THE LANCET Respiratory Medicine

Supplementary appendix

This appendix formed part of the original submission and has been peer reviewed. We post it as supplied by the authors.

Supplement to: Spertini F, Audran R, Chakour R, et al. Safety of human immunisation with a live-attenuated *Mycobacterium tuberculosis* vaccine: a randomised, double-blind, controlled phase I trial. *Lancet Respir Med* 2014; published online Nov 16. http://dx.doi.org/10.1016/S2213-2600(15)00435-X.

SUPPLEMENTARY APPENDIX

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Spertini F., Audran R., Chakour R. et al. Safety of human immunisation with a live-attenuated *Mycobacterium tuberculosis* vaccine: a randomised, double-blind, controlled phase I trial. **The Lancet Respiratory Medicine**

SUPPLEMENTARY FIGURES LEGENDS

Fig. S1. Gating strategy for intracellular cytokine staining (ICS) in whole blood assay. Numbers within the various gates indicate the percentage of T cells from a representative volunteer.

Fig. S2. BCG specific CD4+ responses. CD4 T-cells expressing IFNγ (Panel A), IL-2 (Panel B), TNFα (Panel C) or all 3 cytokines (Panel D) were assessed using intracellular cytokine staining in whole blood assay. Data are expressed as % total $CD4^+$ T-cells at D0, D28, D90 and D210. Numbers above the *x* axis indicate the number of responders per group at a given time point, when present. Friedman ANOVA test with Dunn's post-test was used to compare responses within groups at different time-points with D0. Numbers on top of the panels indicate *p* values of ANOVA, stars indicate *p* values of post-tests, * $p<0.05$, ** $p<0.01$, *** $p<0.001$. Between groups comparisons (Kruskall-Wallis test) did not show any significant difference between BCG and MTBVAC groups.

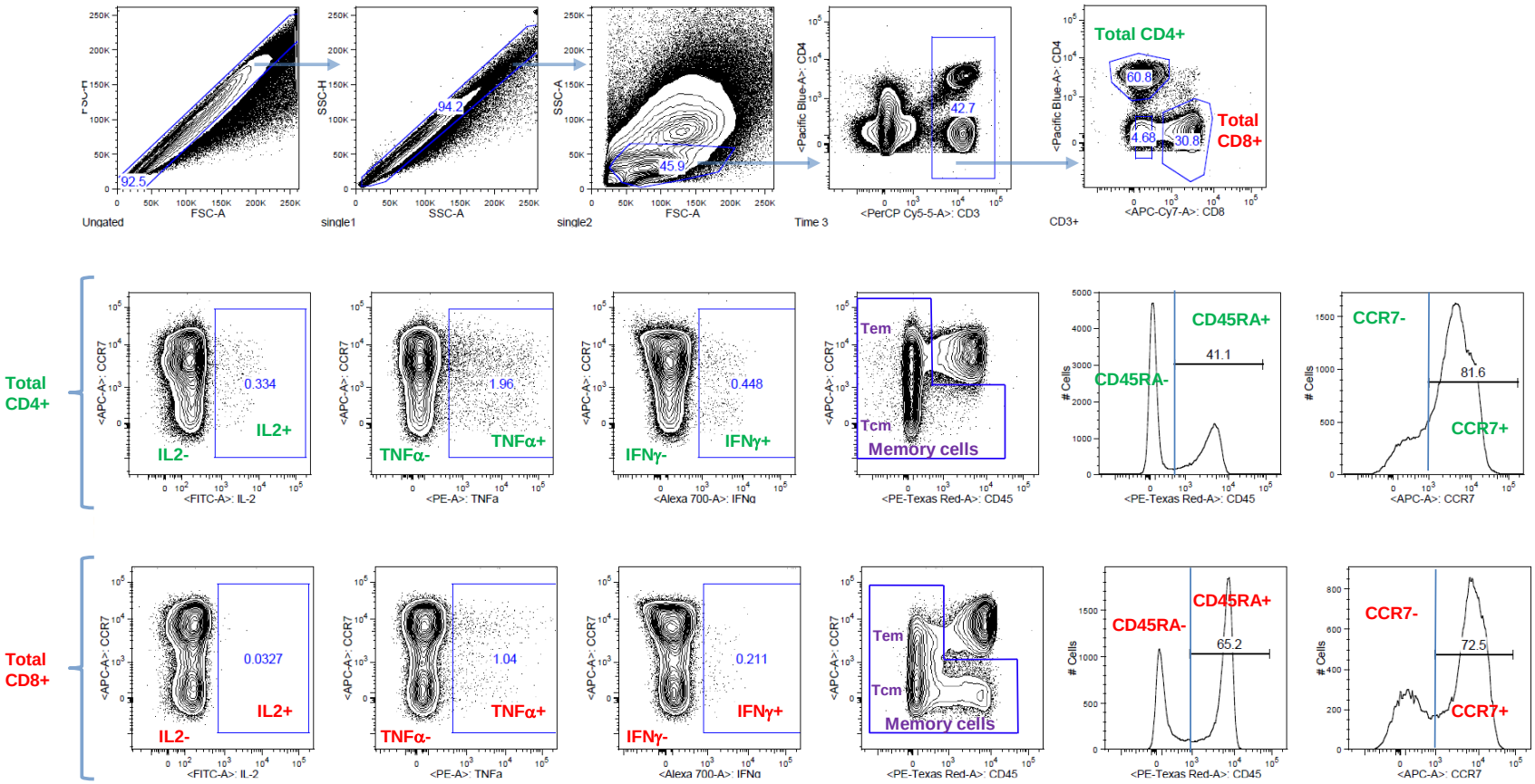
Fig. S3. Polyfunctional responses in $5x10^5$ CFU MTBVAC and $5x10^5$ CFU BCG groups. Pies show the proportions of CD4+ T-cells producing any combination of IFNγ, IL-2 and TNFα in response to a stimulation with live MTBVAC or live BCG.

Fig. S4. Phenotype of $CD4^+$ T-cell memory responses in $5x10^5$ CFU MTBVAC and $5x10^5$ CFU BCG groups. MTBVAC- (upper panels) or BCG- (lower panels) specific $CD4^+$ T-cells expressing at least one cytokine were differentiated upon their expression of CD45RA and CCR7 in T central memory (T_{CM}, CD45RA- CCR7+, right column) and effector memory (T_{EM} CD45RA- CCR7-, left column). Data are expressed as % total $CD4^+$ T-cells at D0, D28, D90 and D210. Numbers above the *x* axis indicate the number of responders per group at a given time point, when present. Friedman ANOVA test with Dunn's post-tests was used to compare responses within groups at different time-points with D0. Numbers on top

of the panels indicate *p* values of ANOVA, stars indicate *p* values of post-test * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$. Between groups comparisons (Kruskall-Wallis test) did not show any significant difference between BCG and MTBVAC groups.

SUPPLEMENTARY TABLE AND FIGURES

Fig. S1



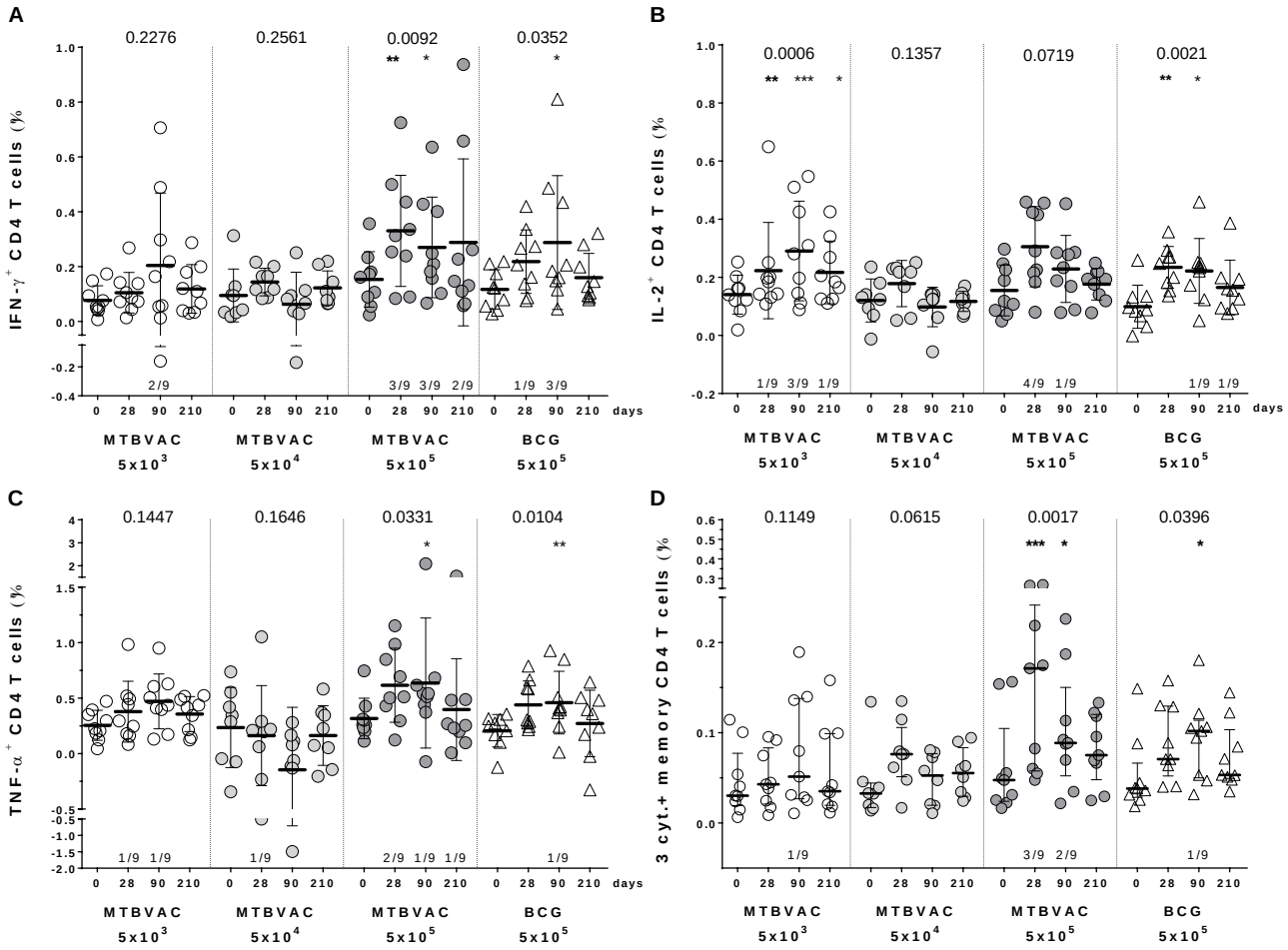

A
B
C
D
IFN-γ+ CD4 T cells (%
IL-2+ CD4 T cells (%
TNF-α+ CD4 T cells (%
3 cyt.+ memory CD4 T cells (%
0.2276
0.2561
0.0092
0.0352
0.0006
0.1357
0.0719
0.0021
0.1447
0.1646
0.0331
0.0104
0.1149
0.0615
0.0017
0.0396
0 28 90 210
days
MTBVAC 5x10^3
MTBVAC 5x10^4
MTBVAC 5x10^5
BCG 5x10^5

Fig. S2

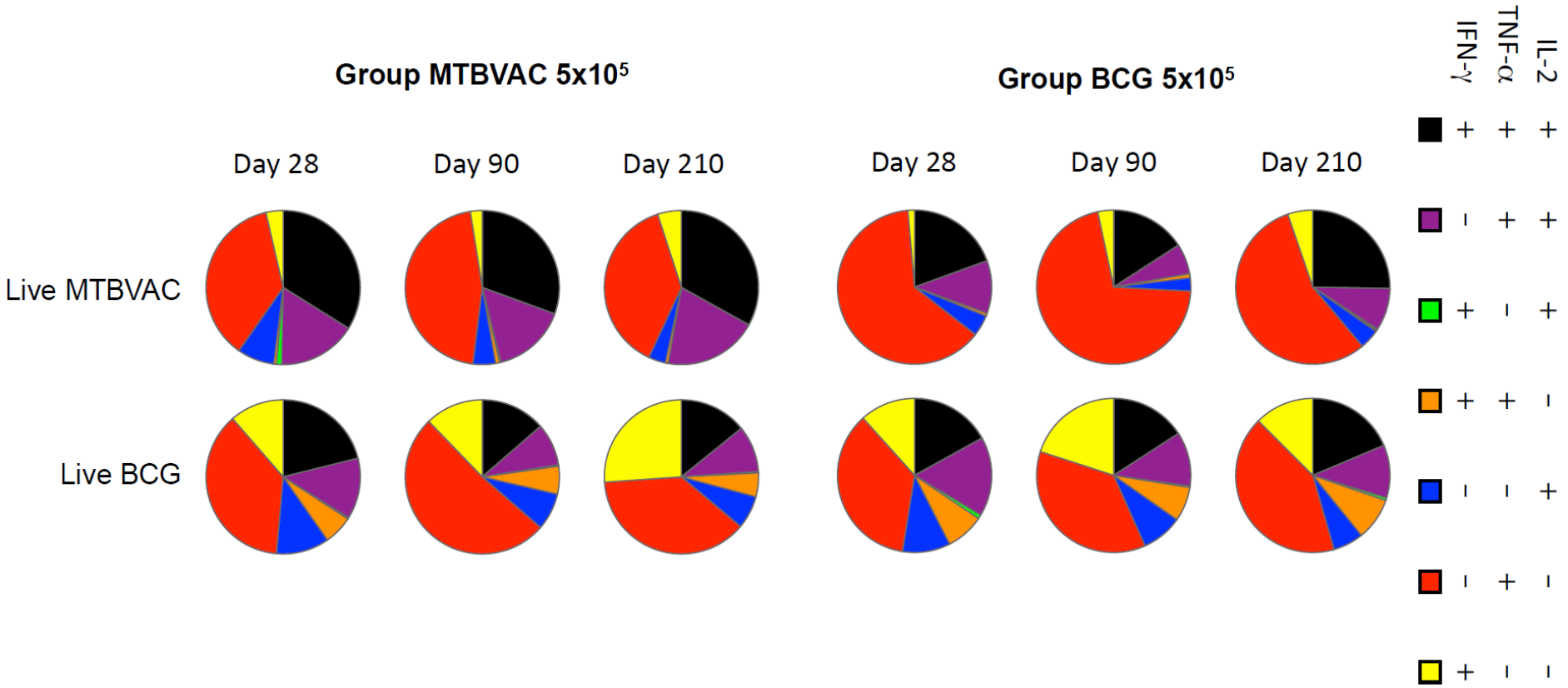

Group MTBVAC 5x10⁵
Group BCG 5x10⁵
Day 28
Day 90
Day 210
Day 28
Day 90
Day 210
Live MTBVAC
Live BCG
IFN-γ
TNF-α
IL-2
+ + +
- + +
+ - +
+ + -
- - +
- + -
+ - -

Fig S3

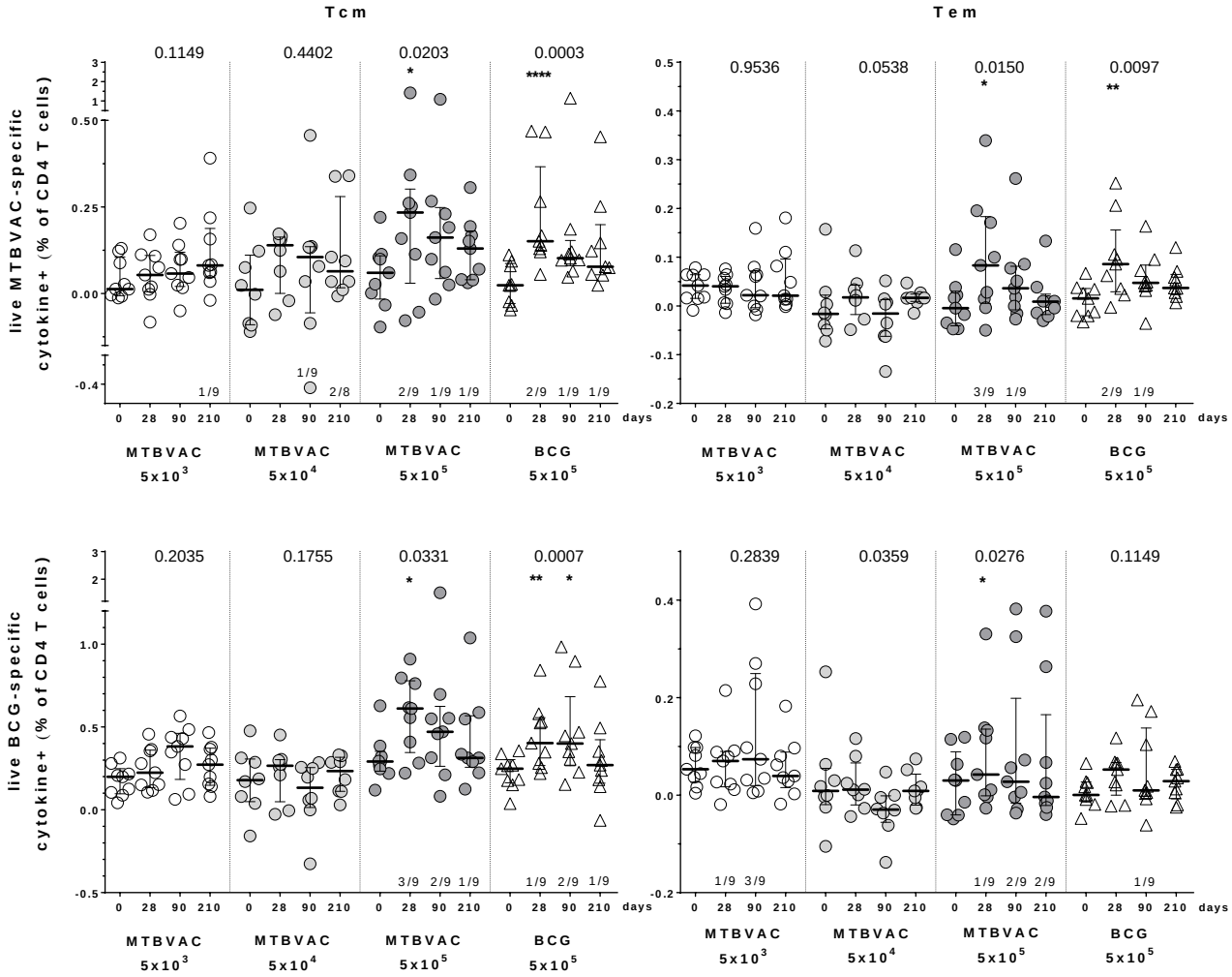

Tcm
Tem
live MTBVAC-specific cytokine+ (% of CD4 T cells)
live BCG-specific cytokine+ (% of CD4 T cells)
0.1149
0.4402
0.0203
0.0003
0.9536
0.0538
0.0150
0.0097
0.2035
0.1755
0.0331
0.0007
0.2839
0.0359
0.0276
0.1149
days
MTBVAC 5x10^3
MTBVAC 5x10^4
MTBVAC 5x10^5
BCG 5x10^5

Fig. S4

Clinical Trial Protocol

Phase I Double Blind, Randomized, Controlled, Dose-Escalation Study to Evaluate the Safety and Immunogenicity of MTBVAC in Comparison with BCG in Elispot Tb (ESAT-6, CFP10, PPD)- and HIV-negative volunteers

Trial Acronym	MTBVAC
Short title:	A new tuberculosis vaccine MTBVAC
Version:	V104
Date	31-01-2013

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STUDY SYNOPSIS

Title Phase I Double Blind, Randomized, Controlled, Dose-Escalation Study to Evaluate the Safety and Immunogenicity of MTBVAC in Comparison with BCG in Elispot Tb (ESAT-6, CFP10, PPD)- and, HIV-negative volunteers

Indication/Study population Prophylactic vaccination against tuberculosis (TB)/ adults, aged 18 to 45 years, BCG naïve, HIV-negative volunteers without evidence of active or latent tuberculosis

Rationale In view of the characteristics of the MTBVAC vaccine candidate and of pre-clinical results, the current study aims to test the safety and immunogenicity of MTBVAC as a potential substitute for BCG vaccination. BCG vaccination has indeed demonstrated its major limitation in inducing protection against TB. Novel vaccines are essential to fight against the current world epidemics in tuberculosis and resistance to anti-TB drugs.

