SUM (Ag85+ESAT6+Rv2660)	84-154	Arm 4 vs 2
	All_cytokine CD8 responses to SUM (Ag85+ESAT6+Rv2660)	84-154	Arm 4 vs 2

2.4.4 Hypothesis Test

This study is an exploratory, hypothesis generating phase I trial. There will be no formal statistical hypotheses testing as part of the analyses of this trial. All group comparisons including p-values will be hypothesis supporting and not interpreted as confirmatory. Because of the exploratory nature of

the trial, there will be no adjustment for multiple testing. The importance of the interpretation of the statistical test/results is defined by the hierarchical ranking in Tables 1 to 3. An increase in immune response will be interpreted as the favourable outcome.

2.4.5 Decision Rule

Group comparisons resulting in a p-value below 0.05 will be denoted as a statistically significant difference, but no claim of superiority either way will be made based on such findings.

2.5 Statistical Interim Analyses and Stopping Guidance

There will be no efficacy interim analyses in this trial. The safety of etoricoxib and H56:IC31 was determined when all participants of Group A reached day 98. After the approval of the safety, the enrolment into arm 4 was started.

2.6 Timing of Final Analysis

When all participants have reached day 238.

2.7 Timing of Outcome Assessments

Table 4. Study flow chart gives an overview of the outcomes assessed at the different time points in the study. The detailed methods of all procedures are described in the protocol chapter 10.4.

	Treatment period (etoricoxib)									Follow-up period			End of study
Study day	**Screen**	**0**	**7**	**14**	**28**	**56**	**84**	**98**	**140**	**154**	**182**	**210**	**238**
Window (days)		**NA**	**±2**	**±2**	**±7**	**±7**	**±7**	**±3**	**±7**	**±3**	**±7**	**±14**	**±14**
Informed consent	X												
Inclusion/exclusion evaluation	X												
Demographics/ medical history	X												
AFS/Culture/Xpert[1)]	X	X		X*		X*	X		X		X		X
Radiology[2)]	X					X					X		
TB chemotherapy[3)]		X	X	X	X	X	X	X	X	X	X		
Physical Examination[4)]	X	X	X	X	X	X	X	X	X	X	X	X	X
Vital signs[5)]	X	X	X	X	X	X	X	X	X	X	X	X	X
Symptoms[1)]	X	X	X	X	X	X	X	X	X	X	X	X	X
Treatment (etoricoxib)		X	X	X	X	X	X	X	X				
Haematology/clinical chemistry[6)]	X	X	X	X	X	X	X	X	X	X	X	X	X
Pharmacokinetics[7)]			X										
Adverse events			X	X	X	X	X	X	X	X	X	X	X
Con. Medication	X	X	X	X	X	X	X	X	X	X	X	X	X
Vaccine adm. (T)[8)]							T		T				
Blood sampling for exploratory ssays[9)]		X		X	X	X	X	X	X	X	X	X	X
Vaccine IgG[9)]		X					X	X	X	X			X
RNA analysis[9)]		X					X	X	X	X			X
PBMC ICS[9)]		X					X	X	X	X			X

1). AFS, culture, microscopy, PCR/GeneXpert as part of routine clinical evaluation of TB. 2) Appropriate radiology (chest X-ray/CT/MRI/US/PET) taken as part of clinical evaluation <1 month before entering the study. 3) Anti-TB chemotherapy: standard combination therapy, induction phase day 0-56, continuation phase day 56-182. 4) Physical examination by clinical investigator at every visit, the extent depending on the clinical situation. In the case of glandular TB, the glands are measured. 5) Blood pressure, pulse, temperature. 6) Hb, Lpk, monocytes, lymphocytes,Tpk, ESR, CRP, SR, Na, K, Ca, creatinine, ASAT, ALAT, LDH, bilirubin, amylase, CK, cholesterol, glucose, gGT, urine acid, S-hCG) at every visit. QFT-TB at day 0 and 182. 7). Study

3 Statistical Principles

3.1 Confidence Intervals and p-values

All calculated p-values will be two-sided and compared to a 5% significance level. If a p-value is less than 0.05, the corresponding group difference will be denoted as statistically significant. All estimates will be presented with two-sided 95% confidence intervals.

3.2 Adherence and Protocol Deviations

3.2.1 Adherence to Allocated Treatment

All subjects who have received at least one dose of etoricoxib (Arcoxia) and/or H56:IC31-vaccine are included in the safety analysis. Because of the hypothesis generating nature of the trial (phase I) no subjects will be excluded from the analysis because of lack of adherence.

Below are the definitions of treatment adherence. If the following conditions are reached, a person is considered compliant:

All arms: No corticosteroids/ additional NSAIDs the last 7 days prior to biobank sampling for immunogenicity read-outs.

Additional requirements for the specific treatment arms:

Arm 1, allocated to etoricoxib treatment: during the intervention period (Day 0-140), ≥80% of the expected number of tablets were taken. Number of tablets taken was recorded by asking the participants and having them return the pill container.

Arm 2 allocated to H56:IC31-vaccine: having received ≥1 dose(s) of the H56:IC31-vaccine.

Arm 4 allocated to etoricoxib treatment and H56:IC31-vaccine: 1) during the intervention period (Day 0-140), ≥80% of the expected number of tablets were taken; AND, 2) having received ≥1 dose(s) of the H56:IC31-vaccine. Number of tablets taken was recorded by asking the participants and having them returned the pill container.

The number (and percentage) of patients that were compliant will be summarised by treatment group with details for reasons of non-compliance. The results will be shown for the Randomised Set, Safety Analysis Set and Full Analysis Set (see Section 3.3. Analysis Populations). No formal statistical testing will be undertaken.

3.2.2 Protocol Deviations

The following are pre-defined major protocol deviations. The protocol deviations will be described in the article:

- Entering the trial when the eligibility criteria should have prevented trial entry
- Discontinuation of intervention, thus not adhering to the allocated treatment as described in 3.2.1.
- Use of sporadic COX-inhibitor/NSAIDS not according the allocated treatment as described in 3.2.1.
- Received or used a different intervention than the one allocated to.

