Clinical Study Protocol

Sponsor:
GlaxoSmithKline Biologicals
Rue de l'Institut 89
1330 Rixensart, Belgium

Primary Study vaccine and number	GlaxoSmithKline (GSK) Biologicals' candidate recombinant *Mycobacterium tuberculosis* vaccine, M72/AS01$_E$ (GSK 692342)
Other study vaccine/product	Placebo
eTrack study number and Abbreviated Titles	115616 (TUBERCULOSIS-018)
Date of protocol	Final: 29 May 2012
Date of protocol amendment 1	Amendment 1 Final: 20 July 2012
Date of protocol amendment 2	Amendment 2 Final: 10 September 2012
Date of protocol amendment 3	Amendment 3 Final: 11 December 2012
Date of protocol amendment 4	Amendment 4 Final: 26 March 2013
Date of protocol amendment 5	Amendment 5 Final: 29 January 2014
Title (Amended: 29 January 2014)	Efficacy of GSK Biologicals' candidate tuberculosis (TB) vaccine GSK 692342 against TB disease, in adults living in a TB endemic region
Detailed Title (Amended: 29 January 2014)	A phase IIb, double-blind, randomised, placebo-controlled study to evaluate the efficacy, safety and immunogenicity of GSK Biologicals' candidate tuberculosis (TB) vaccine GSK 692342 against TB disease, in adults aged 18-50 years, living in a TB endemic region.
Co-ordinating author(s)	PPD, ***Scientific Writer***, XPE Pharma & Science for GSK Biologicals
Contributing authors GSK Biologicals (Amended: 29 January 2014)	• PPD, ***Clinical Research and Development Lead*** • PPD, Project Statistician • PPD, Study ***Delivery*** Manager • PPD, ***Project Delivery Lead*** • PPD, Project Manager Clinical Readouts • PPD, Safety ***Physician*** • PPD, Study Data Manager • PPD, Senior Manager Regulatory ***Affairs*** • PPD, ***Global Regulatory Lead***
Contributing Authors Aeras (Amended: 29 January 2014)	• PPD, ***President &*** Chief ***Executive*** Officer • PPD, Director Clinical Development

GlaxoSmithKline Biologicals will act as the regulatory sponsor for this trial.

GSK Biologicals' Protocol DS v 14.0

Protocol Amendment 5 Sponsor Signatory Approval

eTrack study number and Abbreviated Title	115616 (TUBERCULOSIS-018)
Date of protocol amendment	Amendment 5 Final: 29 January 2014
Detailed Title (Amended: 29 January 2014)	A phase IIb, double-blind, randomised, placebo-controlled study to evaluate the efficacy, safety and immunogenicity of GSK Biologicals' candidate tuberculosis (TB) vaccine GSK 692342 against TB disease, in adults aged 18-50 years, living in a TB endemic region.
Sponsor signatory	Didier Lapierre, Vice President Global Vaccine Development - Malaria, Tuberculosis and Adjuvants
Signature	_____
Date	_____

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Protocol Amendment 5 Rationale

Amendment number:	Amendment 5

Rationale/background for changes:

Optimized study design and sample size by increasing event rate by focussing on IGRA positive subjects. Baseline screening for pulmonary TB as precautionary safety measures. Separate protocol for biobanking of blood samples for future research on biological correlates for TB disease.

Protocol Amendment 5 Investigator Agreement

I agree:

- To conduct the study in compliance with this protocol, any future protocol amendments or protocol administrative changes, with the terms of the clinical trial agreement and with any other study conduct procedures and/or study conduct documents provided by GlaxoSmithKline (GSK) Biologicals.
- To assume responsibility for the proper conduct of the study at this site.
- That I am aware of, and will comply with, 'Good Clinical Practice' (GCP) and all applicable regulatory requirements.
- To ensure that all persons assisting me with the study are adequately informed about the GSK Biologicals' investigational vaccine(s)/product(s) and other study-related duties and functions as described in the protocol.
- To acquire the reference ranges for laboratory tests performed locally and, if required by local regulations, obtain the laboratory's current certification or Quality Assurance procedure manual.
- To ensure that no clinical samples (including serum samples) are retained onsite or elsewhere without the approval of GSK Biologicals and the express written informed consent of the subject and/or the subject's legally acceptable representative.
- To perform no other biological assays on the clinical samples except those described in the protocol or its amendment(s).
- To co-operate with a representative of GSK Biologicals in the monitoring process of the study and in resolution of queries about the data.
- That I have been informed that certain regulatory authorities require the sponsor to obtain and supply, as necessary, details about the investigator's ownership interest in the sponsor or the investigational vaccine(s)/product(s), and more generally about his/her financial ties with the sponsor. GSK Biologicals will use and disclose the information solely for the purpose of complying with regulatory requirements.

Hence I:

- Agree to supply GSK Biologicals with any necessary information regarding ownership interest and financial ties (including those of my spouse and dependent children).
- Agree to promptly update this information if any relevant changes occur during the course of the study and for one year following completion of the study.
- Agree that GSK Biologicals may disclose any information it has about such ownership interests and financial ties to regulatory authorities.
- Agree to provide GSK Biologicals with an updated Curriculum Vitae and other documents required by regulatory agencies for this study.

eTrack study number and Abbreviated Title	115616 (TUBERCULOSIS-018)
Date of protocol amendment	Amendment 5 Final: 29 January 2014
Detailed Title	A phase IIb, double-blind, randomised, placebo-controlled study to evaluate the efficacy, safety and immunogenicity of GSK Biologicals' candidate tuberculosis (TB) vaccine GSK 692342 against TB disease, in adults aged 18-50 years, living in a TB endemic region.
Investigator name	_____
Signature	_____
Date	_____

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Sponsor Information

1. Sponsor

GlaxoSmithKline Biologicals
Rue de l'Institut 89
1330 Rixensart, Belgium

2. Sponsor Medical Expert for the Study

Refer to the local study contact information document.

3. Sponsor Study Monitor

Refer to the local study contact information document.

4. Sponsor Study Contact for Reporting of a Serious Adverse Event

GSK Biologicals Central Back-up Study Contact for Reporting SAEs: refer to Protocol Section 9.3.2.

5. GSK Biologicals' Central Safety Physician On-Call Contact information for Emergency Unblinding

GSK Biologicals Central Safety Physician and Back-up Phone contact: refer to Protocol Section 9.7.

SYNOPSIS

Detailed Title (Amended: 29 January 2014)

A phase IIb, double-blind, randomised, placebo-controlled study to evaluate the efficacy, safety and immunogenicity of GSK Biologicals' candidate tuberculosis (TB) vaccine GSK 692342 against TB disease, in adults aged 18-50 years, living in a TB endemic region.

Indication (Amended: 29 January 2014)

Vaccination against tuberculosis (TB) disease in adults aged 18 to 50 years, living in a TB endemic region.

Rationale for the study and study design

- **Rationale for the study**

The burden of *Mycobacterium tuberculosis* (Mtb) disease, particularly when compounded by Human Immunodeficiency virus (HIV)-infection and the emergence of multi-drug resistant (MDR) and extensively-drug resistant strains (XDR), is significant in TB endemic countries/regions. In 2010, approximately 40% of TB cases were reported in India and China, of which up to 80% were pulmonary in nature [WHO, 2011]. Africa accounted for a further 24% of cases (of which 25% were in South Africa), with most cases in young adults.

Prevention of TB disease is not only preferable to treatment of the disease for public health reasons, but an effective prophylactic TB vaccine would also lead to a considerable return in health benefits. ***In TB endemic settings the incidence of pulmonary TB disease is higher in people who have been infected with Mtb as evidenced by a positive Interferon gamma release assay (IGRA). For the present first phase II proof-of-concept trial focussing on this IGRA+ adult population maximizes the trial efficiency by increasing the event rate in the control group.***
(Amended: 29 January 2014)

This Phase IIb study aims at collecting initial efficacy data and gathering further safety and immunogenicity data on the M72/AS01$_E$ vaccine candidate in adults living in a TB endemic region. **(Amended: 29 January 2014)**

- **Rationale for the study design**

This Phase IIb study is designed to evaluate the protective efficacy of the M72/AS01$_E$ candidate ***TB*** vaccine against definite pulmonary TB disease not associated with HIV-

infection, versus placebo, following 2 injections given one month apart. The protective efficacy will be evaluated in ***baseline IGRA positive*** adults living in a TB endemic region aged 18-50 years, i.e. in an age range where the incidence of ***TB*** is high. **(Amended: 29 January 2014)**

This study is an international, multicentre, phase IIb, double-blind, randomized, controlled trial. There will be follow-up for efficacy for ***up to*** 3 years after vaccination via regular visits or contacts to screen for possible TB. Subjects will also be asked to recognise signs and symptoms compatible with TB disease and to self-report for clinical evaluation. Subjects with clinical suspicion of pulmonary TB will be assessed with confirmatory diagnostic testing using a Nucleic Acid Amplification Test (Xpert MTB/RIF assay, Cepheid) and microbiological culture. Subjects diagnosed with TB will be referred for TB treatment according to local clinical practice. **(Amended: 29 January 2014)**

The primary objective of this study is to evaluate the protective efficacy of two doses of the M72/AS01$_E$ candidate vaccine against Definite pulmonary TB disease **not associated with HIV-infection**, as compared to placebo. To minimize the ***potential confounding effect of*** HIV ***infection*** in the study population, HIV testing will be performed at screening ***and only HIV negative subjects will be enrolled***. Additionally, when a subject is diagnosed with TB, an HIV test (and pre-test counselling) will be performed to rule out HIV co-infection. When a subject is diagnosed with HIV at the time of TB diagnosis, additional tests will be performed to measure CD4 cell counts to assess the severity of the infection and decide on the appropriate treatment regimen. HIV-positive subjects will be referred for HIV treatment according to local clinical practice. **(Amended: 29 January 2014)**

Participating sites will use standardised case definitions for efficacy endpoints and a structured approach to case-assessment as outlined in this study protocol will be used.

An Independent Data Monitoring Committee (IDMC) ***has been*** constituted for this study to perform periodic safety reviews of the safety data. **(Amended: 29 January 2014)**

A sub-cohort of subjects will be selected for specific follow-up for safety (solicited adverse events [AE] and selected Biochemistry and Complete Blood Count [CBC]) and immunogenicity. Unsolicited AEs, serious adverse events (SAEs), potential immune-mediated diseases (pIMDs), AEs leading to withdrawal and pregnancies will be recorded for all subjects.**(Amended: 29 January 2014)**

- **Rationale for the use of placebo**

There is currently no established TB vaccine with recognised efficacy in the age range considered for this study. The use of a control group is mandatory for meeting the primary objective.

The use of an active vaccine as control has been evaluated but introduces significant unblinding risks as the presentation, schedule or indications are different from the M72/AS01$_E$ vaccine.

Objectives

(Amended:29 January 2014)

Primary

- To evaluate the protective efficacy of two doses of the M72/AS01$_E$ candidate vaccine against **Definite pulmonary TB disease** not associated with HIV-infection, meeting the first case definition, as compared to placebo.

Success criterion:

*The lower limit of the **90**% two-sided confidence interval (CI) for the VE (using a Cox regression model) against first occurrence of Definite pulmonary TB disease not associated with HIV-infection, meeting the first case definition, is above 0%.* **(Amended: 29 January 2014)**

Secondary

Efficacy:

- To evaluate the protective efficacy of two doses of the M72/AS01$_E$ candidate vaccine against **Definite Xpert MTB/Rif positive pulmonary TB disease** not associated with HIV-infection, meeting the second case definition, as compared to placebo.

If the primary objective is met, this secondary objective will be analysed with the following success criterion:

*The lower limit of the **90**% two-sided CI for the VE (using a Cox regression model) against first occurrence of Definite pulmonary TB disease not associated with HIV-infection, meeting the **second** case definition, is above 0%.* **(Amended: 29 January 2014)**

- To evaluate the protective efficacy of two doses of the M72/AS01$_E$ candidate vaccine against **Definite pulmonary TB disease** not associated with HIV-infection, meeting the third case definition, as compared to placebo.

- To evaluate the protective efficacy of two doses of the M72/AS01$_E$ candidate vaccine against ***Microbiological*** **pulmonary TB disease** meeting the fourth case definition, as compared to placebo.

- To evaluate the protective efficacy of two doses of the M72/AS01$_E$ candidate vaccine against **Clinical TB disease** meeting the fifth case definition, as compared to placebo.

Safety:

- To assess the safety and reactogenicity of two doses of the M72/AS01$_E$ candidate vaccine.

Immunogenicity:

- To assess the immunogenicity of two doses of the M72/AS01$_E$ candidate vaccine.

Study design

- Phase IIB, randomised, double-blind, controlled, multi-centric, efficacy study with two parallel groups.
- **Duration of the study:** the duration of the study will be approximately 37 months for each subject from

screening to end of efficacy follow-up.

  - Epoch 001: Primary starting at Visit 1 (screening visit) and ending at Visit 10 (Month 36).

- **Study groups:** study groups and treatments foreseen in this study are presented in Synopsis Table 1 and Synopsis Table 2.

Synopsis Table 1 Study groups and epochs foreseen in the study (Amended: 29 January 2014)

Study groups	Number of subjects	Age (Min/Max)	Epoch 001
M72AS01	***1753***	18 years - 50 years	x
Control	***1753***	18 years - 50 years	x

Synopsis Table 2 Study groups and treatment foreseen in the study

Treatment name	Vaccine/Product name	Study Groups	
		M72AS01	Control
M72/AS01E	M72	X	
	$AS01_E$	X	
Placebo	Placebo pellet		X
	Placebo adjuvant		X

- **Blinding:** double-blind (Synopsis Table 3).

Synopsis Table 3 Blinding of study epochs

Study Epochs	Blinding
Epoch 001	double-blind

- **Vaccination schedule:** Subjects will receive 2 doses of $M72/AS01_E$ or placebo, according to their random assignment, one month apart (Day 0 and Day 30) by intramuscular injection in the deltoid region of the arm.
- **Treatment allocation:** Randomised 1:1. Randomisation will occur at the day of first vaccination (Day 0).
- **Control:** placebo control.
- **Sampling schedule (Amended: 29 January 2014):**
  - Blood samples will be collected in the safety and immune sub-cohort for evaluation of biochemistry/haematology parameters (Days 0, 7, 30 and 37), CMI responses (Days 0, 60 and Years 1, 2 and 3) and humoral immune responses (Days 0, 60 and Years 1, 2 and 3). ***Vitamin D levels will***

be measured at Day 0.

- Blood samples will be collected for QFTG testing at ***screening*** for all subjects.
- ***A respiratory sputum sample will be collected prior to vaccination in all subjects for nucleic acid amplification test to rule out pulmonary TB.***
- ***Blood samples will be collected for initial HIV testing at screening for all subjects. Repeat HIV testing will be performed at the last study visit V9, unless the subject has been confirmed HIV positive before V9.***
- Whenever a subject is diagnosed with TB disease, blood samples will be collected for HIV testing ***and HbA1c measurement***. In addition, if a subject is HIV-positive, blood samples will be collected to determine CD4 cell counts.

- **Type of study:** self-contained.
- **Data collection:** Electronic Case Report Form (eCRF). - ***Inform*™ (Amended: 29 January 2014)**
- **Safety monitoring:** an Independent Data Monitoring Committee (IDMC), operating under a charter, ***has been*** constituted for this study to oversee the study. They will perform safety reviews twice a year, or more frequently if deemed necessary on an ongoing basis for the duration of the study. **(Amended: 29 January 2014)**

Case Definitions

First case definition – Definite pulmonary TB, not associated with HIV-infection

- A subject with clinical suspicion* of pulmonary TB disease, with Mtb complex identified from a sputum specimen, taken before initiation of TB treatment, by Xpert MTB/RIF and/or microbiological culture and confirmed HIV-negative at the time of TB diagnosis.

Second case definition –Definite Xpert MTB/Rif positive pulmonary TB, not associated with HIV-infection

- A subject with clinical suspicion* of pulmonary TB disease, with Mtb complex identified from a sputum specimen, taken before initiation of TB treatment, by Xpert MTB/RIF and confirmed HIV-negative at the time of TB diagnosis.

Third case definition – Definite pulmonary TB, not associated with HIV-infection

- A subject with clinical suspicion* of pulmonary TB disease, with Mtb complex identified from a sputum specimen, taken up to four weeks after initiation of TB treatment, by Xpert MTB/RIF and/or microbiological culture and confirmed HIV-negative at the time of TB diagnosis.

Fourth case definition –*Microbiological* pulmonary TB (Amended: 29 January 2014)

- A subject with clinical suspicion* of pulmonary TB disease, with Mtb complex identified from a sputum specimen, taken up to four weeks after initiation of TB treatment, by Xpert MTB/RIF and/or microbiological culture.

*Clinical suspicion of pulmonary TB is defined as a subject presenting with one or more of the following symptoms: unexplained cough > 2 weeks, unexplained fever > 1 week, night sweats, unintentional weight loss, pleuritic chest pains, haemoptysis, fatigue or shortness of breath on exertion.

Fifth case definition – Clinical TB

A subject for whom a clinician has diagnosed TB disease and has decided to treat the patient with TB treatment.

Number of subjects Approximately ***3506***. **(Amended: 29 January 2014)**

Endpoints

Primary

- Incident cases of **Definite Pulmonary TB disease** not associated with HIV-infection, meeting the first case definition.

  Over a period starting 1 month post-dose 2 and lasting up to 35 months post last vaccination.

Secondary

Efficacy

- Incident cases of **Definite Xpert MTB/Rif positive Pulmonary TB disease** not associated with HIV-infection, meeting the second case definition.

  Over a period starting 1 month post-dose 2 and lasting up to 35 months post last vaccination.

- Incident cases of **Definite Pulmonary TB disease** meeting the third case definition.

  Over a period starting 1 month post-dose 2 and lasting up to 35 months post last vaccination.

- Incident cases of ***Microbiological* Pulmonary TB disease** meeting the fourth case definition.

  Over a period starting 1 month post-dose 2 and lasting up to 35 months post last vaccination

- Incident cases of **Clinical TB disease** meeting the fifth case definition.

  Over a period starting 1 month post-dose 2 and lasting up to 35 months post last vaccination

Safety

- Occurrence of SAEs.

  During the entire study period
- Occurrence of unsolicited AEs.

  During the 30 day follow-up period following vaccination (day of vaccination and 29 subsequent days after each vaccine dose)
- Occurrence of solicited local and general AEs in the safety and immune sub-cohort.

  During the 7 day follow-up period following vaccination (day of vaccination and 6 subsequent days after each vaccine dose).
- Occurrence of all pIMDs.

  Over a period starting at Day 0 until 6 months post-dose 2.
- Occurrence of grade ≥ 2 haematological and biochemical***, in the safety and immune sub-cohort,*** levels at:

  Days 0, 7, 30 and 37

  (Amended: 29 January 2014)

Immunogenicity

- Evaluation of CMI responses with respect to components of the study vaccine, in the safety and immune sub-cohort:
  - ***Determined by the frequency of M72-specific CD4+/CD8+ T-cells per million cells identified after in vitro stimulation, as expressing any combination of immune markers among CD40L, IL-2, IFN-γ and TNF-α. (Amended: 29 January 2014)***

Timepoints: prior to dose 1 (Day 0) and post-dose 2 (Day 60, Years 1, 2 and 3)

- Evaluation of humoral immune responses with respect to components of the study vaccine, in the safety and immune sub-cohort:
  - Determined by M72-specific antibody titres as measured by ELISA.
  - Determined by seropositivity rates as measured by ELISA.

Timepoints: prior to dose 1 (Day 0) and post-dose 2 (Day 60, Years 1, 2 and 3).
(Amended: 29 January 2014)

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LIST OF ABBREVIATIONS

AB	Antibody
AE	Adverse Event
AIDS	Acquired Immune Deficiency Syndrome
ALT	Alanine aminotransferase
AP	Alkaline Phosphatase
AST	Aspartate aminotransferase
ATP	According-To-Protocol
β-hCG	Beta-human chorionic gonadotropin
BCG	Bacille Calmette-Guérin
bkg	Background
CBC	Complete Blood Count
CD 4/8/40	Cluster of Differentiation 4/8/40
CD-40L	Cluster of Differentiation-40 Ligand
CFP-10	Culture Filtrate Protein-10
CI	Confidence Interval
CMI	Cell-Mediated Immune(ity)
DMSO	Dimethyl Sulfoxide
eCRF	electronic Case Report Form
EDD	Estimated Date of Delivery
EGA	Estimated Gestational Age
ELISA	Enzyme-Linked Immunosorbent Assay
EMA	European Medicine Agency
ESAT-6	Early Secretory Antigen Target-6
eTDF	electronic Temperature excursion Decision Form

EU/mL	ELISA units/millilitre
GCP	Good Clinical Practice
GMT	Geometric Mean Titre
GSK	GlaxoSmithKline
GVCL	Global Vaccines Clinical Laboratories
H0	Null Hypothesis
Ha	Alternative Hypothesis
Hb	Haemoglobin
HbA1c	***Glycated haemoglobin***
HIV	Human Immunodeficiency Virus
IB	Investigator Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonisation
ICS	Intracellular Cytokine Staining
IEC	Independent Ethics Committee
IFN-γ	Interferon-gamma
IGRA	Interferon-Gamma Release Assay
IM	Intramuscularly
IMP(s)	Investigational Medicinal Product(s)
IDMC	Independent Data Monitoring Committee
IL-2	Interleukin-2
IL-13	Interleukin-13
IL-17	Interleukin-17
IPT	***Izoniazid Preventive Therapy*** **(Amended: 29 January 2014)**
IRB	Institutional Review Board

LMP	Last Menstrual Period
M72/AS01$_E$	*Mycobacterium tuberculosis* fusion protein M72 with GSK's proprietary adjuvant system containing MPL, QS21 with liposomes
MACDP	Metropolitan Atlanta Congenital Defects Program
MDR	Multi-drug resistant
MedDRA	Medical Dictionary for Regulatory Activities
MPL	3-O-desacyl-4'-MonophosPhoryl Lipid A
Mtb	*Mycobacterium tuberculosis*
PBMC	Peripheral Blood Mononuclear Cell
PI	Product Information
pIMD(s)	potential Immune-Mediated Disease(s)
PLT	Platelets
PPD	Purified Protein Derivative
PRE	Population Related Event
QFTG	QuantiFERON® TB Gold
QS21	A triterpene glycoside purified from the bark of the *Quillaja saponaria* tree
RR	Poisson Rate Ration
SAE	Serious Adverse Event
SAS	Statistical Analysis System
SBIR	Randomisation System on Internet
SDV	Source Document Verification
SMS	Short Message Service
SOC	***System Organ Class***
SPC	Summary of Product Characteristics
SPM	Study Procedures Manual

SRT	Safety Review Team
TB	Tuberculosis
TNF-α	Tumour Necrosis Factor-alpha
TVC	Total Vaccinated Cohort
VA	Verbal Autopsy
VE	Vaccine Efficacy
WBC	White Blood Cells
WHO	World Health Organisation
XDR	Extensively drug-resistant

(Amended: 29 January 2014)

GLOSSARY OF TERMS

Adequate contraception:

Adequate contraception is defined as a contraceptive method with failure rate of less than 1% per year when used consistently and correctly and when applicable, in accordance with the product label for example:

- abstinence from penile-vaginal intercourse, when this is their preferred and usual lifestyle,
- oral contraceptives, either combined or progestogen alone,
- injectable progestogen,
- implants of etenogestrel or levonorgestrel,
- estrogenic vaginal ring,
- percutaneous contraceptive patches,
- intrauterine device or intrauterine system,
- male partner sterilisation prior to the female subject's entry into the study, and this male is the sole partner for that subject,

  The information on the male sterility can come from the site personnel's review of the subject's medical records; or interview with the subject on her medical history.
- male condom combined with a vaginal spermicide (foam, gel, film, cream or suppository),
- male condom combined with a female diaphragm, either with or without a vaginal spermicide (foam, gel, film, cream, or suppository).

Adequate contraception does not apply to subjects of child bearing potential with same sex partners, when this is their preferred and usual lifestyle.

Adverse event:

Any untoward medical occurrence in a patient or clinical investigation subject, temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

An adverse event (AE) can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of a medicinal product. For marketed medicinal products, this also includes failure to produce expected benefits (i.e. lack of efficacy), abuse or misuse.

Blinding:	A procedure in which one or more parties to the trial are kept unaware of the treatment assignment in order to reduce the risk of biased study outcomes. The level of blinding is maintained throughout the conduct of the trial, and only when the data are cleaned to an acceptable level of quality will appropriate personnel be unblinded or when required in case of a serious adverse event. In a double blind study, the subject, the investigator and sponsor staff who are involved in the treatment or clinical evaluation of the subjects and the review or analysis of data are all unaware of the treatment assignment.
Clinical suspicion of pulmonary TB:	A subject has clinical suspicion of pulmonary TB when he/she presents with one or more of the following symptoms: unexplained cough > 2 weeks, unexplained fever > 1 week, night sweats, unintentional weight loss, pleuritic chest pains, haemoptysis, fatigue or shortness of breath on exertion.
Eligible:	Qualified for enrolment into the study based upon strict adherence to inclusion/exclusion criteria.
Epoch:	An epoch is a self-contained set of consecutive time points or a single time point from a single protocol. Self-contained means that data collected for all subjects at all time points within that epoch allows to draw a complete conclusion to define or precise the targeted label of the product. Typical examples of epochs are primary vaccinations, boosters, yearly immunogenicity follow-ups, and surveillance periods for efficacy or safety.
eTrack:	GSK's tracking tool for clinical trials.
Evaluable:	Meeting all eligibility criteria, complying with the procedures defined in the protocol, and, therefore, included in the according-to-protocol (ATP) analysis (see Sections 7.6.2 and 11.4 for details on criteria for evaluability).
QFTG **(Amended: 29 January 2014)**	The QFTG assay detects memory T-cell responses against Mtb as a surrogate for exposure to/infection with Mtb. The QFTG does not directly measure the presence of Mtb. The QFTG does not indicate whether the immune response of a subject ***was*** able to eliminate the bacteria or whether Mtb is latently present
Immunological correlate of protection:	The defined humoral antibody response above which there is a high likelihood of protection in the absence of any host factors that might increase susceptibility to the infectious agent.
Investigational vaccine/product:	A pharmaceutical form of an active ingredient or placebo being tested or used as a reference in a clinical trial,

(Synonym of Investigational Medicinal Product)	including a product with a marketing authorisation when used in a way different from the approved form, or when used for an unapproved indication, or when used to gain further information about an approved use.
Menarche:	Menarche is the onset of menses for the first time in a young female and is preceded by several changes associated with puberty including breast development and pubic hair growth. Menarche usually occurs within 1-2 years of breast development, thelarche. However, a young female can become pregnant before her first menses. Thus, a conservative definition of non-childbearing potential in a pre-menarcheal female is a young female who has not yet entered puberty as evidenced by lack of breast development (palpable glandular breast tissue).
Menopause:	Menopause is the age associated with complete cessation of menstrual cycles, menses and implies the loss of reproductive potential by ovarian failure. A practical definition accepts menopause after 1 year without menses with an appropriate clinical profile at the appropriate age e.g. > 45 years.
Potential Immune-Mediated Disease:	Potential immune-mediated diseases (pIMDs) are a subset of AEs that include autoimmune diseases and other inflammatory and/or neurologic disorders of interest which may or may not have an autoimmune aetiology.
Primary completion date:	The date that the final subject was examined or received an intervention for the purpose of final collection of data for the primary outcome, whether the clinical trial concluded according to the pre-specified protocol or was terminated.
Protocol amendment:	The International Conference on Harmonisation (ICH) defines a protocol amendment as: 'A written description of a change(s) to or formal clarification of a protocol.' GSK Biologicals further details this to include a change to an approved protocol that affects the safety of subjects, scope of the investigation, study design, or scientific integrity of the study.
Protocol administrative change:	A protocol administrative change addresses changes to only logistical or administrative aspects of the study. Note: Any change that falls under the definition of a protocol amendment (e.g. a change that affects the safety of subjects, scope of the investigation, study design, or scientific integrity of the study) MUST be prepared as an amendment to the protocol.
Randomisation:	Process of random attribution of treatment to subjects in order to reduce bias of selection.

Self-contained study:	Study with objectives not linked to the data of another study.
Site Monitor:	An individual assigned by the sponsor who is responsible for assuring proper conduct of clinical studies at one or more investigational sites.
Solicited adverse event:	AEs to be recorded as endpoints in the clinical study. The presence/occurrence/intensity of these events is actively solicited from the subject or an observer during a specified post-vaccination follow-up period.
Sub-cohort:	A group of subjects for whom specific study procedures are planned as compared to other subjects.
Subject:	Term used throughout the protocol to denote an individual who has been contacted in order to participate or participates in the clinical study, either as a recipient of the vaccine(s)/product(s) or as a control.
Subject number:	A unique number identifying a subject, assigned to each subject consenting to participate in the study.
"The Collaboration":	Refers to the joint collaboration between GSK Biologicals and Aeras. GSK will act as the sponsor of this study whereas Aeras will provide financial and operational support.
Treatment:	Term used throughout the clinical study to denote a set of investigational product(s) or marketed product(s) or placebo intended to be administered to a subject, identified by a unique number, according to the study randomisation or treatment allocation.
Treatment number:	A number identifying a treatment to a subject, according to the study randomisation or treatment allocation.
Unsolicited adverse event:	Any AE reported in addition to those solicited during the clinical study. Also any 'solicited' symptom with onset outside the specified period of follow-up for solicited symptoms will be reported as an unsolicited AE.
Xpert MTB/RIF:	The Xpert MTB/RIF is a Nucleic Acid Amplification Test to detect Mtb complex and resistance to rifampicin in sputum samples using the GeneXpert® technology from Cepheid.

TRADEMARKS

The following trademarks are used in the present protocol.

Note: In the body of the protocol (including the synopsis), the names of the vaccines will be written without the superscript symbol ™ or ® and in ***italics.***

Trademarks of the GlaxoSmithKline group of companies	Generic description
Havrix™	Hepatitis A vaccine
Boostrix™	Tetanus, Diphtheria, acellular Pertussis vaccine
Ditanrix™	Diphtheria and Tetanus vaccine
Cervarix™	Human Papillomavirus vaccine
Typherix™	Typhoid vaccine

1. INTRODUCTION

1.1. Background

TB is the second leading cause of mortality worldwide, leading to about ***1.3*** million deaths a year [WHO, ***2013***]. It is estimated that a third of the world's population is infected ***latently*** with the *Mycobacterium tuberculosis* (Mtb) bacilli. Although not more than ***5-20***% of infected individuals will become ill, the high number of infected people poses a great risk to individuals with reduced immunity who are at a higher risk for TB. The TB burden is compounded by the emergence of multi-drug resistant (MDR) and extensively-drug resistant (XDR) TB. Approximately ***8.6*** million incident cases of TB were reported in ***2012*** globally. Most TB cases were in Asia (mainly India and China) ***and*** Africa (***58***% ***and 27***% respectively). ***Asia refers to the WHO Regions of South-East Asia and the Western Pacific.*** Of these incident cases, an estimated 1.1 million (13%) were Human Immunodeficiency Virus (HIV) positive [WHO, ***2013***]. TB is the most common co-existing condition in people who die from Acquired Immune Deficiency Syndrome (AIDS). **(Amended: 29 January 2014)**

Prevention of TB disease is not only preferable to treatment of the disease for public health reasons, but an effective prophylactic TB vaccine would also lead to a considerable return in health benefits.

Bacille Calmette-Guerin (BCG), the only existing vaccine against TB, has been widely available for several decades. It is easy and cheap to produce, and when given to neonates or young children it is effective in preventing severe manifestations of disease such as meningeal TB and miliary TB. BCG appears to be less effective in preventing adult-onset TB. Since it became available, more than 3 billion people have received BCG. However, studies have shown that protection against adult pulmonary TB in vaccinated individuals varies between 0 and 80% [Fine, 1995]. Indeed, protection against TB by BCG wanes over time and there is no evidence that it provides protection more than 10 years after vaccination [Sterne, 1998]. Also, BCG is contraindicated for individuals with impaired immunity including symptomatic HIV-infection, where the need to prevent TB is high [WHO, 2004].

Currently, TB is typically treated with a combination of antibiotics taken daily for a long period of time (6-12 months). Compliance is often incomplete, resulting in an increasing incidence of MDR- and XDR-TB. The 'Directly Observed Treatment Strategy' (DOTS) programme in TB endemic countries is currently providing treatment based on supervised therapy.

Drug resistant TB requires a multi-drug combination that is based on drug sensitivity testing results. Injectable drugs may be required and long durations of treatment may also be needed.

Taken together, the development of an efficacious prophylactic vaccine that prevents TB disease and development of active disease in individuals latently infected with Mtb may be the key element for successful control of TB in the long-term.

GlaxoSmithKline (GSK) Biologicals is developing a vaccine to provide protective immunity against TB disease due to Mtb in children, adolescents and adults. The candidate vaccine selected for development is M72/AS01$_E$. M72/AS01$_E$ is a recombinant fusion protein M72, derived from two immunogenic Mtb antigens, adjuvanted with GSK's proprietary Adjuvant System AS01$_E$.

To date, M72/AS01$_E$ has been evaluated in a series of Phase II studies in Purified Protein Derivative (PPD)-negative and -positive adults in South Africa, PPD-positive adults (BCG vaccinated) in The Philippines, healthy BCG vaccinated adolescents in South Africa and HIV-positive adults on Anti Retroviral Therapy (ART) in Switzerland. The vaccine was shown to have a clinically acceptable safety profile and was highly immunogenic producing antigen specific humoral and cellular immune responses in these populations. ***In subjects with concurrent Pulmonary TB disease receiving treatment the vaccination seemed to induced a higher incidence of grade 3 injection site reactions; this observation is under futher investigation.*** **(Amended: 29 January 2014)**

Please refer to the current Investigator Brochure for information regarding the pre-clinical and clinical studies and the potential risks and benefits of M72/AS01$_E$.

1.2. Collaboration between GSK Biologicals and Aeras

This study is a joint collaboration between GSK Biologicals and Aeras, referred to as "The Collaboration" in the remainder of this document.

Established in 2003, Aeras is a non-profit biotechnology organization developing new generation tuberculosis vaccines and biologics with the aim of making products that are both efficacious and affordable in order to maximize public health impact. Aeras utilizes its broad capabilities and technologies in collaboration with a global consortium of partners, and has established expertise in vaccine development – from discovery and preclinical development through manufacturing, clinical testing and global regulatory affairs. Aeras' highly skilled staff consists of nearly 150 in its headquarters in Rockville, Maryland and an additional 16 staff in its office in Cape Town, South Africa.

For this study, GSK Biologicals and Aeras are both providing financial support. GSK Biologicals will act as the regulatory sponsor while Aeras personnel are providing operational support for this study on behalf of the sponsor.

1.3. Rationale for the study and study design

1.3.1. Rationale for the study

The burden of Mtb disease, particularly when compounded by HIV-infection and the emergence of MDR and XDR strains, is significant in TB endemic countries/regions. In 2010, approximately 40% of TB cases were reported in India and China, of which up to 80% were pulmonary in nature [WHO, ***2013***]. Africa accounted for a further 24% of cases (of which 25% were in South Africa), with most cases in young adults.

Prevention of TB disease is not only preferable to treatment of the disease for public health reasons, but an effective prophylactic TB vaccine would also lead to a

considerable return in health benefits. ***In TB endemic settings the incidence of clinical TB disease is highest in people who have been infected with Mtb as evidenced by a positive Interferon gamma release assay (IGRA). For the present first phase II proof-of-concept trial focussing on this IGRA+ adult population maximizes the trial efficiency by increasing the event rate in the control group.*** **(Amended: 29 January 2014)**

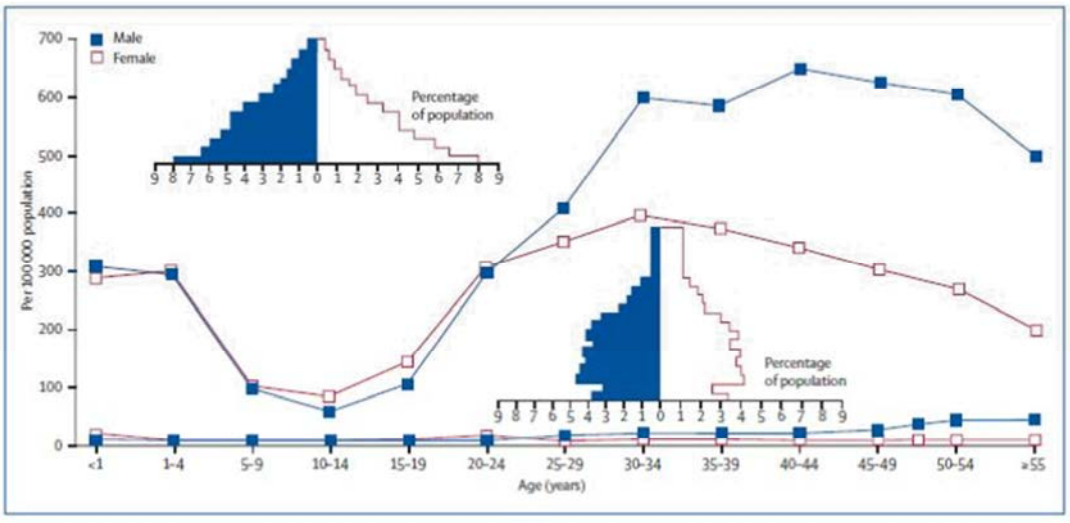
This Phase IIb study aims at collecting initial efficacy data and gathering further safety and immunogenicity data on the M72/AS01$_E$ vaccine candidate in ***IGRA+*** adults, ***healthy or with stable chonic medical condition(s),*** living in a TB endemic region. **(Amended: 29 January 2014)**

All study participants may be offered to consent to participate in a second and parallel study Sponsored by AERAS which aims to collect a supplemental set of pre- and post-vaccination blood samples to constitute a Biobank. After completion of this study this Biobank will serve for investigations into biological correlates of risk for TB disease and potentially correlates of protection. Please refer to this separate protocol for further information. **(Amended:29 January 2014)**

1.3.2. Rationale for the study design

This study is designed to evaluate the protective efficacy of the M72/AS01$_E$ candidate TB vaccine against definite pulmonary TB disease, versus placebo, following 2 injections given one month apart. The protective efficacy will be evaluated in ***IGRA+*** adults living in a TB endemic region aged 18-50 years, i.e. in an age range where the incidence of TB is high (Figure 1). **(Amended: 29 January 2014)**

Figure 1 Age-related and sex-related incidence of tuberculosis



Age- and gender- related incidence of TB in a hypothetical high tuberculosis incidence community with a large number of children and a low TB incidence community with a relatively small number of children [Donald, 2004].

This study is an international, multicentre, phase IIb, double-blind, randomized, controlled trial. There will be follow-up for efficacy for approximately 3 years after vaccination via regular visits or contacts to screen for possible TB. Subjects will also be asked to recognise signs and symptoms compatible with TB disease and to self-report for

clinical evaluation. Subjects with clinical suspicion of pulmonary TB will be assessed with confirmatory diagnostic testing using a Nucleic Acid Amplification Test (Xpert MTB/RIF assay, Cepheid) and microbiological culture. Subjects diagnosed with TB will be referred for TB treatment according to local clinical practice.

The primary objective of this study is to evaluate the protective efficacy of two doses of the M72/AS01$_E$ candidate vaccine against definite pulmonary TB disease **not associated with HIV-infection**, as compared to placebo. To ***avoid the potential confounding effect of*** HIV ***infection*** in the study population at baseline, HIV testing will be ***required to be negative*** at screening. Additionally, when a subject is diagnosed with TB, an HIV test (and pre-test counselling) will be performed to rule out HIV co-infection. When a subject is diagnosed with HIV at the time of TB diagnosis, additional tests will be performed to measure CD4 counts to assess the severity of the infection and decide on the appropriate treatment regimen. HIV-positive subjects will be referred for HIV treatment according to local clinical practice. **(Amended: 29 January 2014)**

Participating sites will use standardised case definitions for efficacy endpoints and a structured approach to case-assessment as outlined in this study protocol will be used.

An Independent Data Monitoring Committee (IDMC) ***has been*** constituted for this study to perform periodic safety reviews of the safety data. **(Amended: 29 January 2014)**

A sub-cohort of subjects will be selected for specific follow-up for safety (solicited adverse events (AE) and selected Biochemistry and Complete Blood Count [CBC]) and immunogenicity. Unsolicited AEs ***and large swelling reactions,*** serious adverse events (SAEs), potential immune-mediated diseases (pIMDs), AEs leading to withdrawal and pregnancies will be recorded for all subjects. **(Amended: 29 January 2014)**

1.3.3. Rationale for the use of placebo

There is currently no established TB vaccine with recognised efficacy in the age range considered for this study. The use of a control group is mandatory for meeting the primary objective, i.e. evaluating the protective efficacy of the M72/AS01$_E$ vaccine candidate against definite pulmonary TB disease not associated with HIV-infection.

The use of an active vaccine as control has been evaluated but it introduces significant unblinding risks as the presentation, schedule or indications are different for vaccines that could have been considered such as:

- *Havrix:* 2 doses needed with a minimal 6 months interval, different appearance, and different volume per dose (1 mL)
- Hepatitis B: 3-dose primary schedule needed, different appearance; 1-dose booster (not registered) required
- *Boostrix*: 1-dose booster schedule, different appearance
- *Ditanrix*: 3-dose primary schedule needed or 1-dose for booster, different appearance
- *Cervarix*: only indicated in females, different appearance
- Influenza: not recommended year round, 1-dose schedule, different appearance

- *Typherix*: 1-dose schedule, different appearance

When using these vaccines as active control, an additional placebo (double-dummy placebo) would be required to match the dosing schedules of the investigational vaccine and comparator vaccine, in order to maintain the blinding. Therefore, a placebo will be used as control.

Use of the placebo control and the double-blind, randomised study design, will allow to control for potential biases in the conduct of the study.

2. OBJECTIVES

2.1. Primary objective

- To evaluate the protective efficacy of two doses of the M72/AS01$_E$ candidate vaccine against **Definite pulmonary TB disease** not associated with HIV-infection, meeting the first case definition, as compared to placebo.

  Success criterion to be used for the primary objective:

  *The lower limit of the **90**% two-sided confidence interval (CI) for the VE (using a Cox regression model) against first occurrence of Definite pulmonary TB disease not associated with HIV-infection, meeting the first case definition, is above 0%.* **(Amended: 29 January 2014)**

Refer to Section 4 for case definitions and Section 11.1 for the definition of the primary endpoint.

2.2. Secondary objectives

Efficacy:

- To evaluate the protective efficacy of two doses of the M72/AS01$_E$ candidate vaccine against **Definite Xpert MTB/Rif positive pulmonary TB disease** not associated with HIV-infection, meeting the second case definition, as compared to placebo.

  If the primary objective is met, this secondary objective will be analysed using the following success criterion:

  *The lower limit of the **90**% two-sided CI for the VE (using a Cox regression model) against first occurrence of Definite pulmonary TB disease not associated with HIV-infection, meeting the **second** case definition, is above 0%.* **(Amended: 29 January 2014)**

- To evaluate the protective efficacy of two doses of the M72/AS01$_E$ candidate vaccine against **Definite pulmonary TB disease** not associated with HIV-infection, meeting the third case definition, as compared to placebo.

- To evaluate the protective efficacy of two doses of the M72/AS01$_E$ candidate vaccine against ***Microbiological*** **pulmonary TB disease** meeting the fourth case definition, as compared to placebo.

- To evaluate the protective efficacy of two doses of the M72/AS01$_E$ candidate vaccine against **Clinical TB disease** meeting the fifth case definition, as compared to placebo.

Safety:

- To assess the safety and reactogenicity of two doses of the M72/AS01$_E$ candidate vaccine.

Immunogenicity:

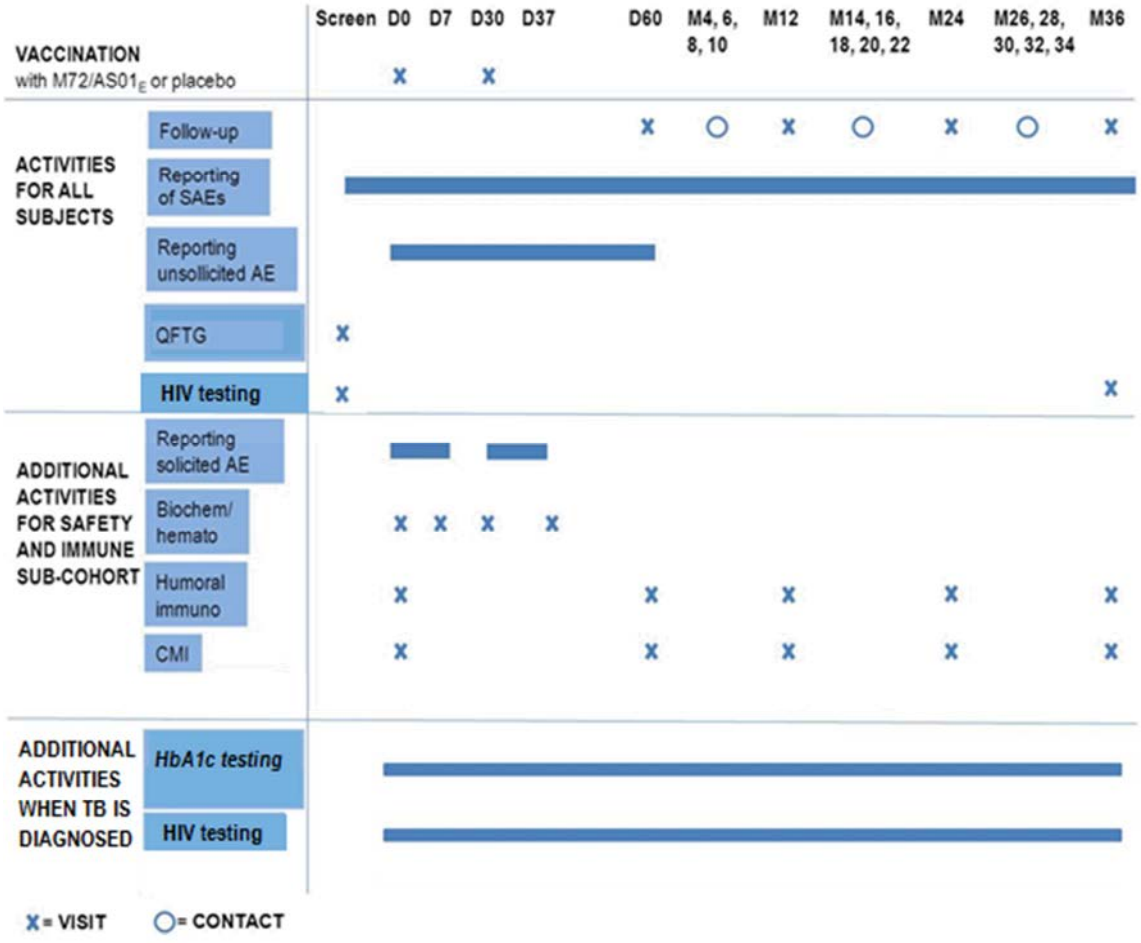
- To assess the immunogenicity of two doses of the M72/AS01$_E$ candidate vaccine.