Objectives **Primary**

- To evaluate the safety and reactogenicity of live vaccine MTBVAC when given as primary vaccination to adults aged 18 to 45 years, Elispot Tb (ESAT-6, CFP10, PPD)- and, HIV-negative adults aged 18 to 45 years

Secondary

- To evaluate the cell-mediated immune (CMI) response induced by the candidate vaccine MTBVAC as determined by ELISPOT and Intracellular Cytokine Staining (ICS)

Exploratory

- To characterize the cell-mediated immune (CMI) response induced by the candidate vaccine MTBVAC as determined by cell phenotyping by FACS and Intracellular Cytokine Staining (ICS)
- To evaluate the cell-mediated immune (CMI) response induced by the candidate vaccine MTBVAC as determined by cytokine secretion into supernatant (Luminex assay)
- To evaluate the humoral immune response induced by the candidate vaccine MTBVAC as measured by ELISA.
- To perform a transcriptomic analysis of the immune response to the candidate vaccine MTBVAC

Study design

- A Phase I, double blind, randomised, controlled single-centre study in Elispot Tb (ESAT-6, CFP10, PPD)- and HIV-negative adults, aged 18 to 45.

- Type of study: safety and immunogenicity.
- All subjects who are enrolled into the study must
  - be HIV (-1 & -2) negative
  - have no history of extra-pulmonary TB
  - have no active disease on chest X-ray
  - have no documented history of BCG vaccination or a BCG scar, and be ELISPOT TB negative
  - have no history of prophylaxis or chemoprophylaxis for TB.
- Treatment allocation: up to 36 subjects (4 groups of 9) will be enrolled and allocated to BCG (single dose level) or to MTBVAC at different dose levels (3 dose levels):
  - Subjects will be randomised by group of 4 (3 verum: 1 control) into three cohorts to receive either the study vaccine MTBVAC (3 different dose levels) or BCG as a control.
- Vaccination schedule: Single intradermal vaccination in the non dominant arm at D0
  - Each vaccine dose will be administered staggered by cohort, starting with the cohort with the lowest MTBVAC dose level
  - After at least 35 days of follow-up within each cohort there will be a safety review and evaluation. Vaccination of the subsequent cohorts will occur if there are no safety issues as defined by preset stopping rules.

Safety follow up

- A 7-day (Day 0-6) follow-up for solicited local and general symptoms after vaccination (diary card).
- Follow-up visits at day 0, 2, 7, 28, 56, 90, 150, 210 for unsolicited local and general adverse events (AEs) after vaccination.
- Serious adverse events will be collected during the entire study period.
- Duration of the study per subject: 9-10 months (screening, vaccination and 6 months follow-up).
- Data collection: on paper CRF.

Number of subjects Target enrolment of 36 healthy Elispot Tb (ESAT-6, CFP10, PPD)- and HIV-negative volunteers, aged 18 to 45.

Primary endpoints Safety and reactogenicity for all subjects as determined by:

- Occurrence of solicited symptoms during the 7-day follow-up period following vaccination (day of vaccination and 6 subsequent days).

- Occurrence of unsolicited symptoms during the 210-day follow-up period following vaccination (day of vaccination and 209 subsequent days).
- Occurrence of grade 3 vaccine related local and general symptoms during the 210-day follow-up period following vaccination (day of vaccination and 209 subsequent days).
- Occurrence of serious adverse events throughout the entire study period. Subjects will be instructed to contact the Investigator if they experience any SAEs during the follow-up phase
- Haematological and biochemical safety test levels prior and after vaccination (Days 0, 2, 7, 28, 56, 90, 150, 210) in each group

Safety review process

- Within each cohort, all AEs and biochemical and haematological parameters collected up until Day 35 after vaccination of the last subject of the cohort will be reviewed by the data safety monitoring board to authorize progression to the next sequential dose and sequential group/cohort.
- Any safety signals as defined by pre-set stopping rules warranting further discussions as per protocol definitions will be reviewed by the data safety monitoring board. Further vaccination will be suspended/put on hold pending review of unblinded data by data safety monitoring board.
- Further vaccination will only continue if following this review there are no safety issues.

Secondary endpoints

Immunogenicity assessment for all subjects as determined by:

- The cell-mediated immune (CMI) response induced by the candidate vaccine MTBVAC as determined by ELISPOT on peripheral blood mononuclear cells (PBMC): frequency of IFNγ positive mononuclear cells in response to vaccine (ESAT-6, CFP10, PPD, BCG and MTBVAC) at screening, and at days 28, 210 in all cohorts

- The cell-mediated immune (CMI) response induced by the candidate vaccine MTBVAC as determined by Intracellular Cell Staining (ICS): frequency of positive mononuclear cells for IL-2, TNFα and IFNγ in response to whole BCG and MTBVAC (overnight stimulation) at Day 0, 28, 90, 210 in all cohorts on blood cells.

Exploratory endpoints

Immunogenicity assessment for all subjects as determined by

- The cell-mediated immune (CMI) response induced by the candidate vaccine MTBVAC as determined by Intracellular Cell Staining (ICS): frequency of positive mononuclear cells expressing markers for memory, effector and regulatory subsets in response to whole BCG and MTBVAC at Day 0, 28, 90, 210 in all cohorts.
- The cell-mediated immune (CMI) response induced by the candidate vaccine MTBVAC as determined by cytokine secretion into supernatant (Luminex assay) of IL-2, IFNγ, TNFα, IL-5, IL-13, GMCSF, IL-10, TGFβ, IL-17, IL-22 in response to whole BCG and whole MTBVAC (overnight culture) as well as against selected MTB antigens (long-term stimulation) on PBMCs at Day 0, 28, 90, 210 in all cohorts.
- The humoral immune response induced by the candidate vaccine MTBVAC as measured by ELISA against selected MTB antigens, PPD, BCG and MTBVAC at Day 0, 28, 56, 90, 210 in all cohorts.
- The transcriptomic analysis of the immune response to the candidate vaccine MTBVAC at Day -30, -3, 0, 2, 7, 28, 56, 90, 150, 210 in all cohorts..

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LIST OF ABBREVIATIONS

Ab	Antibody
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BCG	Bacillus Calmette-Guérin
BUN	Blood Urea Nitrogen
CBC	Complete Blood Count
CRF	Case Report Form
ELISA	Enzyme-linked immunosorbent assay
ELISPOT	Enzyme-linked immuno-spot assay
HBsAg	Hepatitis B Surface Antigen
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
ICS	Intracellular Cytokine Staining
IFN-γ	Interferon-Gamma
Ig	Immunoglobulin
IL	Interleukin
MedDRA	Medical Dictionary for Regulatory Activities
Mtb	*Mycobacterium tuberculosis*
MTBVAC	*Recombinant Mycobacterium tuberculosis*
PBMC	Peripheral Blood Mononuclear Cell
PBS	Phosphate Buffered Saline
PPD	Purified Protein Derivative
SSRI	Selective serotonin re-uptake inhibitor
SUSAR	Suspected Unexpected Serious Adverse Reaction
Th1/Th2	T helper cell (type 1 or type 2)
TNF-α	Tumor necrosis factor – alpha
TB	Tuberculosis
WHO	World Health Organization

GLOSSARY OF TERMS

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 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.
Study Advisor:	An individual assigned by Biofabri who is responsible for advising the sponsor to achieve the study objectives.
Eligible:	Qualified for enrolment into the study based upon strict adherence to inclusion/exclusion criteria.
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 4.4 and 10.4 for details on criteria for evaluability).
Investigational product:	A pharmaceutical form of an active ingredient or placebo being tested or used as a reference in a clinical trial, including a product with a marketing authorization 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.
Clinical and Regulatory Affairs Monitor:	An individual medically qualified to assume the responsibilities of the sponsor (Biofabri) especially in regards to the ethics, clinical safety and regulatory affairs of a study and the assessment of adverse events.
Protocol amendment:	ICH defines a protocol amendment as: "A written description of a change(s) to or formal clarification of a protocol." Biofabri 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. N.B. 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.
Randomization:	Process of random attribution of treatment to subjects in order to reduce bias of selection
Solicited adverse event:	Adverse events (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.
Study Monitor:	An individual assigned by the sponsor who is responsible for assuring proper conduct of a clinical study.
Subject:	Term used throughout the protocol to denote an individual that has been contacted in order to participate in the clinical study, either as a recipient of the investigational product(s) or as a control.
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 randomization or treatment allocation.
Randomization number:	A unique number identifying a treatment to a subject, according to the study randomization for treatment allocation.
Unsolicited adverse event:	Any adverse event (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 adverse event.
Suspected Unexpected Serious Adverse Reaction or SUSAR:	Any unsolicited serious AE that are suspected to be linked to the administration of the investigational product. The investigator will report SUSAR to the Local Ethical Committee and Swiss institute of the therapeutic products "Swissmedic" as soon as possible,

1. INTRODUCTION

1.1. Background

1.1.1. BCG

Despite the widespread use of the current vaccine against TB, Bacillus Calmette Guérin (BCG), the World Health Organization (WHO) declared TB an emerging public health problem in 1993 that has reached alarming proportions with an estimated 9.4 million new cases and 1.7 million deaths reported in 2009. Respiratory (pulmonary) forms of TB are responsible for transmission, while the emergence of life-threatening multi-drug resistant (MDR) and extremely-drug resistant (XDR) strains of *Mycobacterium tuberculosis* is of even more concern, as these strains are virtually untreatable (WHO Report 2011).

BCG is the most widely administered vaccine in humans today with more than 3 billion individuals immunized to date and over 100 million doses administered annually to infants and children worldwide (BCG Vaccine. WHO position paper) (Young and Dye 2006). BCG is a live attenuated strain of *Mycobacterium bovis* effective in reducing the rate of severe forms of TB, including meningitis and miliary TB occurring in infants and children mainly in developing countries (Trunz, Fine et al. 2006). However, this live vaccine is inconsistent in preventing spread of pulmonary TB, the most common form of the disease in adolescents and adults.

1.1.2. A need for new anti-TB vaccines

In the global fight against TB, prevention is vital. There is a profound need for new vaccine(s) both in developing countries, where TB is endemic, and in emerging and industrialized countries. The long history of production, distribution and global use of BCG makes live vaccines cost-effective interventions with a potential of saving millions of lives. Such vaccines, provided they are safe, effective and affordable would have a tremendous impact worldwide on death and morbidity from this widespread disease (BIO Ventures for Global Health, 2006) (Skeiky and Sadoff 2006; Young and Dye 2006).

1.1.3. The recombinant MTBVAC vaccine candidate

1.1.3.1. The live-attenuated SO2 strain

Inside various European TB vaccine projects and the European Tuberculosis Vaccine Initiative (TBVI), Prof. Carlos Martin [University of Zaragoza (UNIZAR), Spain] and Prof. Brigitte Gicquel (Pasteur Institute, France) have collaboratively developed a uniquely attenuated strain (named SO2) from a clinical isolate of M. tuberculosis by inactivation of the *phoP* gene, which encodes the transcription factor PhoP of the two-component system PhoP/PhoR essential for *M. tuberculosis* virulence. It was suspected that the inactivation of this virulence gene would reduce the virulence of the strain, which was demonstrated. The novel strain SO2 was subsequently found to be a spontaneous

mutant unable to synthesize the complex cell-wall lipid phthiocerol dimycocerosate (PDIM), which is one of the most important virulence determinants in *M. tuberculosis* with a role in host immune modulation (Camacho, Ensergueix et al. 1999; Cox, Chen et al. 1999). SO2 has therefore two virulence factors removed: PhoP by genetic engineering, and PDIM as a spontaneous mutation.

The *phoP*-based PDIM deficient (PhoP-/PDIM-) strain SO2 has exhibited promising vaccine characteristics in the relevant animal models potentiating it as reliable prototype vaccine against TB disease after a single dose administration. Rigorous preclinical studies with SO2 have demonstrated proof of principle for adequate attenuation, safety, immunogenicity, and protective efficacy against respiratory forms of TB in stringent animal models (please refer to Investigator Brochure for detailed report).

The protection results in different animal models show that when delivered at a single dose, SO2 presents promising vaccine characteristics of protection and protective immunity against TB disease.

- SO2 confers protection against TB in BALB/c mice comparable to current BCG substrains and generates more potent cell-mediated immune responses.
- In the short-term protection experiment, SO2 and BCG confer comparable reduction in lung bacillary burden in guinea-pigs after low-dose aerosol challenge.
- In the long-term high-dose aerosol challenge study in guinea pigs, SO2 confers superior protective efficacy compared to BCG as measured by survival, reduced severity of disease and lung bacterial burden.
- In non-human primates, SO2 is safe and exhibits improved vaccine characteristics against TB when compared with unvaccinated controls. Following challenge, prototype SO2 confers survival, significant increase in body weight, reduced gross lung lesions and significant decrease in lung bacillary burden, when compared with the current BCG.
- Immunization with SO2 results in significant expansion, differentiation and maintenance of Ag-specific $CD4^+$ T cells compared to BCG-vaccination in mice. These Ag-specific $CD4^+$ T cells are more capable of differentiation into functional effector cells upon challenge with H37Rv. The study published by Triccas *et al*. (Nambiar, Pinto et al.), 2011 provides important information on the immune correlates associated with protective immunity to infection with *M. tuberculosis*.

Safety is one of the main concerns and major challenge for attenuated live vaccines. **The safety studies** conducted with prototype SO2 strain have been extensive to ensure a comprehensive safety profile offering assurance for the intended future use of an SO2-based vaccine (with PhoP-/PDIM- phenotype) in humans. In particular:

- SO2 is fully sensitive to front-line anti-tuberculosis drug treatment indicating that in the hypothetical case of infection with SO2-based vaccines, treatment would be possible.
- SO2 was demonstrated to be completely safe for 6 months after inoculation of guinea pigs with 50 times the standard dose in this species.
- In immunocompromised SCID mice, SO2 is more attenuated than BCG by the intravenous route, and unable to cause disease by the aerosol route.
- From the post-exposure infection experiment in guinea pigs and mice, prototype SO2 does not cause toxic or adverse events.

When combined with the levels of protection achieved in BALB/c mice, guinea pigs and non-human primates, the extended safety and immunogenicity data position the live-attenuated SO2 strain (PhoP-/PDIM- phenotype) as a potential and a reliable vaccine for human use with improved immune-stimulating properties compared with the BCG vaccine. Additionally, the work to date implies that specific factors associated with *phoP* expression may contribute to the relative inhibition of Ag-specific response during wild-type *M. tuberculosis* infection.

1.1.3.2. The live-attenuated MTBVAC strain

Following the Geneva consensus on essential steps towards development of new live attenuated mycobacterial vaccines (Kamath, Fruth et al. 2005), UNIZAR genetically engineered the prototype SO2 strain to have **two independent non-reverting deletion mutations**, without antibiotic markers, in the genes *phoP* and *fadD26*, additionally eliminating PDIM synthesis. The final construct MTBVAC is the first vaccine developed according with and fulfilling the first and second Geneva consensus safety requirements (Walker, Brennan et al. ; Kamath, Fruth et al. 2005) with the aim to deliver on the Millennium Development Goal to combat TB.

Following regulatory requirements, bridging protection and immunogenicity studies were carried out to demonstrate that SO2 and MTBVAC are functionally comparable.

- Bridging short-term protection study in guinea pigs showed the three MTBVAC dose groups intended for use in clinic and SO2 gave statistically equivalent protection, in both the lung and spleen, compared to the BCG Danish control group.
- Bridging immunogenicity data in BALB/c mice provide evidence that MTBVAC and its prototype SO2 are functionally comparable in their ability to induce T_H 1-type $CD4^+$ T cell activation in mice.

Additional safety studies in guinea pigs and SCID mice corroborate the safety profile of MTBVAC relative to BCG.

- In SCID mice subcutaneous inoculation of MTBVAC at 50 times the human dose demonstrated comparable safety to BCG Danish and BCG Pasteur (tested at the same dose).

- In guinea pigs, absence of virulence study carried out on the working seed lot and two final lots showed that MTBVAC complies with Eur. Ph. requirements for lot release of freeze-dried BCG vaccine for human use.

The importance of preclinical data on MTBVAC pharmacodynamics including biodistribution, persistence and shedding was considered and preclinical data was generated providing robust evidence for the similar biological activity between MTBVAC and the BCG vaccine, which is extensively used in clinic.

- Six months biodistribution studies in mice showed that MTBVAC and BCG present comparable biodistribution profile essentially limited to draining lymph nodes and spleen – sites important for initiation of mycobacterial immunity. Both vaccines were cleared at a similar rate from these lymphoid tissues for up to 6 months post vaccination.
- In guinea pigs at 7 weeks post-vaccination MTBVAC and BCG could not be detected in spleen; however, MTBVAC was detected in draining lymph nodes. Since Horwitz *et al*. (Horwitz and Harth 2003) demonstrated that viable BCG was cleared from the lymph nodes between 6 and 10 weeks post-vaccination, it is likely that any BCG present in the animals in this study had already been cleared.
- In gonads of male and female mice MTBVAC and BCG could not be recovered for up to 6 months post-vaccination.
- MTBVAC and BCG are not excreted in urine and stool of BALB/c mice for 6 months or in guinea pigs for up to 7 weeks post-vaccination by the intended clinical route, dose and volume of administration.
- MTBVAC vaccination did not result in any detectable shedding from the site of vaccination of guinea pigs, except immediately following intradermal administration. BCG vaccination resulted in the detection of viable bacilli immediately following vaccination and at site opening event, which resulted in the detection of viable bacilli in 2/10 BCG vaccinated guinea pigs.