The number (and percentage) of patients with major protocol deviations will be summarised by treatment group with details of type of deviation provided. The results will be shown for the Randomised Set, Safety Analysis Set and Full Analysis Set (see Section 3.3. Analysis Populations). No formal statistical testing will be undertaken.

3.3 Analysis Populations

Enrolled Set:
All patients who have provided informed consent and have been entered into the study database.

Randomised Set:
All patients randomly assigned to a treatment group.

Safety Analysis Set (SAS):
All patients of the randomised set having received at least one dose of the allocated treatment (meaning no treatment for the controls and the first phase of the vaccination arm). Subjects who withdrew from the study due to own wish to discontinue his/her participation in the study were not followed-up and no safety information after withdrawal is available. The patient will still be followed and receive standard anti-TB chemotherapy for 26 weeks or as long as needed as determined by the responsible clinician according to clinical guidelines. A list of withdrawn subjects, with the reasons for withdrawal, will be presented in the article. Participants are included in the safety analysis until loss to follow-up or end of study, whatever comes first. Any significant AE will be followed-up by the responsible clinician according to clinical guidelines until the AE is resolved.

The safety analysis will be divided into AE/SAE happening within the following three time periods:

a. Day 0 to day 84 (or date of first vaccination if applicable).
b. Day 85 (or date of first vaccination if applicable) to day 154 (or 14 days after last vaccination/last Arcoxia treatment, if applicable).
c. Day 155 (or 15 days after last vaccination/last Arcoxia treatment, if applicable) to day 238.

If the second vaccination is missing, day 154 is defined as 70 days after the date of day 84.

Full Analysis Set (FAS):
All patients of the randomised set with at least one valid measurement of any of the outcome variables listed in Tables 6-9. We will perform intention to treat (ITT) analysis in the FAS, meaning that we treat participants as they were randomized and independent of adherence. Per protocol analyses will not be performed because of the generally good adherence observed by the study group.

4 Trial Population

4.1 Screening Data, Eligibility, Recruitment, Withdrawal/Follow-up

The total number of screened patients and reasons for not entering the trial will be summarised and tabulated.

A CONSORT flow diagram (Appendix I) will be used to summarise the number of patients who were:

- screened and not eligible
- screened and eligible
- eligible and randomised (corresponding to the Randomised Set)
- eligible but not randomised*
- received the randomised allocation (corresponding to Safety Analysis Set)

- did not receive the randomised allocation*
- lost to follow-up*
- discontinued the intervention*
- withdrew consent*
- randomised and included in the primary analysis (corresponding to Full Analysis Set)
- randomised and excluded from the primary analysis*
- completers

*reasons will be provided.

4.2 Baseline Patient Characteristics

The following patient demographics and baseline characteristics will be summarised (example Table 5): gender (*male, female*), age, ethnicity (*Caucasian, Black, Asian, other*), smoking (*yes, no, unknown*), alcohol use >3units/week (*yes, no, unknown*), drug abuse (*yes, no, unknown*), Bacille Calmette-Guérin (BCG) vaccine scar (*yes, no*), co-morbidities (*allergy, kidney failure, previous gastric ulcer/GI bleeding, liver disease/failure, chronic pulmonary disease, other medical condition)*, previous TB treatment (*yes, no*), TB diagnosis (*pulmonary, pulmonary/extrapulmonary*), Extrapulmonary location (*no EPTB, lymphadenitis, bone/joint/soft tissue, pleuritis, abdominal, urogenital, other*), radiology (*infiltrate, cavity, miliary, pleural fluid, mediastinal changes, extrapulmonary*), symptoms of cough, chest pain, night sweat with grading 0-5 (*no symptoms, 1 symptom, ≥2 symptoms*), QFT result (*positive, intermediate, negative),* vital signs [*weight (kg), BMI (kg/m^2), body temperature (°Celsius)*], and laboratory parameters (*haemoglobin (Hb), Erythrocyte Sedimentation Rate (ESR), CRP and Quantiferon-TB (QFT)*).

Patient demographics and baseline characteristics will be summarised by randomised treatment arm and overall using descriptive statistics: median, interquartile range (IQR), minimum, and maximum for continuous variables, and number and percentages of patients for categorical variables. There will be no formal statistical testing of differences between the arms. Any clinical important imbalance between the treatment groups will be noted.

Table 5 shows a table draft for the baseline patient characteristics to be displayed in the article. The Table will be presented for the FAS (Suppl. Appendix) and the SAS (in the Paper) separately.

Table 5: Draft table of the baseline patient characteristics to be displayed in the article

	Total N=		Arm 1 N=		Arm 2 N=		Arm 3 N=		Arm 4 N=	
Categorical variables	N	%	N	%	N	%	N	%	N	%
Gender										
Male										
Female										
Ethnicity										
Caucasian										
Black										
Asian										
Other										
Smoking status										
Yes										
No										
Unknown										
Alcohol use >3 units/week										
Yes										
No										
Unknown										
Drug abuse										
Yes										
No										
Unknown										
BCG scar										
Yes										
No										
Co-morbidities[a]										
Allergy										
Kidney failure										
Previous gastric ulcer/GI bleeding										
Liver disease										
Chronic pulmonary disease										
Other medical condition										
Previous TB treatment										
Yes										
No										
TB diagnosis										
Pulmonary										
Pulmonary/ extrapulmonary										
Extrapulmonary										
Extrapulmonary location										

Lymphadenitis										
Bone/joints/soft tissue										
Pleuritis										
Abdominal										
Urogenital										
Other										
Radiology										
Infiltrate										
Cavity										
Miliary										
Pleural fluid										
Mediastinal changes										
Extrapulmonary										
Symptoms (cough, chest pain, night sweat)										
No symptoms										
1 symptom										
≥2 symptoms										
QFT result										
Positive										
Indeterminate										
Negative										
Continuous variables	Median	IQR (range)	Median	IQR (range)	Median	IQR (range)	Median	IQR (range)	Median	IQR (range)
Age (years)										
Vital signs										
Weight (kg)										
BMI (kg/m^2)										
Body temperature (°C)										
Hb (g/dl)										
QFT (IU/ml)										
ESR (mm)										
CRP										

Abbreviations: BCG, Bacille Calmette-Guérin; BMI, body mass index; EPTB, extra-pulmonary Tuberculosis; ESR, Erythrocyte Sedimentation Rate; Hb, haemoglobin; IQR, interquartile range; N, number; QFT, Quantiferon. CRP, C-Reactive Protein.