Refer to Section 4 for case definitions and Section 11.2 for the definition of the secondary endpoints.

(Amended: 29 January 2014)

3. STUDY DESIGN OVERVIEW

Figure 2 Overview of the study design (Amended: 29 January 2014)



Protocol waivers or exemptions are not allowed with the exception of immediate safety concerns. Therefore, adherence to the study design requirements, including those specified in the outline of study procedures (Section 6.5), are essential and required for study conduct.

- **Experimental design:** Phase IIb, randomised, double-blind, controlled, multi-centre, efficacy study with two parallel groups.

- **Duration of the study:** the duration of the study will be approximately 37 months for each subject from screening up to end of efficacy follow-up.

  – Epoch 001: Primary starting visit 1 (screening visit) and ending visit 10 (Month 36).

- **Study groups:** Study groups and treatments foreseen in the study are presented in Table 1 and Table 2.

Table 1 Study groups and epochs foreseen in the study (Amended: 29 January 2014)

Study Groups	Number of subjects	Age (Min - Max) (age unit)	Epoch 001
M72AS01	***1753***	18 years - 50 years	x
Control	***1753***	18 years - 50 years	x

Table 2 Study groups and treatment foreseen in the study

Treatment name	Vaccine/Product name	Study Groups	
		M72AS01	Control
M72/AS01$_E$	M72	X	
	AS01$_E$	X	
Placebo	Placebo pellet		X
	Placebo adjuvant		X

- **Control:** placebo control.
- **Vaccination schedule:** Subjects will receive 2 doses of M72/AS01$_E$ or placebo, according to their random assignment, one month apart (Day 0 and Day 30) by intramuscular injection in the deltoid region of the arm.
- **Treatment allocation:** Randomised 1:1. Randomisation will occur at the day of first vaccination (Day 0). Refer to Section 6.2 for a detailed description of the randomisation method.
- **Blinding:** Double-blind (Table 3). Refer to Section 6.3 for method of blinding.

Table 3 Blinding of study epochs

Study Epochs	Blinding
Epoch 001	double-blind

- **Sampling schedule (Amended: 29 January 2014):**
  - Blood samples will be collected in the safety and immune sub-cohort for evaluation of biochemistry/haematology parameters (Days 0, 7, 30 and 37), CMI responses (Days 0, 60 and Years 1, 2 and 3) and humoral immune responses (Days 0, 60 and Years 1, 2 and 3). ***Vitamin D levels will be measured at Day 0.***
  - Blood samples will be collected for QFTG testing at ***screening*** for all subjects.
  - ***A respiratory sputum sample will be collected prior to vaccination in all subjects for nucleic acid amplification test to rule out pulmonary TB.***
  - ***Blood samples will be collected for initial HIV testing at screening for all subjects. Repeat HIV testing will be performed at the last study visit V9, unless the subject has been confirmed HIV positive before V9.***
  - Whenever a subject is diagnosed with TB disease, blood samples will be collected for HIV testing ***and HbA1c measurement***. In addition, if a subject is HIV-positive, blood samples will be collected to determine CD4 cell counts.

- **Type of study:** self-contained.

- **Data collection:** electronic Case Report Form (eCRF – ***Inform*™**). **(Amended: 29 January 2014)**

- **Safety monitoring:** an IDMC, operating under a charter, ***has been*** constituted for this study to oversee the study. They will perform safety reviews twice a year, or more frequently if deemed necessary. **(Amended: 29 January 2014)**

- **Surveillance for efficacy:** efficacy follow-up will be both active and passive (via self-reporting). Refer to Section 6.4.1 for a detailed description of the methods for surveillance for efficacy.

- **Diagnostic procedures for suspected Pulmonary TB:** during the entire study ***(not for screening)***, when a subject presents with clinical suspicion of pulmonary TB, three respiratory sputum samples, preferably taken in the morning, within ***maximum*** a one week interval, must be collected for testing with Xpert MTB/RIF and microbiological culture. **(Amended: 29 January 2014)**

  Refer to Section 6.4.2 for a detailed description of the diagnostic procedures for suspected pulmonary TB.

4. CASE DEFINITIONS

First case definition – Definite pulmonary TB, not associated with HIV-infection

- A subject with clinical suspicion* of pulmonary TB disease, with Mtb complex identified from a sputum specimen, taken before initiation of TB treatment, by Xpert MTB/RIF and/or microbiological culture and confirmed HIV-negative at the time of TB diagnosis.

Second case definition –Definite Xpert MTB/Rif positive pulmonary TB, not associated with HIV-infection

- A subject with clinical suspicion* of pulmonary TB disease, with Mtb complex identified from a sputum specimen, taken before initiation of TB treatment, by Xpert MTB/RIF and confirmed HIV-negative at the time of TB diagnosis**.**

Third case definition – Definite pulmonary TB, not associated with HIV-infection

- A subject with clinical suspicion* of pulmonary TB disease, with Mtb complex identified from a sputum specimen, taken up to four weeks after initiation of TB treatment, by Xpert MTB/RIF and/or microbiological culture and confirmed HIV-negative at the time of TB diagnosis.

Fourth case definition – Microbiological pulmonary TB

- A subject with clinical suspicion* of pulmonary TB disease, with Mtb complex identified from a sputum specimen, taken up to four weeks after initiation of TB treatment, by Xpert MTB/RIF and/or microbiological culture.

*Refer to the GLOSSARY OF TERMS for a definition of clinical suspicion of pulmonary TB.

Fifth case definition – Clinical TB

- A subject for whom a clinician has diagnosed TB disease and has decided to treat the patient with TB treatment.

5. STUDY COHORT

5.1. Number of subjects/centres

Approximately ***3506*** men and women aged 18-50 years will be enrolled in the study. Enrolment will be terminated when target number of subjects is reached. **(Amended: 29 January 2014)**

The study will be conducted in multiple TB endemic countries, in multiple enrolling centres. The study centres will be experienced in detecting TB and have access to TB treatment or have an established referral option for TB patients and HIV-infected individuals.

There will be one sub-cohort for safety/reactogenicity, and immunogenicity. Approximately 450 eligible subjects from selected centres in multiple countries (2 or 3) will be included in this safety and immune sub-cohort (Table 4). Up to 150 enrolled in each contributing country (if 3 contributing countries) or up to 225 subjects enrolled in each contributing country (if 2 contributing countries) may be included in the safety and immune sub-cohort.

To ensure the gender of the study population is equally distributed between females and males, a cap has been put on the number of subjects per gender to be enrolled. A maximum of 60% of subjects from the same gender will be enrolled in the study and enrolment of females/males will be stopped at approximately ***2100*** subjects of any gender maximum. **(Amended: 29 January 2014)**

Refer to section 11.3 for a detailed description of the criteria used in the determination of sample size.

Table 4 Sub-cohorts

Sub-cohort name	Description of additional activities	Estimated number of subjects
Safety and immune sub-cohort	Safety parameters for this sub-cohort will be collected as follows: • Solicited AEs will be collected for 7 days (day of vaccination and 6 subsequent days) following vaccination. • Serum biochemistry/haematology values will be measured at Days 0, 7, 30, and 37. Immunogenicity: • Humoral immunogenicity will be evaluated by measuring M72-specific IgGs at Days 0 and 60 and Years 1, 2 and 3. • CMI will be evaluated by measuring M72-specific CD4+ and CD8+ T cells expressing at least 2 immune markers (CD40-L and/or IFN-γ and/or IL-2 and/or TNF-) at Days 0 and 60 and Years 1, 2 and 3 and any combination among CD40-L, IFN-g, IL-2 and TNF-a. (Amended: 29 January 2014)	450

5.2. Inclusion criteria for enrolment

Deviations from inclusion criteria are not allowed because they can potentially jeopardize the scientific integrity of the study, regulatory acceptability or subject safety. Therefore, adherence to the criteria as specified in the protocol is essential.

All subjects must satisfy ALL the following criteria at study entry:

- Subjects who, in the opinion of the investigator, can and will comply with the requirements of the protocol (e.g. completion of the diary cards, return for follow-up visits).
- A male or female between, and including, 18 and 50 years of age at the time of obtaining informed consent.
- Written (or thumb printed and witnessed) informed consent obtained from the subject.
- ***Baseline positive IGRA test result (Amended: 29 January 2014)***
- ***Baseline negative HIV screen. (Amended: 29 January 2014)***
- ***Baseline negative clinical screening questionare and negative sputum sample for Pulmonary TB disease. (Amended: 29 January 2014)***
- Healthy subjects or those with chronic well-controlled disease as established by medical history and clinical examination.
- Female subjects of non-childbearing potential may be enrolled in the study.
  - Non-childbearing potential is defined as pre-menarche, current tubal ligation, hysterectomy, ovariectomy or post-menopause *
- Female subjects of childbearing potential may be enrolled in the study, if the subject:
  - has practiced adequate contraception* for ***25*** days prior to vaccination, and
  - has a negative pregnancy test on the day of screening and the day of first vaccination, and
  - has agreed to continue adequate contraception during the entire ***vaccination*** period and for 2 months after completion of the vaccination series.

  (Amended: 29 January 2014)

*Please refer to the glossary of terms for the definitions of menarche, menopause and adequate contraception.

5.3. Exclusion criteria for enrolment

Deviations from exclusion criteria are not allowed because they can potentially jeopardize the scientific integrity of the study, regulatory acceptability or subject safety. Therefore, adherence to the criteria as specified in the protocol is essential.

The following criteria should be checked at the time of study entry. If ANY exclusion criterion applies, the subject must not be included in the study:

- Current TB disease or ***history of*** TB ***disease and/or*** treatment ***for TB (including IPT)***. **(Amended: 29 January 2014)**
- Use of any investigational or non-registered product (drug or vaccine) other than the study vaccines within 30 days preceding the first dose of study vaccine, or planned use during the study period.
- Planned administration/administration of a vaccine not foreseen by the study protocol in the period starting 30 days before and ending 30 days after each dose of vaccine.
- History of previous administration of experimental Mtb vaccines.
- Chronic administration (defined as more than 14 days in total) of immunosuppressants or other immune-modifying drugs within six months prior to the first vaccine dose. (For corticosteroids, this will mean prednisone ≥ 20 mg/day or equivalent.). Inhaled and topical steroids are allowed.
- Any condition or illness (acute, chronic or history) or medication, which in the opinion of the Investigator might interfere with the evaluation of the safety or immunogenicity of the vaccine.
- Any confirmed or suspected immunosuppressive or immunodeficient condition, based on medical history and physical examination (no laboratory testing required).
- Planned participation or participation in another experimental protocol during the study.
- Concurrently participating in another clinical study, at any time during the study period, in which the subject has been or will be exposed to an investigational or a non-investigational product (pharmaceutical product or device).
- Administration of immunoglobulins and/or any blood products within the 3 months preceding the first dose of study vaccine or planned administration during the study period.
- History of any reaction or hypersensitivity likely to be exacerbated by any component of the vaccines.
- History of medically confirmed autoimmune disease ***(e.g. Type I diabetes, Lupus)***. **(Amended: 29 January 2014)**
- Pregnant or lactating female.
- Female planning to become pregnant or planning to discontinue contraceptive precautions during the vaccination period and/or before 2 months after completion of the vaccination series.

6. CONDUCT OF THE STUDY

6.1. Regulatory and ethical considerations, including the informed consent process

The study will be conducted in accordance with all applicable regulatory requirements.

The study will be conducted in accordance with the ICH Guideline for Good Clinical Practice (GCP), all applicable subject privacy requirements and the guiding principles of the Declaration of Helsinki.

GSK will obtain favourable opinion/approval to conduct the study from the appropriate regulatory agency, in accordance with applicable regulatory requirements, prior to a site initiating the study in that country.

Conduct of the study includes, but is not limited to, the following:

- Institutional Review Board (IRB)/Independent Ethics Committee (IEC) review and favourable opinion/approval of study protocol and any subsequent amendments.
- Subject informed consent.
- Investigator reporting requirements as stated in the protocol.

GSK will provide full details of the above procedures to the investigator, either verbally, in writing, or both.

Freely given and written (or thumb printed and witnessed) informed consent must be obtained from each subject, as appropriate, prior to participation in the study.

GSK Biologicals will prepare a model Informed Consent Form (ICF) which will embody the ICH GCP and GSK Biologicals required elements. While it is strongly recommended that this model ICF is to be followed as closely as possible, the informed consent requirements given in this document are not intended to pre-empt any local regulations which require additional information to be disclosed for informed consent to be legally effective. Clinical judgement, local regulations and requirements should guide the final structure and content of the local version of the ICF.

The investigator has the final responsibility for the final presentation of the ICF, respecting the mandatory requirements of local regulations. The ICF generated by the investigator with the assistance of the sponsor's representative must be acceptable to GSK Biologicals and be approved (along with the protocol, and any other necessary documentation) by the IRB/IEC.

6.2. Subject identification and randomisation of treatment

6.2.1. Subject identification

Subject identification numbers will be assigned sequentially to the subjects who have consented to participate in the study, according to the range of subject identification numbers allocated to each study centre.

6.2.2. Randomisation of treatment

6.2.2.1. Randomisation of supplies

The randomisation of supplies within blocks will be performed at GSK Biologicals, using MATerial EXcellence (MATEX), a program developed for use in Statistical Analysis System (SAS®) (Cary, NC, USA) by GSK Biologicals. Entire blocks will be shipped to the study centres /warehouse(s).

To allow "The Collaboration" to take advantage of greater rates of recruitment than anticipated at individual centres in this multi-centre study and to thus reduce the overall study recruitment period, an over-randomisation of supplies will be prepared.

6.2.2.2. Treatment allocation to the subject

6.2.2.2.1. Study group and treatment number allocation

The target will be to enrol approximately ***3506*** eligible subjects who will be randomly assigned to two study groups in a 1:1 ratio (approximately ***1753*** subjects in each group). **(Amended: 29 January 2014)**

Allocation of the subject to a study group at the investigator site will be performed using a***n internet based*** randomisation system (SBIR). The randomisation algorithm will use a minimisation procedure accounting for:

- ***Centre*** **(Amended: 29 January 2014)**
- Gender: a maximum of ***2100*** females/males will be recruited. **(Amended: 29 January 2014)**

Minimisation factors will have equal weight in the minimisation algorithm.

The treatment numbers will be allocated by dose at the site of the investigator using SBIR.

After obtaining the ***informed consent*** from the subject and having checked the eligibility of the subject, the site staff in charge of the vaccine administration will access SBIR. Upon providing the subject identification number, the randomisation system will determine the study group and will provide the treatment number to be used for the first dose. The number of each administered treatment must be recorded in the eCRFon the Vaccine Administration screen. **(Amended: 29 January 2014)**

When SBIR is not available, please refer to the SBIR user guide or the Study Procedures Manual (SPM) for specific instructions.

6.2.2.2.2. Treatment number allocation for subsequent doses

For each dose subsequent to the first dose, the study staff in charge of the vaccine administration will access SBIR, provide the subject identification number, and the system will provide a treatment number consistent with the allocated study group.

The number of each administered treatment must be recorded in the eCRF on the Vaccine Administration screen.

6.2.3. Allocation of subjects to assay subsets

Approximately ***450*** subjects will be included in the safety and immune sub-cohort. These subjects will be enrolled at selected centres. Blood sampling and safety assessment will be performed as described in Table 4. ***Distribution of subjects in the sub-cohort is presented in Table 5***. **(Amended: 29 January 2014)**

Table 5 Distribution of subjects in the sub-cohort

Sub-cohort	Treatment group	N to be enrolled
Safety and immune sub-cohort	M72AS01	Approximately 225
	Control	Approximately 225

6.3. Method of blinding

The study will be conducted using double-blind procedures. The vaccine recipient, investigator and any person involved in the clinical conduct of the study will be unaware of which vaccine was administered.

The laboratory in charge of the laboratory testing will be blinded to the treatment, and codes will be used to link the subject and study (without any link to the treatment attributed to the subject) to each sample.

6.4. General study aspects

Supplementary study conduct information not mandated to be present in this protocol is provided in the accompanying SPM. The SPM provides the investigator and the site personnel with administrative and detailed technical ***and operational*** information that does not impact the safety of the subjects. **(Amended: 29 January 2014)**

6.4.1. Surveillance for safety and efficacy

Surveillance for efficacy starts with administration of the first dose of study vaccine or placebo.

6.4.1.1. Active follow-up for safety and efficacy

Subject safety follow-up and timely case capture of incident TB disease is of paramount importance. In addition to study visits at the study facilities, regular contacts (every two

months) with the study subjects must be maintained using one or more of the following methods:

- Regular interval home visits by site staff.
- Phone calls to inquire about current health status, completed by home visits if the subject cannot be reached.
- ***1-way short message service (sms) reminders and/or*** 2-way ***sms*** exchange. **(Amended: 29 January 2014)**

These active follow-up procedures for surveillance of safety and efficacy will be described in local study Standard Operating Procedures and ICFs. ICFs will be submitted to the Ethics Committee for approval prior to implementation.

During study visits and contacts, subjects will be asked if they have signs or symptoms of pulmonary TB. Based on clinical suspicion of TB, and guided by the World Health Organisation (WHO) signs and symptoms algorithm [WHO, 2009], they will be required to provide three sputum samples, preferably taken in the morning and within one week, to be tested for Mtb by Xpert MTB/RIF and microbiological culture.

6.4.1.2. Passive follow-up for efficacy

Subjects will be informed about signs and symptoms compatible with TB at the time of Informed Consent and at each visit/contact. At any time during the study, if a subject suspects that he/she has signs and symptoms of TB, he/she will be required to self-report to the study centre for clinical evaluation (passive follow-up) and if needed confirmatory testing using Xpert MTB/RIF and microbiological culture will be done.

6.4.2. Diagnostic procedures for the detection of suspected pulmonary TB

During the entire study period, when a subject presents with clinical suspicion of pulmonary TB, three respiratory sputum samples, preferably taken in the morning, must be collected, within a one week interval, for testing with Xpert MTB/RIF and microbiological culture. If results are negative but clinical suspicion of TB disease persists, the subject will be cared for according to local clinical practice (e.g. treated with non anti-tuberculosis antibiotics) and will be followed up approximately two weeks later. If clinical suspicion persists, three additional sputum samples, preferably taken in the morning, within a one week interval, will be collected for Xpert MTB/RIF testing and microbiological culture. If TB disease cannot be confirmed with Xpert MTB/RIF and/or microbiological culture, the subject may be given TB treatment based on other diagnostic tests (e.g. smear microscopy) and the judgement of the physician providing care.

Weight of the subject must also be recorded in the subjects' eCRF at the time of TB diagnosis.

Protocol samples for diagnostic testing with Xpert MTB/Rif and/or microbiological culture should preferably be taken before initiation of TB treatment. However, samples for diagnostic testing with Xpert MTB/Rif and/or microbiological culture may be

collected up to four weeks after initiation of TB treatment. Definite Pulmonary TB cases identified from sputum samples taken after initiation of TB treatment will not be included in the primary endpoint.

Testing results per protocol procedures will be communicated expeditiously to the investigator but their availability should not delay any diagnostic or therapeutic intervention as per local routine practice.

Additionally if TB is confirmed a serological test (and pre-test counselling) for HIV-infection must be performed to rule out HIV co-infection. If the subject is HIV-positive, additional testing must be performed to measure CD4 cell counts. ***Diabetes will be screened for by a Hb Ac1 test.*** **(Amended: 29 January 2014)**

Results of HIV testing, including CD4 cell counts if applicable must be recorded in the subjects' eCRF. **(Amended: 29 January 2014)**

6.4.3. Diagnostic procedures for suspected extra-pulmonary TB

During the entire study period, when a subject presents with clinical suspicion of extra-pulmonary TB, a diagnostic procedure must be performed according to local clinical practice and as a minimum, the most recent WHO recommendations in the "International Standards for TB care" should be followed [WHO, 2006].

The diagnostic procedures performed and their results must be recorded in the subjects' eCRF along with the final diagnosis of the attending physician.

Additionally, if extra-pulmonary TB is confirmed, a serological test (and pre-test ***& post-test*** counselling) for HIV-infection must be performed to rule out HIV co-infection must be taken. If the subject is HIV-positive, additional testing must be performed to measure CD4 cell counts. ***Diabetes will be screened for by a Hb Ac1 test.*** **(Amended: 29 January 2014)**

Results of HIV testing, including CD4 cell counts, if applicable must be recorded in the subjects' eCRF. **(Amended: 29 January 2014)**

6.4.4. Procedures for fatalities

In order to diagnose the cause of death of fatalities occurring during this study, it is recommended that a verbal autopsy (VA) will be performed by the study staff for all fatalities, using a standardised VA questionnaire and cause of death qualification [WHO, 2007].

Verbal Autopsy reports that are shared with ***the collaboration or any vendors acting on behalf of the collaboration must*** be anonymised, i.e. all personally identifiable information should be removed. ***In the event that this occurs a GSK data privacy breach form will need to be completed.*** **(Amended: 29 January 2014)**

6.4.5. Safety Monitoring Plan

6.4.5.1. Safety Review Team

The Safety Review Team (SRT) includes as core members the GSK Biologicals' Central Safety Physician, the Clinical Development Manager, Epidemiologist, Clinical Regulatory Affairs representative and the Biostatistician of the project. The SRT will be responsible for ongoing safety monitoring of the study and will meet on a regular basis. In order to keep all people involved in the conduct, cleaning and final analysis of the study blinded, the SRT will monitor safety in a blinded manner.

6.4.5.2. Independent Data Monitoring Committee

In order to ensure the safety of the subjects during the entire study period, an Independent Data Monitoring Committee (IDMC) ***has been*** appointed to monitor the safety and tolerability of the vaccine/placebo administered in the trial and, if necessary, make recommendations to the sponsor concerning the modification or termination of the trial. The IDMC will perform safety reviews twice a year or more frequently if deemed necessary on an ongoing basis during the study period. **(Amended: 29 January 2014)**

An independent statistical team (i.e., comprising members who are neither GSK employees neither Aeras employees and who are not involved in the study management) will be appointed by GSK Biologicals. This team will be unblinded to treatment assignment and provide all necessary tables, listings, figures and individual subject data to the IDMC.

The IDMC consist***s*** of clinical experts who are not involved in the conduct of the study and an independent statistician. The role of the IDMC will be to review the progress of the trial and safety assessments to detect evidence of safety issues for the subjects from the time of first vaccination and throughout the duration of the trial. A report, with recommendations as necessary, will be provided to the sponsor following each IDMC meeting. The frequency of IDMC sessions and other operational details are described in the IDMC charter. In case of a serious safety issue during the study, the sponsor will inform the IDMC as well as fulfil its regulatory obligation expeditiously. **(Amended: 29 January 2014)**

If during the course of enrolment and vaccination two or more related grade 2 or 3 respiratory AE are observed an ad hoc IDMC will be convened. **(Amended: 29 January 2014)**

6.4.5.3. Planned safety review (Amended: 29 January 2014)

A planned safety review after the first 100 subjects have been enrolled and vaccinated will be performed. The SRT will review blinded safety summaries and the IDMC will review unblinded safety summaries.

6.4.5.4. Rules for vaccination (Amended: 29 January 2014)

At individual subject level

- ***No second dose should be administered in a given subject who experiences Grade 3 redness and swelling, i.e. ≥100mm, post dose 1 or Grade 2 or 3 respiratory AE post dose 1.***
- ***No second dose should be administered in a given subject who develops TB disease after dose 1.***

At study level

- ***An ad hoc IDMC review will be called if 2 or more subjects experience related Grade 2 or higher respiratory AEs within 90 days after any dose.***
- ***Further enrolment and vaccination will be suspended if within 90 days after any study vaccine dose any of the following events are observed:***
  - ***Related respiratory AE with Fatal outcome***
  - ***Two or more subjects experiencing Grade 3 related Respiratory AE***
- ***An ad hoc IDMC meeting will be called urgently for review. Vaccination may only be allowed to resume pending final VSMB approval.***

Table 6 Safety holding rules for vaccination (Amended: 29 January 2014)

Event	*Grade*	*Occurrences*	*Action for subject(s)*	*Trial actions*
Redness/Swelling at injection site (within 30 days after dose 1)	*Grade 3 (>100mm)*	*any subject(s)*	*No dose 2 in same subject(s), keep in efficacy follow-up*	*-*
TB disease (after dose 1)	*n/a*	*Any subject(s)*	*No dose 2 in same subject(s) , keep in efficacy follow-up*	*-*
Respiratory SOC AE (within 90 days after dose 1)	*Grade ≥2*	*1 subject*	*No dose 2 in same subject(s), but keep in efficacy follow-up*	*-*
Respiratory SOC AE (within 90 days after dose 1)	*Grade ≥2 related*	*≥2 subjects*	*Clinical assessment & further investigation at investigator discretion*	*Ad hoc IDMC review*
Respiratory SOC SAE	*Related & Fatal*	*≥1 subject*	*-*	*Study Hold,*
Respiratory SOC AE (within 90 days after dose 1)	*Grade ≥3, related*	*≥2 subjects*	*No dose 2 in same subject(s)* *Clinical assessment & further investigation at investigator discretion*	*IMDC Review,* *VSMB*

6.5. Outline of study procedures

Table 7 List of study procedures (Amended: 29 January 2014)

	Visit 1 Screening visit	Visit 2	Visit 3[1]	Visit 4	Visit 5[1]	Visit 6	Contact 1, 2, 3 and 4	Visit 7	Contact 5, 6, 7, 8, 9	Visit *8*	Contact 10, 11, 12, 13, 14	Visit 9
Time point(s)	**D-30 *to D0***	**D 0**	**D 7**	**D 30**	**D 37**	**M 2**	**M 4, 6, 8, 10**	**M 12**	**M 14, 16, 18, 20, 22**	**M 24**	**M 26, 28, 30, 32, 34**	**M 36**
Sampling Time point(s)	**Pre V**	**Pre V**	**Post V1**	**Post V1**	**Post V2**	**Post V2**	**Post V2**	**Post V2**	**Post V2**	**Post V2**	**Post V2**	**Post V2**
Dose		**1**		**2**								
All subjects												
Informed consent	●											
Check inclusion/exclusion criteria	●	●										
Collect demographic data	●											
Medical history	●	●										
Physical examination		●										
History-directed physical examination		O	O	O	O	O						
Resting vital signs		O		O								
HIV testing[2] (up to ~ 2.5 mL/test)	●											●
Record weight[3]		●						●		●		●
Documentation of history of BCG vaccination/presence of scar	●										Visit 1	
Documentation of history of TB household contacts	●											
Pregnancy test (β-HCG urine)	●	●		●								
Study group and treatment number allocation ***for first dose***		O										
Treatment number allocation for second dose				O								

	Visit 1 Screening visit	Visit 2	Visit 3[1]	Visit 4	Visit 5[1]	Visit 6	Contact 1, 2, 3 and 4	Visit 7	Contact 5, 6, 7, 8, 9	Visit *8*	Contact 10, 11, 12, 13, 14	Visit 9
Time point(s)	**D-30 *to D0***	**D 0**	**D 7**	**D 30**	**D 37**	**M 2**	**M 4, 6, 8, 10**	**M 12**	**M 14, 16, 18, 20, 22**	**M 24**	**M 26, 28, 30, 32, 34**	**M 36**
Sampling Time point(s)	**Pre V**	**Pre V**	**Post V1**	**Post V1**	**Post V2**	**Post V2**	**Post V2**	**Post V2**	**Post V2**	**Post V2**	**Post V2**	**Post V2**
Dose		**1**		**2**								
Vaccination		●		●								
Recording of administered treatment number		●		●								
Check contraindications to subsequent vaccination		●		●								
Recording concomitant medication/vaccination		●	●	●	●	●	●	●	●	●	●	●
Recording intercurrent medical condition		●	●	●	●	●	●	●	●	●	●	●
Distribution of diary cards		O		O								
Return of diary cards				O		O						
Recording of unsolicited AEs ***and large swelling reactions***		●	●	●	●	●						
Pre-vaccination assessment (including temperature)		●		●								
Blood sampling for QFTG (~ 3 mL)	●											
Sputum collection for Xpert testing	●											
Reporting SAEs	●[4]	●	●	●	●	●	●[5]	●[4]	●[4]	●[4]	●[4]	●[4]
Reporting pIMDs		●	●	●	●	●	●[6]					
Reporting pregnancy		●	●	●	●	●	●[6]					
Diagnostic procedures for suspected pulmonary TB[7]			●	●	●	●	●	●	●	●	●	●
Documentation of TB cases[8]			●	●	●	●	●	●	●	●	●	●

	Visit 1 Screening visit	Visit 2	Visit 3[1]	Visit 4	Visit 5[1]	Visit 6	Contact 1, 2, 3 and 4	Visit 7	Contact 5, 6, 7, 8, 9	Visit *8*	Contact 10, 11, 12, 13, 14	Visit 9
Time point(s)	**D-30 *to D0***	**D 0**	**D 7**	**D 30**	**D 37**	**M 2**	**M 4, 6, 8, 10**	**M 12**	**M 14, 16, 18, 20, 22**	**M 24**	**M 26, 28, 30, 32, 34**	**M 36**
Sampling Time point(s)	**Pre V**	**Pre V**	**Post V1**	**Post V1**	**Post V2**	**Post V2**	**Post V2**	**Post V2**	**Post V2**	**Post V2**	**Post V2**	**Post V2**
Dose		**1**		**2**								
Safety and immune sub-cohort												
Measuring Vitamin D		●										
Recording of solicited AEs		●	●	●	●							
CBC (Hb, PLT and WBC), ALT, AST, creatinine, bilirubin (~ 8 mL)		●	●	●	●							
Blood sampling for ***Wh.BI.*** CMI (~ 2.5 mL)		●				●		●		●		●
Blood sampling for humoral immunogenicity (~ 2 mL)		●				●		●		●		●
Study conclusions for all subjects												●

Note: The double border at Month 36 indicates the final analyses which will be performed on all data obtained up to month 36.
Pre V: Pre Vaccination; V: Vaccination; Post V: Post Vaccination
● is used to indicate a study procedure that requires documentation in the individual eCRF.
○ is used to indicate a study procedure that does not require documentation in the individual eCRF.
1 Only subjects in the safety and immune sub-cohort need to come for this visit.
2 HIV testing, pre-test ***and post-test*** counselling will be performed ***whenever a HIV test is done***, according to the procedures detailed in Table 10.
3 Weight also needs to be recorded whenever there is suspicion of TB disease.
4 Only SAEs related to study participation or concurrent GSK medication/vaccine.
5 SAEs not related to study participation or concurrent GSK medication/vaccines will be recorded until 6 months post dose 2 (M7).
6 To be recorded until 6 months post dose 2 (M7).
7 Sputum samples for Xpert MTB/RIF testing and microbiological culture will be taken throughout the study if a subject presents with clinical suspicion of pulmonary TB disease. Three sputum samples, preferably taken in the morning and within ***max.*** one week, must be collected. Additionally, when a subject is diagnosed with TB, ***screening for diabetes (HbA1C) and*** HIV testing must be performed according to the procedures detailed in Table 10. Up to 2.5mL of blood will be collected per test. If the subject is confirmed HIV-positive, blood samples must be taken (2.5 mL) to measure CD4 counts.
8 Documentation of TB cases will be done throughout the study, starting at Visit 3 (Day 7); this includes documentation of cases during active and passive follow-up and monitoring of medical facilities and pharmacies.

Table 8 Intervals between study visits for all subjects (Amended: 29 January 2014)

Interval	Allowed interval[1]
Visit 1 - Visit 2	0 days - 30 days
Visit 2 - Visit 4	26 days - 35 days
Visit 4 - Visit 6	26 days - 35 days
Visit 4 -Visit 7	10 months - 12 months
Visit 4 - Visit ***8***	22 months -24 months
Visit 4 - Visit ***9***	34 months - 36 months
Visit n – next contact	6 weeks – 12 weeks
Contact n – contact n+1	6 weeks – 12 weeks

[1] Subjects may not be eligible for inclusion in the ATP cohort for analysis of immunogenicity and efficacy if they ***attend for a*** study visit outside this interval.

Table 9 Intervals between study visits specific for the safety and immune sub-cohort

Interval	Allowed interval[1]
Visit 2 - Visit 3	6 days - 10 days
Visit 4 - Visit 5	6 days - 10 days

[1] Subjects may not be eligible for inclusion in the ATP cohort for analysis of immunogenicity and efficacy if they make the study visit outside this interval.

6.6. Detailed description of study procedures

6.6.1. Informed consent

The signed/witnessed/thumb printed informed consent of the subject must be obtained before study participation. Refer to Section 6.1 for the requirements on how to obtain informed consent as appropriate. Consenting and screening may be repeated, but only once, for subjects who would return out of screening interval but still agree to participate.

6.6.2. Check inclusion and exclusion criteria

Check all inclusion and exclusion criteria as described in Sections 5.2 and 5.3 before enrolment.

6.6.3. Collect demographic data

Record demographic data such as date of birth, gender and ethnicity in the subject's eCRF.

6.6.4. Collect history of TB household contacts

Question the subject about recent exposure (within the last 12 months) to a person (household contact) diagnosed with and/or treated for pulmonary TB disease.

Record history of TB household contacts in the subject's eCRF.

6.6.5. Medical history

Obtain the subject's medical history by interview and/or review of the subject's medical records and record any pre-existing conditions or signs and/or symptoms present in a subject prior to the first study vaccination in the eCRF. In particular, chronic pulmonary conditions (such as COPD, silicosis), history of smoking and diabetes* must be recorded ***(* type I diabetes is an exclusion criterion).*** **(Amended: 29 January 2014)**

6.6.6. Physical examination

Perform a physical examination of the subject at screening, including assessment of body temperature, height, weight and resting vital signs: systolic/diastolic blood pressure, heart rate and respiratory rate after at least 10 minutes of rest. Height and weight of the subject need to be recorded in the eCRF. **(Amended: 29 January 2014)**

Physical examination at each study visit subsequent to the screening visit, will be performed only if the subject indicates during questioning that there might be some underlying pathology(ies) or if deemed necessary by the Investigator or delegate.

Treatment of any abnormality observed during physical examination has to be performed according to local medical practice outside this study or by referral to an appropriate health care provider.

6.6.7. History directed physical examination

Perform a history directed physical examination. If the investigator determines that the subject's health on the day of vaccination temporarily precludes vaccination, the visit will be rescheduled ***within the window period*** (Table 8). **(Amended: 29 January 2014)**

Treatment of any abnormality observed during this examination has to be performed according to local medical practice outside this study or by referral to an appropriate health care provider.

6.6.8. Urine pregnancy test

Female subjects of childbearing potential are to have a urine pregnancy test prior to any study vaccine administration. The study vaccine/placebo may only be administered if the pregnancy test is negative. Additionally, a urine pregnancy test will be performed at screening.

Note: The urine pregnancy test must be performed even if the subject is menstruating at the time of the study visit.

6.6.9. Check contraindications to vaccination

Contraindications to vaccination must be checked at the beginning of each vaccination visit. Refer to Section 7.5 for more details.

6.6.10. Assess pre-vaccination body temperature

The axillary, oral or tympanic body temperature of all subjects needs to be measured prior to any study vaccine administration. The preferred route for recording temperature in this study will be axillary. If the subject has fever [fever is defined as temperature ≥ 37.5°C/99.5°F for oral, axillary or tympanic route] on the day of vaccination, the vaccination visit will be rescheduled within the allowed interval for this visit (see Table 8). **(Amended: 29 January 2014)**

6.6.11. Study group and treatment number allocation

Study group and treatment number allocation will be performed as described in Section 6.2.2. The number of each administered treatment must be recorded in the eCRF.

6.6.12. Sampling

6.6.12.1. Blood sampling for safety *and* immune response assessments (Amended: 29 January 2014)

As specified in the List of Study Procedures in Section 6.5 (Table 7), blood samples are taken during certain study visits. Refer to the Module on Biospecimen Management in the SPM and lab manual for detailed instructions for the collection, handling and processing of the samples.

- For all subjects:
  - At screening, HIV testing must be performed according to the procedures detailed in Table 10. Up to approximately 2.5 mL of blood will be collected for each test.
  - A volume of at least 3 mL of whole blood ***must*** be drawn for QFTG testing at ***screening*** (Table 10)

  (Amended: 29 January 2014)
- For all subjects included in the safety and immune sub-cohort:
  - A volume of at least 2 mL of whole blood should be drawn for analysis of humoral immune responses at each predefined time point (Table 10).
  - A volume of at least 2.5 mL of whole blood should be drawn for analysis of cell-mediated immune (CMI) responses at each predefined time point (Table 10).
  - A volume of at least 8 mL of whole blood should be drawn for tests performed to assay for CBC, ALT, AST, bilirubin and creatinine at each predefined time point (Table 10). Vitamin D levels will be measured at Day 0 only.

  (Amended: 29 January 2014)
- **Whenever a subject is diagnosed with TB disease:**
  - ***Screening for diabetes and*** HIV testing must be performed according to the procedures detailed in Table 10. Up to approximately 2.5 mL of blood will be

collected for each test. Additionally, if HIV is confirmed, at least 2.5 mL of blood will be collected to measure CD4 counts. **(Amended: 29 January 2014)**

6.6.12.2. Sputum sample collection for TB screening (Amended: 29 January 2014)

A single sputum sample must be collected from all subjects going through screening for testing using Xpert.

6.6.12.3. Sputum sample collection for TB diagnosis

Subjects ***suspected with Pulmonary TB*** will be required to provide 3 sputum samples, preferably taken in the morning, within ***maximum*** a one week interval, of ***preferably up to*** 5 mL to test for Mtb using the Xpert MTB/RIF assay and microbiological culture whenever the subject presents with clinical suspicion of pulmonary TB. If needed, induction of sputum through nebulisation of hypertonic saline may be performed. **(Amended: 29 January 2014)**

Refer to the Module on Biospecimen Management in the SPM and lab manual for detailed instructions for the induction of sputum, collection, handling and processing of the samples.

6.6.13. TB disease capture

During active follow-up at regular visits/contacts, the subjects will be questioned for the presence of signs and symptoms of pulmonary TB disease and assessed for clinical suspicion of pulmonary TB disease.

During passive follow-up (i.e. if a subject recognises signs and symptoms of TB and self-reports), the subjects will be assessed for clinical suspicion of pulmonary TB disease.

If clinical suspicion of pulmonary TB disease exists, diagnostic procedures per protocol will be performed (refer to Section 6.4.2).

If clinical suspicion of extra-pulmonary TB disease exists, diagnostic procedures according to local clinical practice will be performed (refer to Section 6.4.3).

At each visit or contact, the subject will be asked if he/she has sought care or received treatment for tuberculosis or suspicion of tuberculosis. Such TB cases fall under the fifth case definition and will be recorded as clinical TB.

Additionally, medical facilities and Pharmacists awareness (if applicable) and surveillance will be put in place in the study area to ensure that TB disease cases occurring in study participants are captured. All Medical facilities and Pharmacists in the study area will be informed of the trial. A team of study personnel/health workers will visit the medical facilities monthly to identify study subjects admitted to or cared for at the hospitals for TB. Relevant staff at the medical facilities will be advised to contact the study personnel for each TB case to ensure that all cases are captured.

6.6.14. Study Vaccine administration

- After completing all prerequisite procedures prior to vaccination, one dose of study vaccine/placebo will be administered intramuscularly (IM) in the deltoid of the arm (refer to Section 7.3 for detailed description of the vaccine administration procedure). If the investigator or delegate determines that the subject's health on the day of administration temporarily precludes vaccine administration, the visit will be rescheduled within the allowed interval for this visit (refer to Table 8).
- The subjects will be observed closely for at least 30 minutes following the administration of the vaccine, with appropriate medical treatment readily available in case of anaphylaxis.

6.6.15. Check and record concomitant medication/vaccination and intercurrent medical conditions

Concomitant medication/vaccination must be checked and recorded in the eCRF as described in Section 7.6.

Intercurrent medical conditions must be checked and recorded in the eCRF as described in Section 7.7.

Recording of AEs, SAEs, pregnancies and pIMDs

- Refer to Section 9.2 for procedures for the investigator to record AEs, SAEs, pregnancies and pIMDs. Refer to Section 9.3 for guidelines on how to submit SAE, pregnancy and pIMD reports to GSK Biologicals.
- The subjects will be instructed to contact the investigator immediately should they manifest any signs or symptoms they perceive as serious.
- At each vaccination visit, diary cards will be provided to the subjects. Any unsolicited AEs (i.e. on the day of vaccination and during the next 29 days) occurring after vaccination will be recorded by all subjects. The subjects included in the safety and immune sub-cohort will also record body (axillary) temperature and any solicited local/general AEs (i.e. on the day of vaccination and during the next 6 days). In case of illiterate subjects, a designated person (such as a family member or a field worker) may provide assistance to complete the diary cards. The subject will be instructed to return the completed diary card to the investigator at a later visit. In case a diary card is lost by the subject or he/she is unable to complete the diary card, the safety information should be captured retrospectively using a descriptive interview. ***The site staff or Investigator should not write on the diary card, this information should be captured in the source notes.*** This process will be documented in a site SOP. Please refer to the SPM for further details. **(Amended: 29 January 2014)**
- Verify completed diary cards during discussion with the subject on Visits 2, 3*, 4, 5* and ***6***. Collect diary cards from the subjects at Visits 4 and ***6***. Any unreturned diary cards will be sought from the subject through telephone call(s) or any other convenient procedure. The investigator will transcribe the collected information into ***Inform™*** in English. *Only for subjects included in the safety and immune sub-cohort. **(Amended: 29 January 2014)**

6.6.16. Study conclusion

The investigator will:

- review data collected to ensure accuracy and completeness.
- complete the Study Conclusion screen in ***Inform*™**. **(Amended:29 January 2014)**

After study conclusion, if vaccine efficacy is demonstrated, recipients of the placebo will be offered the vaccine candidate.

6.7. Biological sample handling and analysis

Please refer to the SPM and lab manual for details on biospecimen management (handling, storage and shipment).

Samples will not be labelled with information that directly identifies the subject but will be coded with the identification number for the subject (subject number).

Under the following circumstances, additional testing on the samples may be performed by GSK Biologicals***, by laboratories working for GSK Biologicals, by laboratories working in collaboration with GSK and by laboratories involved in studies ancillary to TB-018,*** outside the scope of this protocol **(Amended: 29 January 2014)**:

- Collected samples may be used in other assays, for test improvement or development of analytical methods related to the study vaccines and its constituents or the disease under study.
- Collected samples may be used for purposes related to the quality assurance of data generated linked to the study vaccines or the disease under study, such as for maintenance of assays described in this protocol and comparison between analytical methods and/or laboratories.

Information on further investigations and their rationale can be obtained from GSK Biologicals.

Any sample testing will be done in line with the consent of the individual subject.

Refer also to the Investigator Agreement, where it is noted that the investigator cannot perform any other biological assays except those described in the protocol or its amendment(s).

Collected samples will be stored for a maximum of 20 years (counting from when the last subject performed the last study visit), unless local rules, regulations or guidelines require different timeframes or different procedures, which will then be in line with the subject consent. These extra requirements need to be communicated formally to and discussed and agreed with GSK Biologicals.

6.7.1. Use of specified study materials

When materials are provided by "The Collaboration", it is MANDATORY that all clinical samples (including serum samples) be collected and stored exclusively using those materials in the appropriate manner. The use of other materials could result in the exclusion of the subject from the ATP analysis (See Section 11.4 for the definition of study cohorts/ data sets to be analysed). The investigator must ensure that his/her personnel and the laboratory(ies) under his/her supervision comply with this requirement. However, when "The Collaboration" does not provide material for collecting and storing clinical samples, appropriate materials from the investigator's site must be used. Refer to the Module on Clinical Trial Supplies in the SPM and lab manual.

6.7.2. Biological samples

6.7.2.1. Protocol mandated testing and enpoints (Amended: 29 January 2014)

Table 10 Biological samples (Amended: 29 January 2014)

Sample type	Quantity	Unit	Time point	Subjects
Blood sampling for Safety assessment	At least 8	ml	Days 0, 7, 30 and 37[1]	Safety and immune sub-cohort[2]
Blood sampling for Humoral responses	At least 2	ml	Days 0, 60 and Years 1, 2, 3	Safety and immune sub-cohort[2]
Blood sampling for CMI	At least 2.5	ml	Days 0, 60 and Years 1, 2, 3	Safety and immune sub-cohort[2]
Blood sampling for QFTG	At least 3	ml	***At screening***	All subjects
Sputum sampling for TB screening	***Up to 5***	***ml***	***At Screening***	***All subjects***
Sputum sampling for TB diagnosis[3]	Approximately 5	ml	Throughout the study when pulmonary TB is suspected	All subjects with suspicion of pulmonary TB
Blood sampling for initial HIV testing - Step 1 (Rapid test)	Finger prick or at least 2.5	ml	At screening, ***At Visit 9 (study end)***	All subjects
			When TB diagnosis is confirmed	All subjects diagnosed with TB
Blood sample for HIV testing - Step 2 (Rapid test)	Finger prick or at least 2.5	ml	At screening and when TB diagnosis is confirmed	All subjects positive at Step 1[4]
Blood sampling for HIV testing - Step 3 (ELISA)	At least 2.5	ml	At screening, ***At Visit 9 (study end)***	Discordant Rapid test results or if no Rapid test performed[5]
			When TB diagnosis is confirmed	
Blood sampling for PCR - Step 4	At least 2.5	ml	When TB diagnosis is confirmed	Indeterminate ELISA results[5]
Blood sampling for CD4 count	At least 2.5	ml	When TB diagnosis is confirmed and confirmed HIV-positive	HIV positive subjects with TB diagnosis confirmed
Blood sampling for HbA1C	***At least 2.5***	***ml***	***When TB diagnosis is confirmed***	***Subjects with TB diagnosis confirmed***

1 Vitamin D levels will be measured at day 0 only.
2 Refer to Section 5.1 for sub-cohort description.
3 Three sputum samples, preferably taken in the morning, within a one week interval, will be collected whenever a subject presents with clinical suspicion of pulmonary TB.