Skin reactivity and local tolerance studies showed that MTBVAC is less reactogenic than BCG after intradermal administration (intended clinical route, dose and volume) in mice and guinea pigs.

- Unlike the BCG vaccine, no significant reaction was observed at the site of intradermal injection over a period of 7 weeks in MTBVAC vaccinated guinea pigs. A vaccine site ‘opening event’ was observed only in BCG vaccinated animals between 20 and 26 days post-vaccination.
- The 50-day formal intradermal toxicity study in mice showed no evidence of systemic toxicity following vaccination with MTBVAC, and the intradermal injection of MTBVAC on 2 occasions over 3 weeks to BALB/c mice was only associated with local inflammation at the site of administration, noted in-life and histologically, and a higher number of circulating white blood cells. These findings were also observed with BCG. The character of the inflammatory response noted histologically was similar for BCG and MTBVAC, but at Day 4 and Day 25, tended to have a higher severity grade in animals that received BCG.

In conclusion, improved safety and protection against *M. tuberculosis* has been demonstrated in exhaustive preclinical studies with SO2 and MTBVAC compared to BCG. It is hoped that extended studies with MTBVAC in humans, strengthened by the immunological analysis of the mechanism of action of this *phoP*-based PDIM deficient strain at the cellular level, will pave the way for the introduction of a superior vaccine to protect against TB.

1.1.3.3. The live-attenuated MTBVAC manufacturing

The Spanish vaccine manufacturer BIOFABRI, S.L. has produced and characterized the live MTBVAC in compliance with Good Manufacturing Practices as a freeze-dried preparation following the European Pharmacopoeia monograph and the WHO Recommendations to Assure the Quality, Safety and Efficacy of BCG Vaccines (see chapter 6). MTBVAC is thus well-advance to progress to first-in-human clinical evaluation for safety and immunogenicity as projected here.

1.2. Study rationale

In view of the characteristics of the MTBVAC vaccine candidate and of pre-clinical results, the current study aims to test the safety and immunogenicity of MTBVAC as a potential substitute for BCG vaccination. BCG vaccination has indeed demonstrated its major limitation in inducing protection against TB. Novel vaccines are essential to fight against the current world epidemics in tuberculosis and resistance to anti-TB drugs.

The rationale for the three dose levels of MTBVAC relies on the currently used doses for BCG vaccination. Two low dose levels, justified by safety and previous data on animal immunogenicity, will be compared to BCG at its usual dose of 5 x 10^5 cfu, then MTBVAC will be compared at equivalent dose to BCG if safety is judged sufficient.

1.2.1. Safety and Immunogenicity:

The aim of phase Ia clinical vaccine trial is to evaluate the safety of candidate vaccine and characterize the immune responses elicited. Therefore, in this phase I trial, we will address these issues for the vaccine candidate *MTBVAC* administered at doses of either 5 x 10^3, 5 x 10^4 or 5 x 10^5 cfu and compared it to BCG as control.

2. OBJECTIVES

2.1. Primary objectives

- To assess the safety and reactogenicity of MTBVAC candidate tuberculosis vaccine, when given to healthy ELISPOT-negative adults aged 18 to 45 years.

Refer to Section 10.1 for definition of the primary endpoints.

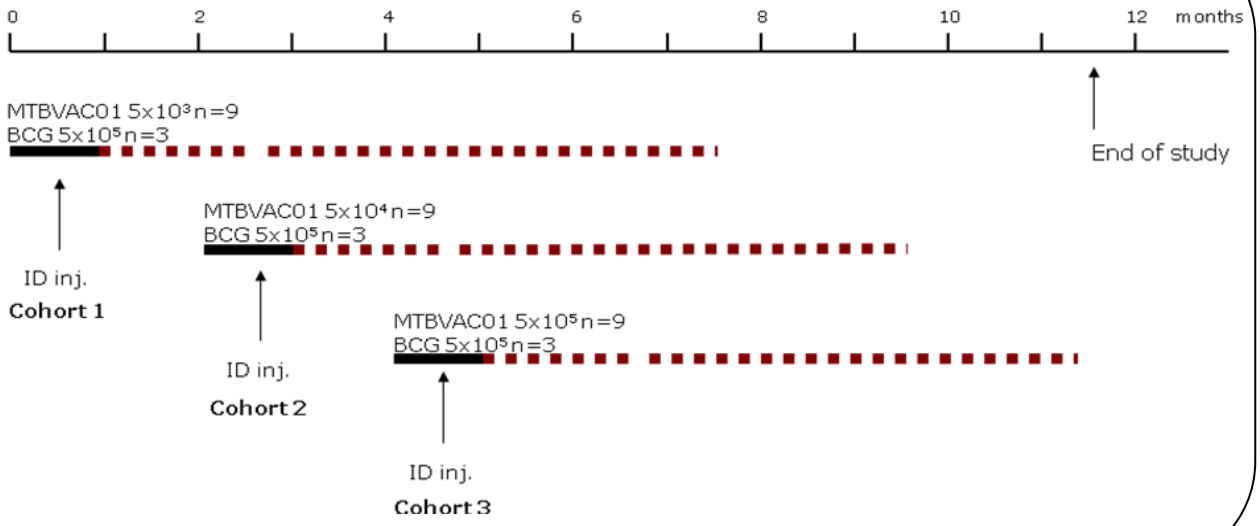
2.2. Secondary objectives

- To assess the immunogenicity of MTBVAC candidate tuberculosis vaccine when given to healthy ELISPOT-negative adults aged 18 to 45 years.

Refer to Section 10.2 for definitions of secondary endpoints.

3. STUDY DESIGN OVERVIEW

Figure 1 Global injection schedule and safety follow up



- A Phase I, open, randomised, controlled, single-centre study in adults, aged 18 to 45, HIV negative, be ELISPOT TB (ESAT-6, CFP 10) negative
- Type of study: safety and immunogenicity.
- All subjects who are enrolled into the study must
  - be HIV (-1 & -2) negative
  - have no history of extrapulmonary TB
  - have no active disease on chest X-ray
  - have no documented history of BCG vaccination or a BCG scar, and ELISPOT PPD less than 100 SFU/mio cell (positive ≥ 100).
  - have no history of prophylaxis or chemoprophylaxis for TB.
- Treatment allocation: up to 36 subjects (4 groups of 9) will be enrolled and allocated to BCG (single dose level) or to MTBVAC at different dose levels (3 dose levels):
  - Subjects will be randomised by block of 4 (3 verum: 1 control) into three cohorts to receive either the study vaccine MTBVAC (3 different dose levels) or BCG as a control.

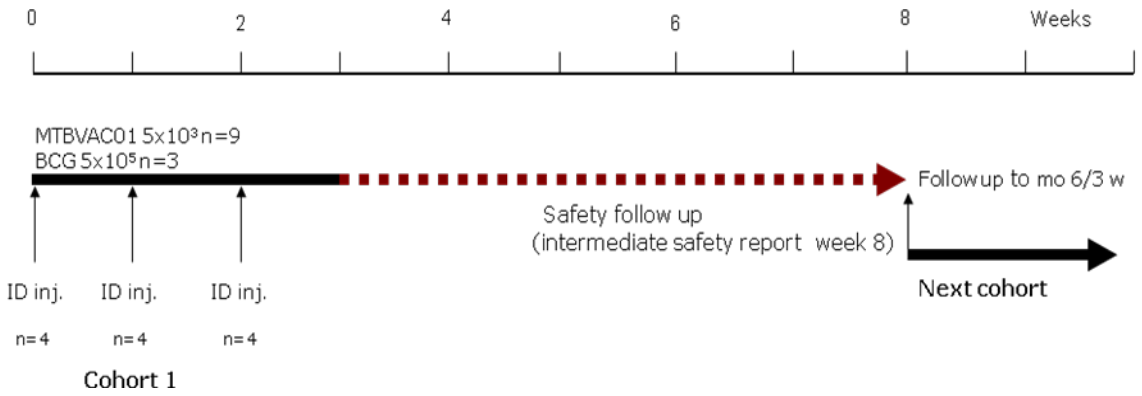
- Vaccination schedule: Single intradermal vaccination in the non dominant arm at D0
  - Each vaccine dose will be administered staggered by cohort, starting with the cohort with the lowest MTBVAC dose level (Figure 1and 2)
  - After at least 35 days of follow-up within each cohort there will be a safety review and evaluation. Vaccination of the subsequent cohorts will occur if there are no safety issues as defined by preset stopping rules (Figure 1 and 2).

Safety follow up

- A 7-day (Day 0-6) follow-up for solicited local and general symptoms after vaccination (diary card).
- Follow-up visits at day 0, 2, 7, 28, 56, 90, 150, 210 for unsolicited local and general adverse events (AEs) after vaccination. The final visit at day 365 will be made through a telephone call. If needed, a physical examination can be made and control biological sampling done.
- Serious adverse events will be collected during the entire study period.
- Duration of the study per subject: 9-10 months (screening, vaccination and 6 months follow-up).

  Data collection: first on paper CRF then electronic CRF.

Figure 2 Detailed injection schedule and safety follow up



First block of 4 volunteers from each cohort will be vaccinated in 2 groups of 2 volunteers at 2 days interval.

4. STUDY GROUPS AND COHORTS

4.1. Number of subjects / centres

Thirty-six healthy BCG naïve ELISPOT TB-negative adults aged 18 to 45 years will be enrolled to receive either the MTBVAC vaccine or BCG as control.

The study will be performed at the following centre in Switzerland:

Centre Hospitalier Universitaire Vaudois
Vaccine and Immunotherapy Center (BT 06)
Av. de Beaumont 29
1011 Lausanne Switzerland

Screening will be completed within 60 days prior to vaccination. Randomization/Enrolment will take place on the day of vaccination.

4.2. Inclusion criteria

All subjects must satisfy the following criteria at study entry. Screening procedures must be completed within 60 days prior to vaccination:

- Subjects who the Investigator believes that they can and will comply with the requirements of the protocol (e.g. completion of the diary cards, return for follow-up visits) should be enrolled in the study.
- Subjects who have no evidence of exposition to BCG as demonstrated by a ELISPOT PPD assay (< 100 SFU/mio cell) along with no history of BCG vaccination and no BCG scar
- A male or female between, and including, 18 and 45 years of age at the time of the vaccination.
- Written informed consent obtained from the subject prior to any study procedure.
- If the subject is female, she must be of non-childbearing potential, i.e. have a current tubal ligation, hysterectomy, ovariectomy or be post-menopausal, or if she is of childbearing potential, she must practice adequate contraception for 30 days prior to vaccination, have a negative pregnancy test and continue such precautions for 2 months after the vaccination.
- Clinically acceptable laboratory values for blood urea nitrogen (BUN), creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatases, total bilirubin, complete blood count (CBC) and differential, haemoglobin, platelet count and urinalysis (leucocytes, RBC, glucose, protein). Clinically acceptable laboratory values prior to randomization.
- Seronegative for human immunodeficiency virus-1 and –2 (HIV-1/2) antibodies, p24 antigen, hepatitis B surface antigen (HBsAg), and hepatitis C virus (HCV) antibodies.
- No evidence of pulmonary pathology (i.e. active acute or chronic pulmonary disease; past TB disease) as confirmed by chest X-ray*.

  **If subjects have documented evidence from a competent authority of a chest X-ray showing a reading of no active pulmonary disease within 6 months prior to vaccination, this need not be repeated (refer to section 5.2)*
- No history of extrapulmonary TB.

- No history of previous contact with *M. tuberculosis* (latent tuberculosis) as demonstrated by a negative ELISPOT Tb (ESAT-6, CFP10) assay.

4.3. Exclusion criteria for enrolment

The following criteria should be checked at the time of study entry. If any apply, the subject must not be included in the study:

- History of allergic reactions (significant IgE-mediated events) or anaphylaxis to previous immunisations (any vaccine).
- History of allergic disease or reactions likely to be exacerbated by any component of the vaccine.
- History of previous administration of experimental *Mycobacterium tuberculosis* vaccines.
- Use of any investigational or non-registered product (drug or vaccine) in another experimental protocol other than the study vaccines within 30 days preceding the vaccination, or planned use during the study period.
- Any chronic drug therapy to be continued during the study period, with the exception of vitamins and/or dietary supplements, birth control pills, anti-histamines for seasonal allergies, SSRIs (e.g. Zoloft etc…).
- Chronic administration (defined as more than 14 days) of immunosuppressors or other immune-modifying drugs within 6 months prior to the first vaccine dose such as systemic steroids, interleukins, systemic interferons (eg local injection of interferon alpha for treatment of HPV is permitted) or systemic chemotherapy. (For corticosteroids, prednisone of equivalent dose $\geq$ 0.5 mg/kg/day are forbidden. Inhaled and topical steroids are allowed).
- Administration of any immunoglobulins, any immunotherapy and/or any blood products within the three months preceding the vaccination, or planned administrations during the study period.
- Any confirmed or suspected immunosuppressive or immunodeficient condition (including HIV) based on medical history and physical examination (no laboratory testing is required).

  Given that BCG is not recommended for use in pregnant or lactating women and the increased risk of BCG dissemination in individuals with underlying immune deficiencies, the exclusion criteria further include volunteers sharing household with pregnant or lactating females, children under two years of age or very old individuals, subjects with family history of congenital or hereditary immunodeficiency, HIV-positive patients or subjects taking any immune-suppressive therapy, including systemic steroids (inhaled or nasal is permitted), interleukins or anti-interleukins, systemic interferons (e.g., local injection of interferon alpha for treatment of HPV is permitted) or systemic chemotherapy.

- Any condition or history of any acute or chronic illness or medication which, in the opinion of the Investigator, may interfere with the evaluation of the study objectives, e.g. the safety and immunogenicity of the vaccine.
- A family history of congenital or hereditary immunodeficiency. Subjects who describe a first-degree relative with clearly documented hereditary autoimmune disease will be excluded.
- **A stay of more than 2 months** in a highly endemic area (*e.g. Eastern Europe (Romania, Bulgaria) and low-income countries)* within 6 months prior to the screening visit **or** travel of more than 2 months foreseen in an area of high endemicity after the enrolment into the study.
- History of any neurologic disorders or seizures.
- History of chronic alcohol consumption and/or drug abuse which in the Investigator's opinion would put the subject at risk.
- Major congenital defects.
- Pregnant or lactating female.
- Female planning to become pregnant or planning to discontinue contraceptive precautions.
- ~~Mantoux test within the last 6 months prior to enrollment.~~

4.4. Elimination criteria

The following criteria should be checked at each visit subsequent to the vaccination visit. If any become applicable during the study, it will not require withdrawal of the subject from the study but may determine a subject's evaluability in the according-to-protocol (ATP) analysis. See Section 10.4 for definition of study cohorts to be evaluated.

- Use of any investigational or non-registered product (drug or vaccine) other than the study vaccines during the study period.
- Use of antibiotics with anti-TB activity anytime during the trial.
- Chronic administration (defined as more than 14 days) of immunosuppressors or other immune-modifying drugs during the study period. (For corticosteroids, this will mean prednisone, or equivalent, ≥ 0.5 mg/kg/day. Inhaled and topical steroids are allowed.).
- Administration of a live vaccine (except study vaccine) not foreseen by the study protocol during the period starting from 30 days before dose of vaccine and ending 30 days after, and an inactivated vaccine within 14 days prior to and after vaccination.
- Administration of immunoglobulins and/or any blood products during the study period.
- Drug and/or alcohol abuse.

4.5. Contraindications to vaccination

The following AEs constitute contraindications to administration of the study vaccine; if any one of these AEs occurs at the time scheduled for vaccination, the subject may be vaccinated at a later date.

- Acute disease at the time of vaccination. (Acute disease is defined as the presence of a moderate or severe illness with or without fever). Vaccines can be administered to persons with a minor illness such as diarrhea, mild upper respiratory infection with or without low-grade febrile illness, i.e. Oral temperature < 37.5°C / Axillary temperature < 37.5°C
- Oral temperature of ≥ 37.5°C / Axillary temperature ≥ 37.5°C.
- Any confirmed or suspected immunosuppressive or immunodeficient condition.

5. CONDUCT OF STUDY

5.1. Ethics and regulatory considerations

The study will be conducted according to Good Clinical Practice (GCP), the 1996 version of the Declaration of Helsinki (Protocol Appendix A), and local rules and regulations of the country.

5.1.1. Institutional Review Board/Independent Ethics Committee (IRB/IEC)

The IRB/IEC must be constituted according to the local laws/customs of participating country. The ICH Harmonized Tripartite Guideline for Good Clinical Practice recommends that the IRB/IEC should include:

a At least five members.

b At least one member whose primary area of interest is in a non-scientific area.

c At least one member who is independent of the institution/ study site.

Only those IRB/IEC members who are independent of the investigator and the sponsor of the study should vote/ provide opinion on a study-related matter.

A list of IRB/IEC members and their qualifications should be obtained by investigator.

This protocol and any other documents that the IRB/IEC may need to fulfil its responsibilities, including subject recruitment procedures and information about payments and compensation available to subjects will be submitted to the IRB/IEC by the investigator. Written unconditional approval of the IRB/IEC must be in the possession of the investigator and Biofabri before commencement of the study. This approval must refer to the study by exact protocol title and number, and should identify the documents reviewed and state the date of review. Relevant Biofabri' data will be supplied by

Biofabri's Clinical Development Manager to the hospital/ university/ independent IRB/IEC for review and approval of the protocol. Verification of IRB/IEC unconditional approval of the protocole and the written informed consent statement will be transmitted by investigator to Biofabri's Clinical Development Manager using the standard notification form, prior to shipment of vaccine supplies to the site.

No deviations from, or changes to, the protocol should be initiated without prior written sponsor and IRB/IEC approval/ favourable opinion of an appropriate amendment, except when necessary to eliminate immediate hazards to the subjects or when the change(s) involves only logistical or administrative aspects of the study (e.g. change of monitor[s], telephone number[s].) Administrative changes are submitted to the IRB/IEC for information only. However, written verification that the administrative change was submitted should be obtained. Approvals/ verifications must be transmitted in writing to (Biofabri's Clinical Development Manager).

The IRB/IEC must be informed by the principal investigator of:

- all subsequent protocol amendments, informed consent changes or revisions of other documents originally submitted for review,
- serious and/or unexpected adverse events occurring during the study, where required,
- all subsequent protocol administrative changes (for information, except for US studies),
- new information that may affect adversely the safety of the subjects or the conduct of the study,
- an annual update and/or request for re-approval, where required,
- when the study has been completed, where required.
- If a trial is prematurely terminated or suspended for reasons including, but not limited to, safety or ethical issues or severe non-compliance, the sponsor will promptly inform the regulatory authorities of the termination or suspension and the reason(s) for the termination or suspension. If required by applicable regulations, the investigator must inform the IEC/IRB promptly and provide the reason for the suspension or termination (see Appendix B for further details).