[a] Numbers and proportions of patients having the respective co-morbidity.

5 Analysis

5.1 Outcome Definitions

5.1.1 General Definitions and Derived Variables

5.1.1.1 Baseline characteristics:

All baseline characteristics variables are listed and described in **Table 5** and section 4.2 Baseline Patient Characteristics.

There is only one derived variable among them:

- Body Mass Index (BMI) at baseline is calculated as follows from weight and height assessed at Screening visit/Day 0:

  BMI=weight in kg/(height in m)^2

5.1.1.2 Safety outcomes:

General definitions and derived variables for safety measurements are listed under 4.1.2 Primary Outcome Definition.

5.1.1.3 Immunogenicity read-outs:

The calculation and definition of the immunogenicity outcomes are described in Tables 6-9. The immunogenicity outcomes were generated by three analysis methods, briefly explained below:

1. **FluoroSpot:** FluoroSpot is a variation of the enzyme-linked immune absorbent spot (ELISpot) assay. It measures the number of thawed peripheral blood mononuclear cells (PBMC) secreting IFN-γ or IL-2 or both cytokines (spot forming units (SFC) per 300.000 PBMCs by adding 2 different fluorophore tags to the antibodies targeting IFN-γ (wavelength 250) and IL-2 (wavelength 630). For each time-point analysed per study subject, these repeated measurements are performed for each of 4 different stimulatory conditions (ESAT6, Ag85b, CFP10, H56) as well as a negative and a positive (CD3) control. The derived outcomes are defined in **Table 6**. The unit for all FluoroSpot outcomes is SFC/300.000 PBMC.

2. **Whole-blood intracellular cytokine staining and flowcytometry (WB-ICS):** Freshly drawn whole blood (WB) from study participants at each time-point was separated in aliquots with various stimulation (ESAT6, Ag85b, Rv2660c, PPD) prior to freezing and storage. Samples are then thawed, stained with fluorescent antibodies and analysed by flow cytometry characterizing phenotypic markers and cytokine production in CD4+ and CD4- (=CD8+) T cells. The derived outcomes are defined in **Table 7** (CD4+) and **Table 8** (CD8+).

3. **Serology analysis:** IgG antibodies against the vaccine fusion protein H56:IC31 was analysed in singlets by enzyme-linked immunosorbent assay (ELISA). Variables are used as given in the dataset, no derived outcome variables or calculations. The serology outcomes are listed in **Table 9**.

Table 6: Definition of derived outcomes from the *FluoroSpot* (FS) analysis

Outcome priority group	Immunology readout	Stimulation	Derived outcome
Tertiary	tot_IFNγ (FS)	ESAT6	Mean(triplicates ESAT6) – mean(triplicates negative control)
Tertiary	tot_IFNγ (FS)	Ag85	Mean(triplicates Ag85) – mean(triplicates negative control)
Tertiary	tot_IFNγ (FS)	CFP10	Mean(triplicates CFP10) – mean(triplicates negative control)
Primary H2[2]/H3[3]	tot_IFNγ (FS)	H56	Mean(triplicates H56) – mean(triplicates negative control)
Primary H1[1]	tot_IFNγ (FS)	SUM (ESAT6+ Ag85+CFP10)	SUM of (tot_IFNγ) for ESAT6, Ag85,CFP10
Secondary H2[2]/H3[3]	tot_IFNγ (FS)	SUM (ESAT6+ Ag85)	SUM of (tot_IFNγ) for ESAT6, Ag85
Tertiary	tot_IL2 (FS)	ESAT6	Mean(triplicates ESAT6) – mean(triplicates negative control)
Tertiary	tot_IL2 (FS)	Ag85	Mean(triplicates Ag85) – mean(triplicates negative control)
Tertiary	tot_IL2 (FS)	CFP10	Mean(triplicates CFP10) – mean(triplicates negative control)
Tertiary	tot_IL2 (FS)	H56	Mean(triplicates H56) – mean(triplicates negative control)
Tertiary	Tot_IL2 (FS)	SUM (ESAT6+ Ag85+CFP10)	SUM of (tot_IL2) for ESAT6, Ag85
Tertiary	Tot_IL2 (FS)	SUM (ESAT6+ Ag85)	SUM of (tot_IL2) for ESAT6, Ag85, CFP10
Tertiary	duo_IFNγ/IL2 (FS)	ESAT6	Mean(triplicates ESAT6) – mean(triplicates negative control)
Tertiary	duo_IFNγ/IL2 (FS)	Ag85	Mean(triplicates Ag85) – mean(triplicates negative control)
Tertiary	duo_IFNγ/IL2 (FS)	CFP10	Mean(triplicates CFP10) – mean(triplicates negative control)
Secondary H2[2]/H3[3], Tertiary H[1]	duo_IFNγ/IL2 (FS)	H56	Mean(triplicates H56) – mean(triplicates negative control)
Secondary H[1]	duo_IFNγ/IL2 (FS)	SUM (ESAT6+ Ag85+CFP10)	SUM of (duo_IFNγ/IL2) for ESAT6, Ag85, CFP10
Secondary H[2]/H[3]	duo_IFNγ/IL2 (FS)	SUM ESAT6+ Ag85)	SUM of (duo_IFNγ/IL2) for ESAT6, Ag85
Secondary	all_ Cytokine (FS)	ESAT6	tot_IFNγ + tot_IL2 – duo_IFNγ/IL2 for ESAT6
Secondary	all_ Cytokine (FS)	Ag85	tot_IFNγ + tot_IL2 – duo_IFNγ/IL2 for Ag85
Secondary	all_ Cytokine (FS)	CFP10	tot_IFNγ + tot_IL2 – duo_IFNγ/IL2 for CFP10
Primary H2[2]/H3[3]	all_Cytokine (FS)	H56	tot_IFNγ + tot_IL2 – duo_IFNγ/IL2 for H56
Primary H2[2]/H3[3]	all_Cytokine (FS)	SUM (ESAT6+ Ag85)	SUM of (tot_IFNγ + tot_IL2 – duo_IFNγ/IL2) for ESAT6, Ag85
Primary H1[1]	all_Cytokine (FS)	SUM (ESAT6+ Ag85+CFP10)	SUM of (tot_IFNγ + tot_IL2 – duo_IFNγ/IL2) for ESAT6, Ag85, CFP10