4 HIV positive subjects at screening will be referred for confirmatory HIV diagnosis/management as per site SOPs.
5 Only the final conclusive diagnosis is to be recorded in the CRF.

6.7.3. Laboratory assays

Please refer to APPENDIX A for a detailed description of the assays performed in the study. Please refer to APPENDIX B for the address of the clinical laboratories used for sample analysis.

The main laboratory assays will be performed at GSK Biologicals' laboratory or in a laboratory designated by "The Collaboration" using standardised and/or qualified procedures (Table 11 - Table 15).

Table 11 Humoral Immunity (Antibody determination)

System	Component	Method	Kit/Manufacturer	Unit	Cut-off	Laboratory*
Serum	Mycobacterium tuberculosis.M72 Ab.IgG	Enzyme Linked Immuno Sorbent Assay	NA	enzyme linked immunosorbent assay unit per milliliter	2.8	CEVAC**

*Refer to APPENDIX B for the laboratory addresses.
**Or as designated by GSK Biologicals.

Table 12 Cell-Mediated Immunity (CMI) (Amended: 29 January 2014)

System	Component	Challenge	Method	Unit	Cut-off	Laboratory*
Whole blood	CD4+/CD8+ T cells expressing at least two immune markers (CD40L and/or IL-2 and/or TNF-α and/or IFN-γ) ***and any combination among CD40-L, IFN-g, IL-2 and TNF-a***	M72	ICS - intraCellular Cytokine Staining	number of events per million cells	N/A	***GSK or as designated by GSK***

*Refer to APPENDIX B for the laboratory addresses.

Table 13 Diagnostic tests for pulmonary TB disease (Amended: 29 January 2014)

System	Component	Method	Unit	Laboratory*	Component priority ranking
Sputum	Mycobacterium tuberculosis DNA	Xpert MTB/RIF assay	NA	Central Laboratory	1
Sputum	Mycobacterium tuberculosis	Microbiological culture	NA	Central Laboratory	2
Sputum	***Mtb***	***Strain Typing***	***NA***	***Central lab***	***3***

*Refer to APPENDIX B for the laboratory addresses.

In case of insufficient sputum sample volume to perform both Xpert MTB/RIF and microbiological culture, the samples will be analysed according to priority ranking provided in Table 13.

Table 14 Diagnostic tests for HIV (Amended: 29 January 2014)

System	Component	Method	Unit	Laboratory*
Whole Blood or Serum	Anti-HIV antibodies, screening step 1, step 2	HIV Rapid Test**	NA	At the Investigator's lab
Serum	Anti-HIV antibodies, screening step 3	ELISA	NA	At the Central Laboratory
Whole Blood	HIV screening step 4	HIV PCR	NA	At the Central Laboratory
Whole blood	CD4 cells	Flow cytometry	Cells/mm^3	At the Central Laboratory

*Refer to APPENDIX B for the laboratory addresses.
**Two different Rapid HIV tests to be used sequentially

Table 15 Haematology, Serum Chemistry, Urine tests

System	Component	Method	Scale
Whole blood	Alanine amino transferase	As per laboratory standard procedures	Quantitative
	Aspartate amino transferase		
	Creatinine		
	Bilirubin (total)		
	Platelets		
	Leucocytes		
	Erythrocytes		
	Haemoglobin		
	Vitamin D		
	IFN-γ release by T-cells	QuantiFERON® TB Gold Assay	Quantitative
Urine	β-HCG	As per laboratory standard procedures	Qualitative

The GSK Biologicals' clinical laboratories have established a Quality System supported by procedures. The activities of GSK Biologicals' clinical laboratories are audited regularly for quality assessment by an internal (sponsor-dependent) but laboratory-independent Quality Department.

6.7.4. Biological samples evaluation *per protocol* (Amended: 29 January 2014)

6.7.4.1. Diagnostic assays

Xpert MTB/RIF testing (Cepheid) and microbiological culture will be performed on three sputum samples, preferably taken in the morning, within a ***maximum*** one week interval, for all subjects presenting with clinical suspicion of pulmonary TB throughout the entire study. **(Amended: 29 January 2014)**

HIV testing and pre-test ***and post-test*** counselling will be performed at screening. HIV positive subjects at screening will be referred for confirmatory HIV diagnosis/management as per site SOPs ***and won't be enrolled (exclusion criterion)***. **(Amended: 29 January 2014)**

Whenever a subject is diagnosed with TB disease, an HIV test (and pre-test counselling) must be performed to rule out HIV co-infection. ***HbA1c measurement will also be performed.*** If a subject is diagnosed with HIV, additional tests to measure CD4 cell counts will be performed. Subjects with a positive test result will be referred for HIV treatment. **(Amended: 29 January 2014)**

All diagnostic assays will be carried out at the ***designated central*** laboratory ***as*** designated by "The Collaboration". **(Amended: 29 January 2014)**

6.7.4.2. Immunological read-outs

Blood will be collected at specific time points to assay for immunological readouts. Table 16 details the main immunological read-outs.

After collection, whole blood samples will be incubated with pool(s) of overlapping peptides covering the M72 antigen sequence and with the stimulation controls for 2 hours in the presence of anti-CD28 and anti-CD49d antibodies. Subsequently, flow cytometry using a short-term stimulation Intracellular Cytokine Staining (ICS) assay will be used to characterise M72-specific CD4+/CD8+ T cells expressing at least two immune markers ***or any combination of markers, i.e. single, double, triple, quadruple,*** (CD40L and/or IL-2 and/or TNF-α and/or IFN-γ) on Days 0, 60, and Years 1, 2 and 3. **(Amended: 29 January 2014)**

The humoral immune response will be evaluated by assessment of antibody titres to M72 by specific ELISA on Days 0 and 60, and Years 1, 2 and 3.

Table 16 Immunological read-outs (Amended: 29 January 2014)

Blood sampling time point		Sub-cohort Name	No. subjects	Component
Type of contact and time point	Sampling time point			
Visit 2 (Day 0)	Pre Vacc	Safety and immune sub-cohort	450	M72-specific T-cells (ICS on whole blood) Anti M72 Ab (ELISA on serum)
Visit ***6*** (Day 60)	Post Vacc 2			M72-specific T-cells (ICS on whole blood) Anti M72 Ab (ELISA on serum)
Visit ***7*** (Year 1)	LTFU			M72-specific T-cells (ICS on whole blood) Anti M72 Ab (ELISA on serum)
Visit ***8*** (Year 2)	LTFU			M72-specific T-cells (ICS on whole blood) Anti M72 Ab (ELISA on serum)
Visit ***9*** (Year 3)	LTFU			M72-specific T-cells (ICS on whole blood) Anti M72 Ab (ELISA on serum)

Vacc: Vaccination
LTFU: Long Term Follow-up

6.7.4.3. Haematology/Biochemistry

A urine pregnancy test will be performed on all female subjects at screening and prior to each vaccination. Urine pregnancy tests may be performed by clinical staff on the premises of the clinic/out-patient facility. The results of the pregnancy test must be recorded in the subjects' eCRF.

Serum biochemistry and haematology tests (Hb, PLT, WBC, ALT, AST, bilirubin, creatinine) will be performed in the safety and immune sub-cohort on Days 0, 7, 30 and 37. To characterise the study population, vitamin D levels will be measured at baseline in the sub-cohort. The tests will be performed at the laboratory of the investigator's site or in a laboratory designated by "The Collaboration". Results for WBCs, haemoglobin, creatinine, ALT, AST, total bilirubin and vitamin D will be recorded in the subject's eCRF or transferred directly to the clinical database. Table 17 details the biochemistry/ haematology readouts.

Table 17 Biochemistry/haematology readouts

Blood sampling time point		Sub-cohort Name	No. subjects	Component
Type of contact and timepoint	Sampling timepoint			
Visit 2 (Day 0)	Pre-Vacc	Safety and immune sub-cohort	450	Hb, PLT, WBC ALT AST Creatinine Bilirubin (total) Vitamin D
Visit 3 (Day 7)	Post-Vacc 1	Safety and immune sub-cohort	450	Hb, PLT, WBC ALT AST Creatinine Bilirubin (total)
Visit 4 (Day 30)	Post-Vacc 1	Safety and immune sub-cohort	450	Hb, PLT, WBC ALT AST Creatinine Bilirubin (total)
Visit 5 (Day 37)	Post Vacc 2	Safety and immune sub-cohort	450	Hb, PLT, WBC ALT AST Creatinine Bilirubin (total)

Vacc: Vaccination

6.7.5. Immunological correlates of protection

No immunological correlate of protection has been demonstrated so far for the antigen(s) used in the candidate vaccine and/or licensed vaccines (BCG).

7. STUDY VACCINES AND ADMINISTRATION

7.1. Description of study vaccines

The candidate vaccine and placebo to be used have been developed and manufactured by GSK Biologicals.

The Quality Control Standards and Requirements for the candidate vaccine are described in separate Quality Assurance documents (e.g. release protocols, certificate of analysis) and the required approvals have been obtained.

The vaccines are labelled and packed according to applicable regulatory requirements.

Table 18 Study vaccines

Treatment name	Vaccine/product name	Formulation	Presentation	Volume to be administered	Number of doses
M72/AS01$_E$	M72	M72 (10 µg); sucrose; polysorbate; TRIS	Lyophilised cake in vial	0.5 ml	2
	AS01$_E$	25µg MPL, 25 µg QS21 in a liposome-based formulation	Liquid in vial		
Placebo	Placebo pellet	5 % Sucrose in phosphate buffer	Lyophilised cake in vial	0.5 ml	2
	Placebo adjuvant	23.8 µg α-Tocopherol, 21.4 µg squalene and 9.7 µg Tween 80	Liquid in vial		

7.2. Storage and handling of study vaccines

The study vaccines must be stored at the respective label storage temperature conditions in a safe and locked place. Access to the storage space should be limited to authorized study personnel. The storage conditions will be assessed during pre-study activities under the responsibility of the sponsor study contact. The storage temperature should be continuously monitored with calibrated (if not validated) temperature monitoring device(s) and recorded. Refer to the Module on Clinical Trial Supplies in the SPM for more details on storage of the study vaccines.

Temperature excursions must be reported in degree Celsius.

Any temperature excursion outside the range of 0.0 to +8.0°C (for +2 to +8°C/+36 to +46°F label storage condition) impacting investigational medicinal products (IMPs) must be reported in the appropriate (electronic) temperature excursion decision form (eTDF). The impacted IMPs must not be used and must be stored in quarantine at label temperature conditions until usage approval has been obtained from the sponsor.

In case of temperature excursion below +2.0°C down to 0.0°C impacting IMP(s) there is no need to report in (e)TDF, but adequate actions must be taken to restore the +2 to +8°C/+36 to +46°F label storage temperature conditions. The impacted IMP(s) may still be administered, but the site should avoid re-occurrence of such temperature excursion.

Refer to the Module on Clinical Trial Supplies in the SPM for more details on actions to take.

Refer to the Module on Clinical Trial Supplies in the SPM for details and instructions on the temperature excursion reporting and usage decision process, packaging and accountability of the study vaccines.

7.3. Dosage and administration of study vaccines

Subjects will be administered the candidate vaccine M72/AS01$_E$ or placebo according to their random assignment:

- Subjects randomised to receive M72/AS01$_E$ will be administered two doses of M72/AS01$_E$ according to a 0, 1 month schedule (Day 0 and Day 30).
- Subjects randomised to receive the placebo will be administered two doses of placebo according to a 0, 1 month schedule (Day 0 and Day 30).

Each dose will be administered intramuscularly in the deltoid region of the arm.

Table 19 summarises the dosage and administration of study vaccine and placebo.

Table 19 Dosage and administration

Type of contact and time point	Volume to be administered	Study group	Treatment name	Route	Site	Side
Visit 2 (day 0)	0.5ml 0.5ml	M72AS01	M72/AS01$_E$	IM	Deltoid	NA
		Control	Placebo	IM	Deltoid	NA
Visit 4 (Day 30)	0.5ml 0.5ml	M72AS01	M72/AS01$_E$	IM	Deltoid	NA
		Control	Placebo	IM	Deltoid	NA

IM: Intramuscular

Refer to the Module on Clinical Trial Supplies in the SPM for details and instructions for reconstitution of M72 antigen/Placebo pellet with AS01$_E$ Adjuvant System/Placebo adjuvant and administration of the reconstituted study vaccines.

7.4. Replacement of unusable vaccine doses

In addition to the vaccine doses provided for the planned number of subjects (including over-randomisation when applicable), at least 5% additional vaccine doses will be supplied to replace those that are unusable.

The investigator will use SBIR to obtain the replacement vial number. The replacement numbers will be allocated by dose. The system will ensure, in a blinded manner, that the replacement vial matches the formulation the subject was assigned to by randomisation.

7.5. Contraindications to subsequent vaccination

The following events constitute absolute contraindications to further administration of M72/AS01$_E$. If any of these events occur during the study, the subject must not receive additional doses of vaccine but may continue other study procedures at the discretion of the investigator (see Section 9.4).

- Anaphylaxis following the administration of vaccine(s).
- Pregnancy (see Section 9.1.6).
- Any confirmed or suspected immunosuppressive or immunodeficient condition, including HIV-infection.
- Active TB disease.
- ***Grade 3 local symptoms redness and swelling, i.e. >100mm or Grade ≥2 respiratory AE following the administration of vaccine(s).*** **(Amended: 29 January 2014).**

The following events constitute contraindications to administration of M72/AS01$_E$ at that point in time; if any of these events occur at the time scheduled for vaccination, the subject may be vaccinated at a later date, within the time window specified in the protocol (see Section 6.5), or the subject may be withdrawn at the discretion of the investigator (see Section 9.4).

- Acute disease and/or fever at the time of vaccination.
  - Acute disease is defined as the presence of a moderate or severe illness with or without fever.
  - Fever is defined as temperature ≥ 37.5°C/99.5°F on oral, axillary or tympanic setting. The preferred route for recording temperature in this study will be axillary. **(Amended: 29 January 2014)**

Subjects with a minor illness (such as mild diarrhoea, mild upper respiratory infection) without fever can be administered all vaccines.

7.6. Concomitant medication/product and concomitant vaccination

At each study visit/contact, the investigator should question the subject about any medication/product taken and vaccination received by the subject.

7.6.1. Recording of concomitant medications/products and concomitant vaccination

The following concomitant medications/products/vaccines must be recorded in the eCRF if administered during the indicated recording period:

- All concomitant medications/products, except vitamins and dietary supplements, administered starting within 30 days following each dose of study vaccine.
- Any concomitant vaccination administered in the period starting 30 days before the first dose of study vaccine and ending at the last study visit.

- Prophylactic medication (i.e. medication administered in the absence of ANY symptom and in anticipation of a reaction to the vaccination).

  E.g. an anti-pyretic is considered to be prophylactic when it is given in the absence of fever and any other symptom, to prevent fever from occurring [fever is defined as temperature ≥ 37.5°C/99.5°F for oral, axillary or tympanic route, or ≥ 38.0°C/100.4°F for rectal route].
- Any concomitant medications/products/vaccines listed in Section 7.6.2.
- Any concomitant medication/product/vaccine relevant to a SAE* or administered at any time during the study period for the treatment of a SAE*.

  *SAEs that are required to be reported per protocol.
- Any medication administered for TB treatment throughout the entire study period.

7.6.2. Concomitant medications/products/vaccines that may lead to the elimination of a subject from ATP analyses

The use of the following concomitant medications/products/vaccines will not require withdrawal of the subject from the study but may determine a subject's evaluability in the ATP analysis. See Section 11.4 for study cohorts/data sets to be analysed.

- Any investigational or non-registered product (drug or vaccine) other than the study vaccines used during the study period.
- Immunosuppressants or other immune-modifying drugs administered chronically (i.e. more than 14 days) during the study period. For corticosteroids, this will mean prednisone ≥ 20 mg/day, or equivalent. Inhaled and topical steroids are allowed.
- A vaccine not foreseen by the study protocol administered during the period starting from 30 days before each dose of vaccine and ending 30 days after.

  In case an emergency mass vaccination for an unforeseen public health threat (e.g.: a pandemic) is organised by the public health authorities, outside the routine immunisation program, the time period described above can be reduced if necessary for that vaccine provided it is licensed and used according to its SPC or PI and according to the local governmental recommendations and provided a written approval of the Sponsor is obtained.
- Immunoglobulins and/or any blood products administered during the study period.

7.7. Intercurrent medical conditions that may lead to elimination of a subject from ATP analyses

At each study visit subsequent to the first vaccination visit, it must be verified if the subject has experienced or is experiencing any intercurrent medical condition. If it is the case, the condition(s) must be recorded in the eCRF.

Subjects may be eliminated from the ATP cohort for immunogenicity if, during the study, they incur tuberculosis disease or any other condition that has the capability of altering their immune response or are confirmed to have an alteration of their initial immune status.

8. HEALTH ECONOMICS

Not applicable.

9. SAFETY

The investigator or site staff is/are responsible for the detection, documentation and reporting of events meeting the criteria and definition of an adverse event (AE) or serious adverse event (SAE) as provided in this protocol.

Each subject will be instructed to contact the investigator immediately should they manifest any signs or symptoms they perceive as serious.

9.1. Safety definitions

9.1.1. Definition of an adverse event

An AE is any untoward medical occurrence in a clinical investigation subject, temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product.

An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of a medicinal product. For marketed medicinal products, this also includes failure to produce expected benefits (i.e. lack of efficacy), abuse or misuse.

Examples of an AE include:

- Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition.
- New conditions detected or diagnosed after investigational vaccine(s)/product(s) administration even though they may have been present prior to the start of the study.
- Signs, symptoms, or the clinical sequelae of a suspected interaction.
- Signs, symptoms, or the clinical sequelae of a suspected overdose of either investigational vaccine(s)/product(s) or a concurrent medication (overdose per se should not be reported as an AE/SAE).
- Signs, symptoms temporally associated with vaccine administration.
- Pre- or post-treatment events that occur as a result of protocol-mandated procedures (i.e. invasive procedures, modification of subject's previous therapeutic regimen).

AEs to be recorded as endpoints (solicited AEs) are described in Section 9.1.3. All other AEs will be recorded as UNSOLICITED AEs.

If subjects observe any large injection site reaction, they should contact the study personnel and visit the investigator's office for evaluation as soon as possible.

A large injection site reaction is:

- ***a swelling that measures more than 100 mm across where the vaccine was given, or***
- ***a noticeable irregular/uneven swelling where the vaccine was given, or***
- ***a noticeable increase in size of the arm that interferes with or prevents everyday activities (e.g., writing, use of computer, school attendance, sleeping, etc.).***

In case of questions or uncertainties, the subject/subject's parent(s)/guardian(s) should contact the investigator by phone and the investigator will determine whether or not a visit should be arranged.

The investigator will record detailed information describing the AE on a specific large injection site reaction screen in the eCRF. An SAE report should also be completed if the large injection site reaction meets the definition of SAE. **(Amended: 29 January 2014)**

Examples of an AE DO NOT include:

- Medical or surgical procedures (e.g. endoscopy, appendectomy); the condition that leads to the procedure is an AE/SAE.
- Situations where an untoward medical occurrence did not occur (e.g. social and/or convenience admission to a hospital, admission for routine examination).
- Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen.
- Pre-existing conditions or signs and/or symptoms present in a subject prior to the first study vaccination. These events will be recorded in the medical history section of the eCRF.

9.1.2. Definition of a serious adverse event

A serious adverse event is any untoward medical occurrence that:

a. Results in death,

b. Is life-threatening,

   Note: The term 'life-threatening' in the definition of 'serious' refers to an event in which the subject was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, had it been more severe.

c. Requires hospitalisation or prolongation of existing hospitalisation,

   Note: In general, hospitalisation signifies that the subject has been admitted at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or in an out-patient setting. Complications that occur during hospitalisation are also considered AEs. If a complication prolongs hospitalisation or fulfils any other serious criteria, the event will also be considered

serious. When in doubt as to whether 'hospitalisation' occurred or was necessary, the AE should be considered serious.

Hospitalisation for elective treatment of a pre-existing condition (known or diagnosed prior to informed consent signature) that did not worsen from baseline is NOT considered an AE.

d. Results in disability/incapacity, OR

Note: The term disability means a substantial disruption of a person's ability to conduct normal life functions. This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhoea, influenza like illness, and accidental trauma (e.g. sprained ankle) which may interfere or prevent everyday life functions but do not constitute a substantial disruption.

e. Is a congenital anomaly/birth defect in the offspring of a study subject.

Medical or scientific judgement should be exercised in deciding whether reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalisation but may jeopardise the subject or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These should also be considered serious. Examples of such events are invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalisation.

9.1.3. Solicited adverse events

The following local (injection site) and general AEs will be solicited:

Table 20 Solicited adverse events (Amended: 29 January 2014)

Solicited local (injection site) AEs	Solicited general AEs
Pain at injection site	Fatigue
Redness at injection site*	Fever
Swelling at injection site	***Respiratory*** Symptoms†
	Headache
	Malaise
	Myalgia

*In case the principal investigator or designate is unable to determine the extent of redness on darkly pigmented skin, it will be reported as non-interpretable in the eCRF and Diary Card.
†***Respiratory*** symptoms include ***cough, blood in sputum, purulent sputum, shortness of breath or difficulties breathing, chestwall pain***

Note: Temperature will be recorded in the evening. Should additional temperature measurements be performed at other times of day, the highest temperature will be recorded in the eCRF.

9.1.4. Clinical laboratory parameters and other abnormal assessments qualifying as adverse events or serious adverse events

In absence of diagnosis, abnormal laboratory findings (e.g. clinical chemistry, haematology, urinalysis) or other abnormal assessments that are judged by the investigator to be clinically significant will be recorded as AE or SAE if they meet the definition of an AE or SAE (refer to Sections 9.1.1 and 9.1.2). Clinically significant abnormal laboratory findings or other abnormal assessments that are present at baseline and significantly worsen following the start of the study will also be reported as AEs or SAEs.

The investigator will exercise his or her medical and scientific judgement in deciding whether an abnormal laboratory finding or other abnormal assessment is clinically significant.

Toxicity grading scales for laboratory parameters [FDA, 2007] are presented in Table 21.

Table 21 Toxicity grading table for laboratory abnormalities (Amended: 29 January 2014)

	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)*
Serum				
Sodium – Hyponatremia mEq/L	132 – 134	130 – 131	125 – 129	< 125
Sodium – Hypernatremia mEq/L	144 – 145	146 – 147	148 – 150	> 150
Potassium – Hyperkalemia mEq/L	5.1 – 5.2	5.3 – 5.4	5.5 – 5.6	> 5.6
Potassium – Hypokalemia mEq/L	3.5 – 3.6	3.3 – 3.4	3.1 – 3.2	< 3.1
Glucose – Hypoglycemia mg/dL	65 – 69	55 – 64	45 – 54	< 45
Glucose – Hyperglycemia Fasting – mg/dL Random – mg/dL	 100 – 110 110 – 125	 111 – 125 126 – 200	 >125 >200	Insulin requirements or hyperosmolar coma
Blood Urea Nitrogen BUN mg/dL	23 – 26	27 – 31	> 31	Requires dialysis
Creatinine – mg/dL	1.5 – 1.7	1.8 – 2.0	2.1 – 2.5	> 2.5 or requires dialysis
Calcium – hypocalcemia mg/dL	8.0 – 8.4	7.5 – 7.9	7.0 – 7.4	< 7.0
Calcium – hypercalcemia mg/dL	10.5 – 11.0	11.1 – 11.5	11.6 – 12.0	> 12.0
Magnesium – hypomagnesemia mg/dL	1.3 – 1.5	1.1 – 1.2	0.9 – 1.0	< 0.9
Phosphorous – hypophosphatemia mg/dL	2.3 – 2.5	2.0 – 2.2	1.6 – 1.9	< 1.6
CPK – mg/dL	1.25 – 1.5 x ULN***	1.6 – 3.0 x ULN	3.1 –10 x ULN	> 10 x ULN
Albumin – Hypoalbuminemia g/dL	2.8 – 3.1	2.5 – 2.7	< 2.5	--
Total Protein – Hypoproteinemia g/dL	5.5 – 6.0	5.0 – 5.4	< 5.0	--
Alkaline phosphate – increase by factor	1.1 – 2.0 x ULN	2.1 – 3.0 x ULN	3.1 – 10 x ULN	> 10 x ULN
Liver Function Tests –ALT, AST increase by factor	1.1 – 2.5 x ULN	2.6 – 5.0 x ULN	5.1 – 10 x ULN	> 10 x ULN
Bilirubin – when accompanied by any increase in Liver Function Test increase by factor	1.1 – 1.25 x ULN	1.26 – 1.5 x ULN	1.51 – 1.75 x ULN	> 1.75 x ULN
Bilirubin – when Liver Function Test	1.1 – 1.5 x ULN	1.6 – 2.0 x ULN	2.0 – 3.0 x ULN	> 3.0 x ULN

	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)*
is normal; increase by factor				
Cholesterol	201 – 210	211 – 225	> 226	---
Pancreatic enzymes - amylase, lipase	1.1 – 1.5 x ULN	1.6 – 2.0 x ULN	2.1 – 5.0 x ULN	> 5.0 x ULN
Haematology				
Hemoglobin (Female) - g/dL	11.0 – 12.0	9.5 – 10.9	8.0 – 9.4	< 8.0
Hemoglobin (Female) change from baseline value - g/dL	Any decrease – 1.5	1.6 – 2.0	2.1 – 5.0	> 5.0
Hemoglobin (Male) - g/dL	12.5 – 13.5	10.5 – 12.4	8.5 – 10.4	< 8.5
Hemoglobin (Male) change from baseline value – g/dL	Any decrease – 1.5	1.6 – 2.0	2.1 – 5.0	> 5.0
WBC Increase - cell/mm^3	10,800 – 15,000	15,001 – 20,000	20,001 – 25,000	> 25,000
WBC Decrease - cell/mm^3	2,500 – 3,500	1,500 – 2,499	1,000 – 1,499	< 1,000
Lymphocytes Decrease - cell/mm^3	750 – 1,000	500 – 749	250 – 499	< 250
Neutrophils Decrease - cell/mm^3	1,500 – 2,000	1,000 – 1,499	500 – 999	< 500
Eosinophils - cell/mm^3	650 – 1500	1501 - 5000	> 5000	Hypereosinophilic
Platelets Decreased - cell/mm^3	125,000 – 140,000	100,000 – 124,000	25,000 – 99,000	< 25,000
PT – increase by factor (prothrombin time)	1.0 – 1.10 x ULN**	1.11 – 1.20 x ULN	1.21 – 1.25 x ULN	> 1.25 ULN
PTT – increase by factor (partial thromboplastin time)	1.0 – 1.2 x ULN	1.21 – 1.4 x ULN	1.41 – 1.5 x ULN	> 1.5 x ULN
Fibrinogen increase - mg/dL	400 – 500	501 – 600	> 600	--
Fibrinogen decrease - mg/dL	150 – 200	125 – 149	100 – 124	< 100 or associated with gross bleeding or disseminated intravascular coagulation (DIC)
Urine				
Protein	Trace	1+	2+	Hospitalization or dialysis
Glucose	Trace	1+	2+	Hospitalization for hyperglycemia
Blood (microscopic) – red blood cells per high power field (rbc/hpf)	1 - 10	11 – 50	> 50 and/or gross blood	Hospitalization or packed red blood cells (PRBC) transfusion

ULN is the upper limit of the normal range and is dependent on normal reference ranges per institutional parameters.
*The clinical signs or symptoms associated with laboratory abnormalities might result in characterization of the laboratory abnormalities as Potentially Life Threatening (Grade 4). For example, a low sodium value that falls within a grade 3 parameter (125-129 mEq/L) should be recorded as a Grade 4 hyponatremia event if the subject had a new seizure associated with the low sodium value ***(source: FDA, 2007).*** **(Amended: 29 January 2014)**

9.1.5. Adverse events of specific interest

9.1.5.1. Potential immune-mediated diseases

Potential immune-mediated diseases (pIMDs) are a subset of AEs that include autoimmune diseases and other inflammatory and/or neurologic disorders of interest which may or may not have an autoimmune aetiology. AEs that need to be recorded and reported as pIMDs include those listed in Table 22.

However, the investigator will exercise his/her medical and scientific judgement in deciding whether other diseases have an autoimmune origin (i.e. pathophysiology involving systemic or organ-specific pathogenic autoantibodies) and should also be recorded as a pIMD.

Table 22 List of potential immune-mediated diseases

Neuroinflammatory disorders	Musculoskeletal disorders	Skin disorders
• Cranial nerve disorders, including paralyses/paresis (e.g. Bell's palsy) • Optic neuritis • Multiple sclerosis • Transverse myelitis • Guillain-Barré syndrome, including Miller Fisher syndrome and other variants • Acute disseminated encephalomyelitis, including site specific variants: e.g. non-infectious encephalitis, encephalomyelitis, myelitis, myeloradiculomyelitis • Myasthenia gravis, including Lambert-Eaton myasthenic syndrome • Immune-mediated peripheral neuropathies and plexopathies, (including chronic inflammatory demyelinating polyneuropathy, multifocal motor neuropathy and polyneuropathies associated with monoclonal gammopathy) • Narcolepsy	• Systemic lupus erythematosus • Scleroderma, including diffuse systemic form and CREST syndrome • Systemic sclerosis • Dermatomyositis • Polymyositis • Antisynthetase syndrome • Rheumatoid arthritis, • Juvenile chronic arthritis, (including Still's disease) • Polymyalgia rheumatic • Spondyloarthritis, including ankylosing spondylitis, reactive arthritis (Reiter's Syndrome) and undifferentiated spondyloarthritis • Psoriatic arthropathy • Relapsing polychondritis • Mixed connective tissue disorder	• Psoriasis • Vitiligo • Erythema nodosum • Autoimmune bullous skin diseases (including pemphigus, pemphigoid and dermatitis herpetiformis) • Cutaneous lupus erythematosus • Alopecia areata • Lichen planus • Sweet's syndrome • Morphoea
Liver disorders	**Gastrointestinal disorders**	**Metabolic diseases**
• Autoimmune hepatitis • Primary biliary cirrhosis • Primary sclerosing cholangitis • Autoimmune cholangitis	• Crohn's disease • Ulcerative colitis • Ulcerative proctitis • Celiac disease	• Autoimmune thyroiditis (including Hashimoto thyroiditis) • Grave's or Basedow's disease • Diabetes mellitus type I • Addison's disease

Vasculitides	Others
• Large vessels vasculitis including: giant cell arteritis such as Takayasu's arteritis and temporal arteritis. • Medium sized and/or small vessels vasculitis including: polyarteritis nodosa, Kawasaki's disease, microscopic polyangiitis, Wegener's granulomatosis, Churg–Strauss syndrome (allergic granulomatous angiitis), Buerger's disease (thromboangiitis obliterans), necrotizing vasculitis and anti-neutrophil cytoplasmic antibody (ANCA) positive vasculitis (type unspecified), Henoch-Schonlein purpura, Behcet's syndrome, leukocytoclastic vasculitis.	• Autoimmune hemolytic anemia • Autoimmune thrombocytopenia • Antiphospholipid syndrome • Pernicious anemia • Autoimmune glomerulonephritis (including IgA nephropathy, glomerulonephritis rapidly progressive, membranous glomerulonephritis, membranoproliferative glomerulonephritis, and mesangioproliferative glomerulonephritis) • Uveitis • Autoimmune myocarditis/cardiomyopathy • Sarcoidosis • Stevens-johnson syndrome • Sjögren's syndrome • Idiopathic pulmonary fibrosis • Goodpasture syndrome • Raynaud's phenomenon

When there is enough evidence to make any of the above diagnoses, the AE must be reported as a pIMD. Symptoms, signs or conditions which might (or might not) represent the above diagnoses, should be recorded and reported as AEs but not as pIMDs until the final or definitive diagnosis has been determined, and alternative diagnoses have been eliminated or shown to be less likely.

In order to facilitate the documentation of pIMDs in the eCRF, a pIMD standard questionnaire and a list of preferred terms (PTs) and PT codes corresponding to the above diagnoses will be available to investigators at study start.

9.1.6. Pregnancy

Female subjects who are pregnant or lactating at the time of vaccination must not receive additional doses of study vaccine/placebo but may continue other study procedures at the discretion of the investigator.

While pregnancy itself is not considered an AE or SAE, any adverse pregnancy outcome or complication or elective termination of a pregnancy for medical reasons will be recorded and reported as an AE or a SAE.

Note: The pregnancy itself should always be recorded on an electronic pregnancy report.

The following should always be considered as SAE and will be reported as described in Sections 9.3.1 and 9.3.3:

- Spontaneous pregnancy loss, including:
  - spontaneous abortion, (spontaneous pregnancy loss before/at 22 weeks of gestation).
  - ectopic and molar pregnancy.
  - stillbirth (intrauterine death of foetus after 22 weeks of gestation).

  Note: the 22 weeks cut-off in gestational age is based on WHO-ICD 10 noted in the EMA Guideline on pregnancy exposure [EMA, 2006]. It is recognized that national regulations might be different.
- Any early neonatal death (i.e. death of a live born infant occurring within the first 7 days of life).
- Any congenital anomaly or birth defect (as per [CDC MACDP] guidelines) identified in the offspring of a study subject (either during pregnancy, at birth or later) regardless of whether the foetus is delivered dead or alive. This includes anomalies identified by prenatal ultrasound, amniocentesis or examination of the products of conception after elective or spontaneous abortion.

Furthermore, any SAE occurring as a result of a post-study pregnancy AND considered by the investigator to be reasonably related to the investigational vaccine(s)/product(s) will be reported to GSK Biologicals as described in Section 9.3.3. While the investigator is not obligated to actively seek this information from former study participants, he/she may learn of a pregnancy through spontaneous reporting.

9.2. Detecting and recording adverse events, serious adverse events and pregnancies

9.2.1. Time period for detecting and recording adverse events, serious adverse events and pregnancies

All AEs starting within 30 days following administration of each dose of study vaccine/placebo must be recorded into the appropriate section of the eCRF, irrespective of intensity or whether or not they are considered vaccination-related.

The time period for collecting and recording SAEs will begin at the first receipt of study vaccine/placebo and will end 180 days following administration of the last dose of study vaccine/placebo for each subject. See Section 9.3 for instructions on reporting of SAEs.

All AEs/SAEs leading to withdrawal from the study will be collected and recorded from the time of the first receipt of study vaccine/placebo.

SAEs that are related to the investigational vaccine/product will be collected and recorded from the time of the first receipt of study vaccine/placebo until the subject is discharged from the study.

In addition to the above-mentioned reporting requirements and in order to fulfil international reporting obligations, SAEs that are related to study participation (i.e. protocol-mandated procedures, invasive tests, a change from existing therapy) or are related to a concurrent GSK medication/vaccine will be collected and recorded from the time the subject consents to participate in the study until she/he is discharged from the study.

The time period for collecting and recording pregnancies will begin at the first receipt of study vaccine/placebo and will end 180 days following administration of the last dose of study vaccine/placebo. See section 9.3 for instructions on reporting of pregnancies.

The time period for collecting and recording of pIMDs will begin at the first receipt of study vaccine/placebo and will end 180 following administration of the last dose of study vaccine/placebo. See section 9.3 for instructions on reporting of pIMDs.

An overview of the protocol-required reporting periods for AEs, SAEs, and pregnancies is given in Table 23.

Table 23 Reporting periods for adverse events, serious adverse events and pregnancies

Study activity	Pre-vacc*	Dose 1 (V1) D 0	7 d post V1 D 6	30 d post V1 D 29	Dose 2 (V2) D 30	7 d post V2 D 36	30 d post V2 D 59	6 months post dose 2 M 7	Study Conclusion M36
Reporting of solicited AEs†									
Reporting of unsolicited AEs									
Reporting of SAEs									
Reporting of SAEs related to the investigational product									
Reporting of SAEs related to study participation or concurrent GSK medication/vaccine									
Reporting of pregnancies									
Reporting of pIMDs									

* i.e. consent obtained.
Pre-Vacc: pre-vaccination; V: vaccination; Post-V: post-vaccination; D: Day, M: Month
† For the safety and immune sub-cohort only

9.2.2. Post-Study adverse events and serious adverse events

A post-study AE/SAE is defined as any event that occurs outside of the AE/SAE reporting period defined in Table 23. Investigators are not obligated to actively seek AEs or SAEs in former study participants. However, if the investigator learns of any SAE at any time after a subject has been discharged from the study, and he/she considers the event reasonably related to the investigational vaccine/product, the investigator will promptly notify the Study Contact for Reporting SAEs.

9.2.3. Evaluation of adverse events and serious adverse events

9.2.3.1. Active questioning to detect adverse events and serious adverse events

As a consistent method of collecting AEs, the subject should be asked a non-leading question such as: "*Have you felt different in any way since receiving the vaccine or since the previous visit?"*

When an AE/SAE occurs, it is the responsibility of the investigator to review all documentation (e.g. hospital progress notes, laboratory and diagnostics reports) relative to the event. The investigator will then record all relevant information regarding an AE/SAE in the eCRF. The investigator is not allowed to send photocopies of the subject's medical records to GSK Biologicals instead of appropriately completing the eCRF. However, there may be instances when copies of medical records for certain cases are requested by GSK Biologicals. In this instance, all subject identifiers will be blinded on the copies of the medical records prior to submission to GSK Biologicals.

The investigator will attempt to establish a diagnosis pertaining to the event based on signs, symptoms, and/or other clinical information. In such cases, the diagnosis should be documented as the AE/SAE and not the individual signs/symptoms.

9.2.3.2. Assessment of adverse events

9.2.3.2.1. Assessment of intensity

The intensity of the following solicited AEs will be assessed as described:

Table 24 Intensity scales for solicited symptoms in adults (Amended: 29 January 2014)

Adults		
Adverse Event	**Intensity grade**	**Parameter**
Pain at injection site	0	None
	1	Mild: Any pain neither interfering with nor preventing normal every day activities.
	2	Moderate: Painful when limb is moved and interferes with every day activities.
	3	Severe: Significant pain at rest. Prevents normal every day activities.
Redness at injection site		Record greatest surface diameter in mm
Swelling at injection site		Record greatest surface diameter in mm
Fever*		Record temperature in °C/°F
Headache	0	Normal
	1	Mild: Headache that is easily tolerated
	2	Moderate: Headache that interferes with normal activity
	3	Severe: Headache that prevents normal activity
Fatigue	0	Normal
	1	Mild: Fatigue that is easily tolerated
	2	Moderate: Fatigue that interferes with normal activity
	3	Severe: Fatigue that prevents normal activity
Respiratory symptoms (cough, blood in sputum, purulent sputum, shortness of breath or difficulties breathing, chestwall pain)	0	***No*** symptoms
	1	Mild: symptoms that are easily tolerated
	2	Moderate: symptoms that interfere with normal activity
	3	Severe: symptoms that prevent normal activity
Malaise	0	Normal
	1	Malaise that is easily tolerated
	2	Malaise that interferes with normal activity
	3	Malaise that prevents normal activity
Myalgia	0	Normal
	1	Myalgia that is easily tolerated
	2	Myalgia that interferes with normal activity
	3	Myalgia that prevents normal activity

*Fever is defined as temperature ≥ 37.5°C / 99.5°F for oral, axillary or tympanic route, or ≥ 38.0°C / 100.4°F for rectal route. The preferred route for recording temperature in this study will be axillary.

The investigator will assess the maximum intensity that occurred over the duration of the event for all unsolicited AEs (including SAEs) recorded during the study. The assessment will be based on the investigator's clinical judgement.

The intensity should be assigned to one of the following categories:

1 (mild)	=	An AE which is easily tolerated by the subject, causing minimal discomfort and not interfering with everyday activities.
2 (moderate)	=	An AE which is sufficiently discomforting to interfere with normal everyday activities.
3 (severe)	=	An AE which prevents normal, everyday activities In adults, such an AE would, for example, prevent attendance at work and would necessitate the administration of corrective therapy.

An AE that is assessed as Grade 3 (severe) should not be confused with a SAE. Grade 3 is a category used for rating the intensity of an event; and both AEs and SAEs can be assessed as Grade 3. An event is defined as 'serious' when it meets one of the pre-defined outcomes as described in Section 9.1.2.

9.2.3.2.2. Assessment of causality

The investigator is obligated to assess the relationship between investigational vaccine/ product and the occurrence of each AE/SAE. The investigator will use clinical judgement to determine the relationship. Alternative plausible causes, such as natural history of the underlying diseases, concomitant therapy, other risk factors, and the temporal relationship of the event to the investigational vaccine/product will be considered and investigated. The investigator will also consult the IB to determine his/her assessment.

There may be situations when a SAE has occurred and the investigator has minimal information to include in the initial report to GSK Biologicals. However, it is very important that the investigator always makes an assessment of causality for every event prior to submission of the SAE report to GSK Biologicals. The investigator may change his/her opinion of causality in light of follow-up information and update the SAE information accordingly. The causality assessment is one of the criteria used when determining regulatory reporting requirements.

In case of concomitant administration of multiple vaccines, it may not be possible to determine the causal relationship of general AEs to the individual vaccines administered. The investigator should, therefore, assess whether the AE could be causally related to vaccination rather than to the individual vaccines.

All solicited local (injection site) reactions will be considered causally related to vaccination. Causality of all other AEs should be assessed by the investigator using the following question:

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

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

If an event meets the criteria to be determined as 'serious' (see Section 9.1.2), additional examinations/tests will be performed by the investigator in order to determine ALL possible contributing factors for each SAE.

Possible contributing factors include:

- Medical history.
- Other medication.
- Protocol required procedure.
- Other procedure not required by the protocol.
- Lack of efficacy of the vaccines, if applicable.
- Erroneous administration.
- Other cause (specify).

9.2.3.3. Assessment of outcomes

The investigator will assess the outcome of all unsolicited AEs (including SAEs) recorded during the study as:

- Recovered/resolved.
- Recovering/resolving.
- Not recovered/not resolved.
- Recovered with sequelae/resolved with sequelae.
- Fatal (SAEs only).

9.2.3.4. Medically attended visits

For each solicited and unsolicited symptom the subject experiences, the subject will be asked if he/she received medical attention defined as hospitalisation, or an otherwise unscheduled visit to or from medical personnel for any reason, including emergency room visits. This information will be recorded in the eCRF.

9.3. Reporting of serious adverse events, pregnancies, and other events

9.3.1. Prompt reporting of serious adverse events, pregnancies, and other events to GSK Biologicals

SAEs that occur in the time period defined in Section 9.2.1 will be reported promptly to GSK within the timeframes described in Table 25, once the investigator determines that the event meets the protocol definition of a SAE.

Pregnancies that occur in the time period defined in Section 9.2.1 will be reported promptly to GSK within the timeframes described in Table 25, once the investigator becomes aware of the pregnancy.

pIMDs that occur in the time period defined in Section 9.2.1 will be reported promptly to GSK within the timeframes described in Table 25, once the investigator becomes aware of the pIMD.

Table 25 Timeframes for submitting serious adverse event, pregnancy and other events reports to GSK Biologicals

Type of Event	Initial Reports		Follow-up of Relevant Information on a Previous Report	
	Timeframe	Documents	Timeframe	Documents
SAEs	24 hours*	Electronic SAE report	24 hours*	Electronic SAE report
Pregnancies	2 weeks*	Electronic pregnancy report	2 weeks*	Electronic pregnancy report
pIMDs	24 hours**	Electronic SAE report	24 hours*	Electronic SAE report

* Timeframe allowed after receipt or awareness of the information.
**Timeframe allowed after the diagnosis is established and known to the investigator.

9.3.2. Contact information for reporting serious adverse events and other events to GSK Biologicals

Study Contact for Reporting SAEs
Back-up Study Contact for Reporting SAEs
24/24 hour and 7/7 day availability: GSK Biologicals Clinical Safety & Pharmacovigilance Fax: PPD or PPD

9.3.3. Completion and transmission of SAE reports to GSK Biologicals

Once an investigator becomes aware that a SAE has occurred in a study subject, the investigator (or designate) must complete the information in the electronic SAE report WITHIN 24 HOURS. The SAE report will always be completed as thoroughly as possible with all available details of the event. Even if the investigator does not have all information regarding a SAE, the report should still be completed within 24 hours. Once additional relevant information is received, the report should be updated WITHIN 24 HOURS. The investigator will always provide an assessment of causality at the time of the initial report.

9.3.3.1. Back-up system in case the electronic SAE reporting system does not work

If the electronic SAE reporting system does not work, the investigator (or designate) must complete, then date and sign a paper SAE report and fax it to the GSK Biologicals Clinical Safety and Pharmacovigilance department within 24 hours.

This back-up system should only be used if the electronic SAE reporting system is not working and NOT if the system is slow. As soon as the electronic SAE reporting system is working again, the investigator (or designate) must complete the electronic SAE report within 24 hours. The final valid information for regulatory reporting will be the information reported through the electronic SAE reporting system.

9.3.4. Completion and transmission of pregnancy reports to GSK Biologicals

Once the investigator becomes aware that a subject is pregnant, the investigator (or designate) must complete the required information onto the electronic pregnancy report WITHIN 2 WEEKS.

Note: Conventionally, the estimated gestational age (EGA) of a pregnancy is dated from the first day of the last menstrual period (LMP) of the cycle in which a woman conceives. If the LMP is uncertain or unknown, dating of EGA and the estimated date of delivery (EDD) should be estimated by ultrasound examination and recorded in the pregnancy report.