5.1.2. Informed consent

In obtaining and documenting informed consent, the investigator should comply with the applicable regulatory requirement(s), and should adhere to GCP and to the ethical principles that have their origin in the 1996 version of the Declaration of Helsinki. Prior to the beginning of the trial, the investigator should have the IRB/IEC's written approval/favourable opinion of the written informed consent form and any other written information to be provided to the subjects.

Information should be given in both oral and written form whenever possible and as deemed appropriate by the IRB/IEC.

An investigator or designate will describe the protocol to potential subjects face to face. The Informed Consent Form may be read to the subjects, but, in any event, the investigator or designate shall give the subjects ample opportunity to inquire about details of the study and ask any questions before dating and signing the Informed Consent Form.

Informed Consent Form must be in a language fully comprehensible to the prospective subjects. Informed consent shall be documented by the use of a written consent form approved by the IRB/IEC and signed and dated by the subjects and by the person who conducted the informed consent discussion on the screening visit day. The signature confirms the consent is based on information that has been understood. All illiterate individuals will have the study, the Informed Consent Form explained to them point by point by the interviewer in the presence of an impartial witness. The witness will personally sign and date the consent form. Oral witnessed consent will replace written consent only in countries where the local custom is contrary or if the subject's incapacity precludes this and provided that the local legal obligations are fulfilled.

Each subject's signed informed consent form must be kept on file by the investigator for possible inspection by Regulatory Authorities and/or Biofabri's collaborators. The subjects should receive a copy of the signed and dated written informed consent form and any other written information provided to the subjects, and should receive copies of any signed and dated consent form updates. Any amendments to the written information will be provided to subjects.

Both the informed consent discussion and the written informed consent form and any other written information to be provided to the subjects should include explanations of the following:

a That the trial involves research.

b The purpose of the trial.

c The trial treatment(s) and the probability for random assignment to each treatment.

d The trial procedures to be followed, including all invasive procedures.

e The subject's responsibilities.

f Those aspects of the trial that are experimental.

g The reasonably foreseeable risks or inconveniences to the subjects and, when applicable, to an embryo, fetus or nursing infant.

h The reasonable expected benefits. When there is no intended clinical benefit to subjects, the subjects should be made aware of this.

i The alternative procedure(s) or course(s) of treatment/ methods of prevention that may be available to subjects, and their important potential benefits and risks.

j The compensation and/or treatment available to subjects in the event of trial-related injury.

k The anticipated prorated payment, if any, to subjects for participating in the trial.

l The anticipated expenses, if any, to subjects for participating in the trial.

m That the subjects' participation in the trial is voluntary and subjects may refuse to participate or withdraw from the trial, at any time, without penalty or loss of benefits to which subjects are otherwise entitled.

n That the monitor(s), the auditor(s), the IRB/IEC, and the regulatory authority(ies) will be granted direct access to the subject's original medical records for verification of clinical trial procedures and/or data, without violating the confidentiality of subjects, to the extent permitted by the applicable laws and regulations and that, by signing a written informed consent, the subject is authorizing such access.

o That records identifying subjects will be kept confidential and, to the extent permitted by the applicable laws and/or regulations, will not be made publicly available. If the results of the trial are published, subjects' identity will remain confidential.

p That the subjects will be informed in a timely manner if information becomes available that may be relevant to the subjects' willingness for continued participation in the trial.

q The person(s) to contact for further information regarding the trial and the rights of trial subjects, and who to contact in the event of trial-related injury.

r The foreseeable circumstances and/or reasons under which a subject's participation in the trial may be terminated.

s The expected duration of a subject's participation in the trial.

t The number of subjects involved in the trial.

u The implication for future tuberculosis testing. Upon vaccination with MTBVAC or BCG, volunteers may become positive in screening tests such as Mantoux tests, Elispot TB or interferon release assays because of an immune response against antigens such as ESAT-6, CFP-10 (post MTBVAC) or PPD (post BCG and MTBVAC). Detailed results and information on the vaccine administered will be indicated in the certificate delivered to all volunteers.

The investigator will prepare a model Informed Consent Form which will embody all the elements described above. While it is strongly recommended that this model document 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 document.

The investigator has the final responsibility for the final presentation of Informed Consent Form, respecting the mandatory requirements of local regulations. The consent form generated by the investigator, must be approved (along with the protocol, and any other necessary documentation) by the IRB/IEC and be acceptable to Biofabri.

5.2. General study aspects

Healthy adult volunteers will be recruited by non-coercive means via poster advertising in the CHUV, the Lausanne Medical School, the University of Lausanne and EPFL or via the CHUV internal web system "intranet". Moreover advertising letters will be sent to CHUV employees through "Médecine du Personnel".

5.2.1. Screening process

At screening, volunteers will provide a medical history (with special attention to a review of pulmonary, cardiac and renal systems and prior vaccine reactions) and undergo physical examination and routine standard laboratory screening tests. Volunteers found to be seropositive for HIV and hepatitis will be counselled and referred to their regular health care provider for further evaluation.

All screening tests will be performed prior to entry into the study and at any other time during the course of the trial judged necessary by the investigators. At screening, subjects will be allocated a **screening number**. Eligible subjects will be allocated a **randomization number at randomization by the pharmacist**. The screening number will also be used as patient identification number (PID) for all data collected on the subject during the study.

Subjects should not have received BCG, and should be negative by ELISPOT TB.

Chest radiographs will be used to eliminate subjects who have active Pulmonary Disease. In persons with latent TB infection chest radiograph is usually normal but may show some apical scarring. Classification as outlined by the American Thoracic Society (American Thoracic Society, 2000) will be used to distinguish between latent infection and active disease. During screening, chest X-ray will not be done for subjects who have had a chest X-ray done within 6 months prior to administration of the first vaccine dose, with a reading of no active pulmonary disease from a competent authority.

5.2.2. Vaccination process

The vaccine MTBVAC and the BCG control will be stored at the CHUV Central Pharmacy. The unique treatment number will identify the unique vaccine dose administered to subject. After randomization the randomization number assigns the subject to one group or another in a blinded way. The randomization number will be recorded on the Case Report Form.

All vaccines will be administered intradermally in the non-dominant arm. Vaccinations will take place at the CHUV Vaccine and Immunotherapy Center.

For each vaccination during the course of the study, a pharmacist or a qualified person will prepare the vaccines. The pharmacist will randomize subject to one of the treatment arm, and attribute to each subject a randomisation number. He will fill a blind syringe according to this study protocol from the vaccine vial (refer to section 6.2). The pharmacist will play no part in the assessment of adverse events reported by subjects.

The clinical investigator qualified person, i.e. a vaccinator, will give all vaccinations in a blind manner. The Vaccinator will receive the syringes with the appropriate vaccine dose as prepared and randomized by the CHUV pharmacist and will administer the vaccine to an enrolled subject.

Subjects, who cannot be vaccinated on the originally scheduled date (due to an acute illness on the judgment of the PI or designate or scheduling conflicts), will be vaccinated within two months. If they cannot be vaccinated within this time period, they will have to pass a new screening visit.

A staff member experienced in the resuscitation of subjects will be available at all vaccination sessions. Facilities and equipment will be available to give emergency treatment in the case of an anaphylactic reaction following administration of vaccines. All subjects will be observed for at least 60 minutes after the administration of vaccine to evaluate and treat any acute adverse events.

Following vaccination subjects will be given diary cards to record any adverse events that may occur post vaccination. These diary cards are to be returned 6 days after vaccination. On receipt of these diary cards the investigator or designate will verify the contents and transcribe the information into the appropriate sections of the CRF. Any unreturned diary cards will be sought from the subjects through a convenient procedure by the investigator.

In addition subjects will be given an '**adverse event and medication form**' to record any other adverse events that may occur outside the follow-up period for solicited symptoms and any medication they may take up until 240 days after each vaccination. The form will have a section to record symptoms with their intensity and start and stop date. There will also be a medication section with information on the trade name, reasons for taking medication, the dosage and start and stop dates. The subject must be reminded to bring this form for each follow up visit to be discussed with the investigative team and the form will be returned to the investigator at subsequent vaccinations or 1 month after the last vaccination.

5.2.3. Subject identification

Screening numbers will be assigned sequentially to subjects consenting to participate in the study, according to the range of subject numbers allocated.

5.2.4. Safety Monitoring Plan

5.2.4.1. Safety Monitor

A local safety monitor (LSM) from Centre Hospitalier Universitaire Vaudois (CHUV) will review volunteer safety information after each dose. This will include a systematic review of grade 3 and serious adverse events after each vaccination. He will not be part of the investigative team. The LSM will offer a recommendation following an assessment of adverse events and/or accompanying safety data if suspension of vaccination of following subjects will be required.

The Local Safety Monitor (LSM) will be an experienced clinician qualified to evaluate safety data from clinical trials of Tuberculosis vaccines. All SAEs will be reported to him/her. In exceptional circumstances, for example a death possibly related to vaccination, or under conditions as outlined below in the safety monitoring plan, he/she will have the authority to suspend vaccination pending discussion with the sponsor and collaborators. He will have the authority to request the code-break information from Biofabri (safety study contact for emergency code break) to unblind individuals at any time if a set of medical events requires unblinding to enable treatment or death related to vaccination. All unblinding should be reported immediately to Biofabri. Refer to section 6.5 for information on study unblinding.

Biofabri will also appoint a Clinical Trial and Regulatory Affairs Monitor, a physician qualified to evaluate safety data from clinical trials of new vaccines who will review in parallel any blinded safety information sent to the LSM.

5.2.4.2. Review of Safety Data by the Clinical Trial Medical and Regulatory Affairs Monitor

Vaccinations per dose level of MTBVAC or control BCG are scheduled at Day 0, and additional safety evaluations at days 2, 7, 28, 56, 90, 150, 210 and 365 after vaccination. The following events must occur within the specified timeframe to ensure proceeding to the next vaccination as per schedule.

- The investigative site must complete paper CRF from the day of vaccination up to Day 7 for each subject by Day 9.
- LSM will receive the data by FAX or electronic doc or paper.
- The Clinical Trial Medical and Regulatory Affairs Monitor must review the demographic and safety data for each subject within one week after the last subject has completed the 7-day follow-up after vaccination. If warranted, the CTMRAM recommend to the site to suspend further vaccination at least 1 business day prior to the next volunteers' vaccination in case a safety signal is observed or the predefined holding rules are met.

5.2.4.3. Vaccination process and Safety Review Team

Subjects will be allocated to 4 groups according to randomization schedule and vaccine type and doses:

D0 dose vaccination will be spread out starting initially by **cohort 1** (9 volunteers receiving the first dose level of MTBVAC plus 3 BCG controls). The volunteers will be randomized by block of four (3 volunteers receiving MTBVAC plus 1 BCG control). The vaccination of the second and third block of four will start one week after the vaccination of the first member of the previous block. Vaccination within **cohort 2** (9 volunteers with second dose level of 9 MTBVAC plus 3 BCG controls) and **cohort 3** (9 volunteers with third dose level of MTBVAC plus 3 BCG controls) will be completed in a similar fashion (see figure 1 and 2, section 3).

Vaccinations of the three cohorts will be staggered by at least six weeks. Vaccination after each cohort will be followed by a safety review of data (reactogenicity, biochemical and haematological safety tests) collected up until 56 days post vaccination of the first volunteers, i.e. at least 5 weeks post vaccination of the last subject. All safety and reactogenicity data collected up until 5 weeks post vaccination of the last subject will be reviewed blinded by the Investigator group and a safety review team (SRT) to see if holding/suspension criteria are met. If there are no safety issues then vaccination of the second cohort will commence.

The investigative site will be required to complete paper CRF from Day 0 to 56 following vaccination for each cohort by two weeks after the visit 7.

A safety review team (SRT), including as core members the Clinical and Regulatory Affairs Monitor and Principal Investigator will be responsible for reviewing blinded safety data 'in-stream' (i.e. throughout the course of the study). The SRT must review the demographic and safety data for each cohort after vaccination within one week after the last subject has completed an at least 5 week follow-up after vaccination. If warranted, the SRT may instruct the site to suspend further vaccination of the next volunteers or the next cohort at least 1 business day prior to vaccination in agreement with the LSM and the investigator.

In case a safety signal is observed or the predefined holding rules are met, the SRT leader is responsible for the urgent communication and escalation to Biofabri.

5.2.4.4. Holding Rules

5.2.4.4.1. Holding rules for vaccination (temporary suspension of vaccination)

Vaccination will be put on hold/ suspended pending review of data with the SRT if greater than 3 vaccinated subjects from a same cohort develop severe or unexpected AEs judged to be related to vaccination by the Investigator within 5 weeks after the last subject of each cohort has been vaccinated;

for a death-or-life threatening SAE judged to be related to vaccination by the Investigator at any time during the study;

for an anaphylactic shock reaction in an enrolled subject following vaccination.

Activation of the Holding Rule requires a thorough review by the Investigators and Sponsor. Vaccinations will only continue following recommendations from the SRT and in agreement with the Investigator.

If for any reason, the study is stopped, the National Regulatory Agency will be informed by letter via the Sponsor and the Ethics Committee via the Investigator.

Vaccination can be put on hold by Local Safety Monitor, subject to discussion with the investigators, BIOFABRI Clinical Development Manager, Clinical and Regulatory

Affairs Monitor, if after reviewing blinded safety and reactogenicity data after each vaccination the following criteria are met:

- Vaccinations will be put on hold

- if at least 3 subjects per cohort develop a Grade 3 general solicited adverse event during the 7 follow-up days after vaccination which persists at Grade 3 for > 48 hours (2 days). The 7 days includes the day of vaccination and 6 subsequent days. The general solicited adverse events are Fatigue, Fever, Gastrointestinal symptoms (includes nausea, vomiting, diarrhea or abdominal pains) and Headache **or**

- if 3 subjects per cohort develop any other Grade 3 unsolicited adverse event considered to be associated with vaccination during the 7 follow-up days after vaccination and persisting at Grade 3 for > 48 hours (2 days). The 7 days includes the day of vaccination and 6 subsequent days.

- Vaccination can be put on hold for the three cohorts if any individual shows large ulceration (necrosis of the dermis, >1 cm in diameter) at the injection site following vaccination.

5.2.4.4.2. Holding Rules for the whole study (temporary suspension of the study)

The study should be put on hold by Local Safety Monitor at any time during the study, subject to discussion with the investigators, the BIOFABRI Clinical Development Manager, or the Clinical and Regulatory Affairs Monitor, if any subject develops one or more of the following

- Hepato/splenomegaly

- Acute lymphadenitis (swelling and or tenderness of the lymph nodes including cervical, axillary, inguinal and epitrochlear lymph nodes).

- Acute pulmonary disease associated with an abnormal chest x-ray.

- New onset of fever, weight loss or chronic cough (Fever for >1week, cough lasting >2months, weight loss of >10kg)

In the onset of any of the above, the subject will undergo a thorough laboratory and clinical examination to establish a diagnosis.

5.2.4.5. Discussion Process after a vaccination or a clinical study hold

BIOFABRI will be informed through the Clinical Development Manager or the Clinical and Regulatory Affairs Monitor on the same day that the LSM makes a decision to temporarily suspend any part of the study. Within 5 working days of placing vaccination of a cohort or the clinical trial on hold, the Clinical Development Manager or the Clinical and Regulatory Affairs Monitor will organise a meeting (via teleconference, videoconference, or face-to-face) to review and discuss the safety data and the events leading to the hold order. Three or more days prior to this meeting, BIOFABRI will disseminate copies of all relevant safety data to all meeting participants.

5.2.4.6. Process for restarting vaccination/trial

Although the vaccination may be put on hold temporarily, further vaccination may restart only if all parties (the Clinical and Regulatory Affairs Monitor, the Clinical Development Manager, the Sponsor's Signatory, the LSM, the SRT and the Principal Investigator) agree to a resumption of vaccination.

5.2.4.7. Process for stopping of trial

In the event that the trial is stopped, BIOFABRI will inform the IRB through the investigator and Swissmedic through its representative in Switzerland. A report will be written detailing the rationale used for reaching this decision.

5.3. Outline of study procedures

The outline of study procedures is presented in Table 1.

Table 1 List of study procedures

Phase		Screen		Vaccination phase				Post-vaccination FU				Tel
Month		-2		0			1	2	3	5	7	12
Day		-60 to -3	-3	0	2	7	28	56	90	150	210	365
Visit		1	2	3	4	5	6	7	8	9	10	
Dose				1								
Informed consent		●										
Check of inclusion & exclusion criteria		○		●								
Check of elimination criteria					●	●	●	●	●	●	●	
Check of contraindications				●								
Medical history		●										
Current medical history (anamnesis)		●		●	●	●	●	●	●	●	●	●
Physical examination		●		●	●	●	●	●	●	●	●	(●)
Resting vital signs (blood pressure, heart rate, temperature)		●		●	●	●	●	●	●	●	●	(●)
Chest X-ray		●*									●**	(●)
Recording of concomitant medication pre-/post-vaccination		●		●	●	●	●	●	●	●	●	●
Pre- and post-vaccination assessment incl. temperature measurement		●		●	●	●	●	●	●	●	●	
Vaccination				●								
Diary cards	Distribution			●								
	Return					●						
AE and medication form	Distribution			●	(●)	(●)	(●)	(●)	(●)	(●)		
	Return				(●)	(●)	(●)	(●)	(●)	(●)	●	
Post-vaccination recording of solicited symptoms				●	●	●						
Recording of non-serious AEs post vaccination by PI				●	●	●	●	●	●	●	●	●
Reporting of serious AEs throughout the study by PI				●	●	●	●	●	●	●	●	●
Microbiological analyses Swabs at injections site, urine and stools culture					●	●	●	(●)***	(●)***	(●)***	(●)***	(●)
Pregnancy test: β-HCG-serum *(4.5 ml)*		●										
β-HCG –urine				●							●	
Biochemical and haematological analysis and virology (blood) BUN, creatinine, AST, ALT, bilirubin, alc. Phosp. (*3 ml)*		●		●		●	●	●	●	●	●	
CBC (*2 ml)*		●		●		●	●	●	●	●	●	
HIV, HBsAg, anti-HCV (*10 ml)*		●										
Urinalysis (proteins, leucocytes, RBC, glucose)		●		●	●	●	●	●	●	●	●	
Immunogenicity 2ary endpoints ELISPOT TB (ESAT-6 / CFP10 / PPD) testing# (*18 ml*)		●##					●				●	
ICS IL-2, TNFa, IFNg (WBA) *(9 ml)* @				●			●		●		●	
Exploratory endpoints ELISPOT (PBMC) ###		●					●				●	
Cytokines in culture (PBMC) *(18 ml)* $				●			●		●		●	
Tem/Tcm/polyfct, FACS subsets *(9ml)* £				●			●		●		●	
anti–MTBVAC/MTB antibodies *(3 ml)*				●			●	●	●		●	
Transcriptomics *(8 ml)* &		●	●	●	●	●	●	●	●	●	●	
Blood volume per visit in ml:		45	8	52	8	13	70	16	52	13	70	

Phase	Screen		Vaccination phase				Post-vaccination FU				Tel
Month	-2		0			1	2	3	5	7	12
Day	-60 to -3	-3	0	2	7	28	56	90	150	210	365
Visit	1	2	3	4	5	6	7	8	9	10	
Dose			1								
Cumulative blood volume (ml):	45	53	105	113	126	196	212	264	277	347	
Study intermediate safety report / cohort							•				
Study conclusion										•	

•is used to indicate a study procedure that requires documentation in the individual CRF

(•) is used to indicate a study procedure that requires documentation in the individual CRF If necessary

*To be done only if the subject has no evidence of the procedure (chest X-ray) performed in the past six months

**chest X-rays at the end of the study will be performed in all subjects.

***Not done if two previous consecutive four weeks culture have been negative

#ELISPOT IFNγ to Mtb PPD, ESAT-6/CFP10 made at CHUV routine lab on PBMC.