[1]Hypothesis 1 (etoricoxib). [2]Hypothesis 2 (H56:IC31 vaccine). [3]Hypothesis 3 (etoricoxib + H56:IC31 vaccine).

Note: For a valid IFNγ result, the following conditions must be fulfilled: the SFU/SFC value (mean of duplicates) for IFNγ at CD3 (positive control) is 1) >12 AND 2) ≥2 times the SFU/SFC value (mean of triplets) for IFNγ at the negative control.
Colour code: blue=secondary priority; red=primary priority; white=tertiary priority.

Table 7: Definition of derived outcomes from the *whole-blood* (WB) intracellular cytokine staining and flow cytometry analysis for CD4+ (eg. CD3+ CD4+ CD8-)*

Outcome priority group	Immunology readout	Stimulation	Derived outcome
Tertiary	sin_IFNγ (WB)	ESAT6	%(CD4+ESAT6) – %(CD4+ Unstim)
Tertiary	sin_IFNγ (WB)	Ag85	%(CD4+ Ag85b) – %(CD4+ Unstim)
Tertiary	sin_IFNγ (WB)	Rv2660c	%(CD4+ RV2660c) – %(CD4+ Unstim)
Tertiary	sin_IFNγ (WB)	PPD	%(CD4+ PPD) – %(CD4+ Unstim)
Tertiary	sin_IL2 (WB)	ESAT6	%(CD4+ ESAT6) – %(CD4+ Unstim)
Tertiary	sin_IL2 (WB)	Ag85	%(CD4+ Ag85b) – %(CD4+ Unstim)
Tertiary	sin_IL2 (WB)	Rv2660c	%(CD4+ RV2660c) – %(CD4+ Unstim)
Tertiary	sin_IL2 (WB)	PPD	%(CD4+ PPD) – %(CD4+ Unstim)
Tertiary	sin_TNFα (WB)	ESAT6	%(CD4+ ESAT6) – %(CD4+ Unstim)
Tertiary	sin_TNFα (WB)	Ag85	%(CD4+ Ag85b) – %(CD4+ Unstim)
Tertiary	sin_TNFα (WB)	Rv2660c	%(CD4+ RV2660c) – %(CD4+ Unstim)
Tertiary	sin_TNFα (WB)	PPD	%(CD4+ PPD) – %(CD4+ Unstim)
Tertiary	duo_IFNγ/IL2 (WB)	ESAT6	%(CD4+ ESAT6) – %(CD4+ Unstim
Tertiary	duo_IFNγ/IL2 (WB)	Ag85	%(CD4+ Ag85b) – %(CD4+ Unstim)
Tertiary	duo_IFNγ/IL2 (WB)	Rv2660c	%(CD4+ RV2660c) – %(CD4+ Unstim)
Secondary H1	duo_IFNγ/IL2 (WB)	PPD	%(CD4+ PPD) – %(CD4+ Unstim)
Tertiary	duo_IFNγ/TNFα (WB)	ESAT6	%(CD4+ ESAT6) – %(CD4+ Unstim)
Tertiary	duo_IFNγ/TNFα (WB)	Ag85	%(CD4+ Ag85b) – %(CD4+ Unstim)
Tertiary	duo_IFNγ/TNFα (WB)	Rv2660c	%(CD4+ RV2660c) – %(CD4+ Unstim)
Tertiary	duo_IFNγ/TNFα (WB)	PPD	%(CD4+ PPD) – %(CD4+ Unstim)
Tertiary	duo_TNFα/IL2 (WB)	ESAT6	%(CD4+ ESAT6) – %(CD4+ Unstim)
Tertiary	duo_TNFα/IL2 (WB)	Ag85	%(CD4+ Ag85b) – %(CD4+ Unstim)
Tertiary	duo_TNFα/IL2 (WB)	Rv2660c	%(CD4+ RV2660c) – %(CD4+ Unstim)
Tertiary	duo_TNFα/IL2 (WB)	PPD	%(CD4+ PPD) – %(CD4+ Unstim)
Tertiary	tri_IFNγ/IL2/TNFα (WB)	ESAT6	%(CD4+ ESAT6) – %(CD4+ Unstim)
Tertiary	tri_IFNγ/IL2/TNFα (WB)	Ag85	%(CD4+ Ag85) – %(CD4+ Unstim)
Tertiary	tri_IFNγ/IL2/TNFα (WB)	Rv2660c	%(CD4+ RV2660c) – %(CD4+ Unstim)
Secondary H1	tri_IFNγ/IL2/TNFα (WB)	PPD	%(CD4+ PPD) – %(CD4+ Unstim)
Tertiary	tot_IFNγ (WB)	ESAT6	%(CD4+ ESAT6) – %(CD4+ Unstim)
Tertiary	tot_IFNγ (WB)	Ag85	%(CD4+ Ag85) – %(CD4+ Unstim)
Tertiary	tot_IFNγ (WB)	Rv2660c	%(CD4+ RV2660c) – %(CD4+ Unstim)
Tertiary	tot_IFNγ (WB)	PPD	%(CD4+ PPD) – %(CD4+ Unstim)
Tertiary	tot_IFNγ (WB)	SEB*	%(CD4+ SEB) – %(CD4+ Unstim)