9.3.5. Reporting of pIMDs to GSK Biologicals

Once onset of a new pIMD or exacerbation of a pre-existing pIMD is diagnosed (serious or non-serious) in a study subject, the investigator (or designate) must complete the information in the electronic SAE report WITHIN 24 HOURS after he/she becomes aware of the diagnosis. A field on the SAE report allows to specify that the event is a pIMD and whether it is serious or non serious. The SAE report will always be completed as thoroughly as possible with all available details of the event, in accordance with the pIMD standard questionnaire provided. Even if the investigator does not have all information regarding a pIMD, the report should still be completed within 24 hours. Once additional relevant information is received, the report should be updated WITHIN 24 HOURS. The investigator will always provide an assessment of causality at the time of the initial report.

Refer to Section 9.3.3.1 for back-up system in case the electronic SAE reporting system does not work.

9.3.6. Updating of SAE, pregnancy, and pIMD information after freezing of the subject's eCRF

When additional SAE, pregnancy, or pIMD information is received after freezing of the subject's eCRF, new or updated information should be recorded on a paper report, with all changes signed and dated by the investigator. The updated report should be faxed to the GSK Biologicals Clinical Safety and Pharmacovigilance department or to the Study Contact for Reporting SAEs (refer to the Sponsor Information Sheet) within the designated reporting time frames specified in Table 25.

9.3.7. Regulatory reporting requirements for serious adverse events

The investigator will promptly report all SAEs to GSK in accordance with the procedures detailed in Section 9.3.1. GSK Biologicals has a legal responsibility to promptly notify, as appropriate, both the local regulatory authority and other regulatory agencies about the safety of a product under clinical investigation. Prompt notification of SAEs by the investigator to the Study Contact for Reporting SAEs is essential so that legal obligations and ethical responsibilities towards the safety of other subjects are met.

Investigator safety reports are prepared according to the current GSK policy and are forwarded to investigators as necessary. An investigator safety report is prepared for a SAE(s) that is both attributable to the investigational vaccine/product and unexpected. The purpose of the report is to fulfil specific regulatory and GCP requirements, regarding the product under investigation.

9.4. Follow-up of adverse events, serious adverse events, and pregnancies

9.4.1. Follow-up of adverse events and serious adverse events

9.4.1.1. Follow-up during the study

After the initial AE/SAE report, the investigator is required to proactively follow each subject and provide additional relevant information on the subject's condition to GSK Biologicals (within 24 hours for SAEs; refer to Table 25).

All SAEs and pIMDs (serious or non-serious) documented at a previous visit/contact and designated as not recovered/not resolved or recovering/resolving will be reviewed at subsequent visits/contacts until the end of the study.

All AEs documented at a previous visit/contact and designated as not recovered/not resolved or recovering/resolving will be reviewed at subsequent visits/contacts until 30 days after the last vaccination.

9.4.1.2. Follow-up after the subject is discharged from the study

The investigator will follow subjects:

- with SAEs, pIMDs (serious or non-serious), or subjects withdrawn from the study as a result of an AE, until the event has resolved, subsided, stabilised, disappeared, or until the event is otherwise explained, or the subject is lost to follow-up.

If the investigator receives additional relevant information on a previously reported SAE, he/she will provide this information to GSK Biologicals using a paper SAE and/or pregnancy report as applicable.

GSK Biologicals may request that the investigator performs or arranges the conduct of additional clinical examinations/tests and/or evaluations to elucidate as fully as possible the nature and/or causality of the AE or SAE. The investigator is obliged to assist. If a subject dies during participation in the study or during a recognised follow-up period, GSK Biologicals will be provided with any available post-mortem findings, including histopathology.

9.4.2. Follow-up of pregnancies

Pregnant subjects will be followed to determine the outcome of the pregnancy. At the end of the pregnancy, whether full-term or premature, information on the status of the mother and child will be forwarded to GSK Biologicals using the electronic pregnancy report and the SAE report if applicable. Generally, the follow-up period doesn't need to be longer than six to eight weeks after the estimated date of delivery.

Regardless of the reporting period for SAEs for this study, if the pregnancy outcome is a SAE, it should always be reported as SAE.

9.5. Treatment of adverse events

Treatment of any AE is at the sole discretion of the investigator and according to current good medical practice. Any medication administered for the treatment of an AE should be recorded in the subject's eCRF (refer to Section 7.6).

9.6. Unblinding

GSK Biologicals' policy (which incorporates ICH E2A guidance, EU Clinical Trial Directive and US Federal Regulations) is to unblind the report of any SAE which is unexpected and attributable/suspected to be attributable to the investigational vaccine/product, prior to regulatory reporting. The GSK Biologicals' Central Safety Physician is responsible for unblinding the treatment assignment in accordance with the specified timeframes for expedited reporting of SAEs (refer to Section 9.3.1).

9.7. Emergency unblinding

Unblinding of a subject's individual treatment code should occur only in the case of a medical emergency, or in the event of a serious medical condition, when knowledge of the study treatment is essential for the clinical management or welfare of the subject, as judged by the investigator.

The emergency unblinding process consists of the automated system SBIR that allows the investigator to have unrestricted, immediate and direct access to the subject's individual study treatment.

The investigator has the option of contacting a GSK Biologicals' On-call Central Safety Physician (or Backup) if he/she needs medical advice or needs the support of GSK to perform the unblinding (i.e. he/she cannot access the automated Internet-based system).

Any emergency unblinding must be fully documented by using the Emergency Unblinding Documentation Form, which must be appropriately completed by the investigator and sent within 24 hours to GSK Biologicals.

GSK Biologicals' Contact information for Emergency Unblinding 24/24 hour and 7/7 day availability
GSK Biologicals' Central Safety Physician: PPD (GSK Biologicals Central Safety Physician on-call) **GSK Biologicals' Central Safety Physician Back-up:** PPD **Emergency Unblinding Documentation Form transmission:** Fax: PPD or PPD

9.8. Subject card

Study subjects must be provided with the address and telephone number of the main contact for information about the clinical study.

The investigator (or designate) must therefore provide a "subject card" to each subject. In an emergency situation this card serves to inform the responsible attending physician that the subject is in a clinical study and that relevant information may be obtained by contacting the investigator.

Subjects must be instructed to keep subject cards in their possession at all times.

10. SUBJECT COMPLETION AND WITHDRAWAL

10.1. Subject completion

A subject who returns for the concluding visit/is available for the concluding contact foreseen in the protocol is considered to have completed the study.

10.2. Subject withdrawal

Withdrawals will not be replaced.

10.2.1. Subject withdrawal from the study

From an analysis perspective, a 'withdrawal' from the study refers to any subject who did not come back for the concluding visit/was not available for the concluding contact foreseen in the protocol.

All data collected until the date of withdrawal/last contact of the subject will be used for the analysis.

A subject is considered a 'withdrawal' from the study when no study procedure has occurred, no follow-up has been performed and no further information has been collected for this subject from the date of withdrawal/last contact.

Investigators will make an attempt to contact those subjects who do not return for scheduled visits or follow-up by means of phone calls and/or home visits by field workers.

Information relative to the withdrawal will be documented in the eCRF.The investigator will document whether the decision to withdraw a subject from the study was made by the subject himself/herself, or by the investigator, as well as which of the following possible reasons was responsible for withdrawal:

- Serious adverse event.
- Non-serious adverse event.
- Protocol violation (specify).
- Consent withdrawal, not due to an adverse event*.
- Moved from the study area.
- Lost to follow-up.
- Other (specify).

*In case a subject is withdrawn from the study because he/she has withdrawn consent, the investigator will document the reason for withdrawal of consent, if specified by the subject, in eCRF.

Subjects who are withdrawn from the study because of SAEs/AEs must be clearly distinguished from subjects who are withdrawn for other reasons. Investigators will follow subjects who are withdrawn from the study as result of a SAE/AE until resolution of the event (see Section 9.4.1.2).

10.2.2. Subject withdrawal from investigational vaccine

A 'withdrawal' from the investigational vaccine refers to any subject who does not receive the complete treatment, i.e. when no further planned dose is administered from the date of withdrawal. A subject withdrawn from the investigational vaccine may not necessarily be withdrawn from the study as further study procedures or follow-up may be performed (safety or immunogenicity) if planned in the study protocol.

Information relative to premature discontinuation of the investigational vaccine will be documented on the Vaccine Administration screen of the eCRF. The investigator will document whether the decision to discontinue further vaccination/treatment was made by the subject himself/herself, or by the investigator, as well as which of the following possible reasons was responsible for withdrawal:

- Serious adverse event.
- Non-serious adverse event.
- Other (specify).

10.3. Extension study

At the end of the study (study conclusion visit/contact), the investigator will ask each subject if they are interested to participate in a long-term study. If a subject is not interested in participating in the long-term study the reason for refusal will be documented in the subject's eCRF.

10.4. Screen and baseline failures

Upon completion of all screening procedures (Refer to Table 7), the investigator, or designee, will review the inclusion/exclusion criteria for each subject. Subjects meeting all eligibility criteria will be scheduled to come for their baseline (Day 0) visit. Their screening information will be recorded on the appropriate screen of eCRF.

If the investigator believes there is a reasonable reason to do so, screening procedures (including, but not limited to, the QTF screening in the case of an intermediate result) may only be repeated once, either individually or in their entirety. **(Amended: 29 January 2014)**

11. STATISTICAL METHODS

11.1. Primary endpoint

- Incident cases of **Definite Pulmonary TB disease** not associated with HIV-infection, meeting the first case definition.

  Over a period starting 1 month post-dose 2 and lasting up to 35 months post last vaccination.

Refer to section 4 for case definitions

11.2. Secondary endpoints

Efficacy

- Incident cases of **Definite Xpert MTB/Rif positive Pulmonary TB disease** not associated with HIV-infection, meeting the second case definition.

  Over a period starting 1 month post-dose 2 and lasting up to 35 months post last vaccination.

- Incident cases of **Definite Pulmonary TB disease** meeting the third case definition.

  Over a period starting 1 month post-dose 2 and lasting up to 35 months post last vaccination.

- Incident cases of ***Microbiological*** **Pulmonary TB disease** meeting the fourth case definition.

  Over a period starting 1 month post-dose 2 and lasting up to 35 months post last vaccination

- Incident cases of **Clinical TB disease** meeting the fifth case definition.

  Over a period starting 1 month post-dose 2 and lasting up to 35 months post last vaccination

Refer to section 4 for case definitions.

Safety

- Occurrence of SAEs.

  During the entire study period

- Occurrence of unsolicited AEs.

  During the 30 day follow-up period following vaccination (day of vaccination and 29 subsequent days after each vaccine dose)

- Occurrence of solicited local and general AEs in the safety and immune sub-cohort.

  During the 7 day follow-up period following vaccination (day of vaccination and 6 subsequent days after each vaccine dose).

- Occurrence of all pIMDs.

  Over a period starting at Day 0 until 6 months post-dose 2.

- Occurrence of grade ≥ 2 haematological and biochemical, ***in the safety and immune sub-cohort***, levels at:

  Days 0, 7, 30 and 37

  (Amended: 29 January 2014)

Immunogenicity

- Evaluation of CMI responses with respect to components of the study vaccine, in the safety and immune sub-cohort:
  - ***Determined by the frequency of M72-specific CD4+/CD8+ T-cells per million cells identified after in vitro stimulation, as expressing any combination of immune markers among CD40L, IL-2, IFN-γ and TNF-α.*** **(Amended: 29 January 2014)**

  Timepoints: prior to dose 1 (Day 0) and post-dose 2 (Day 60, Years 1, 2 and 3)

- Evaluation of humoral immune responses with respect to components of the study vaccine, in the safety and immune sub-cohort:
  - Determined by M72-specific antibody titres as measured by ELISA.
  - Determined by seropositivity rates as measured by ELISA.

  Timepoints: prior to dose 1 (Day 0) and post-dose 2 (Day 60, Years 1, 2 and 3).

11.3. Determination of sample size

The primary objective is to demonstrate that the lower limit of the ***90***% CI of vaccine efficacy (VE), obtained from the Cox proportional hazards regression model (VE=1-estimated hazard ratio) associated to the M72/AS01$_E$ vaccine in preventing first episodes of definite pulmonary TB disease meeting the first case definition is >0. **(Amended: 29 January 2014)**

Using a logrank test with 80% power for a true VE of 70% (hazard ratio of 30%)and a two-sided ***10***% significance level, the number of cases required for a fixed sample design is ***21***cases, based on the assumption that the hazard rates are proportional. **(Amended: 29 January 2014)**

To obtain these ***21*** cases in total at final efficacy analysis, assuming a mean yearly attack rate of 0.***55***% in the control group, ***2*** years of follow-up for each subject and a ***rate of*** loss ***to*** follow-up of ***15***% due to drop out over the ***2*** years period, ***3506*** subjects need to be enrolled based on the logrank test and parameters described above. ***A planned follow-up time of 3 years for each subject allows to mitigate any lower than expected incidence rate and alternative to achieve a higher precision of the VE estimate at the final analysis.*** **(Amended: 29 January 2014)**

11.4. Study cohorts/data sets to be analysed

11.4.1. Total vaccinated cohort

The Total Vaccinated Cohort (TVC) will include all vaccinated subjects for whom data are available.

The TVC for efficacy will include all vaccinated subjects and will include all TB cases occurring post-dose 1.

The TVC analysis will be performed per treatment actually administered at dose 1.

(Amended: 29 January 2014)

11.4.2. According-to-protocol cohort for analysis of safety

The ATP cohort for analysis of safety will include all vaccinated and eligible subjects:

- Who have received at least one dose of study vaccine according to their random assignment.
- For whom study vaccines have been administered according to protocol (administration site and route).
- With sufficient data to perform an analysis of safety (at least one dose administered with safety follow-up).
- Who have not received a vaccine that may lead to elimination from an ATP analysis.
- For whom the randomisation code has not been broken.
- Without randomisation failure.

For the analysis of solicited adverse events and clinical laboratory observations, the ATP cohort for safety will include subjects from the safety and immune sub-cohort only.

11.4.3. According-to-protocol cohort for analysis of immunogenicity

The ATP cohort for immunogenicity will include all subjects from the safety and immune sub-cohort that:

- Received two vaccinations according to protocol procedures within specified intervals.
- Who complied with blood sampling schedules.
- Who had biochemistry and haematology values within normal range before any vaccination (exclude only if all values were out of range).
- For whom post vaccination blood samples are available.
- Who did not receive a vaccine/medication that may lead to elimination of an ATP analysis.

- Who did not present with a medical condition that may lead to elimination of an ATP analysis.
- For whom the randomization code has not been broken.
- ***Without randomisation failure. (*****Amended: 29 January 2014)**

11.4.4. According-to-protocol cohort for analysis of efficacy

The ATP cohort for efficacy will include all subjects included in the Total Vaccinated cohort who received all vaccinations according to protocol procedures within specified intervals that contribute time at risk in the follow-up period starting one month post dose 2 (Day 60).

The ATP cohort for efficacy will include all subjects:

- Who received 2 doses of M72/AS01$_E$ or placebo according to their random assignment.
- Received both vaccinations according to protocol procedures within specified intervals.
- Who entered the evaluation period for efficacy starting one month post dose 2, i.e. who have entered into the efficacy surveillance period.
- Who did not present with TB disease, according to any case definition, during the vaccination period (day of first vaccination up to 1 month post dose 2).
- For whom the randomization code has not been broken.
- Who did not receive a vaccine/medication that may lead to elimination of an ATP analysis.
- Who did not present with a medical condition that may lead to elimination of an ATP analysis.
- ***Without randomisation failure*****. (Amended: 29 January 2014)**

11.5. Derived and transformed data

- The cut-off value for M72-specific antibody titres is 2.8 EU/mL.
- A seronegative subject is a subject whose antibody titre is below the cut-off value of the assay, while a seropositive subject is a subject whose antibody titre is greater than or equal to the cut-off value.
- The Geometric Mean Titre (GMT) calculations are performed by taking the anti-log of the mean of the log10 titre transformations. For descriptive statistics purposes only, antibody titres below the cut-off value of the assay will be given an arbitrary value of half the cut-off value for the purpose of GMT calculation.
- For a given subject and a given immunogenicity measurement, missing or non-evaluable measurements will not be replaced.

- For the analysis of solicited symptoms, missing or non-evaluable measurements will not be replaced. Therefore the analysis of the solicited symptoms based on the total vaccinated cohort will include only subjects with documented safety data (i.e. symptom screen completed).
- For the analysis of unsolicited AEs/SAEs/concomitant medication, all vaccinated subjects will be considered and subjects who did not report an event will be considered as subjects without an event.

11.6. Analysis of demographics

Demographics and baseline characteristics will be described by group and for each of the cohorts described above.

Demographic characteristics (age at first study vaccination, gender, and ethnicity), will be summarised as a whole and by group using descriptive statistics:

- Frequency tables will be generated for categorical variable such as centre.
- Mean, median, standard deviation will be provided for continuous data such as age.

11.7. Analysis of efficacy

The ATP cohort for efficacy will be used for the primary analysis of efficacy. If the percentage of enrolled subjects excluded from the ATP cohort for analysis of efficacy is more than 5%, a second analysis based on the TVC will be performed to complement the primary analysis.

Vaccine efficacy will be estimated from a Cox proportional hazard regression model (VE=1-hazard ratio) and ***90***% CIs and Wald p-value will be derived. The primary analysis will be unadjusted but secondary analyses will evaluate the effect of potential covariates. **(Amended: 29 January 2014)**

At final efficacy analysis, the success criterion for the primary objective is the following:

*The lower limit of the **90**% two-sided confidence interval (CI) for the VE (using a Cox regression model) against first occurrence of Definite pulmonary TB disease not associated with HIV-infection, meeting the first case definition, is above 0%*
(Amended:29 January 2014)

Kaplan-Meier survival curves for the vaccine and control groups will be presented together with p-values from the logrank test.

If the primary objective is met, the confirmatory secondary objective will be evaluated with the following success criterion:

*The lower limit of the **90**% two-sided CI for the VE (using a Cox regression model) against first occurrence of Definite pulmonary TB disease not associated with HIV-infection, meeting the **second** case definition, is above 0%. (Amended: 29 January 2014)*
(Amended: 29 January 2014)

For all other secondary efficacy objectives, Kaplan-Meier survival curves will be plotted and compared by means of the logrank test. The magnitude of the vaccine efficacy will be estimated using Cox regression.

(Amended: 29 January 2014)

11.8. Analysis of immunogenicity

The primary analysis will be based on the ATP cohort for analysis of immunogenicity, which includes subjects from the safety and immune sub-cohort only. If, in any vaccine group, the percentage of vaccinated subjects with immunogenicity results excluded from the ATP cohort for analysis of immunogenicity is 5% or more, a second analysis based on the Total vaccinated cohort, including subjects from the safety and immune sub-cohort only, will be performed to complement the ATP analysis.

11.8.1. CMI and humoral immune responses

11.8.1.1. Within groups assessment

The following parameters will be tabulated at each scheduled time point, for each study group:

- Descriptive statistics of the frequency of M72-specific CD4+ T cells per million cells identified after *in vitro* stimulation, as expressing at least two immune markers among CD40L, IL-2, TNF-α and IFN-γ, after background subtraction.
- Descriptive statistics of the frequency of M72-specific CD8+ T cells per million cells identified after *in vitro* stimulation, as expressing at least two immune markers among CD40L, IL-2, TNF-α and IFN-γ, after background subtraction.
- Description statistics of the frequency of M72-specific CD4+ T cells per million cells identified after *in vitro* stimulation, as expressing any combination of immune markers among CD40L, IL-2, TNF-α and IFN-γ, after background subtraction.
- Description statistics of the frequency of M72-specific CD8+ T cells per million cells identified after *in vitro* stimulation, as expressing any combination of immune markers among CD40L, IL-2, TNF-α and IFN-γ, after background subtraction.
- For anti-M72 antibodies,
  - Seropositivity rate with exact 95% CI.
  - GMTs with exact 95% CI.

(Amended: 29 January 2014)

11.9. Analysis of safety

The primary analysis will be based on the TVC for analysis of safety. If, in any vaccine group, the percentage of vaccinated subjects excluded from the ATP cohort for analysis of safety is 5% or more, a second analysis based on the ATP cohort for analysis of safety will be performed to complement the TVC analysis.

Analyses of solicited (local and general) AEs and clinical laboratory observations will be performed for the safety and immune sub-cohort only. **(Amended: 29 January 2014)**

The following analyses will be performed:

- The percentage of subjects with at least one local AE (solicited and/or unsolicited), with at least one general AE (solicited and/or unsolicited) and with any AE during the solicited follow-up period will be tabulated with exact 95% CI after each vaccine dose and overall. The percentage of doses followed by at least one local AE (solicited and/or unsolicited), by at least one general AE (solicited and/or unsolicited) and by any AE will be tabulated, overall vaccination course, with exact 95% CI.
- The percentage of subjects reporting each individual solicited local and general AE during the solicited follow-up period will be tabulated with exact 95% CI. The percentage of doses, followed by each individual solicited local and general AE will be tabulated, overall vaccination course, with exact 95% CI.
- Injection site swelling and redness will be analysed using a 3-grade evaluation system [0 mm ***to 20 mm***(grade 0); ≥***20*** to ***50*** mm (grade 1); > ***50*** to ***100*** mm (grade 2); > ***100*** mm (grade 3)]. **(Amended: 29 January 2014)**
- Occurrence of fever will be reported per 0.5°C cumulative increments. Duration and prevalence of fever will be presented. Fever will also be analysed using a 3-grade evaluation system [< 37.5°C (grade 0); ≥ 37.5°C but ≤ 38.0°C (grade 1); > 38.0°C but ≤ 39.5°C (grade 2); > 39.5°C (grade 3)].
- The same tabulation will be performed for grade 3 AEs and for AEs with relationship to vaccination.
- The percentage of subjects with at least one report of unsolicited AE classified by the Medical Dictionary for Regulatory Activities (MedDRA) and reported up to 30 days after vaccination will be tabulated with exact 95% CI. The same tabulation will be performed for grade 3 unsolicited AEs and for unsolicited AEs with a relationship to vaccination.
- The percentage of subjects reporting AEs resulting in a medically attended visit will also be tabulated.
- The number of subjects with grade ≥ 2 laboratory abnormalities will be tabulated.

SAEs, pIMDs, and withdrawal from the study due to an AE will be described.

11.10. Interpretation of analyses

For the analysis of the primary objective, a predefined success criterion and an appropriate type I error control are defined (see Section 11.7). The confirmatory secondary objective will be analysed only if the primary objective is met. Because of this hierarchy, the type I error is controlled. All other analyses will be descriptive with the aim to characterise the difference in TB incidence, reactogenicity and immunogenicity between groups. These descriptive analyses should not be interpreted.

11.11. Conduct of analyses

Any deviation(s) or change(s) from the original statistical plan outlined in this protocol will be described and justified in the final study report.

11.11.1. Sequence of analyses

All analyses (including interim analysis) will be conducted on data as clean as possible.

(Amended: 29 January 2014)

- ***Primary a***nalysis of efficacy will be performed when ***21*** cases of definite pulmonary TB disease, meeting the first case definition, have accumulated in the ATP cohort for efficacy, during the efficacy surveillance period starting 1 month post dose 2 (Day 60) or at the latest when all subjects have completed the Month ***24*** visit. **(Amended: 29 January 2014)**
- A final analysis of the primary epoch including all efficacy, safety and immunology data will be performed at the end of the study, i.e. when all subjects have completed their Month 36 visit.
- A pooled analysis of efficacy data generated in this study and efficacy data generated in future efficacy studies is foreseen.

(Amended: 29 January 2014)

12. ADMINISTRATIVE MATTERS

To comply with ICH GCP administrative obligations relating to data collection, monitoring, archiving data, audits, confidentiality and publications must be fulfilled.

12.1. *eCRF* instructions (Amended: 29 January 2014)

***Inform*™** , a validated computer application, will be used as the method for data collection.

In all cases, subject initials will not be collected nor transmitted to GSK. Subject data necessary for analysis and reporting will be entered/transmitted into a validated database or data system. Clinical data management will be performed in accordance with applicable GSK standards and data cleaning procedures.

While completed eCRFs are reviewed by a Site Monitor designated by "The Collaboration" at the study site, omissions or inconsistencies detected by subsequent eCRF review may necessitate clarification or correction of omissions or inconsistencies with documentation and approval by the investigator or appropriately qualified designee. In all cases, the investigator remains accountable for the study data.

The investigator will be provided with a CD-ROM of the final version of the data generated at the investigational site once the database is archived and the study report is complete and approved by all parties.

12.2. Study Monitoring by "The Collaboration"

GSK and ***its partners in***"The Collaboration" will monitor the study to verify that, amongst others, the:

- Data are authentic, accurate, and complete.
- Safety and rights of subjects are being protected.
- Study is conducted in accordance with the currently approved protocol, any other study agreements, GCP and all applicable regulatory requirements. **(Amended: 29 January 2014)**

The investigator and the head of the medical institution (where applicable) agrees to allow the monitor direct access to all relevant documents.

The investigator must ensure provision of reasonable time, space and qualified personnel for monitoring visits.

Direct access to all study-site related and source data is mandatory for the purpose of monitoring review. The monitor will perform a***n eCRF*** review and a Source Document Verification (SDV). By SDV we understand verifying ***eCRF*** entries by comparing them with the source data that will be made available by the investigator for this purpose. **(Amended: 29 January 2014)**

The Source Documentation Agreement Form describes the source data for the different data in the ***eCRF***. This document should be completed and signed by the site monitor and investigator and should be filed in the monitor's and investigator's study file. Any data item for which the ***eCRF*** will serve as the source must be identified, agreed and documented in the source documentation agreement form. **(Amended: 29 January 2014)**

For ***eCRF***, the monitor will mark completed and approved screens at each visit. **(Amended: 29 January 2014)**

Upon completion or premature discontinuation of the study, the monitor will conduct site closure activities with the investigator or site staff, as appropriate, in accordance with applicable regulations, GCP, and "The Collaboration" procedures.

12.3. Record retention

Following closure of the study, the investigator must maintain all site study records (except for those required by local regulations to be maintained elsewhere) in a safe and secure location. The records must be easily accessible, when needed (e.g. audit or inspection), and must be available for review in conjunction with assessment of the facility, supporting systems, and staff. Where permitted by applicable laws/regulations or institutional policy, some or all of these records can be maintained in a validated format other than hard copy (e.g. microfiche, scanned, electronic); however, caution needs to be exercised before such action is taken. The investigator must ensure that all reproductions are legible and are a true and accurate copy of the original and meet accessibility and retrieval standards, including re-generating a hard copy, if required. Furthermore, the investigator must ensure that an acceptable back-up of the reproductions exists and that there is an acceptable quality control procedure in place for making these reproductions.

"The Collaboration" will inform the investigator/institution of the time period for retaining these records to comply with all applicable regulatory requirements. However, the investigator/institution should seek the written approval of the sponsor before proceeding with the disposal of these records. The minimum retention time will meet the strictest standard applicable to a particular site, as dictated by ICH GCP, any institutional requirements, applicable laws or regulations, or GSK standards/procedures; otherwise, the minimum retention period will default to 15 years.

The investigator/institution must notify "The Collaboration" of any changes in the archival arrangements, including, but not limited to archival at an off-site facility, transfer of ownership of the records in the event the investigator leaves the site.

12.4. Quality assurance

To ensure compliance with GCP and all applicable regulatory requirements, GSK or "The Collaboration" may conduct a quality assurance audit. Regulatory agencies may also conduct a regulatory inspection of this study. Such audits/inspections can occur at any time during or after completion of the study. If an audit or inspection occurs, the investigator and institution agree to allow the auditor/inspector direct access to all relevant documents and to allocate his/her time and the time of his/her staff to the auditor/inspector to discuss findings and any relevant issues.

12.5. Posting of information on publicly available clinical trial registers and publication policy

Study information from this protocol will be posted on publicly available clinical trial registers before enrolment of subjects begins.

Summaries of the results of GSK interventional studies (phase I-IV) are posted on publicly available results registers within 12 months of the primary completion date for studies of authorised vaccines and 18 months for studies of non-authorised vaccines.

GSK also aims to publish the results of these studies in the searchable, peer reviewed scientific literature. Manuscripts are submitted for publication within 24 months of the last subject's last visit.

12.6. Provision of study results to investigators

Where required by applicable regulatory requirements, an investigator signatory will be identified for the approval of the study report. The investigator will be provided reasonable access to statistical tables, figures, and relevant reports and will have the opportunity to review the complete study results at a GSK site or other mutually-agreeable location.

GSK Biologicals will also provide the investigator with the full summary of the study results. The investigator is encouraged to share the summary results with the study subjects, as appropriate.

13. COUNTRY SPECIFIC REQUIREMENTS

Not applicable.

14. REFERENCES

Centers for Disease Control and Prevention Metropolitan Atlanta Congenital Defects Program (CDC MACDP) guidelines. Birth defects and genetic diseases branch 6-digit code for reportable congenital anomalies; http://www.cdc.gov/ncbddd/birthdefects/documents/MACDPcode0807.pdf

Donald PR. Childhood tuberculosis: the hidden epidemic. *Int J Tuberc Lung Dis*. 2004: 8 (5); 627–629.

EMA Guideline on the exposure to medicinal products during pregnancy: need for post-authorization data (Doc. Ref. EMEA/CHMP/313666/2005) 'adopted at Community level in May 2006; http://www.ema.europa.eu/docs/en_GB/document_library/Regulatory_and_procedural_guideline/2009/11/WC500011303.pdf

Food and Drug Administration [FDA], 2007. Guidance for Industry, Toxicity Grading Scale for Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials. http://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Vaccines/ucm091977.pdf, accessed March 2012.

Fine PEM. Variation in protection by BCG: implications of and for heterologous immunity. *Lancet.* 1995; 346: 1339–1345.

Sterne JAC, Rodrigues LC, Guedes IN. Does the efficacy of BCG decline with time since Vaccination? *Int J Tuberc Lung Dis*.1998; 2 (3): 200–207.

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World Health Organisation [WHO], 2007. Verbal Autopsy Standards, Ascertaining and Attributing cause of death. http://www.who.int/healthinfo/statistics/verbal_autopsy_standards2.pdf, accessed April 2012.

World Health Organisation [WHO], 2009. Treatment of tuberculosis guidelines, fourth edition. http://whqlibdoc.who.int/publications/2010/9789241547833_eng.pdf, accessed April 2012.

World Health Organisation [WHO], ***2013***. ***Global tuberculosis report 2013***; ***http://www.who.int/tb/publications/global_report/en/, accessed 02 December 2013.***
(Amended: 29 January 2014)

APPENDIX A LABORATORY ASSAYS

Haematology/biochemistry

A complete blood count (CBC), renal and liver function tests will be measured at regular intervals throughout the study period. Laboratory reference values will be maintained by the Investigator in the study file and made available to GlaxoSmithKline Biologicals' Global Study Manager prior to study start.

Serum preparation

Serum for antibody determination will be collected at indicated time points. Blood for analysis of antibody responses will be obtained from each volunteer and allowed to clot for 30 minutes to 1 hour at room temperature, and serum will be separated and frozen at -70°C (+/- 5°C) or below until tested. All blood samples will be labelled with the study number and date obtained.

Whole blood (by capillary or venous route) should be collected observing appropriate aseptic conditions. It is recommended that Vacutainer® tubes WITH integrated serum separator (e.g. Becton-Dickinson Vacutainer® SST or Corvac® Sherwood Medical) be used so as to minimize the risk of haemolysis and to avoid blood cell contamination of the serum when transferring to standard serum tubes.

Serology (Antibody responses)

Immunogenicity (antibody levels) will be determined by evaluating antibody (IgG) responses to M72 as measured using standard ELISA. The enzyme-linked immunoassay (ELISA) utilises antibody and antigen interactions to test for either the presence of specific antibodies or antigens in an unknown sample. The antigens are chosen from the evaluated vaccine based on what is likely to be an immunogenic component. Briefly, partially purified M72 antigen is pre-coated onto a 96-well plate. Serum samples are added directly to the plate followed by a secondary anti-human antibody conjugated with an enzyme. The addition of substrate provides a means of detecting the serum antibody that is specific for the antigens in question. Positive control, calibrators are run on each plate in order to assess the relative titre of each test sample. Negative controls are also run on each plate to ensure specificity.

The cut-off of the anti-M72 ELISA is defined as 2.8 EU/mL. The laboratory designated by GSK uses standard and validated techniques and maintains a quality assurance log that outlines the performance of this particular assay over time.

Cell-mediated immune responses

Stimulation of whole blood

After collection, whole blood samples will be stimulated at 37°C with pool(s) of overlapping peptides covering the M72 antigen sequence and with the stimulation controls for 2 hours in the presence of anti-CD28 and anti-CD49d antibodies. Then, cytokine secretion inhibitor (Brefeldin A) will be added for an additional overnight incubation. The day after, red blood cells will be lysed and white blood cells will be washed and fixed prior to cryopreservation and kept in storage for subsequent cytometry analysis.

Intracellular Cytokine Staining (ICS)

Intracellular fluorescent staining for cytokines (ICS) provides information on the frequency of cells responding to one particular antigen; it allows to define the frequency of CD4+ and CD8+ T lymphocytes expressing molecules involved in immunity such as IFN-γ, IL-2, TNF-α, and/or CD40L (conventional CD4+ and CD8+ T cell response markers). Thawed cells will be stained for surface markers (e.g. CD4+ or CD8+ for T cells) and then fixed. Fixed cells will then be made permeable and stained with labelled cytokine-specific antibody. Cells will then be washed, re-suspended in PBS and analysed by flow cytometry.

Xpert MTB/RIF for Mtb detection

The Xpert MTB/RIF assay and the GeneXpert instrument consists of a single-use multi-chambered plastic cartridge preloaded with liquid buffers and lyophilized reagent beads necessary for sample processing, DNA extraction, and hemi-nested real-time PCR. Clinical sputum samples are treated with a NaOH and isopropanol-containing sample reagent (SR). The SR is added at a 2:1 ratio to the sputum sample or sputum pellet and incubated for 15 min at room temperature. The treated sample is transferred into the cartridge, the cartridge is loaded into the GeneXpert instrument, and an automatic process completes the remaining assay steps. The assay cartridge also contains lyophilized *Bacillus globigii* spores which serve as an internal sample processing and PCR control. The spores are automatically resuspended and processed during the sample processing step, and the resulting *B. globigii* DNA is amplified during the PCR step. The standard user interface indicates the presence or absence of *M. tuberculosis*, the presence or absence of RIF resistance, and a semi quantitative estimate of *M. tuberculosis* concentration (high, medium, low, and very low). Assays that are negative for *M. tuberculosis* and also negative for the *B. globigii* internal control are reported as invalid. The PCR assay amplifies a 192-bp segment of the *M. tuberculosis* rpoB gene in a heminested real-time PCR. The internal control heminested *B. globigii* assay is multiplexed with the *M. tuberculosis* assay. *M. tuberculosis* is detected using five overlapping molecular beacon probes (probes A to E) that are complementary to the entire 81-bp RIF resistance-determining “core” region of the wild-type rpoB gene (5, 7, 14). Mutations in the rpoB gene target inhibit hybridization of one or more of the rpoB-specific molecular beacons, reducing or eliminating the signal from the corresponding probes. *M. tuberculosis* is identified when at least two of the five rpoB-specific molecular beacons give a positive signal with cycle threshold (CT) values that are ≤ 38 and that differ by no more than two cycles. *B. globigii* DNA is detected when the single *B. globigii* molecular beacon produces a CT of < 38 cycles (adapted from: Blakemore, R.; Story, E.; Helb, D.; Kop, J.; Banada, P.; Owens, M.R.; Chakravorty, S.; Jones, M.; Alland, D. Evaluation of the Analytical Performance of the Xpert MTB/RIF assay. *J Clin Microbiol.* 2010, 48(7), 2495-501].

QuantiFERON® TB Gold assay

For evaluation of Mtb complex infection, whether tuberculosis disease, latent TB infection or past TB infection, the QuantiFERON®-TB Gold In-Tube Assay (Cellestis) will be used. QuantiFERON®-TB Gold In-Tube is an in vitro diagnostic test using peptide cocktails simulating ESAT-6, CFP-10 and TB7.7 proteins to stimulate cells in heparinised whole blood. These proteins are absent from all BCG strains and from most non-tuberculosis mycobacteria with the exception of M. kansarii, M. szulgai and M. marinum. The assay will be performed according to the manufacturer's instructions. Briefly, blood is collected directly into QuantiFERON®-TB Gold collection tubes including a Nil Control tube, TB antigen tube and Mitogen Control tube. The tubes should be incubated for 16-24 hours at 37°C prior to harvesting plasma. IFN-γ concentrations in plasma are determined using the QuantiFERON®-TB Gold ELISA kit.

APPENDIX B CLINICAL LABORATORIES

Table 26 GSK Biologicals' laboratories

Laboratory	Address
GSK Biologicals Global Vaccine Clinical Laboratory, Rixensart	Biospecimen Reception - B7/44 Rue de l'Institut, 89 - B-1330 Rixensart - Belgium
GSK Biologicals Global Vaccine Clinical Laboratory, North America- Laval	Biospecimen Reception - Clinical Serology 525 Cartier blvd West - Laval - Quebec - Canada - H7V 3S8
GSK Biologicals Global Vaccine Clinical Laboratory, Wavre-Nord Noir Epine	Avenue Fleming, 20 - B-1300 Wavre - Belgium

Table 27 Outsourced laboratories (Amended: 29 January 2014)

Laboratory	Address
CEVAC - University of Gent	De Pintelaan, 185 Gent Belgium
Central laboratories	
BARC South Africa Pty Ltd	Napier Road POB 8475 2000 Johannesburg Republic of South Africa
KEMRI/CDC Research Station, Kenya	Busia Road POB 1578-40100 Kisumu Kenya
CIDRZ Zambia	Alick Nkhata Road Kalingalinga Clinic Grounds Lusaka Zambia

APPENDIX C AMENDMENTS AND ADMINISTRATIVE CHANGES TO THE PROTOCOL

GlaxoSmithKline Biologicals Clinical Research & Development **Protocol Amendment 1**	
eTrack study number and Abbreviated Title(s)	115616 (TUBERCULOSIS-018)
Amendment number:	Amendment 1
Amendment date:	20 July 2012
Co-ordinating author:	PPD, Project Manager Scientific Writing XPE Pharma & Science for GSK Biologicals

Rationale/background for changes:
The protocol was amended due to a change in the formulation of the placebo to be used in the study.
Also the maximum percentage of females/males to be enrolled in the study was changed to allow for rapid enrolment while assuring a balanced study population in terms of gender distribution.
Additional changes for recording height and weight were made in the study procedures table.

Amended text has been included in *bold italics* and deleted text in ~~strikethrough~~ in the following sections:

Section 5.1 Number of subjects/centres

To ensure the gender of the study population is equally distributed between females and males, a cap has been put on the number of subjects per gender to be enrolled. A maximum of ***60*** ~~55~~% of subjects from the same gender will be enrolled in the study and enrolment of females/males will be stopped at approximately ***4200*** ~~3850~~subjects of any gender maximum***.***

Section 6.5 Outline of study procedures

Table 5 List of study procedures

	Visit 1 Screening visit	Visit 2	Visit 3[1]	Visit 4	Visit 5[1]	Visit 6[2]	Visit 7	Contact 1, 2, 3 and 4	Visit 8	Contact 5, 6, 7, 8, 9	Visit 9	Contact 10, 11, 12, 13, 14	Visit 10
Time point (s)	**D -30**	**D 0**	**D 7**	**D 30**	**D 37**	**D 44**	**M 2**	**M 4, 6, 8, 10**	**M 12**	**M 14, 16, 18, 20, 22**	**M 24**	**M 26, 28, 30, 32, 34**	**M 36**
Sampling Time point(s)	**Pre V**	**Pre V**	**Post V1**	**Post V1**	**Post V2**	**Post V2**	**Post V2**	**Post V2**	**Post V2**	**Post V2**	**Post V2**	**Post V2**	**Post V2**
Dose		**1**		**2**									
All subjects													
Informed consent	●												
Check inclusion/exclusion criteria	●	●											
Collect demographic data	●												
Medical history	●	●											
Physical examination	●												
History-directed physical examination		O	O	O	O	O	O						
Resting vital signs	O	O		O									
HIV testing[3] (~ 2.5 mL/test)	●												
Record weight ~~and height~~[4]		●							●		●		●
Documentation of history of BCG vaccination/presence of scar	●												
Pregnancy test (β-HCG urine)	●	●		●									
Study group and treatment number allocation		O											
Treatment number allocation for second dose				O									
Vaccination		●		●									
Recording of administered treatment number		●		●									
Check contraindications to subsequent vaccination		●		●									

	Visit 1 Screening visit	Visit 2	Visit 3 [1]	Visit 4	Visit 5[1]	Visit 6[2]	Visit 7	Contact 1, 2, 3 and 4	Visit 8	Contact 5, 6, 7, 8, 9	Visit 9	Contact 10, 11, 12, 13, 14	Visit 10
Time point (s)	**D -30**	**D 0**	**D 7**	**D 30**	**D 37**	**D 44**	**M 2**	**M 4, 6, 8, 10**	**M 12**	**M 14, 16, 18, 20, 22**	**M 24**	**M 26, 28, 30, 32, 34**	**M 36**
Sampling Time point(s)	**Pre V**	**Pre V**	**Post V1**	**Post V1**	**Post V2**	**Post V2**	**Post V2**	**Post V2**	**Post V2**	**Post V2**	**Post V2**	**Post V2**	**Post V2**
Dose		**1**		**2**									
Recording concomitant medication/vaccination	●	●	●	●	●	●	●	●	●	●	●	●	●
Recording intercurrent medical condition		●	●	●	●	●	●	●	●	●	●	●	●
Distribution of diary cards		O		O									
Return of diary cards				O			O						
Recording of unsolicited AEs		●	●	●	●	●	●						
Pre-vaccination assessment (including temperature)		●		●									
Blood sampling for RNA expression profiling (~2.5 mL)[5]		●				●							
Reporting SAEs	●[6]	●	●	●	●	●	●	●[7]	●[6]	●[6]	●[6]	●[6]	●[6]
Reporting pIMDs		●	●	●	●	●	●	●[8]					
Reporting pregnancy		●	●	●	●	●	●	●[8]					
Diagnostic procedures for suspected pulmonary TB[9]	●	●	●	●	●	●	●	●	●	●	●	●	●
Documentation of TB cases[10]			●	●	●	●	●	●	●	●	●	●	●
Safety and immune sub-cohort													
Measuring Vitamin D		●											
Recording of solicited AEs		●	●	●	●								
CBC (Hb, PLT and WBC), ALT, AST, creatinine, bilirubin (~ 8 mL)		●	●	●	●								
Blood sampling for CMI (~ 18 mL)		●	●	●	●		●		●		●		●
Blood sampling for humoral immunogenicity (~ 2 mL)		●		●			●		●		●		●

	Visit 1 Screening visit	Visit 2	Visit 3 [1]	Visit 4	Visit 5[1]	Visit 6[2]	Visit 7	Contact 1, 2, 3 and 4	Visit 8	Contact 5, 6, 7, 8, 9	Visit 9	Contact 10, 11, 12, 13, 14	Visit 10
Time point (s)	**D -30**	**D 0**	**D 7**	**D 30**	**D 37**	**D 44**	**M 2**	**M 4, 6, 8, 10**	**M 12**	**M 14, 16, 18, 20, 22**	**M 24**	**M 26, 28, 30, 32, 34**	**M 36**
Sampling Time point(s)	**Pre V**	**Pre V**	**Post V1**	**Post V1**	**Post V2**	**Post V2**	**Post V2**	**Post V2**	**Post V2**	**Post V2**	**Post V2**	**Post V2**	**Post V2**
Dose		**1**		**2**									
Blood sampling for QuantiFERON assay (~ 3 mL)		●							●		●		●
Study conclusions for all subjects													●

Note: The double border at Month 36 indicates the final analyses which will be performed on all data obtained up to month 36.
Pre V: Pre Vaccination; V: Vaccination; Post V: Post Vaccination
● is used to indicate a study procedure that requires documentation in the individual eCRF.
○ is used to indicate a study procedure that does not require documentation in the individual eCRF.
1 Only subjects in the safety and immune sub-cohort need to come for this visit.
2 The most informative time point for collection of blood samples for RNA expression profiling post dose 2 will be determined in a separate study (116777 [TUBERCULOSIS-019]). Depending on the results of this study, the exact time point for blood sampling post dose 2 (Visit 6) will be determined. The exact time point will be communicated to the investigators appropriately at the latest at study initiation.
3 HIV testing and pre-test counselling will be performed at screening in areas were HIV-prevalence > 1%.
4 Weight ~~and height~~ also need***s*** to be recorded ~~at the time of first occurrence of~~ ***whenever TB disease is confirmed*** (definite and clinical).
5 Samples for RNA expression profiling will also be taken at the time of TB diagnosis.
6 Only SAEs related to study participation or concurrent GSK medication/vaccine.
7 SAEs not related to study participation or concurrent GSK medication/vaccines will be recorded until 6 months post dose 2 (M7).
8 To be recorded until 6 months post dose 2 (M7).
9 Sputum samples for Xpert MTB/RIF testing and microbiological culture will be taken throughout the study if a subject presents with clinical suspicion of pulmonary TB disease. At screening, only samples for Xpert MT/RIF will be collected when a subject presents with clinical suspicion of TB disease. Three sputum samples, preferably taken in the morning and within one week, must be collected. Additionally, when a subject is diagnosed with TB, an HIV test must be performed (~2.5 mL/test). If the subject is confirmed HIV-positive, blood samples must be taken (~2.5 mL) to measure CD4 counts.
10 Documentation of TB cases will be done throughout the study; this includes documentation of cases during active and passive follow-up and monitoring of medical facilities and pharmacies.