Results important for inclusion/exclusion in the study.

ELISPOT IFNγ to other Mtb antigens than PPD, ESAT-6/CFP10.

@: stimulation of blood cells (whole blood or PBMC) with whole mycobacteria (BCG and MTBVAC, live or lysed). Evaluation of the frequency of specific cells that secrete cytokine in response to the stimulation.

$: stimulation of PBMC or whole blood with various TB preparations and antigens: lysates of rMtb, Mtb, BCG ; HBHA, Ag85, ESAT-6, CFP-10. Analysis by Luminex of IL-2, IFNg, TNFa, IL-5, IL-13, GMCSF, IL-10, TGFb, IL-17, IL-22.

£: stimulation of blood cells (whole blood or PBMC) with whole mycobacteria (BCG and MTBVAC, live or lysed). Characterization by FACS of the various subsets of Mtb specific cells, memory and polyfunctional, by analysis of the markers: CD3, CD4, CD8, IL-2, TNF-a, IFNg, CD45RA, CCR7.

&: on whole blood. Include Foxp3.

It is the investigator's responsibility to ensure that the intervals between visits/contacts are strictly followed. These intervals determine each subject's evaluability in the according-to-protocol analyses (see Sections 4.4 and 10.4 for details of criteria for evaluability and cohorts to be analyzed).

Table 2 Intervals between study visits

Visit Intervals (Vaccination phase)	Size of interval
Visit 1 (Screening)	-60 to -3 days
Visit 2 (Day -3)	+/- 1 day
Visit 3 (Day 0)	+/- 1 day
Visit 4 (Day 2)	+/- 1 day
Visit 5 (Day 7)	+/- 1 day
Visit 6 (Day 28)	+/- 3 days
Visit 7 (Day 56)	+/- 3 days
Visit 8 (Day 90)	+/- 3 days
Visit 9 (Day 150)	+/- 7 days
Visit 10 (Day 210)	+/- 7 days
Final visit (telephone call at Day 365)	+/- 14 days

5.4. Detailed description of study stages/visits

A description of each stage of the study, as summarized in the outline of study procedures (Section 5.3) is given below.

Visit 1: Screening (Day – 60 to Day – 3)

- Written informed consent obtained from the subjects prior to any study procedure.
- Check of inclusion/ exclusion criteria before enrolment (See Sections 4.2 and 4.3).
- Recording of medical history and physical examination.
- Assess resting (resting is defined as at least 10 minutes of rest prior to obtaining the vital signs) vital signs (heart rate, blood pressure & temperature).
- Chest X-ray
  Note: If the subjects have a chest-X ray done with documented results within 6 months prior to vaccination, they do not have to get an X-ray done at the screening visit.
- Collect a total of 45 ml whole venous blood for haematology, serum chemistry, pregnancy and serology testing, TB status and transcriptomics.
  - 14 ml for CBC, serology testing (HIV-1/2, p24 antigen, HBsAg and HCV antibody) and for biochemical and haematological analyses (creatinine, ALT, AST, total bilirubin, alkaline phosphatases and BUN).
  - 5 ml for quantitative Beta HCG (pregnancy testing) for all female subjects.
  - 18 ml for ELISPOT TB (ESAT-6/CFP10) testing
  - 8 ml for transcriptomic analysis
- Urinalysis (proteins, glucose, leucocytes, RBC)
- Recording of SAEs that are related to study participation (e.g. procedures or invasive test) or to a concurrent medication (see section 8.4)

Visit 2: Day-3 (±1 day) – Transcriptomic baseline

- Collection of a of 8 ml whole venous blood for transcriptomic analysis.

Visit 3: Day 0 – Vaccination day

- Check of inclusion/ exclusion criteria (See Sections 4.2 and 4.3).
- Check contra-indication to vaccination.
- Physical examination.
- Assess resting (resting is defined as at least 10 minutes of rest prior to obtaining the vital signs) vital signs (heart rate, blood pressure).
- Recording of any concomitant medication/vaccination.
- Pre-vaccination assessment including temperature measurement.
- Urine pregnancy test for all female subjects.

- Collection of a total of 52 ml whole venous blood prior to vaccination for the following analyses:
  - 5 ml for biochemical and haematological analysis (CBC, creatinine, ALT, AST, total bilirubin, alc. phosph. and BUN)
  - 39 ml for CMI and humoral response (refer to Table 1 for details)
  - 8 ml for transcriptomic analysis
- Urinalysis (proteins, glucose, leucocytes, RBC)
- Vaccination: Intradermal administration of one dose of study vaccine or control at the level of the deltoid muscle. See Section 6.2 for details.

  The vaccinees will be observed closely for at least 60 minutes, with appropriate medical treatment readily available in case of a rare anaphylactic reaction following the administration of vaccines. The subjects will also be instructed to contact the investigator immediately should they manifest any signs or symptoms they perceive as serious.
- Recording of any post vaccination solicited and unsolicited adverse event.
- Recording of any Serious Adverse Events (SAEs).
- Diary cards will be provided to the subjects to record body temperature (axillary temperature) and any solicited local, general occurring after vaccination, on the day of vaccination and 6 subsequent days. The subjects will be instructed to return the completed diary card to the investigator on Day 7.
- Distribution of an adverse events and medication form to the subjects to record any unsolicited adverse events and medication taken occurring throughout the study. The subjects will be instructed to bring the form to all subsequent visits to be discussed with the investigative team.
- Distribution of material for stool and urine collection.

Visit 4: Day 2 (±1 day) - Post vaccination follow-up visit

- Check elimination criteria.
- Physical examination.
- Assess resting (resting is defined as at least 10 minutes of rest prior to obtaining the vital signs) vital signs (heart rate, blood pressure, temperature).
- Recording of any concomitant medication/vaccination.
- Post-vaccination recording of solicited and unsolicited symptoms by the investigator.
- Recording of any SAEs.
- Collection of 8 ml whole venous blood for transcriptomic analysis.
- Urinalysis (proteins, glucose, RBC, leucocytes)

- Swab of injection site for MTBVAC culture
- Collection of urine and stools for MTBVAC culture and distribution of material.
- Verification of the diary card and the AE/medication forms

Visit 5: Day 7 (±1 day) - Post vaccination follow-up visit

- Check elimination criteria.
- Physical examination.
- Assess resting (resting is defined as at least 10 minutes of rest prior to obtaining the vital signs) vital signs (heart rate, blood pressure, temperature).
- Recording of any concomitant medication/vaccination.
- Collection and verification of completed diary cards.
- Verification the AE/medication forms
- Recording of unsolicited symptoms reported since the last vaccination.
- Recording of non-serious adverse events.
- Recording of any SAEs.
- Collection of a total of 13 ml whole venous blood for the following analyses:
  - 5 ml whole venous blood for biochemical and haemotological analysis (CBC, creatinine, ALT, AST, alc. phosph., total bilirubin and BUN).
  - 8 ml for transcriptomics analysis
- Urinalysis (proteins, glucose, leucocytes, RBC)
- Swab of injection site for MTBVAC culture
- Collection of urine and stools for MTBVAC culture and distribution of material

Visit 6: Day 28±3 days (Month 1 post- vaccination)

- Check elimination criteria.
- Physical examination.
- Collection and verification of adverse events and medication form.
- Assess resting (resting is defined as at least 10 minutes of rest prior to obtaining the vital signs) vital signs (temperature, heart rate and blood pressure).
- Recording of any concomitant medication/vaccination.
- Recording of unsolicited symptoms reported since vaccination.
- Recording of any post vaccination solicited and unsolicited adverse events
- Recording of any SAEs.

- Collection of a total of 70 ml whole venous blood for the following analyses:
  - 5 ml for biochemical and haemotological analysis (CBC, creatinine, ALT, AST, alc. phosph., total bilirubin and BUN)
  - 57 ml for CMI and humoral response (refer to Table 1 for details)
  - 8 ml for transcriptomics analysis
- Urinalysis (proteins, glucose, RBC, leucocytes)
- Swab of injection site for MTBVAC culture
- Collection of urine and stools for MTBVAC culture and distribution of material
- Verification the AE/medication forms

Visit 7: Day 56 ± 3 days (Month 2 post-vaccination visit)

- Check elimination criteria.
- Physical examination.
- Collection and verification of adverse events and medication form.
- Assess resting (resting is defined as at least 10 minutes of rest prior to obtaining the vital signs) vital signs (temperature, heart rate, blood pressure).
- Recording of any concomitant medication/vaccination.
- Recording of unsolicited symptoms reported since vaccination.
- Recording of any post vaccination solicited and unsolicited adverse events
- Recording of any SAEs.
- Collection of a total of 16 ml whole venous blood for the following analyses:
  - 5 ml for biochemical and haemotological analysis (CBC, creatinine, ALT, AST, alc. phosph., total bilirubin and BUN)
  - 3 ml for humoral response (refer to Table 1 for details)
  - 8 ml for transcriptomics analysis
- Urinalysis (proteins, glucose, RBC, leucocytes)
- Swab of injection site for MTBVAC culture if necessary (the cultures for *M. tuberculosis* will stop if the results of two consecutive four weeks cultures are negative. Cultures will be reinstituted if local leakage reappears).
- Collection of urine and stools for MTBVAC culture and distribution of material if necessary (the cultures for *M. tuberculosis* will stop as soon as the results of two consecutive four weeks cultures are negative)
- Verification the AE/medication forms

Visit 8: Day 90 ± 3 days (Month 3 post-vaccination follow-up visit)

- Check elimination criteria.
- Physical examination.
- Recording of any concomitant medication/vaccination.
- Recording of unsolicited symptoms reported since vaccination.
- Recording of any post vaccination solicited and unsolicited adverse events
- Recording of any SAEs.
- Collection of a total of 52 ml whole venous blood for the following analyses:
  - 5 ml for biochemical and haemotological analysis (CBC, creatinine, ALT, AST, alc. phosph., total bilirubin and BUN)
  - 39 ml for CMI and humoral response (refer to Table 1 for details).
  - 8 ml for transcriptomics analysis
- Urinalysis (proteins, glucose, leucocytes, RBC)
- Swab of injection site for MTBVAC culture if necessary (the cultures for *M. tuberculosis* will stop if the results of two consecutive four weeks cultures are negative. Cultures will be reinstituted if local leakage reappears)
- Collection of urine and stools for MTBVAC culture and distribution of material if necessary (the cultures for *M. tuberculosis* will stop as soon as the results of two consecutive four weeks cultures are negative)
- Verification the AE/medication forms

Visit 9: Day 150 ± 7 days (Month 5 post-vaccination follow-up visit)

- Check elimination criteria.
- Physical examination.
- Recording of any concomitant medication/vaccination.
- Recording of unsolicited symptoms reported since vaccination.
- Recording of any post vaccination solicited and unsolicited adverse events
- Recording of any SAEs.
- Collection of a total of 13 ml whole venous blood for the following analyses:
  - 5 ml for biochemical and haematological analyses (CBC, creatinine, ALT, AST, alc. phosph., total bilirubin and BUN)
  - 8 ml for transcriptomics analysis
- Urinalysis (proteins, glucose, leucocytes, RBC)

- Swab of injection site for MTBVAC culture if necessary (the cultures for *M. tuberculosis* will stop if the results of two consecutive four weeks cultures are negative. Cultures will be reinstituted if local leakage reappears)
- Collection of urine and stools for MTBVAC culture and distribution of material if necessary (the cultures for *M. tuberculosis* will stop as soon as the results of two consecutive four weeks cultures are negative)
- Verification the AE/medication forms

Visit 10: Day 210 ± 7 days (Month 7 post-vaccination follow-up visit)

- Check elimination criteria.
- Physical examination.
- Recording of any concomitant medication/vaccination.
- Recording of unsolicited symptoms reported since the last vaccination.
- Recording of any post vaccination solicited and unsolicited adverse events
- Recording of any SAEs.
- Chest X-ray
- Collection of a total of 70 ml whole venous blood for the following analyses:
  - 5.3 ml for biochemical and haemotological analysis (CBC, creatinine, ALT, AST, alc. phosph., total bilirubin and BUN)
  - 57 ml for CMI and humoral response (refer to Table 1 for details)
  - 8 ml for transcriptomics analysis
- Urinalysis (proteins, glucose, leucocytes, RBC)
- Urine pregnancy test for all female subjects.
- Swab of injection site for MTBVAC culture if necessary (the cultures for *M. tuberculosis* will stop if the results of two consecutive four weeks cultures are negative. Cultures will be reinstituted if local leakage reappears)
- Collection of urine and stools for MTBVAC culture if necessary (the cultures for *M. tuberculosis* will stop as soon as the results of two consecutive four weeks cultures are negative)
- Collection and verification of the AE/medication forms

Final visit (telephone call): Day 365 ± 14 days (Month 12 post-vaccination)

- Current medical history (anamnesis) obtained through the telephone call
  - Recording of solicited/unsolicited symptoms
- Physical examination if necessary based on the current history obtained through the telephone call

- Laboratories and/or radiologic investigations if necessary based on the current medical history obtained through the telephone call

5.5. Sample handling and analysis

5.5.1. Treatment and storage of biological samples

Whole blood will be collected at each time point specified in the list of study. Serum will be separated and kept frozen at –70°C until testing.

Whole blood samples for cell-mediated immunity assays will be collected at each time point specified in the list of study procedures and kept at 20-25 °C until transfer to the immunogenicity laboratory for isolation of peripheral blood mononuclear cells (PBMC), freezing and storage until further testing. Isolation of PBMCs needs to be performed within 4 hours after blood collection.

5.5.2. Laboratory assays

5.5.2.1. Screening and safety follow up

Screening

Tests will be performed to assay for HBV (HBsAg), HCV (total anti-HCV Ab) and HIV (anti-HIV1/2 Ab and p24 Ag) as well as for biochemistry, haematology and urinalysis [CBC - complete blood count which includes platelets, white blood cells, haematocrit and haemoglobin, BUN - Blood Urea Nitrogen, Creatinine, ALT -Alanine aminotransferase, AST - Aspartate Aminotransferase, Total bilirubin, alkaline phosphatases, urinalysis (proteins, RBC, leucocytes, glucose)]. A pregnancy test will also be performed on all females.

Safety

Tests will be performed to assay for CBC, BUN, creatinine, ALT, AST, total bilirubin, alc. phosph. and urinalysis (proteins, leucocytes, RBC, glucose) at the following time points : Days 0, 2, 7, 28, 56, 90, 150 and 210.

M. tuberculosis cultures will be made from the injection site, i.d. the deltoid region of the non dominant arm, the urine and the stools separately from visit 4 and on, till two successive 4 week cultures are negative. Practically it means that cultures from each site will be done from V4 to V6. The cultures on visit V7 and after will depend on the result of the previous cultures.

Cellular and humoral immunogenicity

The techniques that will be used to analyse “the immune response” to the vaccine could in the future be updated to the best available approved procedure.

Cellular and humoral immunogenicity

A. PBMCs and/or whole blood will be used to evaluate **secondary outcomes** and will involve:

- ❖ The cell-mediated immune (CMI) response induced by the candidate vaccine MTBVAC as determined by ELISPOT on peripheral blood mononuclear cells (PBMC): frequency of IFNγ positive mononuclear cells in response to vaccine (ESAT-6, CFP10, PPD, whole BCG and whole MTBVAC) at screening, and at days 28, 210 in all cohorts

- ❖ The cell-mediated immune (CMI) response induced by the candidate vaccine MTBVAC as determined by Intracellular Cell Staining (ICS): frequency of positive mononuclear cells for IL-2, TNFα and IFNγ in response to whole BCG and whole MTBVAC (overnight stimulation of whole blood or PBMC) at Day 0, 28, 90, 210 in all cohorts.

B. PBMCs and/or whole blood will be used to evaluate exploratory outcomes and will involve:

- o The cell-mediated immune (CMI) response induced by the candidate vaccine MTBVAC as determined by Intracellular Cell Staining (ICS): frequency of positive mononuclear cells expressing markers for memory, effector and regulatory subsets in response to live BCG and live MTBVAC at Day 0, 28, 90, 210 in all cohorts on whole blood.
- o The cell-mediated immune (CMI) response induced by the candidate vaccine MTBVAC as determined by cytokine secretion into supernatant (Luminex assay) of IL-2, IFNγ, TNFα, IL-5, IL-13, GMCSF, IL-10, TGFβ, IL-17, IL-22 in response to live BCG and live MTBVAC (overnight culture) as well as against selected MTB antigens (long-term stimulation) on PBMCs at Day 0, 28, 90, 210 in all cohorts.

C. The humoral immune response induced by the candidate vaccine MTBVAC as measured by ELISA against selected MTB antigens, PPD, BCG and MTBVAC at Day 0, 28, 56, 90, 210 in all cohorts.

D. The transcriptomic analysis of the immune response to the candidate vaccine MTBVAC at Day -30, -3, 0, 2, 7, 28, 56, 90, 150, 210 in all cohorts (refer to Table 1)

5.5.3. Serology and CMI plan

Serology and CMI plan will be performed according to plan detailed in Table 1.

6. INVESTIGATIONAL PRODUCT AND ADMINISTRATION

6.1. MTBVAC vaccine

The MTBVAC vaccine has been released by BIOFABRI, Porriño, Spain.

The Quality Control Standards and Requirements for the candidate vaccine are described in separate release protocols and the required approvals have been obtained.