Tertiary	tot_IL2 (WB)	ESAT6	%(CD4+ ESAT6) – %(CD4+ Unstim)
Tertiary	tot_IL2 (WB)	Ag85	%(CD4+ Ag85) – %(CD4+ Unstim)
Tertiary	tot_IL2 (WB)	Rv2660c	%(CD4+ RV2660c) – %(CD4+ Unstim)
Tertiary	tot_IL2 (WB)	PPD	%(CD4+ PPD) – %(CD4+ Unstim)
Tertiary	tot_TNFα (WB)	ESAT6	%(CD4+ ESAT6) – %(CD4+ Unstim)
Tertiary	tot_TNFα (WB)	Ag85	%(CD4+ Ag85) – %(CD4+ Unstim)
Tertiary	tot_TNFα (WB)	Rv2660c	%(CD4+ RV2660c) – %(CD4+ Unstim)
Tertiary	tot_TNFα (WB)	PPD	%(CD4+ PPD) – %(CD4+ Unstim)
Secondary	All_Cytokine WB	ESAT6	SUM of (tot_IFNγ, duo_IL2/TNFα, sin_IL2, sin_TNFα) for ESAT6
Secondary	All_Cytokine WB	Ag85	SUM of (tot_IFNγ, duo_IL2/TNFα, sin_IL2, sin_TNFα) for Ag85
Secondary	All_Cytokine WB	Rv2660c	SUM of (tot_IFNγ, duo_IL2/TNFα, sin_IL2, sin_TNFα) for Rv2660c
Primary H1[1], Secondary H2[2]/H3[3]	All_Cytokine WB	PPD	SUM of (tot_IFNγ, duo_IL2/TNFα, sin_IL2, sin_TNFα) for PPD
Primary	All_Cytokine WB	SUM (ESAT6+ Ag85+Rv2660c)	SUM of (tot_IFNγ, duo_IL2/TNFα, sin_IL2, sin_TNFα) for ESAT6, Ag85 and Rv2660c
Secondary	tri_ cytokines (WB)	SUM (ESAT6+ Ag85+Rv2660c)	SUM of (tri_IFNγ/IL2/TNFα) for ESAT6, Ag85 and Rv2660c
Secondary	duo_IFNγ/IL2 (WB)	SUM (ESAT6+ Ag85+Rv2660c)	SUM of (duo_IFNγ/IL2) for ESAT6, Ag85 and Rv2660c
Tertiary	duo_IFNγ/TNFα (WB)	SUM (ESAT6+ Ag85+Rv2660c)	SUM of (duo_IFNγ/TNFα) for ESAT6, Ag85 and Rv2660c
Tertiary	duo_ IL2/TNFα/ (WB)	SUM (ESAT6+ Ag85+Rv2660c)	SUM of (duo_IL2/TNFα) for ESAT6, Ag85 and Rv2660c

[1]Hypothesis 1 (etoricoxib). [2]Hypothesis 2 (H56:IC31 vaccine). [3]Hypothesis 3 (etoricoxib + H56:IC31 vaccine).

Colour code: blue=secondary priority; red=primary priority; white=tertiary priority.

*All samples with an invalid positive control will be excluded from analyses. Invalid positive control is defined as: Invalid if SEB induced tot_IFNγ response by CD4 is < 4 Medians of the tot_IFNγ response by CD4 T cells of the unstimulated controls of all participants in the study at the respective measurement time point.

Table 8: Definition of derived outcomes from the whole-blood (WB) intracellular cytokine staining and flow cytometry analysis for CD8+ (eg.CD3+ CD4- CD8+)

Outcome priority group	Immunology readout	Stimulation	Derived outcome
Tertiary	sin_IFNγ (WB)	ESAT6	%(CD8+ESAT6) – %(CD8+ Unstim
Tertiary	sin_IFNγ (WB)	Ag85	%(CD8+ Ag85) – %(CD8+ Unstim)
Tertiary	sin_IFNγ (WB)	Rv2660c	%(CD8+ RV2660c) – %(CD8+ Unstim)
Tertiary	sin_IFNγ (WB)	PPD	%(CD8+ PPD) – %(CD8+ Unstim)
Tertiary	sin_IL2 (WB)	ESAT6	%(CD8+ ESAT6) – %(CD8+ Unstim)
Tertiary	sin_IL2 (WB)	Ag85	%(CD8+ Ag85) – %(CD8+ Unstim)
Tertiary	sin_IL2 (WB)	Rv2660c	%(CD8+ RV2660c) – %(CD8+ Unstim)