Section 7.1 Description of study vaccines

Table 17 Study vaccines

Treatment name	Vaccine/product name	Formulation	Presentation	Volume to be administered	Number of doses
M72/AS01$_E$	M72	M72 (10 µg); sucrose; polysorbate; TRIS	Lyophilised cake in vial	0.5 ml	2
	AS01$_E$	25µg MPL, 25 µg QS21 in a liposome-based formulation	Liquid in vial		
Placebo	Placebo pellet	5 % Sucrose in phosphate buffer	Lyophilised cake in vial	0.5 ml	2
	Placebo adjuvant	***23.8*** ~~14.8~~µg α-Tocopherol, ***21.4 µg squalene and 9.7 µg Tween 80***	Liquid in vial		

GlaxoSmithKline Biologicals

Clinical Research & Development

Protocol Amendment 2

eTrack study number and Abbreviated Title:	115616 (TUBERCULOSIS-018)
Amendment number:	Amendment 2
Amendment date:	10 September 2012
Co-ordinating author:	PPD, Scientific Writer XPE Pharma&Science, contractor for GSK Biologicals

Rationale/background for changes:

- Less informative time points (Days 7, 30 and 37) for blood sampling for cell-mediated immune responses and humoral immune responses (Day 30) were removed to reduce the logistical burden on the study centres.
- HIV diagnostic testing at screening and at the time of TB diagnosis, was adapted to align with local clinical practice.
- Due to the limitations of the statistical program, the confidence intervals and the two-sided alpha values were adjusted for the interim and final analyses.
- The definition of clinical suspicion of pulmonary TB was updated.
- To ascertain sufficient volume for diagnostic testing, the sputum sample volume was increased.
- Diary card completion guidelines were added.
- Diagnostic testing at screening for subjects with signs and symptoms of TB disease was removed. Subjects with clinical suspicion of TB disease at the time of screening will not be included in the study.
- To illustrate the collaboration between GSK Biologicals and Aeras in the operational conduct of the study the term 'The collaboration' was introduced and inserted where applicable.
- Additional minor corrections were made.

Amended text has been included in *bold italics* and deleted text in ~~strikethrough~~ in the following sections:

Title page

Contributing Authors Aeras	• PPD, Chief Scientific Officer • PPD ***, Director Clinical Development***

Synopsis

Objectives

Primary

Success criterion:

*The lower limit of the ~~95.02~~**95.1**% two-sided confidence interval (CI) for the VE against first occurrence of Definite pulmonary TB disease not associated with HIV-infection, meeting the first case definition, is above 0%.*

Secondary

Efficacy

Success criterion:

*The lower limit of the ~~95.02~~ **95.1**% two-sided confidence interval (CI) for the VE against first occurrence of Definite Xpert MTB/Rif positive pulmonary TB disease not associated with HIV-infection, meeting the second case definition, is above 0%*

Study design

- Sampling schedule:
  - Blood samples will be collected in the safety and immune sub-cohort for evaluation of biochemistry/haematology parameters (Days 0, 7, 30 and 37), CMI responses (Days 0, ~~7, 30, 37,~~ 60 and Years 1, 2 and 3), humoral immune responses (Days 0~~, 30,~~ 60 and Years 1, 2 and 3) and QuantiFERON status (Day 0 and Years 1, 2 and 3).
  - Blood samples for RNA expression profiling will be collected from all subjects (Days 0,~~ and~~ post dose 2 ***and when TB is diagnosed***).

Case definitions

Fourth Case definition – Definite pulmonary TB

*Clinical suspicion of pulmonary TB is defined as ***a subject*** presenting with one or more of the following symptoms: ***unexplained*** cough > ~~1~~ 2 weeks, ***unexplained*** fever > 1 week, night sweats, ***unintentional*** weight loss, pleuritic chest pains, haemoptysis, fatigue or shortness of breath on exertion.

Endpoints

Secondary

Immunogenicity

- Evaluation of CMI responses with respect to components of the study vaccine, in the safety and immune sub-cohort:

  Time points: prior to dose 1 (Day 0), ~~post dose 1 (Days 7 and 30)~~ and post dose 2 (Days~~ 37,~~ 60 and Years 1, 2 and 3).

- Evaluation of humoral immune responses with respect to components of the study vaccine, in the safety and immune sub-cohort:

  Time points: prior to dose1 (Day0)~~, post dose 1 (Day 30)~~ and post dose 2 (Day 60 and Years 1, 2 and 3)

Tertiary

- Description of RNA expression profile signatures in selected samples as a correlate of risk to describe protective and non-protective immune responses after 2 doses of M72/AS01$_E$.

  Time points: Prior to dose 1 (Day 0) and post dose 2 ***and when TB is diagnosed.***

Glossary of terms

Clinical suspicion of pulmonary TB	A subject has clinical suspicion of pulmonary TB when he/she presents with one or more of the following symptoms: ***unexplained*** cough > ~~1~~ 2 weeks, ***unexplained*** fever > 1 week, night sweats, ***unintentional*** weight loss, pleuritic chest pains, haemoptysis, fatigue or shortness of breath on exertion.
"The Collaboration"	***Refers to the joint collaboration between GSK Biologicals and Aeras. GSK will act as the sponsor of this study whereas Aeras will provide financial and operational support.***

1.2. Collaboration between GSK Biologicals and Aeras

This study is a joint collaboration between GSK Biologicals and Aeras, ***referred to as "The Collaboration" in the remainder of this document***.

2.1. Primary objective

Success criterion to be used for the primary objective:

*The lower limit of the ~~95.02~~**95.1**% two-sided confidence interval (CI) for the VE against first occurrence of Definite pulmonary TB disease not associated with HIV-infection, meeting the first case definition, is above 0%.*

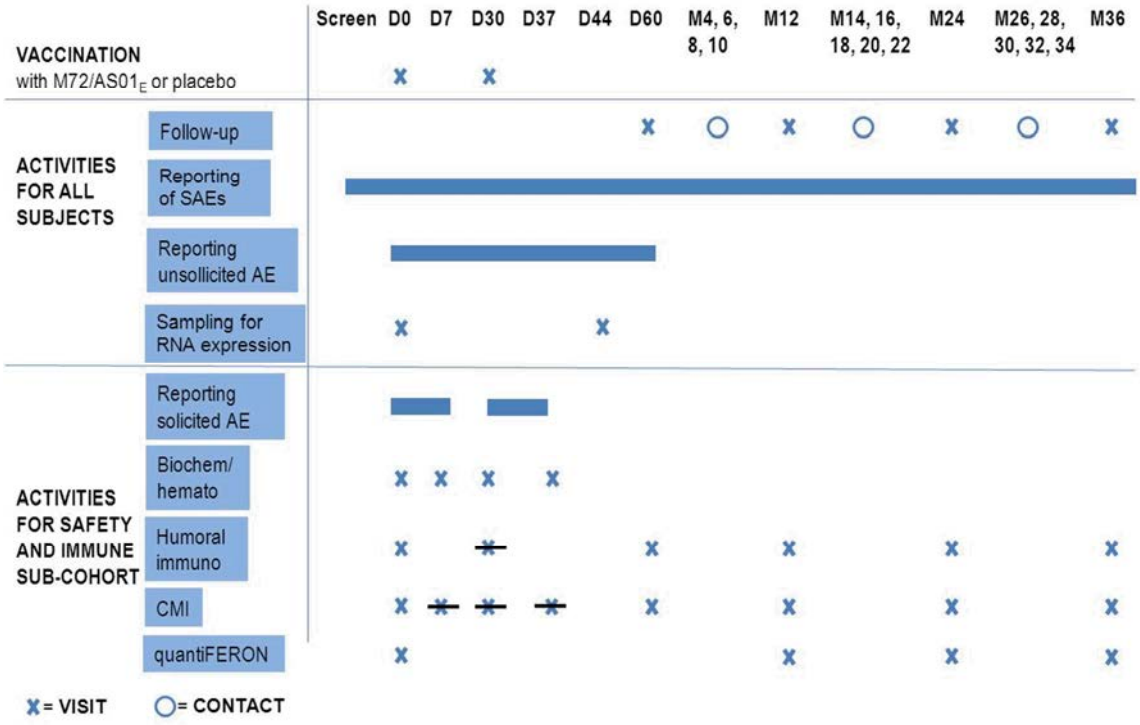
2.2. Secondary objectives

Efficacy

If the primary objective is met, this secondary objective will be analysed using the following success criterion:

*The lower limit of the ~~95.02~~**95.1**% two-sided confidence interval (CI) for the VE against first occurrence of Definite Xpert MTB/Rif positive pulmonary TB disease not associated with HIV-infection, meeting the second case definition, is above 0%.*

3. Study Design Overview



Sampling schedule:

- Blood samples will be collected in the safety and immune sub-cohort for evaluation of biochemistry/haematology parameters (Days 0, 7, 30 and 37), CMI responses (Days 0, ~~7, 30, 37~~ and 60 and Years 1, 2 and 3), humoral immune responses (Days 0~~,~~ ~~30~~ and 60 and Years 1, 2 and 3) and QuantiFERON status (Days 0 and Years 1, 2 and 3).
- Blood samples for RNA expression profiling will be collected from all subjects at predefined time points (Days 0 and Day 44 *****) and whenever a subject is diagnosed with TB disease.

  **** The most informative time point for collection of blood samples for RNA expression profiling post-dose 2 will be determined. The exact time point will be communicated to the investigators appropriately at the latest at study initiation.***

Diagnostic procedures for suspected Pulmonary TB: ~~At screening, when a subject presents with clinical suspicion of pulmonary TB, three respiratory sputum samples, preferably taken in the morning, within a one week interval, must be collected for testing with Xpert MTB/RIF to rule out pulmonary TB disease.~~ during the entire study, when a subject presents with clinical suspicion of pulmonary TB, three respiratory sputum samples, preferably taken in the morning, within a one week interval, must be collected for testing with Xpert MTB/RIF and microbiological culture.

5.1. Number of subjects/centres

There will be one sub-cohort for safety/reactogenicity and immunogenicity. A maximum of 500 eligible subjects from selected centres in India ***and*** South Africa ~~and Kenya~~ will be included in this safety and immune sub-cohort (Table 4).

Table 4 Sub-cohorts

Sub-cohort name	Description	Estimated number of subjects
Safety and immune sub-cohort	Safety parameters for this sub-cohort will be collected as follows: • Solicited AEs will be collected for 7 days (day of vaccination and 6 subsequent days) following vaccination. • Serum biochemistry/haematology values will be measured at Days 0, 7, 30, and 37.	Approximately 450
	Immunogenicity: • Humoral immunogenicity will be evaluated by measuring M72-specific IgGs at Days 0~~, 30~~ and 60 and Years 1, 2 and 3. • CMI will be evaluated by measuring M72-specific CD4+ and CD8+ T cells expressing at least 2 immune markers (CD40-L and/or IFN-γ and/or IL-2 and/or TNF-α) at Days 0, ~~7,30, 37~~ and 60 and Years 1, 2 and 3. • The QuantiFERON status of subjects will be determined at Day 0 and Years 1, 2 and 3.	

5.2. Inclusion criteria for enrolment

- Healthy subjects ***or those with chronic well-controlled disease*** as established by medical history and clinical examination ~~before entering into the study~~.

6.2.2.1. Randomisation of supplies

To allow ~~GSK Biologicals~~ ***"The Collaboration"*** to take advantage of greater rates of recruitment than anticipated at individual centres in this multi-centre study and to thus reduce the overall study recruitment period, an over-randomisation of supplies will be prepared.

6.2.2.2.1. Study group and treatment number allocation

Allocation of the subject to a study group at the investigator site will be performed using a randomisation system on internet (SBIR). The randomisation algorithm will use a minimisation procedure accounting for:

- Gender: a maximum ~~of 3850~~ ***4200*** females/males will be recruited.
- TB disease categorised as history or no history of TB disease.

Minimisation factors will have equal weight in the minimisation algorithm.

6.2.3 Allocation of subjects to assay subsets

Approximately 500 subjects will be included in the safety and immune sub-cohort. These subjects will be enrolled at selected centres in India~~,~~ ***and*** South Africa ~~and Kenya~~. Subjects included in the sub-cohort will be equally distributed between Africa and India. Blood sampling and safety assessment will be performed as described in Table 4.

6.4.1 Surveillance for *safety and* efficacy

Surveillance for efficacy starts with administration of the first dose of study vaccine or placebo.

6.4.1.1. Active follow-up *for safety and efficacy*

Subject ***safety*** follow-up and timely case capture of incident TB disease is of paramount importance. In addition to study visits at the study facilities, regular contacts (every two months) with the study subjects must be maintained using one or more of the following methods:

- Regular interval home visits by ***site staff*** ~~field workers~~
- Phone calls to inquire about current health status, completed by home visits if the subject cannot be reached.
- 2-way short message service (SMS) exchange.

These active follow-up procedures for surveillance of ***safety and*** efficacy will be described in local study Standard Operating Procedures and Informed Consent Forms. Informed Consent Forms will be submitted to the Ethics Committee for approval prior to implementation.

6.4.1.2. Passive follow-up *for efficacy*

6.4.2. Diagnostic procedures for the detection of suspected pulmonary TB

~~At screening, when a subject presents with clinical suspicion of pulmonary TB, three respiratory sputum samples, preferably taken in the morning, must be collected, within a one week interval, for testing with Xpert MTB/RIF to rule out pulmonary TB disease.~~

[…]

~~The method of diagnosis and results must be recorded in the subjects' eCRF.~~

~~Height and w~~***W***eight of the subject must also be recorded in the subjects' eCRF at the time of TB diagnosis.

[…]

~~In case smear microscopy is performed to diagnose pulmonary TB, the results must be recorded in the subjects' eCRF.~~

~~Height and weight of the subject must also be recorded in the subjects' eCRF at the time of TB diagnosis.~~

6.4.5.2. Independent Data Monitoring Committee

An independent statistical team (i.e., comprising members who are ~~not~~ ***neither*** GSK employees ***neither Aeras employees*** and who are not involved in the study management) will be appointed by GSK Biologicals. This team will be unblinded to treatment assignment and provide all necessary tables, listings, figures and individual subject data to the IDMC.

6.5. Outline of study procedures

Table 5 List of study procedures

	Visit 1 Screening visit	Visit 2	Visit 31	Visit 4	Visit 51	Visit 62	Visit 7	Contact 1, 2, 3 and 4	Visit 8	Contact 5, 6, 7, 8, 9	Visit 9	Contact 10, 11, 12, 13, 14	Visit 10
Time point(s)	D-30	D 0	D 7	D 30	D 37	D 44	M 2	M 4, 6, 8, 10	M 12	M 14, 16, 18, 20, 22	M 24	M 26, 28, 30, 32, 34	M 36
Sampling Time point(s)	Pre V	Pre V	Post V1	Post V1	Post V2	Post V2	Post V2	Post V2	Post V2	Post V2	Post V2	Post V2	Post V2
Dose		1		2									
All subjects													
Informed consent	●												
Check inclusion/exclusion criteria	●	●											
Collect demographic data	●												
Medical history	●	●											
Physical examination	●												
History-directed physical examination		O	O	O	O	O	O						
Resting vital signs	O	O		O									
HIV testing3 (***up to*** ~ 2.5 mL/test)	●												
Record weight4		●							●		●		●
Documentation of history of BCG vaccination/presence of scar	●												
Pregnancy test (β-HCG urine)	●	●		●									
Study group and treatment number allocation		O											
Treatment number allocation for second dose				O									
Vaccination		●		●									
Recording of administered treatment number		●		●									
Check contraindications to subsequent vaccination		●		●									

	Visit 1 Screening visit	Visit 2	Visit 31	Visit 4	Visit 51	Visit 62	Visit 7	Contact 1, 2, 3 and 4	Visit 8	Contact 5, 6, 7, 8, 9	Visit 9	Contact 10, 11, 12, 13, 14	Visit 10
Time point(s)	**D-30**	**D 0**	**D 7**	**D 30**	**D 37**	**D 44**	**M 2**	**M 4, 6, 8, 10**	**M 12**	**M 14, 16, 18, 20, 22**	**M 24**	**M 26, 28, 30, 32, 34**	**M 36**
Sampling Time point(s)	**Pre V**	**Pre V**	**Post V1**	**Post V1**	**Post V2**	**Post V2**	**Post V2**	**Post V2**	**Post V2**	**Post V2**	**Post V2**	**Post V2**	**Post V2**
Dose		**1**		**2**									
Recording concomitant medication/vaccination	●	●	●	●	●	●	●	●	●	●	●	●	●
Recording intercurrent medical condition		●	●	●	●	●	●	●	●	●	●	●	●
Distribution of diary cards		O		O									
Return of diary cards				O			O						
Recording of unsolicited AEs		●	●	●	●	●	●						
Pre-vaccination assessment (including temperature)		●		●									
Blood sampling for RNA expression profiling (~2.5 mL)5		●				●							
Reporting SAEs	●6	●	●	●	●	●	●	●7	●6	●6	●6	●6	●6
Reporting pIMDs		●	●	●	●	●	●	●8					
Reporting pregnancy		●	●	●	●	●	●	●8					
Diagnostic procedures for suspected pulmonary TB9	●	●	●	●	●	●	●	●	●	●	●	●	●
Documentation of TB cases10			●	●	●	●	●	●	●	●	●	●	●
Safety and immune sub-cohort													
Measuring Vitamin D		●											
Recording of solicited AEs		●	●	●	●								
CBC (Hb, PLT and WBC), ALT, AST, creatinine, bilirubin (~ 8 mL)		●	●	●	●								
Blood sampling for CMI (~ 18 mL)		●	●	●	●		●		●		●		●
Blood sampling for humoral immunogenicity (~ 2 mL)		●		●			●		●		●		●

	Visit 1 Screening visit	Visit 2	Visit 31	Visit 4	Visit 51	Visit 62	Visit 7	Contact 1, 2, 3 and 4	Visit 8	Contact 5, 6, 7, 8, 9	Visit 9	Contact 10, 11, 12, 13, 14	Visit 10
Time point(s)	**D-30**	**D 0**	**D 7**	**D 30**	**D 37**	**D 44**	**M 2**	**M 4, 6, 8, 10**	**M 12**	**M 14, 16, 18, 20, 22**	**M 24**	**M 26, 28, 30, 32, 34**	**M 36**
Sampling Time point(s)	**Pre V**	**Pre V**	**Post V1**	**Post V1**	**Post V2**	**Post V2**	**Post V2**	**Post V2**	**Post V2**	**Post V2**	**Post V2**	**Post V2**	**Post V2**
Dose		**1**		**2**									
Blood sampling for QuantiFERON assay (~ 3 mL)		●							●		●		●
Study conclusions for all subjects													●

Note: The double border at Month 36 indicates the final analyses which will be performed on all data obtained up to month 36.
Pre V: Pre Vaccination; V: Vaccination; Post V: Post Vaccination
● is used to indicate a study procedure that requires documentation in the individual eCRF.
○ is used to indicate a study procedure that does not require documentation in the individual eCRF.
1 Only subjects in the safety and immune sub-cohort need to come for this visit.
2 The most informative time point for collection of blood samples for RNA expression profiling post dose 2 will be determined ~~in a separate study (116777 [TUBERCULOSIS-019]). Depending on the results of this study, the exact time point for blood sampling post dose 2 (Visit 6) will be determined~~. The exact time point will be communicated to the investigators appropriately at the latest at study initiation.
3 HIV testing and pre-test counselling will be performed at screening in areas were HIV-prevalence > 1%***, according to the procedures detailed in Table 8***.
4 Weight also needs to be recorded whenever TB disease is confirmed (definite or clinical).
5 Samples for RNA expression profiling will also be taken at the time of TB diagnosis.
6 Only SAEs related to study participation or concurrent GSK medication/vaccine.
7 SAEs not related to study participation or concurrent GSK medication/vaccines will be recorded until 6 months post dose 2 (M7).
8 To be recorded until 6 months post dose 2 (M7).
9 Sputum samples for Xpert MTB/RIF testing and microbiological culture will be taken throughout the study if a subject presents with clinical suspicion of pulmonary TB disease. Three sputum samples, preferably taken in the morning and within one week, must be collected. Additionally, when a subject is diagnosed with TB, ***HIV testing must be performed according to the procedures detailed in Table 8. Up to ~ 2.5mL of blood will be collected per test.*** If the subject is confirmed HIV-positive, blood samples must be taken (2.5 mL) to measure CD4 counts.
10 Documentation of TB cases will be done throughout the study, ***starting at Visit 3 (Day 7)***; this includes documentation of cases during active and passive follow-up and monitoring of medical facilities and pharmacies.

Table 6 Intervals between study visits

Interval	Allowed interval[1]
Visit 1 - Visit 2	30 days - 1 days
Visit 2 - Visit 4	26 days - 35 days
Visit 4 - Visit 6	1 days - 25 days [2]
Visit 4 - Visit 7	14 days - 35 days
Visit 4 - Visit 8	10 months - 12 months
Visit 4 - Visit 9	22 months -24 months
Visit 4 - Visit 10	34 months - 36 months
Visit n – next contact	6 weeks – 12 weeks
Contact n – contact n+1	6 weeks – 12 weeks

1 Subjects may not be eligible for inclusion in the ATP cohort for analysis of immunogenicity and efficacy if they make the study visit outside this interval.
2 The most informative time point for collection of blood samples for RNA expression profiling post dose 2 will be determined ~~in a separated study (116777 [TUBERCULOSIS-019]). Depending on the results of this study, the exact time point for blood sampling post dose 2 (Visit 6) will be determined~~. The exact time point will be communicated to the investigators appropriately at the latest at study initiation.

6.6.1. Informed consent

The signed/witnessed/thumb printed informed consent of the subject must be obtained before study participation. Refer to Section 6.1 for the requirements on how to obtain informed consent as appropriate. ***Consenting and screening may be repeated, but only once, for subjects who would return out of screening interval but still agree to participate.***

6.6.11.1. Blood sampling for safety or immune response assessments

- For all subjects included in the safety and immune sub-cohort:
  - A volume of at least 8 mL of whole blood should be drawn for tests performed to assay for CBC, ALT, AST, ~~AP~~, bilirubin and creatinine at each predefined time point (Table 8). Vitamin D levels will be measured at Day 0 only.
- For all subjects:
  - ***At screening, HIV testing must be performed according to the procedures detailed in Table 8 in regions with high HIV prevalence (>1 %). Up to approximately 2.5 mL of blood will be collected for each test.***
  - When a subject is diagnosed with TB disease, ***HIV testing must be performed according to the procedures detailed in Table 8. Up to approximately 2.5 mL of blood will be collected for each test***. ~~at least 2.5 mL of blood will be collected for HIV testing. If the initial HIV test is positive, a confirmatory HIV test must be performed (at least 2.5 mL).~~ Additionally, if HIV is confirmed, at least 2 mL of blood will be collected to measure CD4 counts.
  - *Blood sampling for RNA expression profiling is planned for Day 0 (Visit 2) and Visit 6 (post dose 2). The most informative time point for collecting whole blood samples for RNA expression profiling post dose 2 will be determined ~~in a separate study (116777 [TUBERCULOSIS-019). Depending on the results of this study, the exact time point for blood sampling post dose 2 (Visit 6) will be~~

~~determined~~. The exact time point will be communicated to the Investigators appropriately at the latest at study initiation.

6.6.11.2. Sputum sample collection for TB diagnosis

All subjects will be required to provide 3 sputum samples, preferably taken in the morning, within a one week interval, of ~~at least~~ ***approximately*** ~~1.5~~ ***5*** mL to test for Mtb using the Xpert MTB/RIF assay and microbiological culture whenever the subject presents with clinical suspicion of pulmonary TB. ~~At screening, only Xpert MTB/RIF testing will be performed if the subject presents with clinical suspicion of pulmonary TB, to rule out definite pulmonary TB disease.~~ If needed, induction of sputum through nebulisation of hypertonic saline may be performed.

6.6.15. Recording of AEs, SAEs, pregnancies and pIMDs

- At each vaccination visit, diary cards will be provided to the subjects. Any unsolicited AEs (i.e. on the day of vaccination and during the next 29 days) occurring after vaccination will be recorded by all subjects. The subjects included in the safety and immune sub-cohort will also record body (axillary) temperature and any solicited local/general AEs (i.e. on the day of vaccination and during the next 6 days). ***In case of illiterate subjects, a designated person (such as a family member or a field worker) may provide assistance to complete the diary cards***. The subject will be instructed to return the completed diary card to the investigator at a later visit. ***In case a diary card is lost by the subject or he/she is unable to complete the diary card, the safety information should be captured retrospectively using a descriptive interview. This process will be documented by an Internal Operational Procedure (IOP). Please refer to the SPM for further details.***

6.7.1. Use of specified study materials

When materials are provided by ~~GSK Biologicals~~ ***"The Collaboration"***, it is MANDATORY that all clinical samples (including serum samples) be collected and stored exclusively using those materials in the appropriate manner. The use of other materials could result in the exclusion of the subject from the ATP analysis (See Section 11.5 for the definition of study cohorts/ data sets to be analysed). The investigator must ensure that his/her personnel and the laboratory(ies) under his/her supervision comply with this requirement. However, when ~~GSK Biologicals~~ ***"The Collaboration"*** does not provide material for collecting and storing clinical samples, appropriate materials from the investigator's site must be used.

6.7.2. Biological samples

Table 8 Biological samples

Sample type	Quantity	Unit	Time point	Subjects
Blood sampling for Safety assessment	At least 8	mL	Days 0, 7, 30 and 37[1]	Safety and immune sub-cohort[2]
Blood sampling for Humoral responses	At least 2	mL	Days 0~~, 30~~, 60 and Years 1, 2, 3	Safety and immune sub-cohort[2]
Blood sampling for CMI	At least 18	mL	Days 0~~, 7, 30, 37,~~ 60 and Years 1, 2, 3	Safety and immune sub-cohort[2]
Blood sampling for for QuantiFERON® TB Gold	At least 3	mL	Day 0 and Years 1, 2, 3	Safety and immune sub-cohort[2]
Blood sampling for RNA expression profiling	At least 2.5	mL	Days 0 and Day 44[3] ***and when TB diagnosed is confirmed***	All subjects
Sputum sampling for TB diagnosis[4]	~~at least 1.5~~ ***Approximately 5***	mL	Throughout the study ***when pulmonary TB is suspected***	All subjects ***with suspicion of pulmonary TB***
Blood sampling for initial HIV testing - ***Step 1 (Rapid test)***	***Finger prick or A****~~a~~****t*** least 2.5	mL	At screening~~ and whenever a subject is diagnosed with TB~~	All subjects[5] ***living in a region with HIV prevalence > 1%***
			When TB diagnosis is confirmed	***All subjects diagnosed with TB***
Blood sampling for HIV testing - Step 2 (Rapid test)	***Finger prick or at least 2.5***	***mL***	***At screening and when TB diagnosis is confirmed***	***All subjects positive at step 1[5]***
Blood sampling for confirmatory HIV testing - ***Step 3 (ELISA)***	At least 2.5	mL	~~When initial HIV- test is positive~~ ***At screening[5]***	~~All subjects[5]~~ ***Discordant Rapid test results or if no Rapid test performed[6]***
			When TB diagnosis is confirmed	***At least one positive Rapid test or if no Rapid test performed[6]***
Blood sampling for PCR - Step 4	***At least 2.5***	***mL***	***When TB diagnosis is confirmed***	***Indeterminate ELISA results or no ELISA performed and at least one positive Rapid test[6]***
Blood sampling for CD4 count	At least 2.5	mL	When~~ever a subject is diagnosed with~~ TB ***diagnosis is confirmed*** and confirmed HIV-positive	~~All subjects~~ ***HIV positive subjects with TB diagnosis confirmed***

1 Vitamin D levels will be measured at day 0 only.
2 Refer to Section 5.1 for sub-cohort description.
3 The most informative time point for collection of blood samples for RNA expression profiling post dose 2 will be determined ~~in a separate study (116777 [TUBERCULOSIS-019]). Depending on the results of this study, the exact time point for blood sampling post dose 2 (Visit 6) will be determined~~. The exact time point will be communicated to the investigators appropriately at the latest at study initiation. Additionally, samples for RNA expression profiling must be collected at the time of TB diagnosis.
4 Three sputum samples, preferably taken in the morning, within a one week interval, will be collected whenever a subject presents with clinical suspicion of pulmonary TB.
~~5 HIV testing at screening will only be performed for subjects living in a region with HIV prevalence > 1%.~~
5 HIV positive subjects at screening will be referred for confirmatory HIV diagnosis/management as per site SOPs
6 Only the final conclusive diagnosis is to be recorded in the CRF.

6.7.3. Laboratory assays

The main laboratory assays will be performed at GSK Biologicals' laboratory or in a laboratory designated by ~~GSK Biologicals~~ ***"The Collaboration"*** using standardised and/or qualified procedures (Table 9 – Table 14).

Table 11 RNA expression profiling

System	Component	Method	Unit	Laboratory*
Whole blood	RNA expression	Microarray	NA	As designated by ~~GSK Biologicals~~ ***"The Collaboration"***

*Refer to APPENDIX B for the laboratory addresses.

Table 12 Diagnostic tests for pulmonary TB disease

System	Component	Method	Unit	Laboratory*	Component priority ranking
Sputum	Mycobacterium tuberculosis DNA	Xpert MTB/RIF assay	NA	As designated by ~~GSK Biologicals~~ ***"The Collaboration"***	1
Sputum	Mycobacterium tuberculosis	Microbiological culture	NA	As designated by ~~GSK Biologicals~~ ***"The Collaboration"***	2

*Refer to APPENDIX B for the laboratory addresses.

Table 13 Diagnostic tests for HIV

System	Component	Method	Unit	Laboratory*
Whole Blood or Serum	***Anti-HIV antibodies, screening step 1, step 2***	***HIV Rapid Test****	***NA***	***As designated by "The Collaboration"***
Serum	Anti-HIV antibodies, ***screening step 3***	ELISA~~***~~	NA	As designated by ~~GSK Biologicals~~ ***"The Collaboration"***
Serum	Anti-HIV antibodies, ***screening step 4***	Western Blot~~****~~	NA	As designated by ~~GSK Biologicals~~ ***"The Collaboration"***
Whole Blood	***HIV RNA, screening step 4***	***HIV PCR***	***NA***	***As designated by "The Collaboration"***
Whole blood	CD4 cells	Flow cytometry	Cells/mm^3	As designated by ~~GSK Biologicals~~ ***"The Collaboration"***

*Refer to APPENDIX B for the laboratory addresses.
Two different Rapid HIV tests to be used sequentially
~~***ELISA will be used for initial HIV testing.~~
~~****Western Blot will be used for confirmatory HIV testing.~~

Table 14 Haematology, Serum Chemistry, Urine tests

System	Component	Method	Scale
Whole blood	Alanine amino transferase	As per ***laboratory standard procedures*** ~~Investigator's laboratory~~	Quantitative
	Aspartate amino transferase		
	Creatinine		
	Bilirubin (total)		
	Platelets		
	Leucocytes		
	Erythrocytes		
	Haemoglobin		
	Vitamin D		
	IFN-γ	QuantiFERON® TB Gold Assay	Qualitative
Urine	β-HCG	As per ***laboratory standard procedures*** ~~Investigator's laboratory~~	Qualitative

6.7.4.1. Diagnostic assays

Xpert MTB/RIF testing (Cepheid) and microbiological culture will be performed on three sputum samples, preferably taken in the morning, within a one week interval, for all subjects presenting with clinical suspicion of pulmonary TB throughout the entire study. ~~At screening, only Xpert MTB/RIF testing will be performed if the subject presents with clinical suspicion of pulmonary TB, to rule out definite pulmonary TB disease.~~

HIV testing and pre-test counselling will be performed at screening in regions with high HIV prevalence (> 1%). ***HIV positive subjects at screening will be referred for confirmatory HIV diagnosis/management as per site SOPs.***

[…]

All diagnostic assays will be carried out at the laboratory of the investigator or in a laboratory designated by ~~GSK Biologicals~~ ***"The Collaboration"***. ~~Results of diagnostic assays will be documented in the subjects' eCRF.~~

6.7.4.2. Immunological read-outs

Flow cytometry using a short-term stimulation Intracellular Cytokine Staining (ICS) assay on frozen Peripheral Blood Mononuclear Cells (PBMCs) will be used to characterise M72-specific CD4+/CD8+ T cells expressing at least two immune markers (CD40L and/or IL-2 and/or TNF-α and/or IFN-γ) on Days 0~~, 7, 30, 37~~ and 60, and Years 1, 2 and 3.

[…]

The humoral immune response will be evaluated by assessment of antibody titres to M72 by specific ELISA on Days 0~~, 30~~ and 60, and Years 1, 2 and 3.

Table 15 Immunological read-outs

Blood sampling time point		Sub-cohort Name	No. subjects	Component
Type of contact and time point	Sampling time point			
Visit 2 (Day 0)	Pre Vacc	Safety and immune sub-cohort	500	M72-specific T-cells (ICS on PBMCs) Anti M72 Ab (ELISA on serum)
~~Visit 3 (Day 7)~~	~~Post Vacc 1~~			~~M72-specific T-cells (ICS on PBMCs)~~
~~Visit 4 (Day 30)~~	~~Post Vacc 1~~			~~M72-specific T-cells (ICS on PBMCs)~~ ~~Anti M72 Ab (ELISA on serum)~~
~~Visit 5 (Day 37)~~	~~Post Vacc 2~~			~~M72-specific T-cells (ICS on PBMCs)~~
Visit 7 (Day 60)	Post Vacc 2			M72-specific T-cells (ICS on PBMCs) Anti M72 Ab (ELISA on serum)
Visit 8 (Year 1)	LTFU			M72-specific T-cells (ICS on PBMCs) Anti M72 Ab (ELISA on serum)
Visit 9 (Year 2)	LTFU			M72-specific T-cells (ICS on PBMCs) Anti M72 Ab (ELISA on serum)
Visit 10 (Year 3)	LTFU			M72-specific T-cells (ICS on PBMCs) Anti M72 Ab (ELISA on serum)

6.7.4.3. Haematology/Biochemistry

Serum biochemistry and haematology tests (Hb, PLT, WBC, ALT, AST, bilirubin, creatinine) will be performed in the safety and immune sub-cohort on Days 0, 7, 30 and 37. To characterise the study population, vitamin D levels will be measured at baseline in the sub-cohort. The tests will be performed at the laboratory of the investigator's site or in a laboratory designated by ~~GSK Biologicals~~ ***"The Collaboration"***. Results for WBCs, haemoglobin, creatinine, ALT, AST, total bilirubin and vitamin D will be recorded in the subject's eCRF ***or transferred directly to the clinical database***.

9.1.4. Clinical laboratories parameters and other abnormal assessments qualifying as adverse events or serious adverse events

Table 20 Toxicity grading table for laboratory abnormalities

	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)*
Haematology				
Hemoglobin (Female) - g~~m~~/dL	11.0 – 12.0	9.5 – 10.9	8.0 – 9.4	< 8.0
Hemoglobin (Female) change from baseline value - g~~m~~/dL	Any decrease – 1.5	1.6 – 2.0	2.1 – 5.0	> 5.0
Hemoglobin (Male) - g~~m~~/dL	12.5 – 13.5	10.5 – 12.4	8.5 – 10.4	< 8.5
Hemoglobin (Male) change from baseline value – g~~m~~/dL	Any decrease – 1.5	1.6 – 2.0	2.1 – 5.0	> 5.0

11.2. Secondary endpoints

Immunogenicity

- Evaluation of CMI responses with respect to components of the study vaccine, in the safety and immune sub-cohort.

Time points: prior to dose 1 (Day 0)~~, post dose 1 (Days 7 and 30)~~ and post dose 2 (Days~~ 37,~~ 60 and Years 1, 2 and 3).

- Evaluation of humoral immune responses with respect to components of the study vaccine, in the safety and immune sub-cohort.

  Time points: prior to dose 1 (Day 0)~~, post dose 1 (Day 30)~~ and post dose 2 (Day 60, Years 1, 2 and 3)

11.3. Tertiary endpoints

- Description of RNA expression profile signatures in selected samples as a correlate of risk to describe protective and non-protective immune responses after 2 doses of M72/AS01$_E$.

  Time points: Prior to dose 1 (Day 0) and post dose 2 (post dose 2) ***and when TB is diagnosed.***

11.4. Determination of sample size

The two sided Type I error alpha is divided as ~~0.0002~~ ***0.001*** and ~~0.0498~~ ***0.049*** at interim and final analysis, respectively.

The study will only ***be fully analysed and unblinded early if extremely high efficacy is observed at the interim analysis*** ~~be stopped for efficacy if extremely high efficacy is observed at the interim analysis~~. Therefore a very small alpha is used for the IA.

11.8. Analysis of efficacy

*The lower limit of the ~~99.98~~**99.9**% two-sided confidence interval for the VE against first occurrence of definite pulmonary TB disease, meeting the first case definition, is above 0% (this corresponds to a two-sided alpha of ~~0.02~~**0.1**%).*

*The lower limit of the ~~95.02~~**95.1**% two-sided confidence interval for the VE against first occurrence of definite pulmonary TB disease, meeting the first case definition is above 0% (this corresponds to a two-sided alpha of ~~4.98~~**4.9**%).*

*The lower limit of the ~~95.02~~**95.1**% two-sided confidence interval (CI) for the VE against first occurrence of Definite Xpert MTB/Rif positive pulmonary TB disease not associated with HIV-infection, meeting the second case definition, is above 0%.*

11.10. Analysis of safety

- Injection site swelling ***and redness*** will be analysed using a 3-grade evaluation system [0 mm (grade 0); > 0 to 20 mm (grade 1); > 20 to 50 mm (grade 2); > 50 mm (grade 3)].

11.12.2. Statistical considerations for interim analyses

The analysis will be performed by a statistician external to the investigator group and ~~GSK Biologicals~~ ***"The Collaboration"*** (i.e. from a contract research organisation). The clinical team within GSK Biologicals ***and Aeras***, all participating subjects, study site and ~~GSK~~ personnel directly involved in the conduct of the trial will remain blinded to the

randomisation codes. The GSK statistician (and other ~~GSK~~ personnel directly involved in the conduct of the trial) will not have access to the randomisation list and serology data.

The efficacy results at interim analysis will be looked at by the IDMC in closed session (with prohibited attendance by any member of GSK ***and Aeras*** and by any person involved in the conduct of the study).

12.1 Remote Data Entry instructions

While completed eCRFs are reviewed by a ~~GSK Biologicals'~~ Site Monitor ***designated by "The Collaboration"*** at the study site, omissions or inconsistencies detected by subsequent eCRF review may necessitate clarification or correction of omissions or inconsistencies with documentation and approval by the investigator or appropriately qualified designee. In all cases, the investigator remains accountable for the study data.

12.2. Study Monitoring by GSK Biologicals "*The Collaboration*"

GSK ***and "The Collaboration"*** will monitor the study to verify that, amongst others, the:

[…]

Upon completion or premature discontinuation of the study, the monitor will conduct site closure activities with the investigator or site staff, as appropriate, in accordance with applicable regulations, GCP, and ~~GSK~~ ***"The Collaboration"*** procedures.

12.3. Record retention

~~GSK~~ ***"The Collaboration"*** will inform the investigator/institution of the time period for retaining these records to comply with all applicable regulatory requirements. However, the investigator/institution should seek the written approval of the sponsor before proceeding with the disposal of these records. The minimum retention time will meet the strictest standard applicable to a particular site, as dictated by ICH GCP, any institutional requirements, applicable laws or regulations, or GSK standards/procedures; otherwise, the minimum retention period will default to 15 years.

The investigator/institution must notify ~~GSK~~ ***"The Collaboration"*** of any changes in the archival arrangements, including, but not limited to archival at an off-site facility, transfer of ownership of the records in the event the investigator leaves the site.

12.4. Quality assurance

To ensure compliance with GCP and all applicable regulatory requirements, GSK ***or "The Collaboration"*** may conduct a quality assurance audit. Regulatory agencies may also conduct a regulatory inspection of this study. Such audits/inspections can occur at any time during or after completion of the study. If an audit or inspection occurs, the investigator and institution agree to allow the auditor/inspector direct access to all relevant documents and to allocate his/her time and the time of his/her staff to the auditor/inspector to discuss findings and any relevant issues.

GlaxoSmithKline Biologicals

Clinical Research & Development

Protocol Amendment 3

eTrack study number and Abbreviated Title:	115616 (TUBERCULOSIS-018)
Amendment number:	Amendment 3
Amendment date:	11 December 2012
Co-ordinating author:	PPD, Project Manager Scientific Writing, XPE Pharma&Science, contractor for GSK Biologicals

Rationale/background for changes:

- An additional country (Zambia) was added to the study. In order to avoid protocol amendments in the future whenever a new country is added, all country specificities were removed from the protocol.
- The interval between the screening visit and the first vaccination visit was decreased to allow subjects to be enrolled and screened on the same day as the first vaccination.
- Additional small changes were made to align with the eCRF and available statistical methods.

Amended text has been included in *bold italics* and deleted text in ~~strikethrough~~ in the following sections:

Synopsis:

Rationale for the study and study design

This Phase IIb study is designed to evaluate the protective efficacy of the M72/AS01$_E$ candidate tuberculosis vaccine against definite pulmonary TB disease not associated with HIV-infection, versus placebo, following 2 injections given one month apart. The protective efficacy will be evaluated in adults living in a TB endemic region ~~(India, South Africa and Kenya)~~ aged 18-50 years, i.e. in an age range where the incidence of tuberculosis is high.

Section 1.3.2 Rational for the study design

This study is designed to evaluate the protective efficacy of the M72/AS01$_E$ candidate tuberculosis vaccine against definite pulmonary TB disease, versus placebo, following 2 injections given one month apart. The protective efficacy will be evaluated in adults living in a TB endemic region ~~(India, South Africa and Kenya)~~ aged 18-50 years, i.e. in an age range where the incidence of tuberculosis is high (Figure 1).

Section 5.1 Number of subjects/centres

The study will be conducted in ~~India, Kenya and South Africa~~ ***multiple TB endemic countries***, in multiple enrolling centres. The study centres will be experienced in detecting TB and have access to TB treatment or have an established referral option for TB patients and HIV-infected individuals.

There will be one sub-cohort for safety/reactogenicity and immunogenicity. A maximum of 500 eligible subjects from selected centres ~~in India and South Africa~~ will be included in this safety and immune sub-cohort (Table 4). ~~The subjects included in the safety and immune sub-cohort will be equally distributed between Africa and India.~~

Table 4 Sub-cohorts

Sub-cohort name	Description	Estimated number of subjects
Safety and immune sub-cohort	Safety parameters for this sub-cohort will be collected as follows: • Solicited AEs will be collected for 7 days (day of vaccination and 6 subsequent days) following vaccination. • Serum biochemistry/haematology values will be measured at Days 0, 7, 30, and 37.	~~Approximately~~ ***Up to*** 450
	Immunogenicity: • Humoral immunogenicity will be evaluated by measuring M72-specific IgGs at Days 0 and 60 and Years 1, 2 and 3. • CMI will be evaluated by measuring M72-specific CD4+ and CD8+ T cells expressing at least 2 immune markers (CD40-L and/or IFN-γ and/or IL-2 and/or TNF-α) at Days 0 and 60 and Years 1, 2 and 3. • The QuantiFERON status of subjects will be determined at Day 0 and Years 1, 2 and 3.	

Section 6.2.3 Allocation of subjects to assay subsets

Approximately 500 subjects will be included in the safety and immune sub-cohort. These subjects will be enrolled at selected centres ~~in India and South Africa. Subjects included in the safety and immune sub-cohort will be equally distributed between Africa and India.~~ Blood sampling and safety assessment will be performed as described in Table 4.