All MTBVAC vials should be stored at +2°C to +8°C.

Table 3 Composition of MTBVAC study vaccine and BCG control

Vaccine	Formulation (approximately per standard dose)	Presentation	Volume
MTBVAC	$3 – 17 \times 10^5$ cfu Sucrose 16.5 mg Sodium glutamate 1.3 mg	lyophilised pellet in vials (10 doses)	0.1 mL/dose[a]
BCG	To be provided	To be provided	To be provided

[a]After reconstitution with sterilised water for injections

6.2. Dosage and administration

Subjects will be administered one dose of the study vaccine or BCG control according to vaccination schedule. The vaccine dose will be administered intradermally in the non dominant arm at the deltoid level. A plain plaster (breathable) will be used to cover the site of injection. The plaster will be changed at the first visit, day 2 post-vaccination, and removed at second visit on day 7, provided there are no signs of leakage upon examination by medical practitioner. Subjects will be instructed to protect the plaster and site of vaccination from water. Volunteers will be provided with a waterproof shoulder sleeve that can be used in the shower to protect the vaccination site from getting wet. In the event that the plaster became loose or at risk of falling off, volunteers will be provided with a plastic sample bag and a spare plaster with clear instructions on how to replace the plaster and retain the used plaster in the provided plastic bag and return it to clinic for destruction (refer to Risk Documents for further details).

6.2.1. Reconstitution of MTBVAC

Prefilled syringes will be prepared by the CHUV pharmacy according to the randomization protocol and the following procedure for the different doses.

1. Materials

1.1 MTBVAC, freeze dried vials, 5 x 10^6 cfu/vial

1.2 MTBVAC diluent vials, sterile water for injection

1.3 MTBVAC excipient solution vials (4.5 mL)

2. Cohort 1- Dose 5 x 10^3 cfu/0.1 mL

2.1 - Use MTBVAC diluent (sterile water for injection) for reconstitution of MTBVAC freeze dried vial.

2.2 – The rubber stopper must not be wiped with any antiseptic or detergent. If alcohol is used to swab the rubber stopper of the vial, it must be allowed to evaporate before the stopper is penetrated with the syringe needle.

2.3 – Using a sterile needle and syringe, transfer to the MTBVAC freeze dried vial 1 mL of MTBVAC diluent (sterile water for injections). Carefully invert the vial a few times to resuspend the lyophilised MTBVAC completely. Do not shake.

2.4 – Prepare MTBVAC vaccine dilution 1/10: Draw up into a sterile syringe 0.5 mL of the vaccine suspension and transfer into a vial of MTBVAC sterile excipient solution (content 4.5 mL). Carefully invert the vial a few times to obtain a homogeneous vaccine suspension. Do not shake.

2.5 – Dose 5 x 103 cfu/0.1 mL preparation(MTBVAC vaccine dilution 1/100): Drawn up into a sterile syringe 0.5 mL of the MTBVAC vaccine 1/10 dilution and transfer into a vial of MTBVAC sterile excipients solution (content 4.5 mL). Carefully invert the vial a few times to obtain a homogeneous vaccine suspension. Do not shake. Each vial of 5 mL contains 50 doses of 5 x 10^3 cfu/0.1 mL.

2.6 - MTBVAC vaccine should be administered with a syringe fitted with a short bevel needle (gauge 25 or 26 G).

2.7 – The reconstituted vaccine should be used within 4 hours (storage at +2°-+8°C).

3. Cohort 2 - Dose 5 x 10^4 cfu/0.1 mL

3.1 - Use MTBVAC diluent (sterile water for injections) for reconstitution of MTBVAC freeze dried vial.

3.2 – The rubber stopper must not be wiped with any antiseptic or detergent. If alcohol is used to swab the rubber stopper of the vial, it must be allowed to evaporate before the stopper is penetrated with the syringe needle.

3.3 – Using a sterile needle and syringe, transfer to the MTBVAC freeze dried vial 1 mL of MTBVAC diluent (sterile water for injections). Carefully invert the vial a few times to resuspend the lyophilised MTBVAC completely. Do not shake.

3.4 – Dose 5 x 104 cfu/0.1 mL preparation (MTBVAC vaccine dilution 1/10): Draw up into a sterile syringe 0.5 mL of the vaccine suspension and transfer into a vial of MTBVAC sterile excipient solution (content 4.5 mL). Carefully invert the vial a few times to obtain a homogeneous vaccine suspension. Do not shake. Each vial of 5 mL contains 50 doses of 5 x 104 cfu/0.1 mL.

3.5 - MTBVAC vaccine should be administered with a syringe fitted with a short bevel needle (gauge 25 or 26 G).

3.6 – The reconstituted vaccine should be used within 4 hours (storage at +2°-+8°C).

4. Cohort 3 - Dose 5 x 10^5 cfu /0.1 mL

4.1 - Use MTBVAC diluent (sterile water for injections) for reconstitution of MTBVAC freeze dried vial.

4.2 – The rubber stopper must not be wiped with any antiseptic or detergent. If alcohol is used to swab the rubber stopper of the vial, it must be allowed to evaporate before the stopper is penetrated with the syringe needle.

4.3 – Using a sterile needle and syringe, transfer to the MTBVAC freeze dried vial 1 mL of MTBVAC diluent (sterile water for injections). Carefully invert the vial a few times to resuspend the lyophilised MTBVAC completely. Do not shake. Each vial of 1 mL contains 10 doses of 5 x 10^5 cfu/0.1 mL.

4.4 - MTBVAC vaccine should be administered with a syringe fitted with a short bevel needle (gauge 25 or 26 G).

4.5 – The reconstituted vaccine should be used within 4 hours (storage at +2°-+8°C).

6.2.2. Reconstitution of BCG control

Prefilled syringes will be prepared by the CHUV pharmacy according to randomization protocol using manufacturer´s diluent and following the manufacturer recommendations

6.2.3. Administration of the MTBVAC Vaccine or BCG control

Each 0.1 ml dose of the MTBVAC or the control BCG vaccines should be administered by intradermal injection above the deltoid muscle within 4 hours (storage at +2°- +8°C).

The vaccinees will be observed closely for at least 60 minutes following the administration of vaccines, with appropriate medical treatment readily available in case of a rare anaphylactic reaction following the administration of vaccines.

The vaccines will be administered at the Vaccination and Immunotherapy Center (VIC), CHUV, under the supervision of physicians skilled in the management of anaphylactic reactions.

6.3. Storage

All vaccines must be stored in a safe and locked place with no access for unauthorized personnel (CHUV Pharmacy). They must be kept in the refrigerator (+2°C to +8°C). Storage temperature should be monitored and documented at least once per day, using a calibrated min-max thermometer. It is advisable to have a back-up refrigerator/ freezer in case of power failure/ breakdown. Procedures must be in place to ensure that the vaccines are kept at the indicated temperature range at all times.

The study monitor must be contacted, as soon as possible, if the cold chain is broken (e.g. vaccines become frozen or refrigeration fails).

Storage conditions for transport of vaccines from country medical department or dispatch centre to study sites or between sites are described in Appendix C.

6.4. Treatment allocation and randomization

Subjects within each cohort will be assigned to one of 2 groups (vaccine or control) in the order in which they are enrolled into the study in a 3:1 manner.

The treatment allocation at the investigator site will be performed by the CHUV Pharmacy.

The person in charge of preparing the vaccination (Mrs Beatrice Pellet, CHUV Pharmacy) will have access the randomization program. Upon providing a subject number the randomization system will use the minimization algorithm to determine the

treatment number to be used for the subject. Note that as soon as the target number of subjects in a specific group has been reached, the enrolment will be frozen for this group.

6.5. Method of blinding and breaking the study blind

Data pertaining to the MTBVAC vaccine and to BCG control will be collected in an observer blinded manner. By observer blinded, we mean that during the course of the study the vaccine recipient and those responsible for the evaluation of safety and reactogenicity study parameters, will all be unaware of which vaccine preparation was administered to a particular volunteer. To do so, the MTBVAC vaccine and the BCG control will be prepared and blinded by the pharmacist whereas the vaccination will be done by the medical team (the co-investigator and/or the study nurse). The principal investigator and/or the co-investigator will be responsible for assessing safety and post-vaccination side effects. Blinding will be maintained throughout the vaccination and follow-up portions of the vaccine trial.

The CHUV Pharmacy is in charge of the randomisation process. A set of sealed envelopes with individual randomisation code will be sent to the VIC where they will always be available to the investigators. The code will be broken either by the CHUV Pharmacist (Mrs Pellet, Study Contact for Emergency Code Break) or by the investigators directly only in the case of medical events that the investigator/physician in charge of the subject feels cannot be treated without knowing the identity of the study vaccine(s). Another set will be held at Biofabri's Headquarters as a backup. No code will be broken without the authorisation of the PI. The DSMB will be immediately informed by the PI.

Biofabri' policy (incorporating ICH E2A guidance, EU Clinical Trial Directive and Federal Regulations) is to unblind any serious adverse event (SAE) report associated with the use of the investigational product, which is unexpected and attributable/suspected, prior to regulatory reporting. The CHUV Pharmacist (Mrs Pellet is responsible for unblinding the treatment assignment in accordance with specified time frames for expedited reporting of SAEs (Refer to Section 8.7)

The statistician who will perform the analysis will have access to the randomisation codes at the time of analysis and will be able to break the codes to perform the analysis.

6.6. Replacement of unusable vaccine doses

Additional vaccine doses will be provided to replace those that are unusable (see Appendix C for details of supplies).

In addition to the vaccine doses provided for the planned number of subjects, at least 100% additional doses will be supplied. In case a vaccine dose is broken or unusable, the investigator should replace it with a replacement vaccine dose. Although the sponsor need not be notified immediately in these cases (except in the case of cold-chain failure), documentation of the use of the replacement vaccine must be recorded by the investigator on the vaccine administration page of the CRF and on the vaccine accountability form.

The investigator will access the randomization register via the CHUV Pharmacy to obtain the replacement vial number. The system will ensure, in a blinded manner, that the replacement vial is of the same formulation as the randomized vaccine.

6.7. Packaging

See Appendix C for details

6.8. Vaccine accountability

See Appendix C.

6.9. Concomitant medication/treatment

At each study visit/contact, the investigator should question the subject about any medication(s) taken.

All concomitant medications, with the exception of vitamins and/or dietary supplements, administered at ANY time during the period starting with administration of the study vaccine/control and ending at D210 of the follow up period are to be recorded with generic name of the medication (trade names are allowed for combination drugs, i.e. multi-component drugs), medical indication, total daily dose, route of administration, start and end dates of treatment.

Any treatments and/or medications specifically contraindicated, e.g. any immunoglobulins, antibiotic, other blood products and any immune modifying drugs administered within three months preceding the vaccine administration or at any time during the study period are to be recorded with generic name of the medication (trade names are allowed for combination drugs only), medical indication, total daily dose, route of administration, start and end dates of treatment. Refer to Sections 4.3 and 4.4 .

Any vaccine not foreseen in the study protocol administered in the period beginning 30 days preceding administration of study vaccine/control and ending two months after administration of study vaccine/control is to be recorded with trade name, route of administration and date(s) of administration. Refer to Sections 4.3 and 4.4.

A prophylactic medication is a 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 [axillary temperature < 37.5°C] and any other symptom, to prevent fever from occurring). Any concomitant medication administered prophylactically in anticipation of reaction to the vaccination must be recorded in the CRF with generic name of the medication (trade names are allowed for combination drugs only), total daily dose, route of administration, start and end dates of treatment and coded as 'Prophylactic'.

Concomitant medication administered for the treatment of an AE or SAE within the follow-up period for adverse events must be recorded in the CRF with generic name of the medication (trade names are allowed for combination drugs only), medical indication

(including which AE/SAE), total daily dose, route of administration, start and end dates of treatment. Refer to Section 8.2 for definition of SAE.

7. HEALTH ECONOMICS

Not applicable.

8. ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

The investigator is responsible for the detection and documentation of events meeting the criteria and definition of an adverse event (AE) or serious adverse event (SAE) as provided in this protocol. During the study, when there is a safety evaluation, the investigator or site staff will be responsible for detecting AEs and SAEs, as detailed in this section of the protocol.

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

8.1. 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.

Examples of an AE include:

- Significant or unexpected worsening or exacerbation of the condition/indication under study. See Section 8.3 'Lack of Efficacy' for additional information.
- 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 product administration even though it 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 product or a concurrent medication (overdose per se should not be reported as an AE/SAE).
- Signs, symptoms temporally associated with vaccine administration.

Examples of an AE do not include:

- Medical or surgical procedure (e.g. endoscopy, appendectomy); the condition that leads to the procedure is an AE.
- Situations where an untoward medical occurrence did not occur (social and/or convenience admission to a hospital).
- 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.
- The disease/disorder being studied, or expected progression, signs, or symptoms of the disease/disorder being studied, unless more severe than expected for the subject's condition.

AEs may include 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).

N.B. AEs to be recorded as endpoints (solicited events) are described in Section 8.5.1. All other AEs will be recorded as UNSOLICITED AEs.

Example of events to be recorded in the medical history section of the CRF:

- Pre-existing conditions or signs and/or symptoms present in a subject prior to the start of the study (i.e. prior to the first study procedure) should be recorded in the medical history section of the subject's CRF.

8.2. Definition of a serious adverse event

A serious adverse event (SAE) 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, if it were more severe.

c requires hospitalization or prolongation of existing hospitalization,

NOTE: In general, hospitalization signifies that the subject has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or out-patient setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfils any other serious criteria, the event is serious. When in doubt as to whether "hospitalization" occurred or was necessary, the AE should be considered serious.

Hospitalization for elective treatment of a pre-existing condition 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, 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 hospitalization but may jeopardize 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 hospitalization.

8.3. Definition of Suspected Unexpected Serious Adverse Reaction:

Suspected Unexpected Serious Adverse Reaction (or SUSAR) are any unsolicited serious AE that are suspected to be linked to the administration of the investigational product. The investigator will report any fatal or life threatening SUSAR to the Local Ethical Committee and Swiss institute of the therapeutic products "Swissmedic" as soon as possible, but not later than 7 working days after the event occurred. In case of non life threatening SUSARs, the event will be reported in a 15 days period of time. The report will contain the full description of the event, the identification of the volunteer - including the two digits code, age and sex – and the details of the study product, meaning its posology, its route of administration, the beginning and the end of administration. The announcements of SUSAR to Swissmedic will be sent by post to: "Swissmedic, Institut suisse des produits thérapeutiques, Services d'inspection, Case Manager, section Transplants, Case postale, 3000 Berne 9" using the CIOMS form. If sent also per e-mail, the e-mail address should be to Julia.djonova@swissmedic.ch, using the CIOMS form. The CIOMS form has to be sent by post in any case. It should be noted that, if needed, the code will be break for security reason.

Furthermore, the sponsor has to announce to Swissmedic as per Art 22, §4 OClin, all SAE with presumed causal link with the administered drug (i.e. SADR, serious adverse drug reactions) (http://www.swissmedic.ch/org/00064/00067/00336/00855/index.html?lang=fr, I-315.AA.03-A4_01F Aide-mémoire: Obligation d'annoncer les effets indésirables lors d'essais cliniques avec des transplants standardisés).

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

Abnormal laboratory findings (e.g. clinical chemistry, haematology, urinalysis) or other abnormal assessments (e.g.X-rays, vital signs, etc.) that are judged by the investigator to be clinically significant will be recorded as AEs or SAEs if they meet the definition of an AE, as defined in Section 8.1 or SAE, as defined in Section 8.2 . Clinically significant abnormal laboratory findings or other abnormal assessments that are detected during the study or are present at baseline and significantly worsen following the start of the study will be reported as AEs or SAEs. However, clinically significant abnormal laboratory findings or other abnormal assessments that are associated with the disease being studied, unless judged by the investigator as more severe than expected for the subject's condition, or that are present or detected at the start of the study and do not worsen, will not 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.

8.5. Time period, frequency, and method of detecting adverse events and serious adverse events

All AEs occurring during the 210 day follow up period following administration of study vaccine must be recorded on the Adverse Event form in the subject's CRF, irrespective of severity or whether or not they are considered vaccination-related.

The standard time period for collecting and recording SAEs will begin at randomization or the first receipt of vaccine and will end 210 days following administration of the study vaccine/control for each subject. See Section 8.8 for instructions for reporting and recording SAEs.

Additionally, in order to fulfil international reporting obligations, SAEs that are related to study participation (e.g. procedures, invasive tests, a change from existing therapy) or are related to a concurrent medication will be collected and recorded from the time the subject consents to participate in the study until she/he is discharged.

The investigator will inquire about the occurrence of AEs/SAEs at every visit/contact during the study.

All AEs either observed by the investigator or one of his clinical collaborators or reported by the subject spontaneously or in response to a direct question will be evaluated by the investigator. AEs not previously documented in the study will be recorded in the Adverse Event form within the subject's CRF. The nature of each event, date and time (where appropriate) of onset, outcome, intensity and relationship to vaccination should be established. Details of any corrective treatment should be recorded on the appropriate page of the CRF (Refer to Section 6.9).

As a consistent method of soliciting 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?"

N.B. The investigator should record only those AEs having occurred within the time frame defined above.

AEs already documented in the CRF, i.e. at a previous assessment, and designated as “not recovered/not resolved” or “recovering/resolving” should be reviewed at subsequent visits, as necessary. If these have resolved, the documentation in the CRF should be completed.

N.B. If an AE changes in frequency or intensity during the specified reporting period, a new record of the event will be entered.

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 on the CRF or SAE Report Form as applicable. It is not acceptable for the investigator to send photocopies of the subject’s medical records to Biofabri in lieu of the appropriate completed AE/SAE pages. However, there may be instances when copies of medical records for certain cases are requested by Biofabri. In this instance, all subject identifiers will be blinded on the copies of the medical records prior to submission to Biofabri.

The investigator will attempt to establish a diagnosis of 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.

8.5.1. Solicited adverse events

A 7-day follow-up (Day 0 to 6) of solicited local adverse events at each injection site will be performed after vaccination. Data concerning the following adverse events will be solicited using diary cards provided by the sponsor.

Table 4 Solicited local adverse events

Solicited local adverse events
Pain at injection site
Redness at injection site
Swelling at injection site
Pruritus at injection site
Leakage at injection site

8.5.2. Solicited general AEs

A 7-day follow-up (Day 0 to 6) of solicited general adverse events will be performed after vaccination. Data concerning the following adverse events will be solicited using diary cards provided by the sponsor.

Table 5 Solicited general adverse events

Solicited general adverse events
Fatigue
Fever
Gastrointestinal symptoms*
Headache
Musculo-skeletal pains

*Gastrointestinal symptoms include nausea, vomiting, diarrhea and/or abdominal pain.

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

8.6. Evaluating adverse events and serious adverse events

8.6.1. Assessment of intensity

Intensity of the following AEs will be assessed as described in Table 6.