Tertiary	sin_IL2 (WB)	PPD	%(CD8+ PPD) – %(CD8+ Unstim)
Tertiary	sin_TNFα (WB)	ESAT6	%(CD8+ ESAT6) – %(CD8+ Unstim)
Tertiary	sin_TNFα (WB)	Ag85	%(CD8+ Ag85) – %(CD8+ Unstim)
Tertiary	sin_TNFα (WB)	Rv2660c	%(CD8+ RV2660c) – %(CD8+ Unstim)
Tertiary	sin_TNFα (WB)	PPD	%(CD8+ PPD) – %(CD8+ Unstim)
Tertiary	duo_IFNγ/IL2 (WB)	ESAT6	%(CD8+ ESAT6) – %(CD8+ Unstim
Tertiary	duo_IFNγ/IL2 (WB)	Ag85	%(CD8+ Ag85) – %(CD8+ Unstim)
Tertiary	duo_IFNγ/IL2 (WB)	Rv2660c	%(CD8+ RV2660c) – %(CD8+ Unstim)
Tertiary	duo_IFNγ/IL2 (WB)	PPD	%(CD8+ PPD) – %(CD8+ Unstim)
Tertiary	duo_IFNγ/TNFα (WB)	ESAT6	%(CD8+ ESAT6) – %(CD8+ Unstim)
Tertiary	duo_IFNγ/TNFα (WB)	Ag85	%(CD8+ Ag85) – %(CD8+ Unstim)
Tertiary	duo_IFNγ/TNFα (WB)	Rv2660c	%(CD8+ RV2660c) – %(CD8+ Unstim)
Tertiary	duo_IFNγ/TNFα (WB)	PPD	%(CD8+ PPD) – %(CD8+ Unstim)
Tertiary	duo_TNFα/IL2 (WB)	ESAT6	%(CD8+ ESAT6) – %(CD8+ Unstim)
Tertiary	duo_TNFα/IL2 (WB)	Ag85	%(CD8+ Ag85) – %(CD8+ Unstim)
Tertiary	duo_TNFα/IL2 (WB)	Rv2660c	%(CD8+ RV2660c) – %(CD8+ Unstim)
Tertiary	duo_TNFα/IL2 (WB)	PPD	%(CD8+ PPD) – %(CD8+ Unstim)
Tertiary	tri_IFNγ/IL2/TNFα (WB)	ESAT6	%(CD8+ ESAT6) – %(CD8+ Unstim
Tertiary	tri_IFNγ/IL2/TNFα (WB)	Ag85	%(CD8+ Ag85) – %(CD8+ Unstim)
Tertiary	tri_IFNγ/IL2/TNFα (WB)	Rv2660c	%(CD8+ RV2660c) – %(CD8+ Unstim)
Tertiary	tri_IFNγ/IL2/TNFα (WB)	PPD	%(CD8+ PPD) – %(CD8+ Unstim)
Tertiary	tot_IFNγ (WB)	ESAT6	%(CD8+ ESAT6) – %(CD8+ Unstim)
Tertiary	tot_IFNγ (WB)	Ag85	%(CD8+ Ag85) – %(CD8+ Unstim)
Tertiary	tot_IFNγ (WB)	Rv2660c	%(CD8+ RV2660c) – %(CD8+ Unstim)
Tertiary	tot_IFNγ (WB)	PPD	%(CD8+ PPD) – %(CD8+ Unstim)
Tertiary	tot_IL2 (WB)	ESAT6	%(CD8+ ESAT6) – %(CD8+ Unstim)
Tertiary	tot_IL2 (WB)	Ag85	%(CD8+ Ag85) – %(CD8+ Unstim)
Tertiary	tot_IL2 (WB)	Rv2660c	%(CD8+ RV2660c) – %(CD8+ Unstim)
Tertiary	tot_IL2 (WB)	PPD	%(CD8+ PPD) – %(CD8+ Unstim)
Tertiary	tot_TNFα (WB)	ESAT6	%(CD8+ ESAT6) – %(CD8+ Unstim)
Tertiary	tot_TNFα (WB)	Ag85	%(CD8+ Ag85) – %(CD8+ Unstim)
Tertiary	tot_TNFα (WB)	Rv2660c	%(CD8+ RV2660c) – %(CD8+ Unstim)
Tertiary	tot_TNFα (WB)	PPD	%(CD8+ PPD) – %(CD8+ Unstim)
Secondary	All_Cytokine WB	ESAT6	SUM of (tot_IFNγ, duo_IL2/TNFα, sin_IL2, sin_TNFα) for ESAT6
Secondary	All_Cytokine WB	Ag85b	SUM of (tot_IFNγ, duo_IL2/TNFα, sin_IL2, sin_TNFα) for Ag85
Secondary	All_Cytokine WB	Rv2660c	SUM of (tot_IFNγ, duo_IL2/TNFα, sin_IL2, sin_TNFα) for Rv2660c
Secondary	All_Cytokine WB	PPD	SUM of (tot_IFNγ, duo_IL2/TNFα, sin_IL2, sin_TNFα) for PPD
Secondary	All_Cytokine WB	SUM (ESAT6+ Ag85+Rv2660c)	SUM of (tot_IFNγ, duo_IL2/TNFα, sin_IL2, sin_TNFα) for ESAT6, Ag85 and Rv2660c

Tertiary	tri_IFNγ/IL2/TNFα (WB)	SUM (ESAT6+ Ag85+Rv2660c)	SUM of (tri_IFNγ/IL2/TNFα) for ESAT6, Ag85 and Rv2660c
Tertiary	duo_IFNγ/IL2 (WB)	SUM (ESAT6+ Ag85+Rv2660c)	SUM of (duo_IFNγ/IL2) for ESAT6, Ag85 and Rv2660c
Tertiary	duo_IFNγ/TNFα (WB)	SUM (ESAT6+ Ag85+Rv2660c)	SUM of (duo_IFNγ/TNFα) for ESAT6, Ag85 and Rv2660c
Tertiary	duo_IL2/TNFα (WB)	SUM (ESAT6+ Ag85+Rv2660c)	SUM of (duo_IL2/ TNFα) for ESAT6, Ag85 and Rv2660c

Colour code: blue=secondary priority; red=primary priority; white=tertiary priority.

*All samples with an invalid positive control will be excluded from analyses. Invalid positive control is defined as: Invalid if SEB induced tot_IFNγ response by CD4 cells is < 4 Medians of the tot_IFNγ response by CD4 T cells of the unstimulated controls of all participants in the study at the respective measurement time point.

Table 9: Definition of outcomes from serology: IgG responses to H56:IC31 protein

Outcome priority group	Outcome
Tertiary Hypothesis 1	IgG antibody responses to H56:IC31
Primary Hypothesis 2	IgG antibody responses to H56:IC31
Primary Hypothesis 3	IgG antibody responses to H56:IC31

Colour code: blue=secondary priority; red=primary priority; white=tertiary priority.

5.1.1.4 Pharmacokinetics

Pharmacokintetics of etoricoxib will be measured for selected patients during the first 7-10 days with subsequent measurement of etoricoxib in plasma for patients included in arm 1 and arm 4 at day 14 and day 84. Results will be shown in a Supplementary table.