Section 6.5 Outline of study procedures

Table 5 List of study procedures

	Visit 1 Screening visit	Visit 2	Visit 3[1]	Visit 4	Visit 5[1]	Visit 6[2]	Visit 7	Contact 1, 2, 3 and 4	Visit 8	Contact 5, 6, 7, 8, 9	Visit 8	Contact 10, 11, 12, 13, 14	Visit 9
Time point(s)	**D-30**	**D 0**	**D 7**	**D 30**	**D 37**	**D 44**	**M 2**	**M 4, 6, 8, 10**	**M 12**	**M 14, 16, 18, 20, 22**	**M 24**	**M 26, 28, 30, 32, 34**	**M 36**
Sampling Time point(s)	**Pre V**	**Pre V**	**Post V1**	**Post V1**	**Post V2**	**Post V2**	**Post V2**	**Post V2**	**Post V2**	**Post V2**	**Post V2**	**Post V2**	**Post V2**
Dose		**1**		**2**									
All subjects													
Informed consent	●												
Check inclusion/exclusion criteria	●	●											
Collect demographic data	●												
Medical history	●	●											
Physical examination	●												
History-directed physical examination		O	O	O	O	O	O						
Resting vital signs	O	O		O									
HIV testing[3] (up to ~ 2.5 mL/test)	●												
Record weight[4]		●							●		●		●
Documentation of history of BCG vaccination/presence of scar	●												
Pregnancy test (β-HCG urine)	●	●		●									
Study group and treatment number allocation		O											
Treatment number allocation for second dose				O									
Vaccination		●		●									
Recording of administered treatment number		●		●									

	Visit 1 Screening visit	Visit 2	Visit 3[1]	Visit 4	Visit 5[1]	Visit 6[2]	Visit 7	Contact 1, 2, 3 and 4	Visit 8	Contact 5, 6, 7, 8, 9	Visit 8	Contact 10, 11, 12, 13, 14	Visit 9
Time point(s)	**D-30**	**D 0**	**D 7**	**D 30**	**D 37**	**D 44**	**M 2**	**M 4, 6, 8, 10**	**M 12**	**M 14, 16, 18, 20, 22**	**M 24**	**M 26, 28, 30, 32, 34**	**M 36**
Sampling Time point(s)	**Pre V**	**Pre V**	**Post V1**	**Post V1**	**Post V2**	**Post V2**	**Post V2**	**Post V2**	**Post V2**	**Post V2**	**Post V2**	**Post V2**	**Post V2**
Dose		**1**		**2**									
Check contraindications to subsequent vaccination		●		●									
Recording concomitant medication/vaccination	●	●	●	●	●	●	●	●	●	●	●	●	●
Recording intercurrent medical condition		●	●	●	●	●	●	●	●	●	●	●	●
Distribution of diary cards		O		O									
Return of diary cards				O			O						
Recording of unsolicited AEs		●	●	●	●	●	●						
Pre-vaccination assessment (including temperature)		●		●									
Blood sampling for RNA expression profiling (~2.5 mL)[5]		●				●							
Reporting SAEs	●[6]	●	●	●	●	●	●	●[7]	●[6]	●[6]	●[6]	●[6]	●[6]
Reporting pIMDs		●	●	●	●	●	●	●[8]					
Reporting pregnancy		●	●	●	●	●	●	●[8]					
Diagnostic procedures for suspected pulmonary TB			●	●	●	●	●	●	●	●	●	●	●
Documentation of TB cases			●	●	●	●	●	●	●	●	●	●	●
Safety and immune sub-cohort													
Measuring Vitamin D		●											
Recording of solicited AEs		●	●	●	●								
CBC (Hb, PLT and WBC), ALT, AST, creatinine, bilirubin (~ 8 mL)		●	●	●	●								
Blood sampling for CMI (~ 2.5 mL)		●					●		●		●		●

	Visit 1 Screening visit	Visit 2	Visit 3[1]	Visit 4	Visit 5[1]	Visit 6[2]	Visit 7	Contact 1, 2, 3 and 4	Visit 8	Contact 5, 6, 7, 8, 9	Visit 8	Contact 10, 11, 12, 13, 14	Visit 9
Time point(s)	**D-30**	**D 0**	**D 7**	**D 30**	**D 37**	**D 44**	**M 2**	**M 4, 6, 8, 10**	**M 12**	**M 14, 16, 18, 20, 22**	**M 24**	**M 26, 28, 30, 32, 34**	**M 36**
Sampling Time point(s)	**Pre V**	**Pre V**	**Post V1**	**Post V1**	**Post V2**	**Post V2**	**Post V2**	**Post V2**	**Post V2**	**Post V2**	**Post V2**	**Post V2**	**Post V2**
Dose		**1**		**2**									
Blood sampling for humoral immunogenicity (~ 2 mL)		●					●		●		●		●
									●		●		
Study conclusions for all subjects													●

Note: The double border at Month 36 indicates the final analyses which will be performed on all data obtained up to month 36.
Pre V: Pre Vaccination; V: Vaccination; Post V: Post Vaccination
● is used to indicate a study procedure that requires documentation in the individual eCRF.
○ is used to indicate a study procedure that does not require documentation in the individual eCRF.
1 Only subjects in the safety and immune sub-cohort need to come for this visit.
2 The most informative time point for collection of blood samples for RNA expression profiling post dose 2 will be determined. The exact time point will be communicated to the investigators appropriately at the latest at study initiation.
3 HIV testing and pre-test counselling will be performed at screening in areas were HIV-prevalence > 1%***,*** according to the procedures detailed in Table 9.
4 Weight also needs to be recorded whenever ***there is suspicion of*** TB disease ~~is confirmed (definite and clinical)~~.
5 Samples for RNA expression profiling will also be taken at the time of TB diagnosis.
6 Only SAEs related to study participation or concurrent GSK medication/vaccine.
7 SAEs not related to study participation or concurrent GSK medication/vaccines will be recorded until 6 months post dose 2 (M7).
8 To be recorded until 6 months post dose 2 (M7).
9 Sputum samples for Xpert MTB/RIF testing and microbiological culture will be taken throughout the study if a subject presents with clinical suspicion of pulmonary TB disease. Three sputum samples, preferably taken in the morning and within one week, must be collected. Additionally, when a subject is diagnosed with TB, HIV testing must be performed according to the procedures detailed in Table 9. Up to ~ 2.5mL of blood will be collected per test. If the subject is confirmed HIV-positive, blood samples must be taken (2.5 mL) to measure CD4 counts.
10 Documentation of TB cases will be done throughout the study, starting at Visit 3 (Day 7); this includes documentation of cases during active and passive follow-up and monitoring of medical facilities and pharmacies.

Table 6 Intervals between study visits for all subjects

Interval	Allowed interval[1]
Visit 1 - Visit 2	30 days - ~~1~~***0*** days
Visit 2 - Visit 4	26 days - 35 days
Visit 4 - Visit 6	1 days - 25 days [2]
Visit 4 - Visit 7	14 days - 35 days
Visit 4 - Visit 8	10 months - 12 months
Visit 4 - Visit 9	22 months -24 months
Visit 4 - Visit 10	34 months - 36 months
Visit n – next contact	6 weeks – 12 weeks
Contact n – contact n+1	6 weeks – 12 weeks

Section 11.8 Analysis of efficacy

The ATP cohort for efficacy will be used for the primary analysis of efficacy. If the percentage of enrolled subjects excluded from the ATP cohort for analysis of efficacy is more than 5%, a second analysis based on the TVC will be performed to complement the primary analysis.

As a primary analysis, vaccine efficacy will be estimated using a frequentist approach. In this analysis, vaccine efficacy is defined as 1-RR where RR is the Poisson rate ratio. The rate in the vaccine group is equal to the number of cases in the vaccine group divided by the follow-up time. The rate in the control group is defined the same way. CIs and p-values will be calculated using the exact method implemented in the Poisson procedure of the StatXact package provided by Cytel. ***This method is also available in proc genmod when using the exact option.***

GlaxoSmithKline Biologicals

Clinical Research & Development

Protocol Amendment 4

eTrack study number and Abbreviated Title	115616 (TUBERCULOSIS-018)
Amendment number:	Amendment 4
Amendment date:	29 March 2013
Co-ordinating author:	PPD, Project Manager Scientific Writing, XPE Pharma&Science, contractor for GSK Biologicals

Rationale/background for changes:

The study was initially designed to evaluate the efficacy of two doses of the M72/AS01$_E$ candidate vaccine against definite pulmonary TB disease in multiple countries in Africa and in India to obtain initial efficacy data in these populations. However, to increase the feasibility and reduce the logistical burden and complexity, the study was re-evaluated to include multiple countries in Africa only. As the incidence rate in Africa (overall incidence rate: 262 per 100.000 population[1]) is significantly higher as compared to India (incidence rate: 181 per 100.000 population), a significantly lower number of subjects (4500 versus 7000) will be needed to evaluate the vaccine efficacy (VE).

The sample size was recalculated based on the logrank test for comparison of time to first diagnosis of definite pulmonary TB disease: assuming 80% power for a true VE of 70% and a two-sided 5% significance level, 27 cases of definite pulmonary TB disease, meeting the first case definition would be required instead of 28, as previously determined using the Poisson rate ratio. To obtain these 27 cases, using updated estimated yearly attack rates in the control group (Africa only: 0.35% instead of Africa and India combined: 0.29%), a follow-up of 3 years and a loss of follow-up time of 12% due to drop out, 4500 enrolled subjects will be needed.

The vaccine efficacy defined as 1-estimated hazard ratio and associated 95% CIs and Wald p value will be derived using a Cox proportional hazards regression model.

Initially, the number of subjects in the safety and immune sub-cohort was set at 500, equally distributed between India and Africa in order to bridge the immune responses between these populations. As India will no longer be included in the study and the total number of subjects has decreased from 7000 to 4500, the number of subjects to be included in the safety and immune sub-cohort decreased from 500 to 450 subjects (225 subjects in each treatment group). Subjects from multiple countries (2 or 3) will be included in the sub-cohort to explore potential differences in vaccine take between different countries. Up to 150 subjects enrolled in each contributing country (if 3 contributing countries) or up to 225

[1] World Health Organisation (WHO). Global Tuberculosis Report, 2012
http://apps.who.int/iris/bitstream/10665/75938/1/9789241564502_eng.pdf

subjects enrolled in each contributing country (if 2 contributing countries) will be included in the safety and immune sub-cohort. Assuming a standard deviation of log10(GMT or GM frequency of CD4 T cells expressing at least 2 immune markers) of 0.40, as observed in previous studies, a precision for the 95 % CI for log10(GMT or median frequency of CD4 T cells expressing at least 2 immune markers) of 0.052 or 0.092 will be obtained for a total samples size of 225 subjects vaccinated with M72/AS01$_E$ or 75 subjects vaccinated with M72/AS01$_E$/country respectively.

Cell-mediated immune responses will be evaluated in whole blood samples instead of peripheral blood mononuclear cells (PBMCs), excluding the need for PBMC separation at the investigator's laboratory or central laboratory and decreasing the volume of blood to be collected while obtaining the same results.

In addition, this study offers the opportunity to evaluate whether the M72/AS01$_E$ candidate vaccine might also provide protection against *Mycobacterium tuberculosis* (Mtb) infection. Mtb complex infection, whether tuberculosis disease, latent TB infection or past TB infection, can be detected by interferon-gamma release assays (IGRAs), such as the QuantiFERON® TB Gold (QFTG) assay (Cellestis), by measuring IFN-γ release by memory T cells in response to Mtb antigens. The QuantiFERON® TB Gold In Tube method uses peptide cocktails covering the early secretory antigen target-6 (ESAT-6),culture filtrate protein-10 (CFP-10) and TB 7.7 protein sequences to stimulate T cell lymphocytes. As these proteins are absent from all BCG strains, the test is not confounded by previous BCG vaccination nor is it confounded by exposure to most environmental mycobacteria.

Although yearly QFTG testing is planned for all subjects included in the safety and immune sub-cohort (N=450) to estimate the exposure to TB during the study, QFTG testing will also be performed at baseline for all subjects ,at the end of the study (Year 3) for all subjects QFTG-negative at baseline to evaluate the protective efficacy of the candidate vaccine against Mtb infection, as measured by QFTG conversion. In addition QFTG testing will be performed whenever a subject is diagnosed with TB disease.

To avoid trial progression with a vaccine candidate with very low efficacy, an interim efficacy analysis for futility is planned after approximately 10 Xpert MTB/Rif positive pulmonary TB cases in confirmed HIV-negative subjects (approximately 40% of the target events) have accumulated in the modified Total Vaccinated Cohort (TVC) for interim efficacy analysis at least 1 month post dose two. The modified TVC for interim efficacy will include all subjects who received two vaccine doses according to protocol and did not present with TB disease, according to any case definition, before completion of the vaccination phase (up to 1 month post dose 2) without randomisation failure and without wrong replacement. The interim analysis for futility will be performed by the Independent Data Monitoring Committee (IDMC) statistician and stopping rules for futility will be defined in the statistical analysis plan for interim analysis. If the study is stopped for futility, all subjects will be followed up until completion of the safety follow-up (up to 6 months post dose 2). An interim analysis after accrual of half of the total number of cases is therefore no longer foreseen.

Other changes to the protocol include:

- Names and addresses of laboratories involved in the conduct of the study (previously not identified), are included.
- History of household contacts, smoking and diabetes will be recorded at screening

Amended text has been included in *bold italics* and deleted text in ~~strikethrough~~ in the following sections:

Synopsis: Rationale for the study design

- Rationale for the study design

A sub-cohort of subjects will be selected for specific follow-up for safety (solicited adverse events [AE] and selected Biochemistry and Complete Blood Count [CBC]) and immunogenicity. Unsolicited AEs, serious adverse events (SAEs), potential immune-mediated diseases (pIMDs), AEs leading to withdrawal and pregnancies will be recorded for all subjects. ***An IFN-γ positive T cell response induced by past or present Mtb infection, as measured by the QuantiFERON® TB Gold (QFTG) assay (Cellestis), will be evaluated at baseline for all subjects, at study end (Year 3) for all subjects QFTG-negative at baseline and yearly for subjects in the safety and immune sub-cohort. In addition, a QFTG assay will be performed whenever a subject is diagnosed with TB disease.***

Synopsis: Objectives

Primary

Success criterion:

The lower limit of the 95~~.1~~% two-sided confidence interval (CI) for the VE ***(using a Cox regression model)*** *against first occurrence of Definite pulmonary TB disease not associated with HIV-infection, meeting the first case definition, is above 0%.*

Secondary

If the primary objective is met, this secondary objective will be analysed with the following success criterion:

The lower limit of the 95~~.1~~% two-sided confidence interval (CI) for the VE ***(using a Cox regression model)*** *against first occurrence of Definite pulmonary TB disease not associated with HIV-infection, meeting the first case definition, is above 0%.*

Tertiary

- ***To evaluate the protective efficacy of two doses of the M72/AS01$_E$ candidate vaccine against Mtb infection, as measured by QFTG conversion.***

****QFTG conversion is defined as the change from a negative QFTG test result (value below the cut off for IFN-γ concentration) to a positive QFTG test result (value at or above the cut off for IFN-γ concentration) in two serial samples.***

Synopsis Table 1 Study groups and epochs foreseen in the study

Study groups	Number of subjects	Age (Min/Max)	Epoch 001
M72AS01	~~3500~~ ***2250***	18 - 50 years	x
Control	~~3500~~ ***2250***	18 - 50 years	x

Synopsis: Sampling schedule

- Blood samples will be collected in the safety and immune sub-cohort for evaluation of biochemistry/haematology parameters (Days 0, 7, 30 and 37), CMI responses (Days 0, 60 and Years 1, 2 and 3) ***and*** humoral immune responses (Days 0, 60 and Years 1, 2 and 3). ~~and QuantiFERON status~~
- ***Blood samples will be collected for QFTG testing at baseline (Day 0) for all subjects, at study end (Year 3) for all subjects QFTG-negative at baseline and yearly for subjects included in the safety and immune sub-cohort.***
- Blood samples for RNA expression profiling will be collected from all subjects (Days 0 ***and*** post dose 2~~and when TB is diagnosed~~)
- ***Whenever a subject is diagnosed with TB disease, blood samples will be collected for HIV testing, mRNA expression profiling and QFTG testing. In addition, if a subject is HIV-positive, blood samples will be collected to determine CD4 cell counts.***

Synopsis: safety monitoring

an Independent Data Monitoring Committee (IDMC), operating under a charter, will be constituted for this study to oversee the study. They will perform safety reviews ***three times in the first year of enrolment and*** twice a year, or more frequently if deemed necessary ***on an ongoing basis for the duration of the study*** and evaluate the interim efficacy analysis

Synopsis: Number of subjects

Approximately ~~7000~~***4500***

Synopsis: Secondary endpoints

Immunogenicity

- Evaluation of CMI responses with respect to components of the study vaccine, in the safety and immune sub-cohort:
  - Determined by the frequency of M72-specific CD4+/CD8+ T cells per million cells identified after *in vitro* stimulation, as expressing at least 2 immune markers among CD40L, IL-2, IFN-γ and TNF-α.
  - ~~Determined by the frequency of M72-specific CD4+/CD8+ T cells per million cells identified after in vitro stimulation, as expressing any combination of immune markers among CD40L, IL-2, IFN-γ and TNF-α.~~

  Time points: prior to dose 1 (Day 0) and post dose 2 (Day 60, Years 1, 2 and 3)

Safety

- ***Occurrence of grade ≥ 2 haematological and biochemical levels at:***

  Days 0, 7, 30 and 37

Synopsis: Tertiary endpoints

- ***Evaluation of CMI responses with respect to components of the study vaccine, in the safety and immune sub-cohort:***
  - ***Determined by the frequency of M72-specific CD4+/CD8+ T cells per million cells identified after in vitro stimulation, as expressing any combination of immune markers among CD40L, IL-2, IFN-γ and TNF-α.***

  Time points: prior to dose 1 (Day 0) and post dose 2 (Day 60 and Years 1, 2 and 3)
- ***QFTG test result.***

  Time points: prior to dose 1 (Day 0), post dose 2 (Year 3)

LIST OF ABBREVIATIONS:

IGRA	***Interferon-Gamma Release Assay***
QFTG	***QuantiFERON® TB Gold***

GLOSSARY OF TERMS

QFTG conversion	***The change from a negative QFTG test result (value below the cut off for IFN-γ concentration) to a positive QFTG test result (value at or above the cut off for IFN-γ concentration) in two serial samples.*** ***The QFTG assay detects memory T cell responses against Mtb as a surrogate for exposure to/infection with Mtb. The QFTG does not directly measure the presence of Mtb. The QFTG does not indicate whether the immune response of a subject is able to eliminate the bacteria or whether Mtb is latently present***

Section 1.3.2 Rational for the study design

A sub-cohort of subjects will be selected for specific follow-up for safety (solicited adverse events (AE) and selected Biochemistry and Complete Blood Count [CBC]) and immunogenicity. Unsolicited AEs, serious adverse events (SAEs), potential immune-mediated diseases (pIMDs), AEs leading to withdrawal and pregnancies will be recorded for all subjects. ***An IFN-γ positive T cell response induced by past or present Mtb infection, as measured by the QuantiFERON® TB Gold (QFTG) assay (Cellestis), will be evaluated at baseline for all subjects, at study end (Year 3) for all subjects QFTG-negative at baseline and yearly in safety and immune the sub-cohort. In addition, a QFTG assay will be performed whenever a subject is diagnosed with TB disease.***

Section 2.1 Primary Objective

Success criterion to be used for the primary objective:

The lower limit of the 95.~~1~~% two-sided confidence interval (CI) for the VE against first occurrence of Definite pulmonary TB disease not associated with HIV-infection, meeting the first case definition, is above 0%

Section 2.2 Secondary Objectives

Efficacy:

If the primary objective is met, this secondary objective will be analysed using the following success criterion:

The lower limit of the 95.~~1~~% two-sided confidence interval (CI) for the VE against first occurrence of Definite pulmonary TB disease not associated with HIV-infection, meeting the first case definition, is above 0%

Section 2.3 Tertiary Objectives

- ***To evaluate the protective efficacy of two doses of the M72/AS01$_E$ candidate vaccine against Mtb infection, as measured by QFTG conversion.***

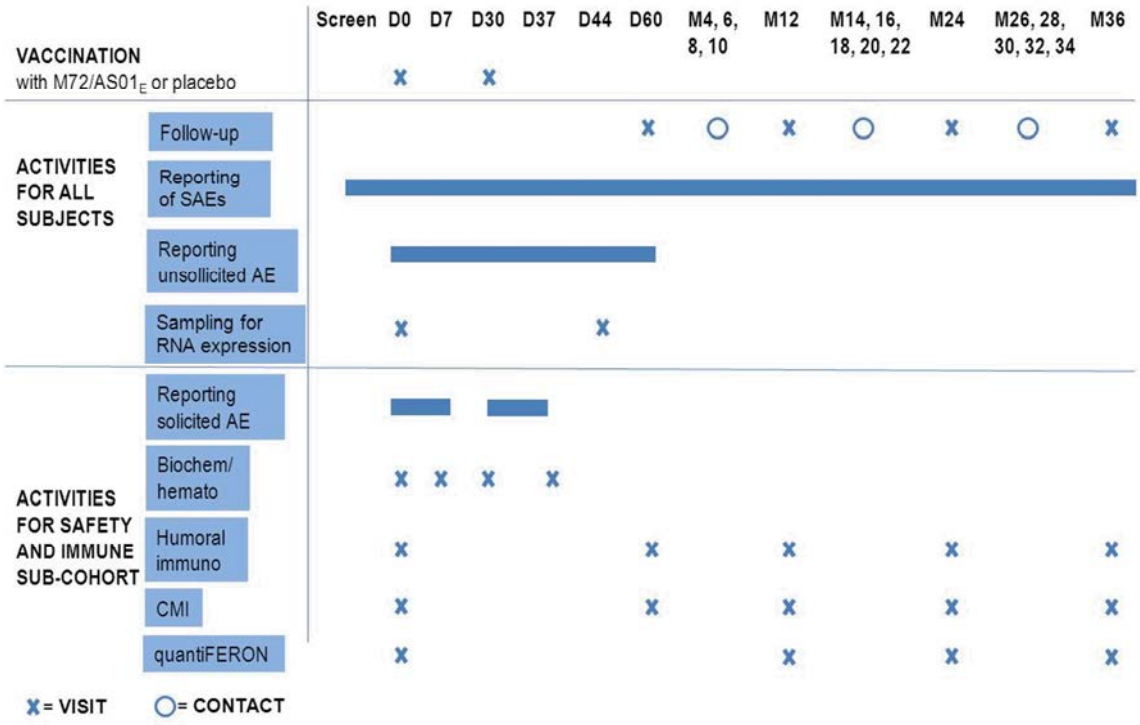
…

Refer to the GLOSSARY OF TERMS for a definition of QFTG conversion.

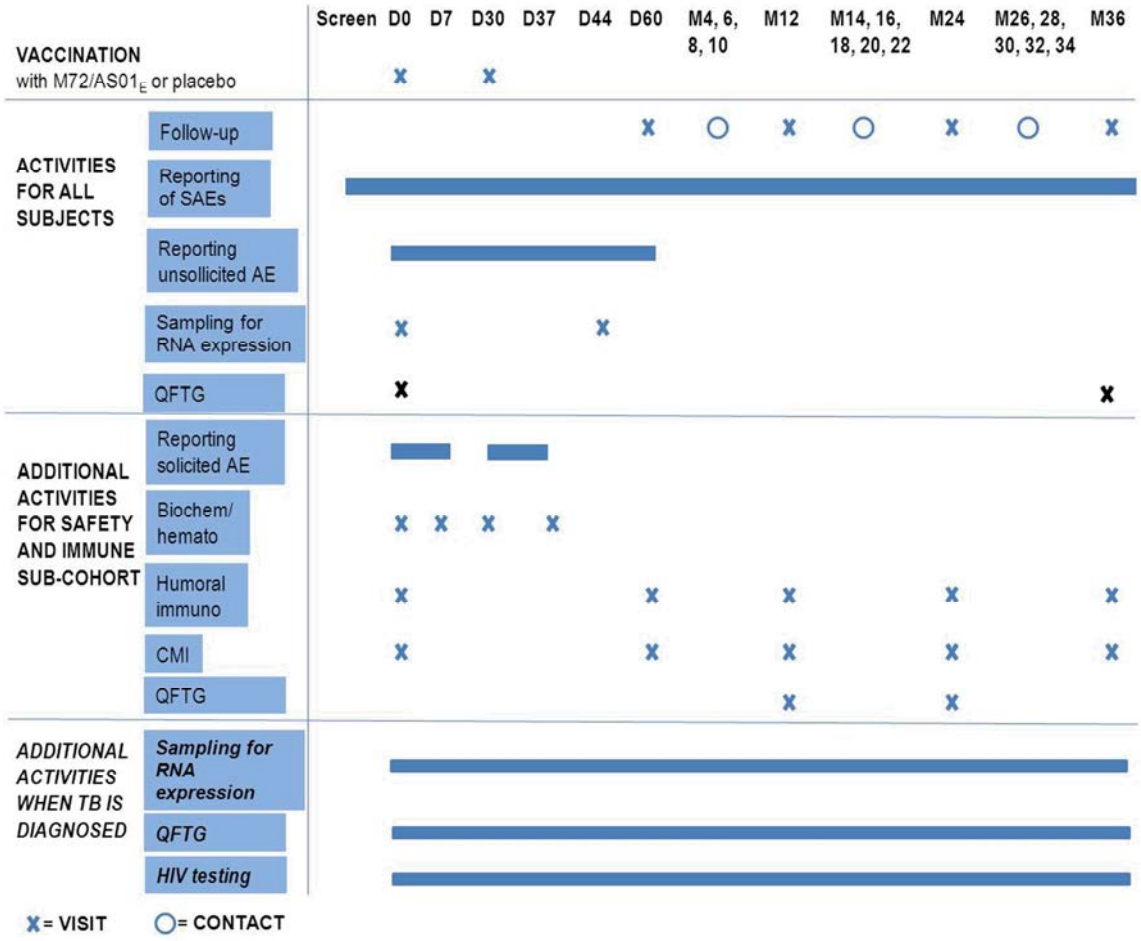
Section 3 Study design overview

Figure 2 Overview of the study design

The following figure:



Was replaced by:



- **Duration of the study:** the duration of the study will be approximately 37 months ***for each subject*** from screening up to end of efficacy follow-up.

Table 1 Study groups and epochs foreseen in the study

Study Groups	Number of subjects	Age (Min - Max) (age unit)	Epoch 001
M72AS01	~~3500~~ ***2250***	18 - 50 years	x
Control	~~3500~~***2250***	18 - 50 years	x

- **Sampling schedule:**
  - Blood samples will be collected in the safety and immune sub-cohort for evaluation of biochemistry/haematology parameters (Days 0, 7, 30 and 37), CMI responses (Days 0, 60 and Years 1, 2 and 3) ***and*** humoral immune responses (Days 0, 60 and Years 1, 2 and 3) ~~and QuantiFERON status.~~
  - ***Blood samples will be collected for QFTG testing at baseline (Day 0) for all subjects, at study end (Year 3) for all subjects QFTG-negative at baseline and yearly for subjects included in the safety and immune sub-cohort.***

- Blood samples for RNA expression profiling will be collected from all subjects at predefined time points (Days 0 and Day 44*)~~and whenever a subject is diagnosed with TB disease~~ .
- ***Whenever a subject is diagnosed with TB disease, blood samples will be collected for HIV testing, mRNA expression profiling and QFTG testing. In addition, if a subject is HIV-positive, blood samples will be collected to determine CD4 cell counts.***

Section 5.1 Number of subjects/centres

Approximately ~~7000~~***4500*** men and women aged 18-50 years will be enrolled in the study. Enrolment will be terminated when target number of subjects is reached.

The study will be conducted in multiple TB endemic countries, in multiple enrolling centres. The study centres will be experienced in detecting TB and have access to TB treatment or have an established referral option for TB patients and HIV-infected individuals.

There will be one sub-cohort for safety/reactogenicity, and immunogenicity. ~~A maximum~~ ***Approximately*** ~~of~~ ~~500~~ ***450*** eligible subjects from selected centres in ***multiple*** countr***ies (2 or 3)*** will be included in this safety and immune sub-cohort (Table 4). ***Up to 150 enrolled in each contributing country (if 3 contributing countries) or up to 225 subjects enrolled in each contributing country (if 2 contributing countries) may be included in the safety and immune sub-cohort.***

To ensure the gender of the study population is equally distributed between females and males, a cap has been put on the number of subjects per gender to be enrolled. A maximum of 60% of subjects from the same gender will be enrolled in the study and enrolment of females/males will be stopped at approximately ~~4200~~ ***2700*** subjects of any gender maximum.

Table 4 Sub-cohorts

Sub-cohort name	***D*escription *of additional activities***	Estimated number of subjects
Safety and immune sub-cohort	Safety parameters for this sub-cohort will be collected as follows: • Solicited AEs will be collected for 7 days (day of vaccination and 6 subsequent days) following vaccination. • Serum biochemistry/haematology values will be measured at Days 0, 7, 30, and 37.	***Approximately*** ~~Up to 500~~***450***
	Immunogenicity: • Humoral immunogenicity will be evaluated by measuring M72-specific IgGs at Days 0 and 60 and Years 1, 2 and 3. • CMI will be evaluated by measuring M72-specific CD4+ and CD8+ T cells expressing at least 2 immune markers (CD40-L and/or IFN-γ and/or IL-2 and/or TNF-α) at Days 0 and 60 and Years 1, 2 and 3. • The QuantiFERON status of subjects will be determined at Day 0 and Years 1, 2 and 3.	

Section 6.2.2.2.1 Study group and treatment number allocation

The target will be to enrol approximately ~~7000~~ ***4500*** eligible subjects who will be randomly assigned to two study groups in a 1:1 ratio (approximately ~~3500~~ ***2250*** subjects in each group).

Allocation of the subject to a study group at the investigator site will be performed using a randomisation system on internet (SBIR). The randomisation algorithm will use a minimisation procedure accounting for:

- Gender: a maximum of ~~4200~~ ***2700*** females/males will be recruited.
- TB disease categorised as history or no history of TB disease.

Section 6.2.3 Allocation of subjects to assay subsets

Approximately ~~500~~ ***450*** subjects will be included in the safety and immune sub-cohort. These subjects will be enrolled at selected centres. Blood sampling and safety assessment will be performed as described in Table 4. ***Distribution of subjects in the sub-cohort is presented in Table 5.***

Table 5 Distribution of subjects in the sub-cohort

Sub-cohort	*Treatment group*	*N to be enrolled*
Safety and immune sub-cohort	*M72AS01*	*Approximately 225*
	Control	*Approximately 225*

Section 6.4.2 Diagnostic procedures for the detection of suspected pulmonary TB

Additionally if TB is confirmed a serological test (and pre-test counselling) for HIV-infection must be performed to rule out HIV co-infection and a blood sample for RNA expression profiling ***and QFTG testing*** must be taken. If the subject is HIV-positive, additional testing must be performed to measure CD4 cell counts.

Results of HIV testing, including CD4 cell counts if applicable, ***and QFTG testing*** must be recorded in the subjects' eCRF.

Section 6.4.3 Diagnostic procedures for suspected extra-pulmonary TB

Additionally, if extra-pulmonary TB is confirmed, a serological test (and pre-test counselling) for HIV-infection must be performed to rule out HIV co-infection and a blood sample for RNA expression profiling ***and QFTG testing*** must be taken. If the subject is HIV-positive, additional testing must be performed to measure CD4 cell counts.

Results of HIV testing, including CD4 cell counts~~,~~ if applicable, ***and QFTG testing*** must be recorded in the subjects' eCRF.

Section 6.4.5.2 Independent Data Monitoring Committee

In order to ensure the safety of the subjects during the entire study period, an Independent Data Monitoring Committee (IDMC) will be appointed to monitor the safety and tolerability of the vaccine/placebo administered in the trial and, if necessary, make

recommendations to the sponsor concerning the modification or termination of the trial. The IDMC will perform safety reviews ***three times in the first year and*** twice a year or more frequently if deemed necessary ***on an ongoing basis during the study period***.
(Amended 26 March 2013)

Section 6.5 Outline of study procedures

Table 6 List of study procedures

	Visit 1 Screening visit	Visit 2	Visit 3[1]	Visit 4	Visit 5[1]	Visit 6[2]	Visit 7	Contact 1, 2, 3 and 4	Visit 8	Contact 5, 6, 7, 8, 9	Visit 9	Contact 10, 11, 12, 13, 14	Visit 10
Time point(s)	**D-30**	**D 0**	**D 7**	**D 30**	**D 37**	**D 44**	**M 2**	**M 4, 6, 8, 10**	**M 12**	**M 14, 16, 18, 20, 22**	**M 24**	**M 26, 28, 30, 32, 34**	**M 36**
Sampling Time point(s)	**Pre V**	**Pre V**	**Post V1**	**Post V1**	**Post V2**	**Post V2**	**Post V2**	**Post V2**	**Post V2**	**Post V2**	**Post V2**	**Post V2**	**Post V2**
Dose		**1**		**2**									
All subjects													
Informed consent	●												
Check inclusion/exclusion criteria	●	●											
Collect demographic data	●												
Medical history	●	●											
Physical examination	●												
History-directed physical examination		O	O	O	O	O	O						
Resting vital signs	O	O		O									
HIV testing[3] (up to ~ 2.5 mL/test)	●												
Record weight[4]		●							●		●		●
Documentation of history of BCG vaccination/presence of scar	●												
Documentation of history of TB household contacts	●												
Pregnancy test (β-HCG urine)	●	●		●									
Study group and treatment number allocation		O											
Treatment number allocation for second dose				O									
Vaccination		●		●									

	Visit 1 Screening visit	Visit 2	Visit 3[1]	Visit 4	Visit 5[1]	Visit 6[2]	Visit 7	Contact 1, 2, 3 and 4	Visit 8	Contact 5, 6, 7, 8, 9	Visit 9	Contact 10, 11, 12, 13, 14	Visit 10
Time point(s)	**D-30**	**D 0**	**D 7**	**D 30**	**D 37**	**D 44**	**M 2**	**M 4, 6, 8, 10**	**M 12**	**M 14, 16, 18, 20, 22**	**M 24**	**M 26, 28, 30, 32, 34**	**M 36**
Sampling Time point(s)	**Pre V**	**Pre V**	**Post V1**	**Post V1**	**Post V2**	**Post V2**	**Post V2**	**Post V2**	**Post V2**	**Post V2**	**Post V2**	**Post V2**	**Post V2**
Dose		**1**		**2**									
Recording of administered treatment number		●		●									
Check contraindications to subsequent vaccination		●		●									
Recording concomitant medication/vaccination	●	●	●	●	●	●	●	●	●	●	●	●	●
Recording intercurrent medical condition		●	●	●	●	●	●	●	●	●	●	●	●
Distribution of diary cards		O		O									
Return of diary cards				O			O						
Recording of unsolicited AEs		●	●	●	●	●	●						
Pre-vaccination assessment (including temperature)		●		●									
Blood sampling for RNA expression profiling (~2.5 mL)[5]		●				●							
Blood sampling for IGRA conversion(~ 3 mL)[6]		●											●[7]
Reporting SAEs	●[6,8]	●	●	●	●	●	●	●[7,9]	●[6,8]	●[6,8]	●[6,8]	●[6,8]	●[6,8]
Reporting pIMDs		●	●	●	●	●	●	●[8,10]					
Reporting pregnancy		●	●	●	●	●	●	●[8]					
Diagnostic procedures for suspected pulmonary TB[9,11]			●	●	●	●	●	●	●	●	●	●	●
Documentation of TB cases[10,12]			●	●	●	●	●	●	●	●	●	●	●

	Visit 1 Screening visit	Visit 2	Visit 3[1]	Visit 4	Visit 5[1]	Visit 6[2]	Visit 7	Contact 1, 2, 3 and 4	Visit 8	Contact 5, 6, 7, 8, 9	Visit 9	Contact 10, 11, 12, 13, 14	Visit 10
Time point(s)	**D-30**	**D 0**	**D 7**	**D 30**	**D 37**	**D 44**	**M 2**	**M 4, 6, 8, 10**	**M 12**	**M 14, 16, 18, 20, 22**	**M 24**	**M 26, 28, 30, 32, 34**	**M 36**
Sampling Time point(s)	**Pre V**	**Pre V**	**Post V1**	**Post V1**	**Post V2**	**Post V2**	**Post V2**	**Post V2**	**Post V2**	**Post V2**	**Post V2**	**Post V2**	**Post V2**
Dose		**1**		**2**									
Safety and immune sub-cohort													
Measuring Vitamin D		●											
Recording of solicited AEs		●	●	●	●								
CBC (Hb, PLT and WBC), ALT, AST, creatinine, bilirubin (~ 8 mL)		●	●	●	●								
Blood sampling for CMI (~ ~~18~~ ***2.5*** mL)		●					●		●		●		●
Blood sampling for humoral immunogenicity (~ 2 mL)		●					●		●		●		●
Blood sampling for IGRA conversion(~ 3 mL)		●							●		●		●
Study conclusions for all subjects													●

Note: The double border at Month 36 indicates the final analyses which will be performed on all data obtained up to month 36.

Pre V: Pre Vaccination; V: Vaccination; Post V: Post Vaccination

● is used to indicate a study procedure that requires documentation in the individual eCRF.

○ is used to indicate a study procedure that does not require documentation in the individual eCRF.

1 Only subjects in the safety and immune sub-cohort need to come for this visit.

2 The most informative time point for collection of blood samples for RNA expression profiling post dose 2 will be determined. The exact time point will be communicated to the investigators appropriately at the latest at study initiation.

3 HIV testing and pre-test counselling will be performed at screening in areas were HIV-prevalence > 1%, according to the procedures detailed in Table 9.

4 Weight also needs to be recorded whenever there is suspicion of TB disease.

5 Samples for RNA expression profiling will also be taken at the time of TB diagnosis.

6 Samples for QFTG testing will also be taken at the time of TB diagnosis.

7 Only for subjects QFTG-negative at baseline.

~~6~~8 Only SAEs related to study participation or concurrent GSK medication/vaccine.

~~7~~9 SAEs not related to study participation or concurrent GSK medication/vaccines will be recorded until 6 months post dose 2 (M7).

~~8~~10 To be recorded until 6 months post dose 2 (M7).

~~9~~11 Sputum samples for Xpert MTB/RIF testing and microbiological culture will be taken throughout the study if a subject presents with clinical suspicion of pulmonary TB disease. Three sputum samples, preferably taken in the morning and within one week, must be collected. Additionally, when a subject is diagnosed with TB, HIV testing must be performed according to the procedures detailed in Table 9. Up to (2.5mL of blood will be collected per test. If the subject is confirmed HIV-positive, blood samples must be taken (2.5 mL) to measure CD4 counts.

~~10~~12 Documentation of TB cases will be done throughout the study, starting at Visit 3 (Day 7); this includes documentation of cases during active and passive follow-up and monitoring of medical facilities and pharmacies.

Section 6.6.4 Collect history of TB household contacts

Question the subject about recent exposure (within the last 12 months) to a person (household contact) diagnosed with and/or treated for pulmonary TB disease.

Record history of TB household contacts in the subject's eCRF.

Section 6.6.5 Medical history

Obtain the subject's medical history by interview and/or review of the subject's medical records and record any pre-existing conditions or signs and/or symptoms present in a subject prior to the first study vaccination in the eCRF. In particular, chronic pulmonary conditions (such as COPD, silicosis), history of smoking and diabetes***** must be recorded ***(* type I diabetes is an exclusion criterion).***

Section 6.6.12.1 Blood sampling for safety, ~~or~~ immune response assessments *and QFTG status*

As specified in the List of Study Procedures in Section 6.5 (Table 6), blood samples are taken during certain study visits. Refer to the Module on Biospecimen Management in the SPM ***and lab manual*** for detailed instructions for the collection, handling and processing of the samples.

- For all subjects included in the safety and immune sub-cohort:
  - A volume of at least 3 mL of whole blood should be drawn for ***QFTG testing*** ~~QuantiFERON® TB Gold Assay~~ at each predefined time point (Table 9).
  - A volume of at least 2 mL of whole blood should be drawn for analysis of humoral immune responses at each predefined time point (Table 9).
  - A volume of at least ~~18~~ ***2.5*** mL of whole blood should be drawn for analysis of cell-mediated immune (CMI) responses at each predefined time point (Table 9).
  - A volume of at least 8 mL of whole blood should be drawn for tests performed to assay for CBC, ALT, AST, bilirubin and creatinine at each predefined time point (Table 9). Vitamin D levels will be measured at Day 0 only.
- For all subjects:
  - A volume of at least 2.5 mL of whole blood will be collected directly in a Paxgene™ Blood RNA Tube providing pure RNA for accurate detection and quantification of RNA expression profiles ("transcriptomics") at each predefined time point* (Table 9).
  - At screening, HIV testing must be performed according to the procedures detailed in Table 9 in regions with high HIV prevalence (> 1%). Up to approximately 2.5 mL of blood will be collected for each test.
  - ***A volume of at least 3 mL of whole blood should be drawn for QFTG testing at each predefined time point** (Table 9)***

  *Blood sampling for RNA expression profiling is planned for Day 0 (Visit 2) and Visit 6 (post dose 2). The most informative time point for collecting whole blood samples for RNA expression profiling post dose 2 will be determined. The exact

time point will be communicated to the Investigators appropriately at the latest at study initiation.

*****At study end (Year 3) QFTG testing will only be performed for all subjects QFTG-negative at baseline.***

- ***Whenever a subject is diagnosed with TB disease:***
  - ~~When a subject is diagnosed with TB disease,~~ HIV testing must be performed according to the procedures detailed in Table 9. Up to approximately 2.5 mL of blood will be collected for each test. Additionally, if HIV is confirmed, at least 2***.5*** mL of blood will be collected to measure CD4 counts.
  - ***A volume of at least 2.5 mL of whole blood will be collected directly in a Paxgene™ Blood RNA Tube providing pure RNA for accurate detection and quantification of RNA expression profiles ("transcriptomics").***
  - ***A volume of at least 3 mL of whole blood should be drawn for QFTG testing.***

Section 6.6.12.2 Sputum sample collection for TB diagnosis

Refer to the Module on Biospecimen Management in the SPM ***and lab manual*** for detailed instructions for the induction of sputum, collection, handling and processing of the samples.

Section 6.6.13 TB disease capture

Additionally, medical facilities and Pharmacists awareness ***(if applicable)*** and surveillance will be put in place in the study area to ensure that TB disease cases occurring in study participants are captured. All Medical facilities and Pharmacists in the study area will be informed of the trial. A team of study personnel/health workers will visit the medical facilities monthly to identify study subjects admitted to or cared for at the hospitals for TB. Relevant staff at the medical facilities will be advised to contact the study personnel for each TB case to ensure that all cases are captured.

Section 6.6.16 Recording of AEs, SAEs, pregnancies and pIMDs

- At each vaccination visit, diary cards will be provided to the subjects. Any unsolicited AEs (i.e. on the day of vaccination and during the next 29 days) occurring after vaccination will be recorded by all subjects. The subjects included in the safety and immune sub-cohort will also record body (axillary) temperature and any solicited local/general AEs (i.e. on the day of vaccination and during the next 6 days). In case of illiterate subjects, a designated person (such as a family member or a field worker) may provide assistance to complete the diary cards. The subject will be instructed to return the completed diary card to the investigator at a later visit. In case a diary card is lost by the subject or he/she is unable to complete the diary card, the safety information should be captured retrospectively using a descriptive interview. This process will be documented ***in a site SOP*** ~~by an Internal Operational Procedure (IOP)~~. Please refer to the SPM for further details.

Section 6.7 Biological sample handling and analysis

Please refer to the SPM ***and lab manual*** for details on biospecimen management (handling, storage and shipment).
(Amended 26 March 2013)

Section 6.7.1 Use of specified materials

When materials are provided by "The Collaboration", it is MANDATORY that all clinical samples (including serum samples) be collected and stored exclusively using those materials in the appropriate manner. The use of other materials could result in the exclusion of the subject from the ATP analysis (See Section 11.5 for the definition of study cohorts/ data sets to be analysed). The investigator must ensure that his/her personnel and the laboratory(ies) under his/her supervision comply with this requirement. However, when "The Collaboration" does not provide material for collecting and storing clinical samples, appropriate materials from the investigator's site must be used. Refer to the Module on Clinical Trial Supplies in the SPM ***and lab manual***.

Section 6.7.2 Biological samples

Table 9 Biological samples

Sample type	Quantity	Unit	Time point	Subjects
Blood sampling for Safety assessment	At least 8	mL	Days 0, 7, 30 and 37[1]	Safety and immune sub-cohort[2]
Blood sampling for Humoral responses	At least 2	mL	Days 0, 60 and Years 1, 2, 3	Safety and immune sub-cohort[2]
Blood sampling for CMI	At least ~~18~~ ***2.5***	mL	Days 0***,*** 60 and Years 1, 2, 3	Safety and immune sub-cohort[2]
Blood sampling for ~~QuantiFERON® TB Gold~~ ***QFTG***	At least 3	mL	Day 0 and Years 1, 2, 3	Safety and immune sub-cohort[2]
Blood sampling for QFTG	***At least 3***	***mL***	***Day 0 and Year 3***	***All subjects[3]***
Blood sampling for RNA expression profiling	At least 2.5	mL	Days 0 and Day 44[34] and when TB diagnosis is confirmed	All subjects
Sputum sampling for TB diagnosis[45]	Approximately 5	mL	Throughout the study when pulmonary TB is suspected	All subjects with suspicion of pulmonary TB
Blood sampling for initial HIV testing - Step 1 (Rapid test)	Finger prick or at least 2.5	mL	At screening	All subjects living in a region with HIV prevalence > 1%
			When TB diagnosis is confirmed	All subjects diagnosed with TB
Blood sampling for HIV testing - Step 2 (Rapid test)	Finger prick or at least 2.5	mL	At screening and when TB diagnosis is confirmed	All subjects positive at Step 1[56]
Blood sampling for HIV testing - Step 3 (ELISA)	At least 2.5	mL	At screening[5]	Discordant Rapid test results or if no Rapid test performed[67]
			When TB diagnosis is confirmed	At least one positive Rapid test ~~or if no Rapid test performed~~[67]

Sample type	Quantity	Unit	Time point	Subjects
Blood sampling for PCR - Step 4	At least 2.5	mL	When TB diagnosis is confirmed	Indeterminate ELISA results ~~or no ELISA performed and at least one positive Rapid test~~[67]
Blood sampling for CD4 count	At least 2.5	mL	When TB diagnosis is confirmed and confirmed HIV-positive	HIV positive subjects with TB diagnosis confirmed

1 Vitamin D levels will be measured at day 0 only.
2 Refer to Section 5.1 for sub-cohort description.
3 At study end (Year 3) QFTG testing will only be performed for subjects QFTG-negative at baseline.
~~3~~4 The most informative time point for collection of blood samples for RNA expression profiling post dose 2 will be determined. The exact time point will be communicated to the investigators appropriately at the latest at study initiation. Additionally, samples for RNA expression profiling must be collected at the time of TB diagnosis.
45 Three sputum samples, preferably taken in the morning, within a one week interval, will be collected whenever a subject presents with clinical suspicion of pulmonary TB.
~~5~~6 HIV positive subjects at screening will be referred for confirmatory HIV diagnosis/management as per site SOPs.
~~6~~7 Only the final conclusive diagnosis is to be recorded in the CRF.

Section 6.7.3 Laboratory assays

Table 11 Cell-Mediated Immunity (CMI)

System	Component	Challenge	Method	Unit	Cut-off	Laboratory*
~~PBMC~~***Whole blood***	CD4+/CD8+ T cells expressing at least two immune markers (CD40L and/or IL-2 and/or TNF-α and/or IFN-γ)	M72	ICS - intraCellular Cytokine Staining	number of events per million cells	N/A	~~CEVAC**~~***GSK Biologicals*

*Refer to APPENDIX B for the laboratory addresses.
~~**Or as designate by GSK Biologicals~~

Table 12 RNA expression profiling

System	Component	Method	Unit	Laboratory*
Whole blood	RNA expression	Microarray	NA	***COVANCE****

*Refer to APPENDIX B for the laboratory addresses.
Or as designated by "The Collaboration".