Table 6 Intensity scales for solicited symptoms

Adverse Event	Intensity grade	Parameter
Pain at injection site	0	Absent
	1	Painful on touch
	2	Painful when limb is moved
	3	Pain that prevents normal activity
Redness at injection site (Greatest diameter in mm)	0	None = 0 mm
	1	0 < redness ≤20 mm
	2	20 < redness ≤ 50 mm
	3	> 50 mm
Prurit at injection site (Greatest diameter in mm)	0	Normal
	1	Easily tolerated
	2	Interferes with normal activity
	3	Prevents normal activity
Swelling at injection site (Greatest diameter in mm)	0	None = 0 mm
	1	0 < swelling ≤20 mm
	2	20 < swelling ≤ 50 mm
	3	> 50 mm
Fluid leakage at injection site	0	None
	1	Weak
	2	Moderate
	3	Strong

Adverse Event	Intensity grade	Parameter
Fever* (Temperature in °C)	0 1 2 3	< 37.5°C 37,5 ≤ temperature < 38,0°C 38,0 ≤ temperature < 39,0°C ≥ 39,0°C
Headache	0 1 2 3	Normal Easily tolerated Interferes with normal activity Prevents normal activity
Fatigue	0 1 2 3	Normal Easily tolerated Interferes with normal activity Prevents normal activity
Gastrointestinal symptoms (nausea, vomiting, diarrhoea and/or abdominal pain)	0 1 2 3	Normal Easily tolerated Interfere with normal activity Prevent normal activity
Musculo-skelettal pains	0 1 2 3	Normal Easily tolerated Interfere with normal activity Prevent normal activity

*Fever is defined as axillary temperature ≥ 37.5 °C

The investigator will make an assessment of intensity for all other AEs, i.e. unsolicited symptoms, including SAEs reported during the study. The assessment will be based on the investigator's clinical judgement. The intensity of each AE and SAE recorded in the CRF or SAE Report Form, as applicable, 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/ adolescents, such an AE would, for example, prevent attendance at work/ school 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 utilized 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 8.2.

8.6.2. Assessment of causality

The investigator is obligated to assess the relationship between investigational product and the occurrence of each AE/SAE. The investigator will use clinical judgement to determine the relationship. Alternative causes, such as natural history of the underlying diseases, concomitant therapy, other risk factors and the temporal relationship of the event to the investigational product will be considered and investigated. The investigator will also consult the Investigator Brochure and/or Product Information, for marketed products, in the determination of 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 Biofabri. However, it is very important that the investigator always makes an assessment of causality for every event prior to transmission of the SAE Report Form to Biofabri. The investigator may change his/her opinion of causality in light of follow-up information, amending the SAE Report Form accordingly. The causality assessment is one of the criteria used when determining regulatory reporting requirements.

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 product?

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

Non-serious and serious AEs will be evaluated as two distinct events. If an event meets the criteria to be determined “serious” (see Section 8.2 for definition of serious adverse event), it will be examined by the investigator to the extent to be able to determine ALL contributing factors applicable to each serious adverse event.

Other possible contributors include:

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

8.6.3. Medically attended visits

For each solicited and unsolicited symptom the subject experiences, the subject will be asked if he received medical attention defined as hospitalization, an emergency room visit or a visit to or from medical personnel (medical doctor) for any reason and this information will be recorded in the CRF.

8.7. Follow-up of adverse events, serious adverse events/ Suspected Unexpected Serious Adverse Reaction (SUSAR) and assessment of outcome

After the initial AE/SAE/SUSAR report, the investigator is required to proactively follow each subject and provide further information to Biofabri on the subject's condition.

All AEs and SAEs/SUSAR documented at a previous visit/contact and designated as not recovered/not resolved or recovering/resolving will be reviewed at subsequent visits/contacts.

Investigators will follow-up subjects:

- with SAEs/SUSARs or subjects withdrawn from the study as a result of an AE, until the event has resolved, subsided, stabilized, disappeared, the event is otherwise explained, or the subject is lost to follow-up;
- or, in the case of other non-serious AEs, until they complete the study or they are lost to follow-up.

Clinically significant laboratory abnormalities will be followed up until they have returned to normal, or a satisfactory explanation has been provided. Additional information (including but not limited to laboratory results) relative to the subsequent course of such an abnormality noted for any subject must be made available to the Study Medical Monitor.

Biofabri may request that the investigator perform or arrange for the conduct of supplemental measurements 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 recognized follow-up period, Biofabri will be provided with a copy of any available post-mortem findings, including histopathology.

New or updated information will be recorded on the originally completed SAE Report Form, with all changes signed and dated by the investigator. The updated SAE report form should be resent to Biofabri within 24 hours of receipt of the follow-up information as outlined in Section8.8.1.

Outcome of any non-serious AE occurring within 210 days post-vaccination (i.e. unsolicited AE) as well as any SAE reported during the entire study will be assessed as:

- Recovered/resolved
- Not recovered/not resolved

- Recovering/resolving
- Recovered with sequelae/resolved with sequelae
- Fatal (SAEs only).

8.8. Prompt reporting of serious adverse events to Biofabri

8.8.1. Time frames for submitting serious adverse event reports to Biofabri

SAEs/SUSARs will be reported promptly to BIOFABRI once the investigator determines that the event meets the protocol definition of an SAE/SUSARs. The investigator or designate will fax the SAE reports to Biofabri' Study Contact for Serious Adverse Event Reporting WITHIN 24 HOURS OF HIS/HER BECOMING AWARE OF THESE EVENTS. Additional or follow-up information relating to the initial SAE report is also to be reported to the Biofabri' Study Contact for Serious Adverse Event Reporting within 24 hours of receipt of such information.

8.8.2. Completion and transmission of serious adverse event reports to Biofabri

Once an investigator becomes aware that a SAE/SUSAR has occurred in a study subject, she/he will report the information to BIOFABRI within 24 hours as outlined in Section 8.8.1. The SAE/SUSAR Report Form will always be completed as thoroughly as possible with all available details of the event, signed by the investigator (or designee), and forwarded to BIOFABRI within the designated time frames. If the investigator does not have all information regarding an SAE, he/she will not wait to receive additional information before notifying BIOFABRI of the event and completing the form. The form will be updated when additional information is received and forwarded to BIOFABRI WITHIN 24 HOURS as outlined in Section 8.8.1.

The investigator will always provide an assessment of causality at the time of the initial report as described in Section 8.6.2.

Facsimile (Fax) transmission of the SAE/SUSAR Report Form is the preferred method to transmit this information to the Study Contact for Reporting SAEs. In rare circumstances and in the absence of facsimile equipment, notification by telephone is acceptable, with a copy of the SAE Report Form sent by overnight mail. Initial notification via the telephone does not replace the need for the investigator to complete and sign the SAE/SUSAR Report Form within 24 hours as outlined in Section 8.8.1.

In the event of a death determined by the investigator to be related to vaccination, sending of the fax must be accompanied by telephone call to the Study Contact for Reporting SAEs/SUSARs.

Primary Study contacts at Biofabri
Clinical Development Manager and Local BIOFABRI Contact person Dr Juana DOCE **BIOFABRI, S.L.** La Relva s/n, 36400 Porriño – Pontevedra. SPAIN Email: j.doce@biofabri.es Tel. office: + 34 986 34 52 16 Fax: + 34 986 34 52 01
Biofabri Clinical and Regulatory Affairs Monitor Dr. Luc HESSEL **Chair of the Clinical Development team of TBVI** **Tuberculosis Vaccine Initiative (TBVI)** Runderweg 6, 8219 Pk Lelystad. THE NETHERLANDS Email: luc.hessel@orange.fr Tel: +31 320 277 550
Study Contact at Biofabri for Reporting Serious Adverse Events Dr Eugenia PUENTES **BIOFABRI, S.L.** La Relva s/n, 36400 Porriño (Pontevedra), SPAIN Email : e.puentes@biofabri.es Tel. office: +34 986 34 52 13 Fax: :+34 986 34 52 01

8.9. Regulatory reporting requirements for serious adverse events

The investigator will promptly report all SAEs/SUSARs to BIOFABRI in accordance with the procedures detailed in Section8.8. Biofabri has a legal responsibility to promptly notify, as appropriate, both the local regulatory authorities and other regulatory agencies about the safety of a product under clinical investigation. Prompt notification of SAEs/SUSARs 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.

The investigator, or responsible person according to local requirements, will comply with the applicable local regulatory requirements related to the reporting of SAEs/SUSARs to the IRB/IEC and, if required, to the applicable government authority.

Investigator safety reports are prepared according to BIOFABRI policy and are forwarded to investigators as necessary. An investigator safety report is prepared for a

SAE(s) that is both attributable to investigational product and unexpected. The purpose of the report is to fulfil specific regulatory and Good Clinical Practice (GCP) requirements, regarding the product under investigation.

An investigator who receives an investigator safety report describing a SAE(s) or other specific safety information (e.g., summary or listing of SAEs) from Biofabri will file it with the Investigator Brochure or other appropriate study documentation and will notify the IRB or IEC, if appropriate according to local requirements

8.10. 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 detection period defined in Section 8.5. Investigators are not obligated to actively seek AEs or SAEs in former study participants.

However, if the investigator learns of any SAE, including a death, at any time after a subject has been discharged from the study, and he/she considers the event reasonably related to the investigational product, the investigator will promptly notify the Study Contact for Reporting SAEs.

8.11. Pregnancy

Subjects who become pregnant during the study must not receive additional doses of study vaccine/ control but may continue other study procedures at the discretion of the investigator. The subject will not have blood drawn or chest X-rays, as normally mandated by the protocol. However, pregnant subjects will undergo all other evaluations according to Protocol.

The investigator, or his/her designee, will collect pregnancy information on any subject who becomes pregnant while participating in this study. The investigator, or his/her designee, will record pregnancy information on the Pregnancy Report Form provided by the Sponsor and submit it to BIOFABRI within 24 hours of learning of a subject's pregnancy. The subject will be followed to determine the outcome of the pregnancy. At the end of the pregnancy, whether that be full-term or prematurely, information on the status of the mother and child will be forwarded to BIOFABRI. Generally, follow-up will be no longer than six to eight weeks following the estimated delivery date.

While pregnancy itself is not considered an AE or SAE, any pregnancy complication or elective termination of a pregnancy for medical reasons will be recorded as an AE or a SAE, as described in Section 8.1 and 8.2, and will be followed as described in Section 8.6.

A spontaneous abortion is always considered to be a SAE and will be reported as described in Section 8.8. Furthermore, any SAE occurring as a result of a post-study pregnancy AND considered reasonably related in time to receipt of the investigational product by the investigator, will be reported to Biofabri as described in Section 8.10. 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.

Information on pregnancies identified during the screening phase/prior to vaccine administration does not need to be collected; this information need not be communicated to safety.

8.12. Treatment of adverse events

Treatment of any adverse event 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 CRF. Refer to Section 6.9.

9. SUBJECT COMPLETION AND WITHDRAWAL

9.1. Subject completion

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

9.2. Subject withdrawal

Subjects who are withdrawn for AEs must be clearly distinguished from subjects who are withdrawn for other reasons. Investigators will follow subjects who are withdrawn as result of a SAE/SUSAR/AE until resolution of the event (see Section 8.6).

9.2.1. Subject withdrawal from the study

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

A subject qualifies as 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.

Information relative to the withdrawal will be documented on the Study Conclusion page of the CRF. The investigator will document whether the decision to withdraw from the study was made by the subject or the investigator and 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)

9.3. Screen and baseline failures

Screening evaluations (see section 0) will occur within 60 days prior to Day 0. Informed consent will be collected from patients willing to participate in the study prior to any study procedure. No study-specific invasive procedures may be performed before informed consent is signed.

Upon completion of all screening procedures, the investigator, or designee, will review the inclusion/exclusion criteria for each subject and will verify that all eligibility criteria are met. Subjects that meet all inclusion/exclusion criteria will be scheduled for their baseline (Day 0) visit. Their screening information will be recorded on the appropriate case report form (CRF).

10. DATA EVALUATION: CRITERIA FOR EVALUATION OF OBJECTIVES

10.1. Primary endpoints

Safety and reactogenicity for all subjects as determined by:

- Occurrence of solicited symptoms during the 7-day follow-up period following vaccination (day of vaccination and 6 subsequent days).
- Occurrence of unsolicited symptoms during the 210-day follow-up period following vaccination (day of vaccination and 209 subsequent days).
- Occurrence of grade 3 vaccine related local and general symptoms during the 210-day follow-up period following vaccination (day of vaccination and 209 subsequent days).
- Occurrence of serious adverse events throughout the entire study period.

10.2. Secondary endpoints

Immunogenicity assessment for all subjects as determined by:

- The cell-mediated immune (CMI) response induced by the candidate vaccine MTBVAC as determined by ELISPOT on peripheral blood mononuclear cells (PBMC): frequency of IFNγ positive mononuclear cells in response to vaccine

(ESAT-6, CFP10, PPD, live BCG and live MTBVAC) at screening, and at days 28, 210 in all cohorts

- The cell-mediated immune (CMI) response induced by the candidate vaccine MTBVAC as determined by Intracellular Cell Staining (ICS): frequency of positive mononuclear cells for IL-2, TNFα and IFNγ in response to live BCG and live MTBVAC (overnight stimulation) at Day 0, 28, 90, 210 in all cohorts on whole blood.

10.3. Immunogenicity assessment for all subjects as determined by estimated sample size

The sample size of 9 subjects per group is based on what is typical and reasonable for a Phase 1 study, and not on statistical consideration of power to observe differences between the cohorts. The sample size will allow Biofabri' to define an initial safety profile for MTBVAC vaccination and to perform an evaluation of immunogenicity specific to MTBVAC.

The number of volunteers in the study should give important clues suggesting large differences in the rate of adverse reactions or immunogenicity between the vaccine candidate and the control within each cohort.

10.4. Study cohorts to be evaluated

10.4.1. Total Vaccinated cohort

The Total Vaccinated cohort will include all vaccinated subjects for whom data are available. Thus, the Total analysis of safety will include all vaccinated subjects and the Total analysis of immunogenicity/efficacy will include vaccinated subjects for whom data concerning immunogenicity/efficacy endpoint measures are available.

10.4.2. According To Protocol (ATP) cohort for analysis of immunogenicity

The ATP cohort for analysis of immunogenicity will include all evaluable subjects (i.e. those meeting all eligibility criteria, complying with the procedures defined in the protocol, with no elimination criteria during the study) for whom data concerning immunogenicity endpoint measures are available.

10.5. Derived and transformed data

For a given subject and a given immunogenicity measurement, missing or non-evaluable measurements will not be replaced. Therefore, an analysis will exclude subjects with missing or non-evaluable measurements.

- For intracellular cytokine staining, the results will be expressed as the number of antigen-specific cytokine-producing cells per million CD4+ T cells or CD8+ T cells after subtraction of background activity.

Subjects who missed reporting symptoms (solicited/unsolicited or concomitant medications) will be treated as subjects without symptoms (solicited/unsolicited or concomitant medications, respectively).

In case of significant non-compliance of study procedures for reporting symptoms, the analysis plan will be reassessed to ensure more accurate reporting of study data by further analysis.

10.6. Final analyses

The final report will include data on safety/reactogenicity and available CMI data collected during the pre-vaccination up until 7 months post-vaccination. Data will be from a cleaned database.

10.6.1. Analysis of demographics/baseline characteristics

Demographic characteristics (age, gender, race) of each study cohort will be tabulated.

The mean age (plus range and standard deviation) by gender of the enrolled subjects, as a whole, and per group, will be calculated.

The distribution of subjects enrolled will be tabulated as a whole and per group.

10.6.2. Analysis of immunogenicity

The primary analysis will be based on the ATP cohort for analysis of immunogenicity. An analysis on the Total Vaccinated cohort will be performed only if more than 10% of the subjects are excluded from the ATP cohort for analysis of immunogenicity.

10.6.2.1. Intracellular cytokine staining data

The frequency of specific CD4+ T cells and CD8+ T cells secreting cytokines will be summarised for each vaccination group, at each time point (descriptive statistics).

10.6.3. Analysis of safety

The primary analysis will be based on the Total Vaccinated cohort.

The percentage of subjects with at least one local adverse event (solicited and unsolicited), with at least one general adverse event (solicited and unsolicited) and with any adverse event during the solicited follow-up period will be tabulated with exact 95% CI overall.

The percentage of subjects reporting each individual solicited local and general adverse event during the solicited follow-up period will be tabulated with exact 95% CI.

Occurrence of fever and related fever will be reported per 0.5°C cumulative increments. Duration and prevalence of fever will be presented.

For all solicited symptoms, the same tabulation will be performed for grade 3 adverse events and for adverse events with relationship to vaccination.

The proportion of subjects with at least one report of unsolicited adverse event classified by the World Health Organization Preferred Terms or by the Medical Dictionary for Regulatory Activities (MedDRA), whenever available, and reported up to 210 days after vaccination will be tabulated with exact 95% CI. The same tabulation will be performed for grade 3 unsolicited adverse events and for unsolicited adverse events with a relationship to vaccination.

Serious adverse events will be described in detail.

The proportion of AEs resulting in a medically attended visit will also be tabulated.

10.7. Planned interim analysis

No interim analyses of data are planned.

11. ADMINISTRATIVE MATTERS

To comply with Good Clinical Practice important administrative obligations relating to investigator responsibilities, monitoring, archiving data, audits, confidentiality and publications must be fulfilled. See Appendix B for details.

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13. APPENDIX A:

WORLD MEDICAL ASSOCIATION DECLARATION OF HELSINKI

Recommendations guiding physicians
in biomedical research involving human subjects

Adopted by the 18th World Medical Assembly
Helsinki, Finland, June 1964

and amended by the
29th World Medical Assembly
Tokyo, Japan, October 1975
35th World Medical Assembly
Venice, Italy, October 1983
41st World Medical Assembly
Hong Kong, September 1989
and the
48th General Assembly
Somerset West, Republic of South Africa, October 1996

INTRODUCTION

It is the mission of the physician to safeguard the health of the people. His or her knowledge and conscience are dedicated to the fulfilment of this mission.

The Declaration of Geneva of the World Medical Association binds the physician with the words, "The health of my patient will be my first consideration," and the International Code of Medical Ethics declares that, "A physician shall act only in the patient's interest when providing medical care which might have the effect of weakening the physical and mental condition of the patient."

The purpose of biomedical research involving human subjects must be to improve diagnostic, therapeutic and prophylactic procedures and the understanding of the etiology and pathogenesis of disease.

In current medical practice most diagnostic, therapeutic or prophylactic procedures involve hazards. This applies especially to biomedical research.

Medical progress is based on research which ultimately must rest in part on experimentation involving human subjects.