5.1.1.5. Concomitant medication

Will be summarised and displayed by term and ATC code for each treatment arm and shown in a Supplementary table of individual study participants including co-morbidities, medical history, symptoms, reason for stopping intervention etc.

The following parameters will be described only in the Clinical Study Report

- **Clinical chemistry:** Sodium (Na), Potassium (K), Calcium (Ca), Creatinine, Creatinine kinase (CK), ASAT, ALAT, Lactate dehydrogenase (LD), Bilirubin, Amylase, GGT, Cholesterol, glucose, HbA1c, Uric acid,
- **Haematology:** Whole blood count (WBC), monocytes, lymphocytes, Platelets.
- **Vital signs**: Respiratory rate, heart rate, systolic blood pressure, diastolic blood pressure.

5.1.2 Primary Outcome Definition

The primary outcome is the occurrence of adverse events (AEs), serious adverse events (SAEs), and suspected unexpected serious adverse reactions (SUSARs) for the following time intervals:

a. Day 0 to day 84 (or date of first vaccination if applicable).

b. Day 85 (or date of first vaccination if applicable) to day 154 (or 14 days after last vaccination/last Arcoxia treatment, if applicable).
c. Day 155 (or 15 days after last vaccination/last Arcoxia treatment, if applicable) to day 238.

If the second vaccination is missing, day 154 is defined as 70 days after the date of day 84.

AEs are defined as any unintended sign, symptom or disease in a participant receiving the treatment and which does not necessarily have to be causally related with the treatment. The AEs will be coded using the Medical Dictionary for Regulatory Activities (MedDRA 22).

SAEs are defined as a medical occurrence that results in death, is life-threatening, requires in-patient hospitalisation or prolongation of the existing hospitalisation, results in disabilities, is a congenital abnormality of birth defect, or requires medical intervention to prevent the outcomes mentioned above. If there is a doubt about an AE being serious or not, it is considered serious.

SUSARs are defined as SAEs that are unexpected as defined in the study protocol (V6_121016), Section 8.1 and 8.2, pages 32-34 and possibly related to the study treatment.

5.1.3 Secondary Outcomes Definitions

All secondary outcomes are listed and defined in the Tables 6 to 9 (Section 5.1 Outcome Definitions).

5.1.4 Overview of Outcomes

Table 10. Overview of outcomes analysed in this SAP

Nr	Level	Outcome	Timeframe	Type
1	Primary	Number of AEs, SAEs, and SUSARs	Day 0-84, 85-154 and 155-238	Counts
2	Secondary, Hypothesis 1, Priority 1	Immunogenicity outcomes of primary priority in Table 1	Day 0-238	Continuous
3	Secondary, Hypothesis 2, Priority 1	Immunogenicity outcomes of primary priority in Table 2	Day 0-238	Continuous
4	Secondary, Hypothesis 3, Priority 1	Immunogenicity outcomes of primary priority in Table 3	Day 0-238	Continuous
5	Secondary, Hypothesis 1, Priority 2	Immunogenicity outcomes of secondary priority in Table 1	Day 0-238	Continuous
6	Secondary, Hypothesis 2, Priority 2	Immunogenicity outcomes of secondary priority in Table 2	Day 0-238	Continuous

7	Secondary, Hypothesis 3, Priority 2	Immunogenicity outcomes of secondary priority in Table 3	Day 0-238	Continuous
8	Secondary, Hypothesis 1 to 3, Priority 3	Immunogenicity outcomes of tertiary priority in Tables 6-9	Day 0-238	Continuous

5.2 Analysis Methods

Because of the explorative nature of the trial and the low number of subjects included, we will mainly use descriptive statistics to present the results of the trial.

5.2.1 Primary outcome (Outcome 1, Table 10)

5.2.1.1 Primary Analysis

We will calculate the numbers and proportions of participants in the Safety Analysis Set that experienced an AE, SAE, and SUSAR (see 3.3 Analysis Populations) by study arm and above defined three time intervals. We will also present the number and proportions of participants with 1, 2, or ≥3 AEs, SAEs, and SUSARs; the number and proportion of participants with any treatment related AE and SAE; and, the number and proportion of participants with an AE/SAE leading to study drug discontinuation in each study arm and above defined three time intervals. No formal statistical testing of differences between the study arms will be performed. Further, we will display the total number of AEs, SAEs, SUSARs, treatment related AEs and SAEs, and AEs/SAEs leading to study drug discontinuation independent of number of participants. In addition, we will describe the absolute, number of participants with and proportion of participants with AE, SAE, SUSAR, severe AEs, and AEs leading to study drug discontinuation by system organ class (SOC) and preferred term (PT), without calculating any p-values.

5.2.1.2 Assumption Checks and Alternative Analyses

There will be no assumption checks or alternative analyses

5.2.1.3 Missing data

We will assess safety as reported.

5.2.1.4 Sensitivity analyses

There will be no sensitivity analyses.

5.2.2 Secondary outcomes (Outcomes Tables 6-9)

5.2.2.1 Main analysis

The following analyses will be performed for all secondary outcomes described in Tables 6-9 (all continuous variables).

Descriptive analysis (graphical display of the data) in the FAS:

All the outcomes listed in Tables 6-9 will be described as medians with IQRs over each study time point from 0 to 238 (day 0, 84, 98, 140, 154, 238) by ITT study arm.

All the outcomes listed in Tables 6-9 will be described for each individual over each study time point from 0 to 238 (day 0, 84, 98, 140, 154, 238), stratified by ITT study arm.

For the IgG antibody response to H56:IC31 (Table 9) we will describe the proportion of participants with a twofold increase in response from baseline at any time point during the study, stratified by study arm.

Additional descriptive Figures will be made to graphically display the secondary outcomes of primary priority according to the hypothesis stated in Tables 1-3.