Table 13 Diagnostic test for pulmonary TB disease

System	Component	Method	Unit	Laboratory*	Component priority ranking
Sputum	Mycobacterium tuberculosis DNA	Xpert MTB/RIF assay	NA	~~As designated by "The Collaboration"~~ ***Central Laboratory***	1
Sputum	Mycobacterium tuberculosis	Microbiological culture	NA	~~As designated by "The Collaboration"~~ ***Central Laboratory***	2

Table 14 Diagnostic tests for HIV

System	Component	Method	Unit	Laboratory*
Whole Blood or Serum	Anti-HIV antibodies, screening step 1, step 2	HIV Rapid Test**	NA	~~As designated by "The Collaboration"~~ ***At the Investigator's lab***
Serum	Anti-HIV antibodies, screening step 3	ELISA	NA	~~As designated by "The Collaboration"~~ ***At the Central Laboratory***
Whole Blood	HIV RNA, screening step 4	HIV PCR	NA	~~As designated by "The Collaboration"~~ ***At the Central Laboratory***
Whole blood	CD4 cells	Flow cytometry	Cells/mm^3	~~As designated by "The Collaboration"~~ ***At the Central Laboratory***

Table 15 Haematology, Serum Chemistry, Urine tests

System	Component	Method	Scale
Whole blood	Alanine amino transferase	As per laboratory standard procedures	Quantitative
	Aspartate amino transferase		
	Creatinine		
	Bilirubin (total)		
	Platelets		
	Leucocytes		
	Erythrocytes		
	Haemoglobin		
	Vitamin D		
	IFN-γ release by T cells	QuantiFERON® TB Gold Assay	~~Qualitative~~Quantitative
Urine	β-HCG	As per laboratory standard procedures	Qualitative

~~The laboratory that will perform Xpert MTB/RIF, Microbiological culture and HIV testing is not yet identified and will be defined before study start.~~

~~The laboratory that will perform RNA expression profiling is not yet identified and will be identified before study end.~~

Section 6.7.4.1 Diagnostic assays

~~For the safety and immune sub-cohort a QuantiFERON® TB Gold assay~~ ***QFTG testing*** will be done ~~prior to dose 1~~ ***at baseline*** (Day 0) ***for all subjects, at study end (Year 3) for all subjects QFTG-negative at baseline and yearly*** ~~and post dose 2~~ (Years 1, 2 and 3) ***for subjects included in the safety and immune sub-cohort***, to detect CMI responses to TB infection by measuring IFN-γ produced ***by memory T cells*** in whole blood after incubation with synthetic peptides of the Mtb-secreted antigens Early Secretory Antigen Target-6 (ESAT-6)***,*** ~~and~~ Culture Filtrate Protein-10 (CFP-10) ***and TB 7.7.*** These TB-specific proteins stimulate a robust and detectable immune response in TB-infected and previously TB-infected people. ***In addition, QFTG testing will be done whenever a subject is diagnosed with TB disease.***

Section 6.7.4.2 Immunological read-outs

After collection, whole blood samples will be incubated with pool(s) of overlapping peptides covering the M72 antigen sequence and with the stimulation controls for 2 hours in the presence of anti-CD28 and anti-CD49d antibodies. Subsequently, ~~F~~*f*low cytometry using a short-term stimulation Intracellular Cytokine Staining (ICS) assay ~~on frozen Peripheral Blood Mononuclear Cells (PBMCs)~~ will be used to characterise M72-specific CD4+/CD8+ T cells expressing at least two immune markers (CD40L and/or IL-2 and/or TNF-α and/or IFN-γ) on Days 0, 60, and Years 1, 2 and 3.

~~Complementary analyses may be performed. Flow cytometry using ICS on PBMCs may be used to evaluate M72-specific expression of other cytokines, including IL-13 and/or Il-17, and markers characterising memory and functionality responses as well as to characterise other TB antigen specific CD4+ and CD8+ T cells (such as PPD, Early Secretory Antigenic Target- 6 [ESAT-6], Culture Filtrate Protein-10 [CFP-10], etc).~~

Table 16 Immunological read-outs

Blood sampling time point		Sub-cohort Name	No. subjects	Component
Type of contact and time point	Sampling time point			
Visit 2 (Day 0)	Pre Vacc	Safety and immune sub-cohort	~~500~~***450***	M72-specific T-cells (ICS on ***whole blood*** ~~PBMCs~~) Anti M72 Ab (ELISA on serum)
Visit 7 (Day 60)	Post Vacc 2			M72-specific T-cells (ICS on ***whole blood*** ~~PBMCs~~) Anti M72 Ab (ELISA on serum)
Visit 8 (Year 1)	LTFU			M72-specific T-cells (ICS on ***whole blood*** ~~PBMCs~~) Anti M72 Ab (ELISA on serum)
Visit 9 (Year 2)	LTFU			M72-specific T-cells (ICS on ***whole blood*** ~~PBMCs~~) Anti M72 Ab (ELISA on serum)
Visit 10 (Year 3)	LTFU			M72-specific T-cells (ICS on ***whole blood*** ~~PBMCs~~) Anti M72 Ab (ELISA on serum)

Section 6.7.4.3 Haematology/Biochemistry

Table 17 Biochemistry/haematology readouts

Blood sampling time point		Sub-cohort Name	No. subjects	Component
Type of contact and time point	Sampling time point			
Visit 2 (Day 0)	Pre-Vacc	Safety and immune sub-cohort	~~500~~ ***450***	Hb, PLT, WBC ALT AST Creatinine Bilirubin (total) Vitamin D
Visit 3 (Day 7)	Post-Vacc 1	Safety and immune sub-cohort	~~500~~ ***450***	Hb, PLT, WBC ALT AST Creatinine Bilirubin (total)

Blood sampling time point		Sub-cohort Name	No. subjects	Component
Type of contact and time point	Sampling time point			
Visit 4 (Day 30)	Post-Vacc 1	Safety and immune sub-cohort	~~500~~ ***450***	Hb, PLT, WBC ALT AST Creatinine Bilirubin (total)
Visit 5 (Day 37)	Post Vacc 2	Safety and immune sub-cohort	~~500~~ ***450***	Hb, PLT, WBC ALT AST Creatinine Bilirubin (total)

Section 11.2 Secondary endpoints

Safety

- ***Occurrence of grade ≥ 2 haematological and biochemical levels at:***

  Days 0, 7, 30 and 37

Immunogenicity

- Evaluation of CMI responses with respect to components of the study vaccine, in the safety and immune sub-cohort:
  - Determined by the frequency of M72-specific CD4+/CD8+ T cells per million cells identified after *in vitro* stimulation, as expressing at least 2 immune markers among CD40L, IL-2, IFN-γ and TNF-α.
  - ~~Determined by the frequency of M72-specific CD4+/CD8+ T cells per million cells identified after in vitro stimulation, as expressing any combination of immune markers among CD40L, IL-2, IFN-γ and TNF-α.~~

Time points: prior to dose 1 (Day 0) and post dose 2 (Day 60, Years 1, 2 and 3)

Section 11.3 Tertiary endpoints

- ***Evaluation of CMI responses with respect to components of the study vaccine, in the safety and immune sub-cohort:***
  - ***Determined by the frequency of M72-specific CD4+/CD8+ T cells per million cells identified after in vitro stimulation, as expressing any combination of immune markers among CD40L, IL-2, IFN-γ and TNF-α.***

  Time points: prior to dose 1 (Day 0) and post dose 2 (Day 60 and Years 1, 2 and 3).
- ***QFTG test result.***

  Time points: prior to dose 1 (Day 0), post dose 2 (Year 3)

Section 11.4 Determination of sample size

The primary objective is to demonstrate that the ***lower limit of the 95% Confidence interval of*** vaccine efficacy (VE), ***obtained from the Cox proportional hazards regression model (VE=1-estimated hazard ratio)*** associated to the M72/AS01$_E$ vaccine

in preventing ~~a~~ first episode***s*** of definite pulmonary TB disease ***meeting the first case definition is >0***, ~~is statistically significant at the 5 % two-sided significance level, to demonstrate that the M72/AS01$_E$ vaccine statistically reduces the number of definite TB cases when compared to the control group (placebo)~~.

Statistical analysis during the course of this study will include an interim analysis (IA) for ~~advanced Phase III preparations and a final efficacy analysis~~ ***futility, i.e. if a too low vaccine efficacy is observed at the time of the interim analysis, the trial will be stopped for futility. Therefore, no alpha adjustment is needed***.

~~Null hypothesis (H0): Vaccine efficacy in prevention of definite pulmonary TB disease ≤ 0% (interim and final analysis).~~

~~Alternative hypothesis (Ha): Vaccine efficacy in prevention of definite pulmonary TB disease > 0% (interim and final analysis).~~

~~Procedures to maintain the overall type I error for the primary objective to 0.05 (two-sided):~~

~~The two sided Type I error alpha is divided as 0.001 and 0.049 at interim and final analysis, respectively.~~

~~The study will only be fully analysed and unblinded early if extremely high efficacy is observed at the interim analysis. Therefore a very small alpha is used for the IA.~~

Using a ~~one proportion test (Ha: p<0.5)~~ ***logrank test*** with 80% power for a ***true*** VE of 70% ***(hazard ratio of 0.3)*** and a two-sided 5% significance level, the number of cases required for a fixed sample design is ~~28~~ ***27*** cases, ***based on the assumption that the hazard rates are proportional. These results are obtained by the program PASS 2005.***

To obtain these ~~28~~ ***27*** cases in total at final efficacy analysis, ~~using~~ ***assuming*** a mean yearly attack rate of ~~0.29~~ ***0.35*** % in the control group, 3 years of follow-up ***for each subject*** and a ~~rate of~~ loss of follow-up time of ~~13~~***12***% due to drop out over the 3 years period, ~~7000~~ ***4500*** subjects need to be enrolled ***based on the logrank test and parameters described above***.

<u>Sample size considerations for the safety and immune sub-cohort:</u>

Approximately 450 subjects will be enrolled in the safety and immune sub-cohort (225 subjects in each treatment group) with up to 150 subjects/contributing country (75 subjects in each treatment group).

Assuming a standard deviation of log10(value*) of 0.40**, as observed in previous studies, a precision for the 95 % CI for log10(value*) of 0.052 or 0.092 will be obtained for a total samples size of 225 subjects vaccinated with M72/AS01$_E$ or 150 subjects vaccinated with M72/AS01$_E$/country respectively.

Precision of the log10(value*) is equal to half of the with of the 95% CI for log10(value*). The precision of the 95% CI and 95% CI can be derived by assuming

various levels of the value*. Precision of the 95% CI for GMT for a sample size of 150 subjects and 450 subjects is presented in Table 26 and Table 27, respectively.

****GMT or median frequency of CD4 T cells expressing at least two immune markers among CD40L, IL-2, IFN-γ and TNF-α***

*****A standard deviation around 0.40 for log10(GMT) and below 0.40 for log10(CD4 T cells expressing at least 2 immune markers) has been observed in previous studies.***

Table 26 Precision for the 95% CI for GMT obtained from the 95% CI build on log10(GMT) assuming various levels of GMT for a sample size of 75 subjects vaccinated with M72/AS01$_E$

GMT	Log10(GMT)	precision for the 95% CI for log10(GMT)	95% CI for the GMT		precision for the 95% CI for the GMT
			LL	UL	
500	2.698970004	0.092	404.5479	617.9737	106.7129
1000	3	0.092	809.0959	1235.947	213.4258
2000	3.301029996	0.092	1618.192	2471.895	426.8515
3000	3.477121255	0.092	2427.288	3707.842	640.2773
4000	3.602059991	0.092	3236.384	4943.79	853.7031
5000	3.698970004	0.092	4045.479	6179.737	1067.129
6000	3.77815125	0.092	4854.575	7415.685	1280.555

CI: Confidence interval; GMT: Geometric Mean Titre; LL: Lower limit; UL: Upper limit

Table 27 Precision for the 95% CI for GMT obtained from the 95% CI build on log10(GMT) assuming various levels of GMT for a sample size of 225 subjects vaccinated with M72/AS01$_E$

GMT	Log10(GMT)	precision for the 95% CI for log10(GMT)	95% CI for the GMT		precision for the 95% CI for the GMT
			LL	UL	
500	2.698970004	0.052	443.578	563.5987	60.01036
1000	3	0.052	887.156	1127.197	120.0207
2000	3.301029996	0.052	1774.312	2254.395	240.0414
3000	3.477121255	0.052	2661.468	3381.592	360.0622
4000	3.602059991	0.052	3548.624	4508.79	480.0829
5000	3.698970004	0.052	4435.78	5635.987	600.1036
6000	3.77815125	0.052	5322.936	6763.185	720.1243

CI: Confidence interval; GMT: Geometric Mean Titre; LL: Lower limit; UL: Upper limit

Section 11.5.1 Total Vaccinated cohort

The Total Vaccinated cohort for efficacy will include all vaccinated subjects ~~for whom data concerning efficacy endpoint measures are available~~ and will include all TB cases occurring post dose 1.

Section 11.5.2 Modified Total vaccinated cohort for interim efficacy analysis

The modified TVC for interim efficacy analysis will include all subjects:

- ***For whom two doses of study vaccines have been administered according to protocol (administration site and route)***
- ***Without randomisation failure***
- ***Without wrong replacment***
- ***Who did not present with TB disease, according to any case definition, during the vaccination period (day of first vaccination up to 1 month post dose 2).***

Section 11.5.4 According-to-protocol cohort for analysis of immunogenicity

The ATP cohort for immunogenicity will include all subjects from the safety and immune sub-cohort that:

- Received ~~all~~ ***two*** vaccinations according to protocol procedures within specified intervals.
- Who complied with blood sampling schedules.
- Who had biochemistry and haematology values within normal range before any vaccination (exclude only if all values were out of range).
- For whom post vaccination blood samples are available.
- Who did not receive a vaccine/medication that may lead to elimination of an ATP analysis.
- Who did not present with a medical condition that may lead to elimination of an ATP analysis.
- For whom the randomization code has not been broken.
- ~~Who completed the whole vaccination course or if not, no blood samples should have been taken after discontinuation of the vaccination course.~~

Section 11.5.5 According-to-protocol cohort for analysis of QFTG status

The ATP cohort for analysis of QFTG status will include all subjects:

- ***Who received two vaccinations according to protocol procedures within specified intervals.***
- ***For whom post vaccination QFTG blood samples are available.***
- ***Who did not receive a vaccine/medication that may lead to elimination of an ATP analysis.***
- ***Who did not present with a medical condition that may lead to elimination of an ATP analysis.***
- ***For whom the randomization code has not been broken.***
- ***With a negative QFTG test result at baseline.***
- ***With no indeterminate QFTG test result at any time point.***

Section 11.6 Derived and transformed data

- The cut-off value for ***M72-specific*** antibody titres is 2.8 EU/mL.

Section 11.8 Analysis of efficacy

Vaccine efficacy will be estimated from a Cox proportional hazard regression model (VE=1-hazard ratio) and 95% CIs and Wald p-value will be derived. The primary analysis will be unadjusted but secondary analyses will evaluate the effect of potential covariates. ~~As a primary analysis, vaccine efficacy will be estimated using a frequentist approach. In this analysis, vaccine efficacy is defined as 1-RR where RR is the Poisson rate ratio.~~

~~The rate in the vaccine group is equal to the number of cases in the vaccine group divided by the follow-up time. The rate in the control group is defined the same way. CIs and p-values will be calculated using the exact method implemented in the Poisson procedure of the StatXact package provided by Cytel. This method is also available in proc genmod when using the exact option.~~

~~At interim analysis, the success criterion for the primary objective is the following:~~

~~*The lower limit of the* 99.9*% two-sided confidence interval for the VE against first occurrence of definite pulmonary TB disease, meeting the first case definition, is above 0% (this corresponds to a two-sided alpha of* 0.1*%).*~~

~~The interim analysis for advanced Phase III preparation will be performed after approximately half of the total number of definite pulmonary TB cases, meeting the first case definition, have accumulated in the ATP cohort for efficacy based on data that are as clean as possible.~~

At final efficacy analysis, the success criterion for the primary objective is the following:

The lower limit of the 95~~.1~~*% two-sided confidence interval for the VE against first occurrence of definite pulmonary TB disease, meeting the first case definition is above 0%* ~~*(this corresponds to a two-sided alpha of* 4.9*%)*~~.

~~As a secondary analysis, the rate ratio will be calculated taking into account country, gender and other co-variables as strata.~~

Kaplan-Meier survival curves for the vaccine and control groups will be presented together with p-values from the logrank test.

If the primary objective is met, the confirmatory secondary objective will be evaluated with the following success criterion:

The lower limit of the 95~~.1~~*% two-sided confidence interval (CI) for the VE against first occurrence of Definite Xpert MTB/Rif positive pulmonary TB disease not associated with HIV-infection, meeting the second case definition, is above 0%.*

For all other secondary efficacy objectives, Kaplan-Meier survival curves will be plotted and compared by means of the logrank test. The magnitude of the vaccine efficacy will be estimated using Cox regression.

For the tertiary efficacy analysis, the analysis will be based on the ATP cohort for analysis of Mtb infection. The vaccine efficacy will be estimated as 1- the Rate Ratio (RR) where the rate is defined as the number of subjects QFTG- positive (converters) at study end divided by the number of subjects QFTG- negative at baseline, in each group. These two proportions will be compared using the Fisher exact test.

Section 11.10

- ***The number of subjects with grade ≥ 2*** ~~Clinical~~ laboratory ~~observations~~ ***abnormalities*** will be ~~described~~ ***tabulated***.

Section 11.12.1 Sequence of analyses

- An interim efficacy analysis ***for futility*** will be performed after ~~approximately half of the total number of~~ ***approximately 10*** ~~definite~~***Xpert MTB/Rif positive*** pulmonary TB cases ***(approximately 40% of the target events) in confirmed HIV-negative subjects***~~, meeting the first case definition,~~ have accumulated in the ~~ATP cohort~~ ***modified TVC*** for ***interim*** efficacy ***analysis***, during the efficacy surveillance period starting 1 month post dose 2 (Day 60). ***If the study is stopped for futility, subjects will continue to be followed-up for safety up to 6 months post dose 2 and a study conclusion visit will be scheduled at the earliest convenient time point. All data (efficacy, safety and immunogenicity) collected up to last subject last visit (LSLV) will be cleaned, unblinded and analysed.***
- Analysis of efficacy will be performed when ~~28~~ ***27*** cases of definite pulmonary TB disease, meeting the first case definition, have accumulated in the ATP cohort for efficacy, during the efficacy surveillance period starting 1 month post dose 2 (Day 60) or at the latest when all subjects have completed the Month 36 visit.

Section 11.12.2 Statistical considerations for interim analyses

~~To start advanced phase III preparations~~ ***In order to avoid trial progression with a candidate vaccine with very low efficacy,*** an interim efficacy analysis ***for futility*** is planned***, i.e. if a too low vaccine efficacy is observed at the time of the interim analysis, the trial will be stopped for futility. Therefore, no alpha adjustment is needed***.

…

Stopping rules for futility will be defined in the statistical analysis plan for interim analysis.

~~Specifications for recommendations by the IDMC will be documented in the IDMC charter.~~

APPENDIX A Laboratory assays

Cell-mediated immune responses

Stimulation of whole blood

After collection, whole blood samples will be stimulated at 37°C with pool(s) of overlapping peptides covering the M72 antigen sequence and with the stimulation controls for 2 hours in the presence of anti-CD28 and anti-CD49d antibodies. Then, cytokine secretion inhibitor (Brefeldin A) will be added for an additional overnight incubation. The day after, red blood cells will be lysed and white blood cells will be washed and fixed prior to cryopreservation and kept in storage for subsequent cytometry analysis.

~~*Preparation of Peripheral Blood Mononuclear Cells*~~

~~PBMCs will be isolated from whole blood cells by Ficoll hypaque gradient following GSK Bio standard procedures. After the washing steps, isolated PBMCs will be re-suspended in 1 mL of 90% cold foetal calf serum and 10% DMSO to be frozen slowly to -70°C (+/-5°C) or colder and transferred to liquid nitrogen for storage up to subsequent CMI analysis is described below.~~

Intracellular Cytokine Staining (ICS) ~~*using frozen PBMCs*~~

Intracellular fluorescent staining for cytokines (ICS) provides information on the frequency of cells responding to one particular antigen; it allows to define the frequency of CD4+ and CD8+ T lymphocytes expressing molecules involved in immunity such as IFN-γ, IL-2, TNF-α, and/or CD40L (conventional CD4+ and CD8+ T cell response markers). ~~ICS will be performed on thawed PBMCs stimulated with pools of overlapping peptides covering the entire M72 sequence and/or other Mtb related antigens for 2 hours. Then an intracellular block (e.g. Brefeldin A) will be added to inhibit cytokine secretion for an additional overnight incubation.~~ ***Thawed*** ~~C~~*c*ells will ~~subsequently~~ be ~~harvested,~~ stained for surface markers (e.g. CD4+ or CD8+ for T cells) and then fixed. Fixed cells will then be made permeable and stained with labelled cytokine-specific antibody. Cells will then be washed, re-suspended in PBS and analysed by flow cytometry.

QuantiFERON® TB Gold assay Amended 21 March 2013)

For evaluation of Mtb complex infection, whether tuberculosis disease, latent TB infection or past TB infection,, the QuantiFERON®-TB Gold In-Tube Assay (Cellestis) will be used. QuantiFERON®-TB Gold In-Tube is an in vitro diagnostic test using peptide cocktails simulating ESAT-6, CFP-10 and TB7.7 proteins to stimulate cells in heparinised whole blood. These proteins are absent from all BCG strains and from most non-tuberculosis mycobacteria with the exception of M. kansarii, M. szulgai and M. marinum. The assay will be performed according to the manufacturer's instructions. Briefly, blood is collected directly into QuantiFERON®-TB Gold collection tubes including a Nil Control tube, TB antigen tube and Mitogen Control tube. The tubes should be incubated for 16-24 hours at 37°C prior to harvesting plasma. IFN-γ concentrations in plasma are determined using the QuantiFERON®-TB Gold ELISA kit.

APPENDIX B Clinical Laboratories

Table 29 Outsourced Laboratories

Laboratory	Address
CEVAC - University of Gent	De Pintelaan, 185 Gent Belgium
COVANCE	***401 Terry Avenue North*** ***Suite 200*** ***Seattle, Washington 98109*** ***USA***
Central laboratories	
BARC South Africa Pty Ltd	***Napier Road POB 8475*** ***2000 Johannesburg*** ***Republic of South Africa***
KEMRI/CDCResearch Station, Kenya	***Busia Road POB 1578-40100*** ***Kisumu*** ***Kenya***
CIDRZ Zambia	***Alick Nkhata Road*** ***Kalingalinga Clinic Grounds*** ***Lusaka*** ***Zambia***

GlaxoSmithKline Biologicals

Clinical Research & Development

Protocol Amendment 5

eTrack study number and Abbreviated Title	115616 (TUBERCULOSIS-018)
Amendment number:	Amendment 5
Amendment date:	29 January 2014
Co-ordinating author:	PPD, Scientific Writer, XPE Pharma&Science, contractor for GSK Biologicals

Rationale/background for changes:

Optimized study design and sample size by increasing event rate by focussing on IGRA positive subjects. Baseline screening for pulmonary TB as precautionary safety measures. Separate protocol for biobanking of blood samples for future research on biological correlates for TB disease.

Amended text has been included in *bold italics* and deleted text in ~~strikethrough~~ in the following sections:

Cover page

Contributing authors GSK Biologicals	• PPD, ***Clinical Research and Development Lead***~~Director Clinical Development~~ • PPD, Project Statistician • PPD, ~~Global Study Manager~~***Study Delivery Manager*** • PPD***, Project Delivery Lead*** • PPD, Project Manager Clinical Readouts • PPD PPD Safety ~~Scientist~~***Physician*** • PPD PPD Study Data Manager • PPD, Senior Manager ~~Clinical~~ Regulatory ***Affairs***~~and Labelling~~ • PPD***, Global Regulatory Lead***
Contributing Authors Aeras	• PPD, ***President &*** Chief ~~Scientific~~***Executive*** Officer • PPD, Director Clinical Development

Sponsor Signatory Approval Page

Detailed Title	A phase IIb, double-blind, randomised, placebo-controlled study to evaluate the efficacy, safety and immunogenicity of GSK Biologicals' candidate tuberculosis (TB) vaccine GSK 692342 against TB disease, in ~~healthy~~ adults aged 18-50 years, living in a TB endemic region.

Investigator Agreement Page

Detailed Title	A phase IIb, double-blind, randomised, placebo-controlled study to evaluate the efficacy, safety and immunogenicity of GSK Biologicals' candidate tuberculosis (TB) vaccine GSK 692342 against TB disease, in ~~healthy~~ adults aged 18-50 years, living in a TB endemic region.

Synopsis

Rationale for the study and study design

- **Rationale for the study**

In TB endemic settings the incidence of pulmonary TB disease is higher in people who have been infected with Mtb as evidenced by a positive Interferon gamma release assay (IGRA). For the present first phase II proof-of-concept trial focussing on this IGRA+ adult population maximizes the trial efficiency by increasing the event rate in the control group.

This Phase IIb study aims at collecting initial efficacy data and gathering further safety and immunogenicity data on the M72/AS01$_E$ vaccine candidate in ~~healthy~~ adults living in a TB endemic region.

- **Rationale for the study design**

This Phase IIb study is designed to evaluate the protective efficacy of the M72/AS01$_E$ candidate ***TB*** vaccine against definite pulmonary TB disease not associated with HIV-infection, versus placebo, following 2 injections given one month apart. The protective efficacy will be evaluated in ***baseline IGRA positive*** adults living in a TB endemic region aged 18-50 years, i.e. in an age range where the incidence of ***TB*** is high.

This study is an international, multicentre, phase IIb, double-blind, randomized, controlled trial. There will be follow-up for efficacy for ~~approximately~~***up to*** 3 years after vaccination via regular visits or contacts to screen for possible TB.

To minimize the ***potential confounding effect of*** HIV ~~prevalence~~***infection*** in the study population ~~at baseline~~, HIV testing will be performed at screening ~~in regions with a high~~***and only*** HIV ~~prevalence (> 1%).~~***negative subjects will be enrolled***.

An Independent Data Monitoring Committee (IDMC) ~~will be~~***has been*** constituted for this study to perform periodic safety reviews of the safety data ~~and evaluate the interim efficacy analysis~~.

~~An IFN-γ positive T-cell response induced by past or present Mtb infection, as measured by the QuantiFERON® TB Gold (QFTG) assay (Cellestis), will be evaluated at baseline for all subjects, at study end (Year 3) for all subjects QFTG-negative at baseline and yearly for subjects in the safety and immune sub-cohort. In addition, a QFTG assay will be performed whenever a subject is diagnosed with TB disease.~~

Objectives

Primary

*The lower limit of the ~~95~~**90**% two-sided confidence interval (CI) for the VE (using a Cox regression model) against first occurrence of Definite pulmonary TB disease not associated with HIV-infection, meeting the first case definition, is above 0%.*

Secondary

The lower limit of the ~~95~~ ***90***% two-sided ~~confidence interval (~~CI~~)~~ for the VE (using a Cox regression model) against first occurrence of Definite pulmonary TB disease not associated with HIV-infection, meeting the ~~first~~***second*** case definition, is above 0%.

~~**Tertiary**~~

~~To evaluate the protective efficacy of two doses of the M72/AS01$_E$ candidate vaccine against Mtb infection, as measured by QFTG conversion.*~~

~~• To identify candidate biological markers linked to the risk for TB disease.~~

~~*QFTG conversion is defined as the change from a negative QFTG test result (value below the cut off for IFN-γ concentration) to a positive QFTG test result (value at or above the cut off for IFN-γ concentration) in two serial samples.~~

Synopsis Table 1 Study groups and epochs foreseen in the study

Study groups	Number of subjects	Age (Min/Max)	Epoch 001
M72AS01	~~2250~~***1753***	18 - 50 years	x
Control	~~2250~~***1753***	18 - 50 years	x

Sampling schedule:

– Blood samples will be collected in the safety and immune sub-cohort for evaluation of biochemistry/haematology parameters (Days 0, 7, 30 and 37), CMI

responses (Days 0, 60 and Years 1, 2 and 3) and humoral immune responses (Days 0, 60 and Years 1, 2 and 3). ***Vitamin D levels will be measured at Day 0.***

- Blood samples will be collected for QFTG testing at ~~baseline (Day 0)~~***screening*** for all subjects~~, at study end (Year 3) for .~~
- ***A respiratory sputum sample will be collected prior to vaccination in*** all subjects ~~QFTG-negative at baseline and yearly for subjects included in the safety and immune sub-cohort~~***for nucleic acid amplification test to rule out pulmonary TB.***
- ~~Blood samples for RNA expression profiling will be collected from all subjects (Days 0 and post dose 2)~~
- ***Blood samples will be collected for initial HIV testing at screening for all subjects. Repeat HIV testing will be performed at the last study visit V9, unless the subject has been confirmed HIV positive before V9.***
- Whenever a subject is diagnosed with TB disease, blood samples will be collected for HIV testing~~, mRNA expression profiling~~ ***and*** ~~QFTG testing~~***HbA1c measurement***.

Data collection: Electronic Case Report Form (eCRF) **-** ***Inform*™**

Safety monitoring: an Independent Data Monitoring Committee (IDMC), operating under a charter,~~ will be~~***has been*** constituted for this study to oversee the study. They will perform safety reviews ~~three times in the first year of enrolment and~~ twice a year, or more frequently if deemed necessary on an ongoing basis for the duration of the study ~~and evaluate the interim efficacy analysis~~.

Case Definitions:
Fourth case definition – ~~Definite~~*Microbiological* pulmonary TB

Number of subjects: Approximately ~~450~~***3506***.
Endpoints: Secondary

Efficacy

- Occurrence of grade ≥ 2 haematological and biochemical***, in the safety and immune sub-cohort,*** levels at:

  Days 0, 7, 30 and 37

Immunogenicity

- Evaluation of CMI responses with respect to components of the study vaccine, in the safety and immune sub-cohort:
- Determined by the frequency of M72-specific CD4+/CD8+ T-cells per million cells identified after in vitro stimulation, as expressing ~~at least 2~~***any combination of*** immune markers among CD40L, IL-2, IFN-γ and TNF-α.

~~**Tertiary**~~

- ~~Evaluation of CMI responses with respect to components of the study vaccine, in the safety and immune sub-cohort:~~
  - ~~Determined by the frequency of M72-specific CD4+/CD8+ T cells per million cells identified after in vitro stimulation, as expressing any combination of immune markers among CD40L, IL-2, IFN-γ and TNF-α.~~

  ~~Time points: prior to dose 1 (Day 0) and post dose 2 (Day 60 and Years 1, 2 and 3)~~
- ~~QFTG test result.~~

  ~~Time points: prior to dose 1 (Day 0), post dose 2 (Year 3)~~
- ~~Description of RNA expression profile signatures in selected samples as a correlate of risk to describe protective and non-protective immune responses after 2 doses of M72/AS01$_E$.~~

  ~~Time points: Prior to dose 1 (Day 0), post dose 2 and when TB is diagnosed.~~

LIST OF ABBREVIATIONS

~~**IA**~~	~~Interim Analysis~~
HbA1c	***Glycated haemoglobin***
IPT	***Izoniazid Preventive Therapy***
~~**mRNA**~~	~~messenger Ribonucleic Acid~~
~~**RDE**~~	~~Remote Data Entry~~
~~**RNA**~~	~~Ribonucleic acid~~
SOC	***System Organ Class***

GLOSSARY OF TERMS

QFTG ~~conversion~~	~~The change from a negative QFTG test result (value below the cut off for IFN-γ concentration) to a positive QFTG test result (value at or above the cut off for IFN-γ concentration) in two serial samples.~~ The QFTG assay detects memory T-cell responses against Mtb as a surrogate for exposure to/infection with Mtb. The QFTG does not directly measure the presence of Mtb. The QFTG does not indicate whether the immune response of a subject ~~is~~***was*** able to eliminate the bacteria or whether Mtb is latently present

INTRODUCTION

TB is the second leading cause of mortality worldwide, leading to about ~~1.45~~***1.3*** million deaths a year [WHO, ~~2011~~***2013]***. It is estimated that a third of the world's population is infected ***latently*** with the *Mycobacterium tuberculosis* (Mtb) bacilli. Although not more than ~~10~~***5-20***% of infected individuals will become ill, the high number of infected people poses a great risk to individuals with reduced immunity who are at a higher risk for TB. The TB burden is compounded by the emergence of multi-drug resistant (MDR) and extensively-drug resistant (XDR) TB. Approximately ~~8.8~~***8.6*** million incident cases of TB were reported in ~~2010~~***2012*** globally. Most TB cases were in ~~the South-East~~ Asia ~~region~~ (mainly India and China~~),~~) ***and*** Africa (***58***% ***and 27***% respectively). ***Asia refers to the WHO Regions of South-East Asia and the Western Pacific*** ~~regions (40%, 24% and 19% respectively).~~ Of these incident cases, an estimated 1.1 million (13%) were Human Immunodeficiency Virus (HIV) positive [WHO, ~~2011~~***2013***]. TB is the most common co-existing condition in people who die from Acquired Immune Deficiency Syndrome (AIDS).

In subjects with concurrent Pulmonary TB disease receiving treatment the vaccination seemed to induced a higher incidence of grade 3 injection site reactions; this observation is under futher investigation.

1.3. Rationale for the study and study design

1.3.1. Rationale for the study

The burden of Mtb disease, particularly when compounded by HIV-infection and the emergence of MDR and XDR strains, is significant in TB endemic countries/regions. In 2010, approximately 40% of TB cases were reported in India and China, of which up to 80% were pulmonary in nature [WHO,~~2011~~***2013***].

In TB endemic settings the incidence of clinical TB disease is highest in people who have been infected with Mtb as evidenced by a positive Interferon gamma release assay (IGRA). For the present first phase II proof-of-concept trial focussing on this IGRA+ adult population maximizes the trial efficiency by increasing the event rate in the control group.

This Phase IIb study aims at collecting initial efficacy data and gathering further safety and immunogenicity data on the M72/AS01E vaccine candidate in ***IGRA+*** ~~healthy~~ adults, ***or with stable chonic medical condition(s)***, living in a TB endemic region.

All study participants may be offered to consent to participate in a second and parallel study Sponsored by AERAS which aims to collect a supplemental set of pre- and post-vaccination blood samples to constitute a Biobank. After completion of this study this Biobank will serve for investigations into biological correlates of risk for TB disease and potentially correlates of protection. Please refer to this separate protocol for further information.

1.3.2. Rationale for the study design

The protective efficacy will be evaluated in ***IGRA+*** adults living in a TB endemic region aged 18-50 years, i.e. in an age range where the incidence of tuberculosisTB is high (Figure 1).

The primary objective of this study is to evaluate the protective efficacy of two doses of the M72/AS01$_E$ candidate vaccine against definite pulmonary TB disease **not associated with HIV-infection**, as compared to placebo. To ~~minimize~~***avoid the potential confounding effect of*** HIV ~~prevalence~~***infection*** in the study population at baseline, HIV testing will be ~~performed~~***required to be negative*** at screening ~~in regions with a high HIV prevalence (>1%)~~.

An Independent Data Monitoring Committee (IDMC) ~~will be~~***has been*** constituted for this study to perform periodic safety reviews of the safety data~~ and evaluate the interim efficacy analysis~~.

A sub-cohort of subjects will be selected for specific follow-up for safety (solicited adverse events (AE) and selected Biochemistry and Complete Blood Count [CBC]) and immunogenicity. Unsolicited AEs ***and large swelling reactions***, serious adverse events (SAEs), potential immune-mediated diseases (pIMDs), AEs leading to withdrawal and pregnancies will be recorded for all subjects.

~~An IFN-γ positive T-cell response induced by past or present Mtb infection, as measured by the QuantiFERON® TB Gold (QFTG) assay (Cellestis), will be evaluated at baseline for all subjects, at study end (Year 3) for all subjects QFTG-negative at baseline and yearly in safety and immune the sub-cohort. In addition, a QFTG assay will be performed whenever a subject is diagnosed with TB disease.~~

2. OBJECTIVES

2.1. Primary objective

*The lower limit of the ~~95~~**90**% two-sided confidence interval (CI) for the VE (using a Cox regression model) against first occurrence of Definite pulmonary TB disease not associated with HIV-infection, meeting the first case definition, is above 0%*

2.2. Secondary objective

Efficacy:

*The lower limit of the ~~95~~**90**% two-sided ~~confidence interval (CI)~~ for the VE (using a Cox regression model) against first occurrence of Definite pulmonary TB disease not associated with HIV-infection, meeting the ~~first~~**second** case definition, is above 0%.*

To evaluate the protective efficacy of two doses of the M72/AS01$_E$ candidate vaccine against ~~**Definite**~~***Microbiological*** **pulmonary TB disease** meeting the fourth case definition, as compared to placebo.

Immunogenicity:

- ~~Tertiary objective~~

- ~~To evaluate the protective efficacy of two doses of the M72/AS01$_E$ candidate vaccine against Mtb infection, as measured by QFTG conversion.~~

- ~~To **identify** candidate biological markers linked to the risk for TB disease.~~

~~Refer to Section 11.3 for the definition of the tertiary endpoints.~~

~~Refer to the GLOSSARY OF TERMS for a definition of QFTG conversion.~~

3. STUDY DESIGN OVERVIEW

Figure 2 was updated to reflect the overview of the study design.

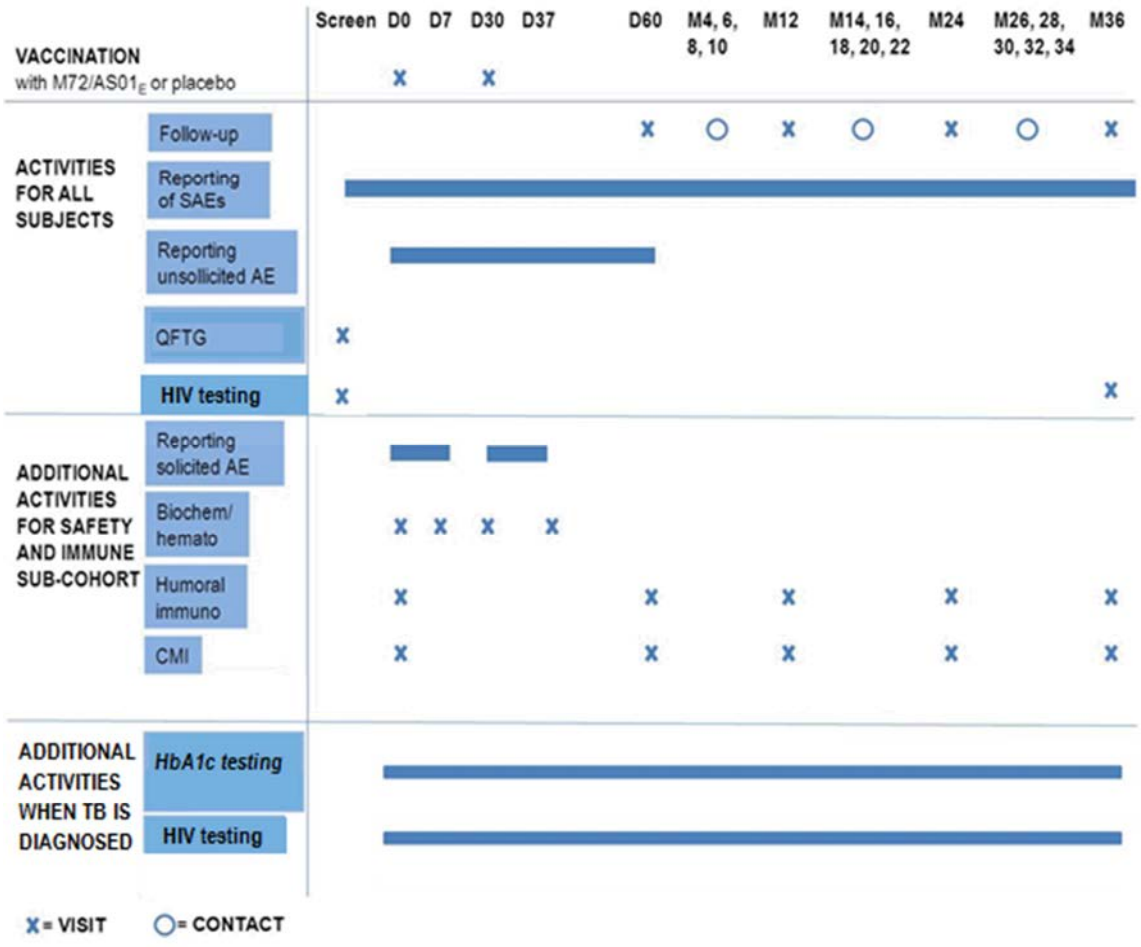


Table 1 Study groups and epochs foreseen in the study

Study Groups	Number of subjects	Age (Min - Max) (age unit)	Epoch 001
M72AS01	~~2250~~***1753***	18 - 50 years	x
Control	~~2250~~***1753***	18 - 50 years	x

- **Sampling schedule;**
  - Blood samples will be collected in the safety and immune sub-cohort for evaluation of biochemistry/haematology parameters (Days 0, 7, 30 and 37), CMI responses (Days 0, 60 and Years 1, 2 and 3) and humoral immune responses (Days 0, 60 and Years 1, 2 and 3). ***Vitamin D levels will be measured at Day 0.***
  - Blood samples will be collected for QFTG testing at ~~baseline (Day 0)~~***screening*** for all subjects~~, at study end (Year 3) for~~
  - ***A respiratory sputum sample will be collected prior to vaccination in all subjects*** ~~QFTG-negative at baseline and yearly for subjects included in the safety and immune sub-cohort~~***for nucleic acid amplification test to rule out pulmonary TB.***
  - ~~Blood samples for RNA expression profiling will be collected from all subjects at predefined time points (Days 0 and Day 44*).~~
  - ***Blood samples will be collected for initial HIV testing at screening for all subjects. Repeat HIV testing will be performed at the last study visit V9, unless the subject has been confirmed HIV positive before V9.***
  - Whenever a subject is diagnosed with TB disease, blood samples will be collected for HIV testing~~, mRNA expression profiling~~ ***and*** ~~QFTG testing~~***HbA1c measurement***. In addition, if a subject is HIV-positive, blood samples will be collected to determine CD4 cell counts.
    ~~*The most informative time point for collection of blood samples for RNA expression profiling post-dose 2 will be determined. The exact time point will be communicated to the investigators appropriately at the latest at study initiation.~~

- **Type of study:** self-contained.

- **Data collection:** electronic Case Report Form (eCRF~~). –~~ ***Inform™***).

- **Safety monitoring:** an IDMC, operating under a charter, ~~will be~~***has been*** constituted for this study to oversee the study. They will perform safety reviews twice a year, or more frequently if deemed necessary ~~and evaluate the interim efficacy analysis.~~

- **Diagnostic procedures for suspected Pulmonary TB:** during the entire study ***(not for screening)***, when a subject presents with clinical suspicion of pulmonary TB, three respiratory sputum samples, preferably taken in the morning, within ***maximum*** a one week interval, must be collected for testing with Xpert MTB/RIF and microbiological culture.

5. STUDY COHORT

5.1. Number of subjects/centres

Approximately ~~4500~~***3506*** men and women aged 18-50 years will be enrolled in the study. Enrolment will be terminated when target number of subjects is reached.

To ensure the gender of the study population is equally distributed between females and males, a cap has been put on the number of subjects per gender to be enrolled. A maximum of 60% of subjects from the same gender will be enrolled in the study and enrolment of females/males will be stopped at approximately ~~2700~~***2100*** subjects of any gender maximum.

Table 4 Sub-cohorts

Sub-cohort name	Description of additional activities	Estimated number of subjects
Safety and immune sub-cohort	Safety parameters for this sub-cohort will be collected as follows: • Solicited AEs will be collected for 7 days (day of vaccination and 6 subsequent days) following vaccination. • Serum biochemistry/haematology values will be measured at Days 0, 7, 30, and 37.	Approximately 450
	Immunogenicity: • Humoral immunogenicity will be evaluated by measuring M72-specific IgGs at Days 0 and 60 and Years 1, 2 and 3. • CMI will be evaluated by measuring M72-specific CD4+ and CD8+ T cells expressing at least 2 immune markers (CD40-L and/or IFN-γ and/or IL-2 and/or TNF-α) at Days 0 and 60 and Years 1, 2 and 3. • ~~The QuantiFERON status of subjects will be determined at Day 0 and Years 1, 2 and 3.~~ ***and any combination among CD40-L, IFN-g, IL-2 and TNF-a***	

5.2. Inclusion criteria for enrolment

All subjects must satisfy ALL the following criteria at study entry:

- ***Baseline positive IGRA test result.***
- ***Baseline negative HIV screen.***
- ***Baseline negative clinical screening questionnaire and negative sputum sample for Pulmonary TB disease.***
- Female subjects of childbearing potential may be enrolled in the study, if the subject:
  - has practiced adequate contraception* for ~~30~~***25*** days prior to vaccination, and
  - has a negative pregnancy test on the day of screening and the day of first vaccination, and

– has agreed to continue adequate contraception during the entire ~~treatment~~***vaccination*** period and for 2 months after completion of the vaccination series.

5.3. Exclusion criteria for enrolment

The following criteria should be checked at the time of study entry. If ANY exclusion criterion applies, the subject must not be included in the study:

- Current TB disease or ***history of*** TB ***disease and/or*** treatment ~~within the last 12 months.~~***for TB (including IPT)***.
- ~~Documented HIV-positive subject*.~~

  ~~*HIV testing and pre-test counselling will be performed in areas with high prevalence of HIV (> 1%).~~
- History of medically confirmed autoimmune disease ***(e.g. Type I diabetes, Lupus)***.

6. CONDUCT OF THE STUDY

6.2.2.2. Treatment allocation to the subject

6.2.2.2.1. Study group and treatment number allocation

The target will be to enrol approximately ~~4500~~***3506*** eligible subjects who will be randomly assigned to two study groups in a 1:1 ratio (approximately ~~2250~~***1753*** subjects in each group).

Allocation of the subject to a study group at the investigator site will be performed using ~~a~~***an internet based*** randomisation system ~~on internet~~ (SBIR). The randomisation algorithm will use a minimisation procedure accounting for:

- ***Centre***
- Gender: a maximum of ~~2700~~***2100*** females/males will be recruited.
- ~~TB disease categorised as history or no history of TB disease.~~

Minimisation factors will have equal weight in the minimisation algorithm.

The treatment numbers will be allocated by dose at the site of the investigator using SBIR.

After obtaining the ~~signed and dated ICF~~***informed consent*** from the subject and having checked the eligibility of the subject, the site staff in charge of the vaccine administration will access SBIR. Upon providing the subject identification number, the randomisation system will determine the study group and will provide the treatment number to be used for the first dose. The number of each administered treatment must be recorded in the eCRFon the Vaccine Administration screen.