Statistical analysis and hypothesis testing (according to the statistical framework described in section 2.4) in the FAS:

The secondary outcomes of primary priority for hypothesis 1 (Section 2.4.1) are the response to treatment defined as the change from day 0 to day 84 in the defined readouts. The outcomes will be analysed using median regression with treatment as only independent explanatory factor. The treatment difference of the combination of Arm 1 and 4 (treated with a COX-2 inhibitor) and the combination of Arm 2 and 3 (control) will be presented as estimated difference in median response with 95% confidence interval based on 1000 bootstrap replications and corresponding two-sided p-value.

The secondary outcomes of primary priority for hypothesis 2 (Section 2.4.2) and hypothesis 3 (Section2.4.3) are the responses to vaccine treatment defined as the change from day 84 to day 154 in the defined readouts. The outcomes for hypothesis 2 and 3 will be analysed and presented as for hypothesis 1.

The seed for the bootstrap replications will be set to the date of the final statistical analysis plan (Version 1.0), in the format yyyymmdd.

5.2.2.2 Assumption Checks and Alternative Analyses

The median regression with bootstrap-estimated standard error and confidence interval is robust to skewed data and with small sample sizes. There will be no alternative analyses.

5.2.2.3 Missing Data

Descriptive statistics and hypothesis testing will be based on available data.

5.2.2.4 Sensitivity Analyses

No sensitivity analyses will be performed.

6 Safety Analyses

Safety is the primary outcome of this trial and all performed safety analyses are described under 5.2.1 Primary Analysis.

7 Statistical Software

All statistical analyses will be done in Stata v16 or newer (StataCorp. 2015. *Stata Statistical Software: Release 16.1* College Station, TX, USA).

8 References

8.1 Literature References

1. Gamble C, Krishan A, Stocken D, et al. Guidelines for the Content of Statistical Analysis Plans in Clinical Trials. *Jama* 2017; **318**: 2337-43.
2. WHO (2018). Global tuberculosis report 2018, L.C.B.-N.-S. IGO., ed. (Geneva: World Health Organization: Geneva: World Health Organization).

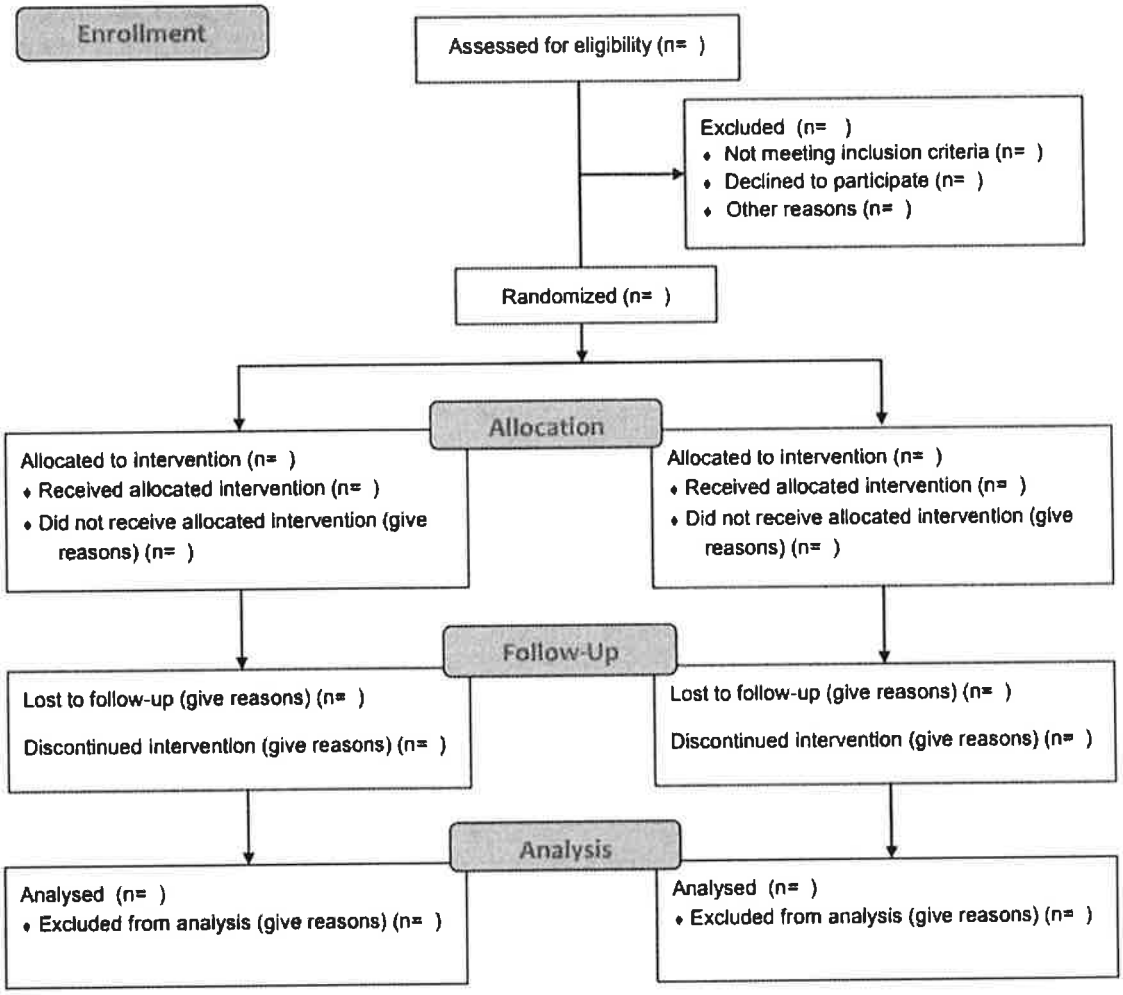
8.2 Reference to Data Handling Plan

The Data Handling Plan is appended to this SAP in Appendix II.

APPENDIX I – The CONSORT flow diagram

CONSORT
TRANSPARENT REPORTING of TRIALS

CONSORT 2010 Flow Diagram



APPENDIX II – Data Handlings Plan