6.4. General study aspects

Supplementary study conduct information not mandated to be present in this protocol is provided in the accompanying SPM. The SPM provides the investigator and the site personnel with administrative and detailed technical ***and operational*** information that does not impact the safety of the subjects.

6.4.1.1. Active follow-up for safety and efficacy

Subject safety follow-up and timely case capture of incident TB disease is of paramount importance. In addition to study visits at the study facilities, regular contacts (every two months) with the study subjects must be maintained using one or more of the following methods:

- Regular interval home visits by site staff.
- Phone calls to inquire about current health status, completed by home visits if the subject cannot be reached.
- ~~2~~***1-way short message service (***~~SMS)~~***sms) reminders and/or*** 2-way ***sms*** exchange.

6.4.3. Diagnostic procedures for the detection of suspected pulmonary TB

- Additionally if TB is confirmed a serological test (and pre-test counselling) for HIV-infection must be performed to rule out HIV co-infection~~ and a blood sample for RNA expression profiling and QFTG testing must be taken~~. If the subject is HIV-positive, additional testing must be performed to measure CD4 cell counts. ***Diabetes will be screened for by a Hb Ac1 test.***
- Results of HIV testing, including CD4 cell counts if applicable~~, and QFTG testing~~ must be recorded in the subjects' eCRF.

6.4.3. Procedures for fatalities

Verbal Autopsy reports that are shared with ~~GSK Biologicals should~~***the collaboration or any vendors acting on behalf of the collaboration must*** be anonymised, i.e. all personally identifiable information should be removed. ***In the event that this occurs a GSK data privacy breach form will need to be completed.***

6.4.5.2. Independent Data Monitoring Committee

In order to ensure the safety of the subjects during the entire study period, an Independent Data Monitoring Committee (IDMC) ~~will be~~***has been*** appointed to monitor the safety and tolerability of the vaccine/placebo administered in the trial and, if necessary, make recommendations to the sponsor concerning the modification or termination of the trial. The IDMC will perform safety reviews ~~three times in the first year and~~ twice a year or more frequently if deemed necessary on an ongoing basis during the study period.

The IDMC ~~will consist~~consist***s*** of clinical experts who are not involved in the conduct of the study and an independent statistician.

If during the course of enrolment and vaccination two or more related grade 2 or 3 respiratory AE are observed an ad hoc IDMC will be convened.

6.4.5.3. Planned safety review

A planned safety review after the first 100 subjects have been enrolled and vaccinated will be performed. The SRT will review blinded safety summaries and the IDMC will review unblinded safety summaries.

6.4.5.4. ***Rules for vaccination***

At individual subject level

- ***No second dose should be administered in a given subject who experiences Grade 3 redness and swelling, i.e. ≥100mm, post dose 1 or Grade 2 or 3 respiratory AE post dose 1.***
- ***No second dose should be administered in a given subject who develops TB disease after dose 1.***

At study level

- ***An ad hoc IDMC review will be called if 2 or more subjects experience related Grade 2 or higher respiratory AEs within 90 days after any dose.***
- ***Further enrolment and vaccination will be suspended if within 90 days after any study vaccine dose any of the following events are observed:***
  - ***Related respiratory AE with Fatal outcome***
  - ***Two or more subjects experiencing Grade 3 related Respiratory AE***
- ***An ad hoc IDMC meeting will be called urgently for review. Vaccination may only be allowed to resume pending final VSMB approval.***

Table 6 was inserted and the table number has been updated. Table 7 was updated with the new outline of study procedures.

Table 6 Safety holding rules for vaccination

Event	*Grade*	*Occurrences*	*Action for subject(s)*	*Trial actions*
Redness/Swelling at injection site (within 30 days after dose 1)	*Grade 3 (>100mm)*	*any subject(s)*	*No dose 2 in same subject(s), keep in efficacy follow-up*	*-*
TB disease (after dose 1)	*n/a*	*Any subject(s)*	*No dose 2 in same subject(s) , keep in efficacy follow-up*	*-*
Respiratory SOC AE (within 90 days after dose 1)	*Grade ≥2*	*1 subject*	*No dose 2 in same subject(s), but keep in efficacy follow-up* *Clinical assessment & further investigation at investigator discretion*	*-*
Respiratory SOC AE (within 90 days after dose 1)	*Grade ≥2 related*	*≥2 subjects*		*Ad hoc IDMC review*
Respiratory SOC SAE	*Related & Fatal*	*≥1 subject*	*-*	*Study Hold,* *IMDC Review,* *VSMB*
Respiratory SOC AE (within 90 days after dose 1)	*Grade ≥3, related*	*≥2 subjects*	*No dose 2 in same subject(s)* *Clinical assessment & further investigation at investigator discretion*	

Table 7 List of study procedures

	Visit 1 Screening visit	Visit 2	Visit 3[1]	Visit 4	Visit 5[1]	~~Visit 6[2]~~	Visit *6*	Contact 1, 2, 3 and 4	Visit *7*	Contact 5, 6, 7, 8~~, 9~~	Visit *8*	Contact 10, 11, 12, 13, 14	Visit 9
Time point(s)	**D-30 *to D0***	**D 0**	**D 7**	**D 30**	**D 37**	**~~D 44~~**	**M 2**	**M 4, 6, 8, 10**	**M 12**	**M 14, 16, 18, 20, 22**	**M 24**	**M 26, 28, 30, 32, 34**	**M 36**
Sampling Time point(s)	**Pre V**	**Pre V**	**Post V1**	**Post V1**	**Post V2**	**~~Post V2~~**	**Post V2**	**Post V2**	**Post V2**	**Post V2**	**Post V2**	**Post V2**	**Post V2**
Dose		**1**		**2**									
All subjects													
Informed consent	●												
Check inclusion/exclusion criteria	●	●											
Collect demographic data	●												
Medical history	●	●											
Physical examination		●											
History-directed physical examination		O	O	O	O	~~O~~	O						
Resting vital signs		O		O									
HIV testing[2] (up to ~ 2.5 mL/test)	●												●
Record weight[3]		●							●		●		●
Documentation of history of BCG vaccination/presence of scar	●												
Documentation of history of TB household contacts	●												
Pregnancy test (β-HCG urine)	●	●		●									
Study group and treatment number allocation ***for first dose***		O											
Treatment number allocation for second dose				O									

	Visit 1 Screening visit	Visit 2	Visit 3[1]	Visit 4	Visit 5[1]	~~Visit 6[2]~~	Visit *6*	Contact 1, 2, 3 and 4	Visit *7*	Contact 5, 6, 7, 8~~, 9~~	Visit *8*	Contact 10, 11, 12, 13, 14	Visit 9
Time point(s)	**D-30 *to D0***	**D 0**	**D 7**	**D 30**	**D 37**	~~D 44~~	**M 2**	**M 4, 6, 8, 10**	**M 12**	**M 14, 16, 18, 20, 22**	**M 24**	**M 26, 28, 30, 32, 34**	**M 36**
Sampling Time point(s)	**Pre V**	**Pre V**	**Post V1**	**Post V1**	**Post V2**	~~Post V2~~	**Post V2**	**Post V2**	**Post V2**	**Post V2**	**Post V2**	**Post V2**	**Post V2**
Dose		**1**		**2**									
Vaccination		●		●									
Recording of administered treatment number		●		●									
Check contraindications to subsequent vaccination		●		●									
Recording concomitant medication/vaccination		●	●	●	●	~~●~~	●	●	●	●	●	●	●
Recording intercurrent medical condition		●	●	●	●	~~●~~	●	●	●	●	●	●	●
Distribution of diary cards		O		O									
Return of diary cards				O			O						
Recording of unsolicited AEs ***and large swelling reactions***		●	●	●	●	~~●~~	●						
Pre-vaccination assessment (including temperature)		●		●									
~~Blood sampling for RNA expression profiling (~ 2.5 mL)~~[5]		~~●~~				~~●~~							
Blood sampling for QFTG (~ 3 mL)	●												
Sputum collection for Xpert testing	●												
Reporting SAEs	●[4]	●	●	●	●	~~●~~	●	●[5]	●[4]	●[4]	●[4]	●[4]	●[4]
Reporting pIMDs		●	●	●	●	~~●~~	●	●[6]					
Reporting pregnancy		●	●	●	●	~~●~~	●	●[8][6]					
Diagnostic procedures for suspected pulmonary TB[7]			●	●	●	~~●~~	●	●	●	●	●	●	●

	Visit 1 Screening visit	Visit 2	Visit 3[1]	Visit 4	Visit 5[1]	~~Visit 6[2]~~	Visit *6*	Contact 1, 2, 3 and 4	Visit *7*	Contact 5, 6, 7, 8~~, 9~~	Visit *8*	Contact 10, 11, 12, 13, 14	Visit 9
Time point(s)	**D-30 *to D0***	**D 0**	**D 7**	**D 30**	**D 37**	~~D 44~~	**M 2**	**M 4, 6, 8, 10**	**M 12**	**M 14, 16, 18, 20, 22**	**M 24**	**M 26, 28, 30, 32, 34**	**M 36**
Sampling Time point(s)	**Pre V**	**Pre V**	**Post V1**	**Post V1**	**Post V2**	~~Post V2~~	**Post V2**	**Post V2**	**Post V2**	**Post V2**	**Post V2**	**Post V2**	**Post V2**
Dose		**1**		**2**									
Documentation of TB cases[8]			●	●	●	~~●~~	●	●	●	●	●	●	●
Safety and immune sub-cohort													
Measuring Vitamin D		●											
Recording of solicited AEs		●	●	●	●								
CBC (Hb, PLT and WBC), ALT, AST, creatinine, bilirubin (~ 8 mL)		●	●	●	●								
Blood sampling for ***Wh.Bl.*** CMI (~ 2.5 mL)		●					●		●		●		●
Blood sampling for humoral immunogenicity (~ 2 mL)		●					●		●		●		●
Study conclusions for all subjects													●

Note: The double border at Month 36 indicates the final analyses which will be performed on all data obtained up to month 36.

Pre V: Pre Vaccination; V: Vaccination; Post V: Post Vaccination

● is used to indicate a study procedure that requires documentation in the individual eCRF.

○ is used to indicate a study procedure that does not require documentation in the individual eCRF.

1 Only subjects in the safety and immune sub-cohort need to come for this visit.

2 ~~The most informative time point for collection of blood samples for RNA expression profiling post dose 2 will be determined. The exact time point will be communicated to the investigators appropriately at the latest at study initiation~~HIV testing, pre-test ~~at screening in areas were~~***and post-test*** counselling will be performed ***whenever a HIV*** ~~prevalence > 1%~~***test is done***, according to the procedures detailed inTable 10.

3 Weight also needs to be recorded whenever there is suspicion of TB disease.

4 Only SAEs related to study participation or concurrent GSK medication/vaccine.

5 ~~Samples for RNA expression profiling will also be taken at the time of TB diagnosis~~ SAEs not related to study participation or concurrent GSK medication/vaccines will be recorded until 6 months post dose 2 (M7).

6 ~~Samples for QFTG testing will also be taken at the time of TB diagnosis~~ To be recorded until 6 months post dose 2 (M7).

7 ~~Only for subjects QFTG-negative at baseline~~ Sputum samples for Xpert MTB/RIF testing and microbiological culture will be taken throughout the study if a subject presents with clinical suspicion of pulmonary TB disease. Three sputum samples, preferably taken in the morning and within ***max.*** one week, must be collected. Additionally, when a subject is diagnosed with TB, ***screening for diabetes (HbA1C) and*** HIV testing must be performed according to the procedures detailed inTable 10. Up to 2.5mL of blood will be collected per test. If the subject is confirmed HIV-positive, blood samples must be taken (2.5 mL) to measure CD4 counts.

8 Documentation of TB cases will be done throughout the study, starting at Visit 3 (Day 7); this includes documentation of cases during active and passive follow-up and monitoring of medical facilities and pharmacies.

Table~~7~~*8* Intervals between study visits for all subjects

Interval	Allowed interval[1]
Visit 1 - Visit 2	0 days - 30 days
Visit 2 - Visit 4	26 days - 35 days
~~Visit 4 - Visit 6~~	~~1 days - 25 days~~[2]
Visit 4 - Visit ~~7~~***6***	~~14~~ ***26 days*** - - 35 days
Visit 4 - Visit ~~8~~***7***	10 months - 12 months
Visit 4 - Visit ~~9~~***8***	22 months -24 months
Visit 4 - Visit ~~10~~***9***	34 months - 36 months
Visit n – next contact	6 weeks – 12 weeks
Contact n – contact n+1	6 weeks – 12 weeks

[1] Subjects may not be eligible for inclusion in the ATP cohort for analysis of immunogenicity and efficacy if they ~~make the~~***attend for a*** study visit outside this interval.

~~[2] The most informative time point for collection of blood samples for RNA expression profiling post dose 2 will be determined. The exact time point will be communicated to the investigators appropriately at the latest at study initiation.~~

6.6.5. Medical history

Obtain the subject's medical history by interview and/or review of the subject's medical records and record any pre-existing conditions or signs and/or symptoms present in a subject prior to the first study vaccination in the eCRF. In particular, history of TB disease***** and/or previous treatment for tuberculosis, chronic pulmonary conditions (such as COPD, silicosis), history of smoking and diabetes***** must be recorded ***(* type I diabetes is an exclusion criterion).***

6.6.7. History directed physical examination

Perform a history directed physical examination. If the investigator determines that the subject's health on the day of vaccination temporarily precludes vaccination, the visit will be rescheduled ***within the window period*** (Table 9).

6.6.10. Assess pre-vaccination body temperature

The axillary~~, rectal~~, oral or tympanic body temperature of all subjects needs to be measured prior to any study vaccine administration. The preferred route for recording temperature in this study will be axillary. If the subject has fever [fever is defined as temperature ≥ 37.5°C/99.5°F for oral, axillary or tympanic route~~, or ≥ 38.0°C/100.4°F for rectal route~~] on the day of vaccination, the vaccination visit will be rescheduled within the allowed interval for this visit (see Table 9).

6.6.12.1. Blood sampling for safety*and* immune response assessments ~~*and QFTG status*~~

- For all subjects:
  - A volume of at least 3 mL of whole blood ~~should~~***must*** be drawn for QFTG testing at ~~each predefined time point (~~***screening*** (Table 11)

- ~~For all subjects:~~

- ~~A volume of at least 2.5 mL of whole blood will be collected directly in a Paxgene™ Blood RNA Tube providing pure RNA for accurate detection and quantification of RNA expression profiles ("transcriptomics") at each predefined time point* (Table 9).~~

- ~~At screening, HIV testing must be performed according to the procedures detailed in Table 9 in regions with high HIV prevalence (> 1%). Up to approximately 2.5 mL of blood will be collected for each test.~~

- ~~A volume of at least 3 mL of whole blood should be drawn for QFTG testing at each predefined time point** (Table 9)~~

~~*Blood sampling for RNA expression profiling is planned for Day 0 (Visit 2) and Visit 6 (post dose 2). The most informative time point for collecting whole blood samples for RNA expression profiling post dose 2 will be determined. The exact time point will be communicated to the Investigators appropriately at the latest at study initiation.~~

~~**At study end (Year 3) QFTG testing will only be performed for all subjects QFTG-negative at baseline.~~

- **Whenever a subject is diagnosed with TB disease:**

  - ***Screening for diabetes and*** HIV testing must be performed according to the procedures detailed in Table 10. Up to approximately 2.5 mL of blood will be collected for each test. Additionally, if HIV is confirmed, at least 2.5 mL of blood will be collected to measure CD4 counts.

  - ~~A volume of at least 2.5 mL of whole blood will be collected directly in a Paxgene™ Blood RNA Tube providing pure RNA for accurate detection and quantification of RNA expression profiles ("transcriptomics").~~

  - ~~A volume of at least 3 mL of whole blood should be drawn for QFTG testing.~~

6.6.12.2. Sputum sample collection for TB screening

A single sputum sample must be collected from all subjects going through screening for testing using Xpert.

6.6.12.3. Sputum sample collection for TB diagnosis

~~All subjects~~ ***S***ubjects ***suspected with Pulmonary TB*** will be required to provide 3 sputum samples, preferably taken in the morning, within ***maximum*** a one week interval, of ~~approximately~~***preferably up to*** 5 mL to test for Mtb using the Xpert MTB/RIF assay and microbiological culture whenever the subject presents with clinical suspicion of pulmonary TB. If needed, induction of sputum through nebulisation of hypertonic saline may be performed.

6.6.15. Check and record concomitant medication/vaccination and intercurrent medical conditions

- ***The site staff or Investigator should not write on the diary card, this information should be captured in the source notes.*** This process will be documented in a site SOP. Please refer to the SPM for further details.
- Verify completed diary cards during discussion with the subject on Visits 2, 3*, 4, 5* and ~~7.~~ ***6***.
- Collect diary cards from the subjects at Visits 4 and ~~7.~~ ***6***.
- Any unreturned diary cards will be sought from the subject through telephone call(s) or any other convenient procedure. The investigator will transcribe the collected information into ~~the eCRF in English.~~***Inform™*** in English. *Only for subjects included in the safety and immune sub-cohort.

6.6.16. Study conclusion

The investigator will:

- review data collected to ensure accuracy and completeness.

complete the Study Conclusion screen in ~~the eCRF.~~***Inform™***.

6.7. Biological sample handling and analysis

Under the following circumstances, additional testing on the samples may be performed by GSK Biologicals***, by laboratories working for GSK Biologicals, by laboratories working in collaboration with GSK and by laboratories involved in studies ancillary to TB-018,*** outside the scope of this protocol.

6.7.2.1. Protocol mandated testing and enpoints

Table ~~9~~*10* Biological samples

Sample type	Quantity	Unit	Time point	Subjects
Blood sampling for Safety assessment	At least 8	mL	Days 0, 7, 30 and 37[1]	Safety and immune sub-cohort[2]
Blood sampling for Humoral responses	At least 2	mL	Days 0, 60 and Years 1, 2, 3	Safety and immune sub-cohort[2]
Blood sampling for CMI	At least 2.5	mL	Days 0, 60 and Years 1, 2, 3	Safety and immune sub-cohort[2]
~~Blood sampling for ***QFTG***~~	~~At least 3~~	~~mL~~	~~Day 0 and Years 1, 2, 3~~	~~Safety and immune sub-cohort[2]~~
Blood sampling for QFTG	At least 3	mL	~~Day 0 and Year 3~~***At screening***	All subjects[3]
~~Blood~~***Sputum sampling for*** ~~RNA expression profiling~~***TB screening***	~~At least 2.~~***Up to 5***	***mL***	~~Days 0 and Day 44[4] and when TB diagnosis is confirmed~~***At Screening***	***All subjects***
Sputum sampling for TB diagnosis[5]~~3~~	Approximately 5	mL	Throughout the study when pulmonary TB is suspected	All subjects with suspicion of pulmonary TB
Blood sampling for initial	Finger prick or at	mL	At screening,	All subjects ~~living in~~

Sample type	Quantity	Unit	Time point	Subjects
HIV testing - Step 1 (Rapid test)	least 2.5		***At Visit 9 (study end)***	~~a region with HIV prevalence > 1%~~
			When TB diagnosis is confirmed	All subjects diagnosed with TB
Blood sampling for HIV testing - Step 2 (Rapid test)	Finger prick or at least 2.5	mL	At screening and when TB diagnosis is confirmed	All subjects positive at Step 1[6]
Blood sampling for HIV testing - Step 3 (ELISA)	At least 2.5	mL	At screening[~~5~~], ***At Visit 9 (study end)***	Discordant Rapid test results or if no Rapid test performed[~~7~~***5***] ~~At least one positive Rapid test[7]~~
			When TB diagnosis is confirmed	
Blood sampling for PCR - Step 4	At least 2.5	mL	When TB diagnosis is confirmed	Indeterminate ELISA results[~~7~~***5***]
Blood sampling for CD4 count	At least 2.5	mL	When TB diagnosis is confirmed and confirmed HIV-positive	HIV positive subjects with TB diagnosis confirmed
Blood sampling for HbA1C	***At least 2.5***	***mL***	***When TB diagnosis is confirmed***	***Subjects with TB diagnosis confirmed***

1 Vitamin D levels will be measured at day 0 only.
2 Refer to Section 5.1 for sub-cohort description.
~~3 At study end (Year 3) QFTG testing will only be performed for subjects QFTG-negative at baseline.~~
~~4 The most informative time point for collection of blood samples for RNA expression profiling post dose 2 will be determined. The exact time point will be communicated to the investigators appropriately at the latest at study initiation. Additionally, samples for RNA expression profiling must be collected at the time of TB diagnosis.~~
~~5~~***3*** Three sputum samples, preferably taken in the morning, within a one week interval, will be collected whenever a subject presents with clinical suspicion of pulmonary TB.
~~6~~***4*** HIV positive subjects at screening will be referred for confirmatory HIV diagnosis/management as per site SOPs.
~~7~~***5*** Only the final conclusive diagnosis is to be recorded in the CRF.

Table~~11~~*12* Cell-Mediated Immunity (CMI)

System	Component	Challenge	Method	Unit	Cut-off	Laboratory*
Whole blood	CD4+/CD8+ T cells expressing at least two immune markers (CD40L and/or IL-2 and/or TNF-α and/or IFN-γ) ***and any combination among CD40-L, IFN-g, IL-2 and TNF-a***	M72	ICS - intraCellular Cytokine Staining	number of events per million cells	N/A	***GSK ~~Biologicals~~or as designated by GSK***

*Refer to APPENDIX B for the laboratory addresses.

Table 12 has been deleted:

Table 12 RNA expression profiling ~~(Amended 26 March 2013)~~

~~System~~	~~Component~~	~~Method~~	~~Unit~~	~~Laboratory*~~
~~Whole blood~~	~~RNA expression~~	~~Microarray~~	~~NA~~	~~***COVANCE****~~

~~*Refer to APPENDIX B for the laboratory addresses.~~
~~****Or as designated by "The Collaboration".***~~

Table13 Diagnostic tests for pulmonary TB disease

System	Component	Method	Unit	Laboratory*	Component priority ranking
Sputum	Mycobacterium tuberculosis DNA	Xpert MTB/RIF assay	NA	Central Laboratory	1
Sputum	Mycobacterium tuberculosis	Microbiological culture	NA	Central Laboratory	2
Sputum	***Mtb***	***Strain Typing***	***NA***	***Central lab***	***3***

*Refer to APPENDIX B for the laboratory addresses.

Table 14 Diagnostic tests for HIV

System	Component	Method	Unit	Laboratory*
Whole Blood or Serum	Anti-HIV antibodies, screening step 1, step 2	HIV Rapid Test**	NA	At the Investigator's lab
Serum	Anti-HIV antibodies, screening step 3	ELISA	NA	At the Central Laboratory
Whole Blood	HIV ~~RNA,~~ screening step 4	HIV PCR	NA	At the Central Laboratory
Whole blood	CD4 cells	Flow cytometry	Cells/mm^3	At the Central Laboratory

*Refer to APPENDIX B for the laboratory addresses.
**Two different Rapid HIV tests to be used sequentially

6.7.4. Biological samples evaluation *per protocol*

6.7.4.1. Diagnostic assays

Xpert MTB/RIF testing (Cepheid) and microbiological culture will be performed on three sputum samples, preferably taken in the morning, within a ***maximum*** one week interval, for all subjects presenting with clinical suspicion of pulmonary TB throughout the entire study.

HIV testing and pre-test ***and post-test*** counselling will be performed at screening ~~in regions with high HIV prevalence (> 1%)~~. HIV positive subjects at screening will be referred for confirmatory HIV diagnosis/management as per site SOPs ***and won't be enrolled (exclusion criterion)***.

Whenever a subject is diagnosed with TB disease, an HIV test (and pre-test counselling) must be performed to rule out HIV co-infection. ***HbA1c measurement will also be performed.*** If a subject is diagnosed with HIV, additional tests to measure CD4 cell counts will be performed. Subjects with a positive test result will be referred for HIV treatment.

~~QFTG testing will be done at baseline (Day 0) for all subjects, at study end (Year 3) for all subjects QFTG-negative at baseline and yearly (Years 1, 2 and 3) for subjects included in the safety and immune sub-cohort, to detect CMI responses to TB infection by measuring IFN-γ produced by memory T cells in whole blood after incubation with synthetic peptides of the Mtb-secreted antigens Early Secretory Antigen Target-6 (ESAT-6), Culture Filtrate Protein-10 (CFP-10) and TB 7.7. These TB-specific proteins stimulate a robust and detectable immune response in TB-infected and previously TB-infected~~

~~people. In addition, QFTG testing will be done whenever a subject is diagnosed with TB disease.~~

All diagnostic assays will be carried out at the ***designated central*** laboratory ~~of the investigator or in a laboratory~~***as*** designated by "The Collaboration".

6.7.4.2. Immunological read-outs

Blood will be collected at specific time points to assay for immunological readouts.Table 16 details the main immunological read-outs.

After collection, whole blood samples will be incubated with pool(s) of overlapping peptides covering the M72 antigen sequence and with the stimulation controls for 2 hours in the presence of anti-CD28 and anti-CD49d antibodies. Subsequently, flow cytometry using a short-term stimulation Intracellular Cytokine Staining (ICS) assay will be used to characterise M72-specific CD4+/CD8+ T cells expressing at least two immune markers ***or any combination of markers, i.e. single, double, triple, quadruple,*** (CD40L and/or IL-2 and/or TNF-α and/or IFN-γ) on Days 0, 60, and Years 1, 2 and 3.

The humoral immune response will be evaluated by assessment of antibody titres to M72 by specific ELISA on Days 0 and 60, and Years 1, 2 and 3.

Table 16 Immunological read-outs

Blood sampling time point		Sub-cohort Name	No. subjects	Component
Type of contact and time point	Sampling time point			
Visit 2 (Day 0)	Pre Vacc	Safety and immune sub-cohort	450	M72-specific T-cells (ICS on whole blood) Anti M72 Ab (ELISA on serum)
Visit ~~7~~***6*** (Day 60)	Post Vacc 2			M72-specific T-cells (ICS on whole blood) Anti M72 Ab (ELISA on serum)
Visit ~~8~~***7*** (Year 1)	LTFU			M72-specific T-cells (ICS on whole blood) Anti M72 Ab (ELISA on serum)
Visit ~~9~~***8*** (Year 2)	LTFU			M72-specific T-cells (ICS on whole blood) Anti M72 Ab (ELISA on serum)
Visit ~~10~~***9*** (Year 3)	LTFU			M72-specific T-cells (ICS on whole blood) Anti M72 Ab (ELISA on serum)

Vacc: Vaccination
LTFU: Long Term Follow-up

~~Microarray analysis will be performed on whole blood samples collected in PAXgene® Blood RNA tubes (PreAnalytiX®). The total purified RNA extract will be converted to cDNA by Reverse Transcriptase PCR and hybridised to a microarray chip that contains probe sets covering a substantial portion of the human transcriptome (which is the collective set of expressed mRNA). Microarray data will be analysed using bioinformatics approaches. Further analysis of mRNA expression profiles and validation might be performed by quantitative Reverse Transcriptase - Polymerase Chain Reaction or any updated technique, such as deep sequencing.~~

~~RNA expression profiling will be performed on a selected number of subjects' samples to compare cases and non-cases within the control group, within the M72AS01 group and between groups.~~

~~The expected outcome of this analysis is to arrive at a RNA expression profile signature as a correlate of risk that could describe non-protective immune responses.~~

7.5. Contraindications to subsequent vaccination

- ***Grade 3 local symptoms redness and swelling, i.e. >100mm or Grade ≥2 respiratory AE following the administration of vaccine(s).***

Fever is defined as temperature ≥ 37.5°C/99.5°F on oral, axillary or tympanic setting~~, or ≥ 38.0°C/100.4°F on rectal setting~~. The preferred route for recording temperature in this study will be axillary

9.1. Safety definitions

Examples of an AE include:

If subjects observe any large injection site reaction, they should contact the study personnel and visit the investigator's office for evaluation as soon as possible.

A large injection site reaction is:

- ***a swelling that measures more than 100 mm across where the vaccine was given, or***
- ***a noticeable irregular/uneven swelling where the vaccine was given, or***
- ***a noticeable increase in size of the arm that interferes with or prevents everyday activities (e.g., writing, use of computer, school attendance, sleeping, etc.).***

In case of questions or uncertainties, the subject/subject's parent(s)/guardian(s) should contact the investigator by phone and the investigator will determine whether or not a visit should be arranged.

The investigator will record detailed information describing the AE on a specific large injection site reaction screen in the eCRF. An SAE report should also be completed if the large injection site reaction meets the definition of SAE.

Table 20 Solicited adverse events

Solicited local (injection site) AEs	Solicited general AEs
Pain at injection site	Fatigue
Redness at injection site*	Fever
Swelling at injection site	~~Gastrointestinal~~***Respiratory*** Symptoms†
	Headache
	Malaise
	Myalgia

*In case the principal investigator or designate is unable to determine the extent of redness on darkly pigmented skin, it will be reported as non-interpretable in the eCRF and Diary Card.

†~~Gastrointestinal~~***Respiratory*** symptoms include ~~nausea, vomiting, diarrhoea and/~~***cough, blood in sputum, purulent sputum, shortness of breath or*** ~~abdominal~~***difficulties breathing, chestwall pain***

Table 21 Toxicity grading table for laboratory abnormalities footnotes updated:

(source:FDA, 2007).

Table 24 Intensity scales for solicited symptoms in adults

Adults		
Adverse Event	**Intensity grade**	**Parameter**
Pain at injection site	0	None
	1	Mild: Any pain neither interfering with nor preventing normal every day activities.
	2	Moderate: Painful when limb is moved and interferes with every day activities.
	3	Severe: Significant pain at rest. Prevents normal every day activities.
Redness at injection site		Record greatest surface diameter in mm
Swelling at injection site		Record greatest surface diameter in mm
Fever*		Record temperature in °C/°F
Headache	0	Normal
	1	Mild: Headache that is easily tolerated
	2	Moderate: Headache that interferes with normal activity
	3	Severe: Headache that prevents normal activity
Fatigue	0	Normal
	1	Mild: Fatigue that is easily tolerated
	2	Moderate: Fatigue that interferes with normal activity
	3	Severe: Fatigue that prevents normal activity
~~Gastrointestinal symptoms~~***Respiratory symptoms (cough, blood in sputum, purulent sputum, shortness of breath or difficulties breathing, chestwall pain)***	0	~~Gastrointestinal~~***No*** symptoms~~ normal~~
	1	Mild: ~~Gastrointestinal~~ symptoms that are easily tolerated
	2	Moderate: ~~Gastrointestinal~~ symptoms that interfere with normal activity
	3	Severe: ~~Gastrointestinal~~ symptoms that prevent normal activity
Malaise	0	Normal
	1	Malaise that is easily tolerated
	2	Malaise that interferes with normal activity
	3	Malaise that prevents normal activity
Myalgia	0	Normal
	1	Myalgia that is easily tolerated
	2	Myalgia that interferes with normal activity
	3	Myalgia that prevents normal activity

10.4. Screen and baseline failures

If the investigator believes there is a reasonable reason to do so, screening procedures (including, but not limited to, the QTF screening in the case of an intermediate result) may only be repeated once, either individually or in their entirety.

11. STATISTICAL METHODS

- Incident cases of ~~**Definite**~~***Microbiological*** **Pulmonary TB disease** meeting the third case definition.

11.2. Secondary endpoints

IMMUNOGENICITY

- Evaluation of CMI responses with respect to components of the study vaccine, in the safety and immune sub-cohort:
  - ~~Determined by the frequency of M72-specific CD4+/CD8+ T-cells per million cells identified after *in vitro* stimulation, as expressing at least 2 immune markers among CD40L, IL-2, IFN-γ and TNF-α.~~ ***Determined by the frequency of M72-specific CD4+/CD8+ T-cells per million cells identified after in vitro stimulation, as expressing any combination of immune markers among CD40L, IL-2, IFN-γ and TNF-α.***

  Timepoints: prior to dose 1 (Day 0) and post-dose 2 (Day 60, Years 1, 2 and 3)

~~**11.3. Tertiary endpoints**~~

- ~~Evaluation of CMI responses with respect to components of the study vaccine, in the safety and immune sub-cohort:~~
  - ~~Determined by the frequency of M72-specific CD4+/CD8+ T-cells per million cells identified after in vitro stimulation, as expressing any combination of immune markers among CD40L, IL-2, IFN-***γ*** and TNF-***α***~~
  - ~~***QFTG test result.***~~

  ~~***Time points: prior to dose 1 (Day 0), post dose 2 (Year 3)***~~
  - ~~Description of RNA expression profile signatures in selected samples as a correlate of risk to describe protective and non-protective immune responses after 2 doses of M72/AS01$_E$.~~

  ~~Time points: Prior to dose 1 (Day 0), post dose 2 and when TB is diagnosed.~~

11.3. Determination of sample size

The primary objective is to demonstrate that the lower limit of the ~~95% Confidence interval~~***90***% CI of vaccine efficacy (VE), obtained from the Cox proportional hazards regression model (VE=1-estimated hazard ratio) associated to the M72/AS01$_E$ vaccine in preventing first episodes of definite pulmonary TB disease meeting the first case definition is >0.

~~Statistical analysis during the course of this study will include an interim analysis (IA) for futility, i.e. if a too low vaccine efficacy is observed at the time of the interim analysis, the trial will be stopped for futility. Therefore, no alpha adjustment is needed.~~

Using a logrank test with 80% power for a true VE of 70% (hazard ratio of 30%)and a two-sided ~~5~~***10***% significance level, the number of cases required for a fixed sample design is ~~27 cases~~***21cases***, based on the assumption that the hazard rates are proportional. ~~These results are obtained by the program PASS 2005.~~

To obtain these ~~27~~***21*** cases in total at final efficacy analysis, assuming a mean yearly attack rate of 0.~~35~~ ***55***% in the control group, ~~3~~***2*** years of follow-up for each subject and a ***rate of*** loss ~~of~~***to*** follow-up ~~time~~ of ~~12~~***15***% due to drop out over the ~~3~~***2*** years period, ~~4500~~***3506*** subjects need to be enrolled based on the logrank test and parameters described above.

~~Sample size considerations for the safety and immune sub-cohort:~~

~~Approximately 450 subjects will be enrolled in the safety and immune sub-cohort (225 subjects in~~ ***A planned follow-up time of 3 years for each*** ~~treatment group) with up~~***subject allows to*** ~~150 subjects/contributing country (75 subjects in each treatment group)~~

~~Assuming a standard deviation of log10(value*) of 0.40**, as observed in previous studies, a~~***mitigate any lower than expected incidence rate and alternative to achieve a higher precision*** ~~for the 95 % CI for log10(value*) of 0.052 or 0.092 will be obtained for a total sample size of 225 subjects vaccinated with M72/AS01$_E$ or 75 subjects vaccinated with M72/AS01$_E$/country respectively.~~***of the VE estimate at the final analysis.***

~~Precision of the log10(value*) is equal to half of the with of the 95% CI for log10(value*). The precision of the 95% CI and 95% CI can be derived by assuming various levels of the value*. Precision of the 95% CI for GMT for a sample size of 150 subjects and 450 subjects is presented in Table 26 and Table 27, respectively.~~

~~*GMT or median frequency of CD4 T cells expressing at least two immune markers among CD40L, IL-2, IFN-γ and TNF-α~~

~~**A standard deviation around 0.40 for log10(GMT) and below 0.40 for log10(CD4 T cells expressing at least 2 immune markers) has been observed in previous studies.~~

Tables 26 and 27 and also Section 11.5.2. have been deleted:

~~Table 26 Precision for the 95% CI for GMT obtained from the 95% CI build on log10(GMT) assuming various levels of GMT for a sample size of 75 subjects vaccinated with M72/AS01$_E$~~

~~GMT~~	~~Log10(GMT)~~	~~precision for the 95% CI for log10(GMT)~~	~~95% CI for the GMT~~		~~precision for the 95% CI for the GMT~~
			~~LL~~	~~UL~~	
~~500~~	~~2.698970004~~	~~0.092~~	~~404.5479~~	~~617.9737~~	~~106.7129~~
~~1000~~	~~3~~	~~0.092~~	~~809.0959~~	~~1235.947~~	~~213.4258~~
~~2000~~	~~3.301029996~~	~~0.092~~	~~1618.192~~	~~2471.895~~	~~426.8515~~
~~3000~~	~~3.477121255~~	~~0.092~~	~~2427.288~~	~~3707.842~~	~~640.2773~~
~~4000~~	~~3.602059991~~	~~0.092~~	~~3236.384~~	~~4943.79~~	~~853.7031~~
~~5000~~	~~3.698970004~~	~~0.092~~	~~4045.479~~	~~6179.737~~	~~1067.129~~
~~6000~~	~~3.77815125~~	~~0.092~~	~~4854.575~~	~~7415.685~~	~~1280.555~~

~~CI: Confidence interval; GMT: Geometric Mean Titre; LL: Lower limit; UL: Upper limit~~

Table 27 Precision for the 95% CI for GMT obtained from the 95% CI build on log10(GMT) assuming various levels of GMT for a sample size of 225 subjects vaccinated with M72/AS01$_E$

GMT	Log10(GMT)	precision for the 95% CI for log10(GMT)	95% CI for the GMT		precision for the 95% CI for the GMT
			LL	UL	
500	2.698970004	0.052	443.578	563.5987	60.01036
1000	3	0.052	887.156	1127.197	120.0207
2000	3.301029996	0.052	1774.312	2254.395	240.0414
3000	3.477121255	0.052	2661.468	3381.592	360.0622
4000	3.602059991	0.052	3548.624	4508.79	480.0829
5000	3.698970004	0.052	4435.78	5635.987	600.1036
6000	3.77815125	0.052	5322.936	6763.185	720.1243

CI: Confidence interval; GMT: Geometric Mean Titre; LL: Lower limit; UL: Upper limit

11.5.2. Modified Total vaccinated cohort for interim efficacy analysis

The modified TVC for interim efficacy analysis will include all subjects:

- ***For whom two doses of study vaccines have been administered according to protocol (administration site and route)***
- ***Without randomisation failure***
- ***Without wrong replacment***

Who did not present with TB disease, according to any case definition, during the vaccination period (day of first vaccination up to 1 month post dose 2).

11.5.3. According-to-protocol cohort for analysis of immunogenicity

Without randomisation failure.

11.5.4. According-to-protocol cohort for analysis of efficacy

Without randomisation failure.

11.5.5. According-to-protocol cohort for analysis of QFTG status

The ATP cohort for analysis of QFTG status will include all subjects:

- Who received two vaccinations according to protocol procedures within specified intervals.
- For whom post vaccination QFTG blood samples are available.
- Who did not receive a vaccine/medication that may lead to elimination of an ATP analysis.
- Who did not present with a medical condition that may lead to elimination of an ATP analysis.

- ~~For whom the randomization code has not been broken.~~

- ~~With a negative QFTG test result at baseline.~~

- ~~With no indeterminate QFTG test result at any time point.~~

11.7. Analysis of efficacy

Vaccine efficacy will be estimated from a Cox proportional hazard regression model (VE=1-hazard ratio) and ~~95~~***90***% CIs and Wald p-value will be derived. The primary analysis will be unadjusted but secondary analyses will evaluate the effect of potential covariates.

*The lower limit of the ~~95~~**90**% two-sided confidence interval (CI) for the VE (using a Cox regression model) against first occurrence of Definite pulmonary TB disease not associated with HIV-infection, meeting the first case definition, is above 0%*

If the primary objective is met, the confirmatory secondary objective will be evaluated with the following success criterion:

*The lower limit of the ~~95~~**90**% two-sided ~~confidence interval (~~CI~~)~~ for the VE (using a Cox regression model) against first occurrence of Definite pulmonary TB disease not associated with HIV-infection, meeting the ~~first~~**second** case definition, is above 0%.*

~~For the tertiary efficacy analysis, the analysis will be based on the ATP cohort for analysis of Mtb infection. The vaccine efficacy will be estimated as 1- the Rate Ratio (RR) where the rate is defined as the number of subjects QFTG- positive (converters) at study end divided by the number of subjects QFTG- negative at baseline, in each group. These two proportions will be compared using the Fisher exact test.~~

~~11.9.2. RNA expression profiling~~

~~In order to attempt to establish a correlate of risk for acquisition of infection, a systems biology approach will be used (refer to Section 6.7.4.2).~~

~~As exploratory, a comprehensive analysis of selected subjects' immune responses following vaccination will be performed by RNA expression profiling using microarray analysis on whole blood samples. Microarray data will be analysed using bioinformatics approaches. The expected outcome of this analysis is to arrive at a RNA expression profile signature as a correlate of risk that could describe non-protective immune responses. Additional outcomes will shed further light on the immune responses induced by the vaccine.~~

~~Sample selection for microarray analysis could be based on the following comparisons:~~

- ~~Comparison of profiles of 'Breakthrough' cases, i.e. M72/AS01$_E$ vaccinated subjects with confirmed pulmonary TB meeting the first, second, third or fourth case definition with comparable group of M72/AS01$_E$ vaccinated subjects with no pulmonary TB. This could define a correlate of risk.~~

- ~~Comparison of cases from M72/AS01$_E$ vaccinated and placebo groups will help deduce if the vaccine had an impact in the breakthrough cases, and if this analysis reveals the same signature in breakthrough cases from both groups.~~
- ~~Comparison of cases and non-cases in the placebo group as a negative control since there should be no difference.~~
- ~~Description of RNA expression profile signature to describe immune responses after dose 2 by comparing samples from vaccinated subjects with non-vaccinated subjects. This analysis is not linked to cases (e.g. comparison between the M72/AS01$_E$ vaccinated versus placebo).~~

11.9. Analysis of safety

Injection site swelling and redness will be analysed using a 3-grade evaluation system [0 mm ***to 20 mm***(grade 0); ~~> 0 to 20 mm (grade 1); >~~ ***≥20*** to ***50*** mm (grade 1); > ***50*** to ***100*** mm (grade 2); > ~~50~~***100*** mm (grade 3)]

11.12.1. Sequence of analyses

All analyses (including interim analysis) will be conducted on data as clean as possible.

~~The analyses will be performed stepwise:~~

- ~~An interim efficacy analysis for futility will be performed after approximately 10 Xpert MTB/Rif positive pulmonary TB cases (aprroximately 40 % of the target events) in confirmed HIV-negative subjects have accumulated in the modified TVC for interim efficacy analysis, during the efficacy surveillance period starting 1 month post dose 2 (Day 60). If the study is stopped for futility, subjects will continue to be followed-up for safety up to 6 months post dose 2 and a study conclusion visit will be scheduled at the earliest convenient time point. All data (efficacy, safety and immunogenicity) collected up to last subject last visit (LSLV) will be cleaned, unblinded and analysed.~~

~~Analysis of efficacy will be performed when 27~~

- ***Primary a***nalysis of efficacy will be performed when ***21*** cases of definite pulmonary TB disease, meeting the first case definition, have accumulated in the ATP cohort for efficacy, during the efficacy surveillance period starting 1 month post dose 2 (Day 60) or at the latest when all subjects have completed the Month ~~36~~***24*** visit.

~~11.12.2. Statistical considerations for interim analyses~~

~~In order to avoid trial progression with a candidate vaccine with very low efficacy, an interim efficacy analysis for futility is planned, i.e. if a too low vaccine efficacy is observed at the time of the interim analysis, the trial will be stopped for futility. Therefore, no alpha adjustment is needed.~~

~~The analysis will be performed by a statistician external to the investigator group and "The Collaboration" (i.e. from a contract research organisation). The clinical team within GSK Biologicals and Aeras, all participating subjects, study site and GSK personnel~~

~~directly involved in the conduct of the trial will remain blinded to the randomisation codes. The GSK statistician (and other personnel directly involved in the conduct of the trial) will not have access to the randomisation list and serology data.~~

~~The efficacy results at interim analysis will be looked at by the IDMC in closed session (with prohibited attendance by any member of GSK and Aeras and by any person involved in the conduct of the study).~~

~~Stopping rules for futility will be defined in the statistical analysis plan for interim analysis.~~

12.1. ~~Remote Data Entry~~*eCRF* instructions

~~Remote Data Entry (RDE),~~***Inform*™** , a validated computer application, will be used as the method for data collection.

12.2. Study Monitoring by "The Collaboration"

GSK and ***its partners in***"The Collaboration" will monitor the study to verify that, amongst others, the:

- Data are authentic, accurate, and complete.
- Safety and rights of subjects are being protected.

Study is conducted in accordance with the currently approved protocol, any other study agreements, GCP and all applicable regulatory requirements.

Direct access to all study-site related and source data is mandatory for the purpose of monitoring review. The monitor will perform ~~a RDE~~a***n eCRF*** review and a Source Document Verification (SDV). By SDV we understand verifying ~~RDE~~***eCRF*** entries by comparing them with the source data that will be made available by the investigator for this purpose.

The Source Documentation Agreement Form describes the source data for the different data in the ~~RDE~~***eCRF***. This document should be completed and signed by the site monitor and investigator and should be filed in the monitor's and investigator's study file. Any data item for which the ~~RDE~~***eCRF*** will serve as the source must be identified, agreed and documented in the source documentation agreement form.

For ~~RDE~~***eCRF***, the monitor will mark completed and approved screens at each visit.

14. REFERENCES

World Health Organisation [WHO], ~~2011. Global Tuberculosis Control: Surveillance, Planning and Financing~~***2013***. ***Global tuberculosis report 2013***; ***http://www.who.int/tb/publications/global_report/en/, accessed*** ~~26 October 2011.~~***02 December 2013.***

APPENDIX B CLINICAL LABORATORIES

Table 27 Outsourced laboratories

Laboratory	Address
CEVAC - University of Gent	De Pintelaan, 185 Gent Belgium
~~COVANCE~~	~~401 Terry Avenue North~~ ~~Suite 200~~ ~~Seattle, Washington 98109~~ ~~USA~~
Central laboratories	
BARC South Africa Pty Ltd	Napier Road POB 8475 2000 Johannesburg Republic of South Africa
KEMRI/CDC Research Station, Kenya	Busia Road POB 1578-40100 Kisumu Kenya
CIDRZ Zambia	Alick Nkhata Road Kalingalinga Clinic Grounds Lusaka Zambia