In the field of biomedical research a fundamental distinction must be recognized between medical research in which the aim is essentially diagnostic or therapeutic for a patient, and medical research, the essential object of which is purely scientific and without implying direct diagnostic or therapeutic value to the person subjected to the research.

Special caution must be exercised in the conduct of research which may affect the environment, and the welfare of animals used for research must be respected.

Because it is essential that the results of laboratory experiments be applied to human beings to further scientific knowledge and to help suffering humanity, the World Medical Association has prepared the following recommendations as a guide to every physician in biomedical research involving human subjects. They should be kept under review in the future. It must be stressed that the standards as drafted are only a guide to physicians all over the world. Physicians are not relieved from criminal, civil and ethical responsibilities under the laws of their own countries.

I. BASIC PRINCIPLES

1. Biomedical research involving human subjects must conform to generally accepted scientific principles and should be based on adequately performed laboratory and animal experimentation and on a thorough knowledge of the scientific literature.
2. The design and performance of each experimental procedure involving human subjects should be clearly formulated in an experimental protocol which should be transmitted for consideration, comment and guidance to a specially appointed committee independent of the investigator and the sponsor provided that this independent committee is in conformity with the laws and regulations of the country in which the research experiment is performed.
3. Biomedical research involving human subjects should be conducted only by scientifically qualified persons and under the supervision of a clinically competent medical person. The responsibility for the human subject must always rest with a medically qualified person and never rest on the subject of research, even though the subject has given his or her consent.
4. Biomedical research involving human subjects cannot legitimately be carried out unless the importance of the objective is in proportion to the inherent risk to the subject.
5. Every biomedical research project involving human subjects should be preceded by careful assessment of predictable risks in comparison with foreseeable benefits to the subject or to others. Concern for the interests of the subject must always prevail over the interests of science and society.
6. The right of the research subject to safeguard his or her integrity must always be respected. Every precaution should be taken to respect the privacy of the subject and to minimize the impact of the study on the subject's physical and mental integrity and on the personality of the subject.
7. Physicians should abstain from engaging in research projects involving human subjects unless they are satisfied that the hazards involved are believed to be predictable. Physicians should cease any investigation if the hazards are found to outweigh the potential benefits.
8. In publication of the results of his or her research, the physician is obliged to preserve the accuracy of the results. Reports of experimentation not in accordance

with the principles laid down in this Declaration should not be accepted for publication.

9. In any research on human beings, each potential subject must be adequately informed of the aims, methods, anticipated benefits and potential hazards of the study and the discomfort it may entail. He or she should be informed that he or she is at liberty to abstain from participation in the study and that he or she is free to withdraw his or her consent to participation at any time. The physician should then obtain the subject's freely-given informed consent, preferably in writing.
10. When obtaining informed consent for the research project the physician should be particularly cautious if the subject is in a dependent relationship to him or her or may consent under duress. In that case the informed consent should be obtained by a physician who is not engaged in the investigation and who is completely independent of this official relationship.
11. In case of legal incompetence, informed consent should be obtained from the legal guardian in accordance with national legislation. Where physical or mental incapacity makes it impossible to obtain informed consent, or when the subject is a minor, permission from the responsible relative replaces that of the subject in accordance with national legislation.
    Whenever the minor child is in fact able to give a consent, the minor's consent must be obtained in addition to the consent of the minor's legal guardian.
12. The research protocol should always contain a statement of the ethical considerations involved and should indicate that the principles enunciated in the present Declaration are complied with.

II. MEDICAL RESEARCH COMBINED WITH PROFESSIONAL CARE (Clinical research)

1. In the treatment of the sick person, the physician must be free to use a new diagnostic and therapeutic measure, if in his or her judgement it offers hope of saving life, re-establishing health or alleviating suffering.
2. The potential benefits, hazards and discomfort of a new method should be weighed against the advantages of the best current diagnostic and therapeutic methods.
3. In any medical study, every patient - including those of a control group, if any - should be assured of the best proven diagnostic and therapeutic method. This does not exclude the use of inert placebo in studies where no proven diagnostic or therapeutic method exists.
4. The refusal of the patient to participate in a study must never interfere with the physician–patient relationship.
5. If the physician considers it essential not to obtain informed consent, the specific reasons for this proposal should be stated in the experimental protocol for transmission to the independent committee (I, 2).

6. The Physician can combine medical research with professional care, the objective being the acquisition of new medical knowledge, only to the extent that medical research is justified by its potential diagnostic or therapeutic value for the patient.

III. NON-THERAPEUTIC BIOMEDICAL RESEARCH INVOLVING HUMAN SUBJECTS
(Non-clinical biomedical research)

1. In the purely scientific application of medical research carried out on a human being, it is the duty of the physician to remain the protector of the life and health of that person on whom biomedical research is being carried out.
2. The subjects should be volunteers - either healthy persons or patients for whom the experimental design is not related to the patient's illness.
3. The investigator or the investigating team should discontinue the research if in his/her or their judgement it may, if continued, be harmful to the individual.

In research on man, the interest of science and society should never take precedence over considerations related to the well being of the subject.

14. APPENDIX B:

INVESTIGATOR'S AND SPONSOR'S RESPONSIBILITIES

GUIDELINE FOR GOOD CLINICAL PRACTICE

CPMP/ICH/1 35/95/Step5, Explanatory Note and Comments to the above, issued as CPMP/768/97

1. INVESTIGATOR

1.1 Investigator's Qualifications and Agreements

1.1.1 The investigator(s) should be qualified by education, training, and experience to assume responsibility for the proper conduct of the trial, should meet all the qualifications specified by the applicable regulatory requirement(s), and should provide evidence of such qualifications through up-to-date curriculum vitae and/or other relevant documentation requested by the sponsor, the IRB/IEC, and/or the regulatory authority(ies).

1.1.2 The investigator should be thoroughly familiar with the appropriate use of the investigational product(s), as described in the protocol, in the current Investigator's Brochure, in the product information and in other information sources provided by the sponsor.

1.1.3 The investigator should be aware of, and should comply with, GCP and the applicable regulatory requirements.

1.1.4 The investigator/institution should permit monitoring and auditing by the sponsor, and inspection by the appropriate regulatory authority(ies).

1.1.5 The investigator should maintain a list of appropriately qualified persons to whom the investigator has delegated significant trial-related duties.

1.2 Adequate Resources

1.2.1 The investigator should be able to demonstrate (e.g., based on retrospective data) a potential for recruiting the required number of suitable subjects within the agreed recruitment period.

1.2.2 The investigator should have sufficient time to properly conduct and complete the trial within the agreed trial period.

1.2.3 The investigator should have available an adequate number of qualified staff and adequate facilities for the foreseen duration of the trial to conduct the trial properly and safely.

1.2.4 The investigator should ensure that all persons assisting with the trial are adequately informed about the protocol, the investigational product(s), and their trial-related duties and functions.

1.3 Medical Care of Trial Subjects

1.3.1 A qualified physician (or dentist when appropriate), who is an investigator or a sub-investigator for the trial, should be responsible for all trial-related medical (or dental) decisions.

1.3.2 During and following a subject's participation in a trial, the investigator/institution should ensure that adequate medical care is provided to a subject for any adverse events, including clinically significant laboratory values, related to the trial. The investigator/institution should inform a subject when medical care is needed for intercurrent illness(es) of which the investigator becomes aware.

1.3.3 It is recommended that the investigator inform the subject's primary physician about the subject's participation in the trial if the subject has a primary physician and if the subject agrees to the primary physician being informed.

1.3.4 Although a subject is not obliged to give his/her reason(s) for withdrawing prematurely from a trial, the investigator should make a reasonable effort to ascertain the reason(s), while fully respecting the subject's rights.

1.4 Communication with IRB/IEC

1.4.1 Before initiating a trial, the investigator/institution should have written and dated approval favourable opinion from the IRB/IEC for the trial protocol, written informed consent fomi, consent form updates, subject recruitment procedures (e.g., advertisements), and any other written information to be provided to subjects.

1.4.2 As part of the investigator's/institution's written application to the IRB/IEC, the investigator/ institution should provide the IRB/IEC with a current copy of the Investigator's Brochure. If the Investigator's Brochure is updated during the trial, the investigator/institution should supply a copy of the updated Investigator's Brochure to the IRB/IEC.

1.4.3 During the trial the investigator/institution should provide to the IRB/IEC all documents subject to review.

1.5 Compliance with Protocol

1.5.1 The investigator/institution should conduct the trial in compliance with the protocol agreed to by the sponsor and, if required, by the regulatory authority(ies) and which was given approval/favourable opinion by the IRB/IEC. The

investigator/institution and the sponsor should sign the protocol, or an alternative contract, to confirm agreement.

1.5.2 The investigator should not implement any deviation from, or changes of the protocol without agreement by the sponsor and prior review and documented approval/favourable opinion from the IRB/IEC of an amendment, except where necessary to eliminate an immediate hazard(s) to trial subjects, or when the change(s) involves only logistical or administrative aspects of the trial (e.g., change in monitor(s), change of telephone number(s)).

1.5.3 The investigator, or person designated by the investigator, should document and explain any deviation from the approved protocol.

1.5.4 The investigator may implement a deviation from, or a change of, the protocol to eliminate an immediate hazard(s) to trial subjects without prior IRB/IEC approval/favourable opinion. As soon as possible, the implemented deviation or change, the reasons for it and, if appropriate, the proposed protocol amendment(s) should be submitted:
(a) to the IRB/IEC for review and approval/favourable opinion,
(b) to the sponsor for agreement and, if required,
(c) to the regulatory authority(ies).

1.6 Investigational Product(s)

1.6.1 Responsibility for investigational product(s) accountability at the trial site(s) rests with the investigator/institution.

1.6.2 Where allowed/required, the investigator/institution may/should assign some or all of the investigator's/ institution's duties for investigational product(s) accountability at the trial site(s) to an appropriate pharmacist or another appropriate individual who is under the supervision of the investigator/institution.

1.6.3 The investigator/institution and/or a pharmacist or other appropriate individual, who is designated by the investigator/institution, should maintain records of the product's delivery to the trial site, the inventory at the site, the use by each subject and the return to the sponsor or alternative disposition of unused product(s). These records should include dates, quantities, batch/serial numbers, expiration dates (if applicable), and the unique code numbers assigned to the investigational product(s) and trial subjects. Investigators should maintain records that document adequately that the subjects were provided the doses specified by the protocol and reconcile all investigational product(s) received from the sponsor.

1.6.4 The investigational product(s) should be stored as specified by the sponsor (see 5.13.2 and 5.14.3) and in accordance with applicable regulatory requirement(s).

1.6.5 The investigator should ensure that the investigational product(s) are used only in accordance with the approved protocol.

1.6.6 The investigator, or a person designated by the investigator/institution, should explain the correct use of the investigational product(s) to each subject and should check at intervals appropriate for the trial, that each subject is following the instructions properly.

1.7 Randomization Procedures and Unblinding

The investigator should follow the trial's randomization procedures, if any, and should ensure that the code is broken only in accordance with the protocol. If the trial is blinded, the investigator should promptly document and explain to the sponsor any premature unblinding (e.g., accidental unblinding, unblinding due to a serious adverse event) of the investigational product(s).

1.8 Informed Consent of Trial Subjects

1.8.1 In obtaining and documenting informed consent, the investigator should comply with the applicable regulatory requirement(s), and should adhere to GCP and to the ethical principles that have their origin in the Declaration of Helsinki. Prior to the beginning of the trial, the investigator should have the IRB/IEC's written approval/favourable opinion of the written informed consent form and any other written information to be provided to subjects.

1.8.2 The written informed consent form and any other written information to be provided to subjects should be revised whenever important new information becomes available that may be relevant to the subject's consent. Any revised written informed consent form, and written information should receive the IRB/IEC's approval/favourable opinion in advance of use. The subject or the subject's legally acceptable representative should be informed in a timely manner if new information becomes available that may be relevant to the subject's willingness to continue participation in the trial. The communication of this information should be documented.

1.8.3 Neither the investigator, nor the trial staff, should coerce or unduly influence a subject to participate or to continue to participate in a trial.

1.8.4 None of the oral and written information concerning the trial, including the written informed consent form, should contain any language that causes the subject or the subject's legally acceptable representative to waive or to appear to waive any legal rights, or that releases or appears to release the investigator, the institution, the sponsor, or their agents from liability for negligence.

1.8.5 The investigator, or a person designated by the investigator, should fully inform the subject or, if the subject is unable to provide informed consent, the subject’s legally acceptable representative, of all pertinent aspects of the trial including the written information and the approval/ favourable opinion by the IRB/IEC.

1.8.6 The language used in the oral and written information about the trial, including the written informed consent form, should be as non-technical as practical and should be understandable to the subject or the subject's legally acceptable representative and the impartial witness, where applicable.

1.8.7 Before informed consent may be obtained, the investigator, or a person designated by the investigator, should provide the subject or the subject's legally acceptable representative ample time and opportunity to inquire about details of the trial and to decide whether or not to participate in the trial. All questions about the trial should be answered to the satisfaction of the subject or the subject's legally acceptable representative.

1.8.8 Prior to a subject's participation in the trial, the written informed consent form should be signed and personally dated by the subject or by the subject's legally acceptable representative, and by the person who conducted the informed consent discussion.

1.8.9 If a subject is unable to read or if a legally acceptable representative is unable to read, an impartial witness should be present during the entire informed consent discussion. After the written informed consent form and any other written information to be provided to subjects, is read and explained to the subject or the subject's legally acceptable representative, and after the subject or the subject's legally acceptable representative has orally consented to the subject's participation in the trial and, if capable of doing so, has signed and personally dated the informed consent form, the witness should sign and personally date the consent form. By signing the consent form, the witness attests that the information in the consent form and any other written information was accurately explained to, and apparently understood by, the subject or the subject's legally acceptable representative, and that informed consent was freely given by the subject or the subject's legally acceptable representative.

1.8.10 Both the informed consent discussion and the written informed consent form and any other written information to be provided to subjects should include explanations of the following:

(a) The trial involved research.

(b) The purpose of the trial.

(c) The trial treatment(s) and the probability for random assignment to each treatment.

(d) The trial procedures to be followed, including all invasive procedures.

(c) The subject's responsibilities.

(f) Those aspects of the trial that are experimental.

(g) The reasonably foreseeable risks or inconveniences to the subject and, when applicable, to an embryo, fetus, or nursing infant.

(h) The reasonably expected benefits. When there is no intended clinical benefit to the subject, the subject should be made aware of this.

(i) The alternative procedure(s) or course(s) of treatment that may be available to the subject and their important potential benefits and risks.

(j) The compensation and/or treatment available to the subject in the event of trial-related injury.
(k) The anticipated prorated payment, if any, to the subject for participating in the trial.
(1) The anticipated expenses, if any, to the subject for participating in the trial.
(m) That the subject's participation in the trial is voluntary and that the subject may refuse to participate or withdraw from the trial, at any time, without penalty or loss of benefits to which the subject is otherwise entitled.
(n) That the monitor(s), the auditor(s), the IRB/IEC, and the regulatory authority(ies) will be granted direct access to the subject's original medical records for verification of clinical trial procedures and/or data, without violating the confidentiality of the subject, to the extent permitted by the applicable laws and regulations and that, by signing a written informed consent form the subject or the subject's legally acceptable representative is authorizing such access.
(o) That records identifying the subject will be kept confidential and, to the extent permitted by the applicable laws and/or regulations, will not be made publicly available. If the results of the trial are published, the subject's identity will remain confidential.
(p) That the subject or the subject's legally acceptable representative will be informed in a timely manner if information becomes available that may be relevant to the subject's willingness to continue participation in the trial.
(q) The person(s) to contact for further information regarding the trial and the rights of trial subjects, and whom to contact in the event of trial-related injury.
(r) The foreseeable circumstances and/or reasons under which the subject's participation in the trial may be terminated.
(s) The expected duration of the subject's participation in the trial.
(t) The approximate number of subjects involved in the trial.

1.8.11 Prior to participation in the trial, the subject or the subject's legally acceptable representative should receive a copy of the signed and dated written informed consent form and any other written information provided to the subjects. During a subject's participation in the trial, the subject or the subject's legally acceptable representative should receive a copy of the signed and dated consent form updates and a copy of any amendments to the written information provided to subjects.

1.8.12 When a clinical trial (therapeutic or non-therapeutic) includes subjects who can only be enrolled in the trial with the consent of the subject's legally acceptable representative (e.g., minors, or patients with severe dementia), the subject should be informed about the trial to the extent compatible with the subject's understanding and, if capable, the subject should sign and personally date the written informed consent.

1.8.13 Except as described in 4.8.14, a non-therapeutic trial (i.e. a trial in which there is no anticipated direct clinical benefit to the subject), should be conducted in subjects who personally give consent and who sign and date the written informed consent form

1.8.14 Non-therapeutic trials may be conducted in subjects with consent of a legally acceptable representative provided the following conditions are fulfilled:
(a) The objectives of the trial can not be met by means of a trial in subjects who can give informed consent personally.
(b) The foreseeable risks to the subjects are low.
(c) Tb c negative impact on the subject's well-being g is minimized and low.
(d) The trial is not prohibited by law.
(e) The approval/favourable opinion of the IRB/IEC is expressly sought on the inclusion of such subjects, and the written approval/ favourable opinion covers this aspect.
Such trials, unless an exception is justified, should be conducted in patients having a disease or condition for which the investigational product is intended. Subjects in these trials should be particularly closely monitored and should be withdrawn if they appear to be unduly distressed.

1.8.15 In emergency situations, when prior consent of the subject is not possible, the consent of the subject's legally acceptable representative, if present, should be requested. When prior consent of the subject is not possible, and the subject's legally acceptable representative is not available, enrolment of the subject should require measures described in the protocol and/or elsewhere, with documented approval/favourable opinion by the IRB/IEC, to protect the rights, safety and well-being of the subject and to ensure compliance with applicable regulatory requirements. The subject or the subject’s legally acceptable representative should be informed about the trial as soon as possible and consent to continue and other consent as appropriate (see 4.8. 10) should be requested.

1.9 Records and Reports

1.9.1 The investigator should ensure the accuracy, completeness, legibility, and timeliness of the data reported to the sponsor in the CRFs and in all required reports.

1.9.2 Data reported on the CRF, that are derived from source documents, should be consistent with the source documents or the discrepancies should be explained.

1.9.3 Any change or correction to a CRF should be dated, initialed, and explained (if necessary) and should not obscure the original entry (i.e. an audit trail should be maintained); this applies to both written and electronic changes or corrections (see 5.18.4 (n)). Sponsors should provide guidance to investigators and/or the investigators' designated representatives on making such corrections. Sponsors should have written procedures to assure that changes or corrections in CRFs made by sponsor's designated representatives are documented, are necessary, and are endorsed by the investigator. The investigator should retain records of the changes and corrections.

1.9.4 The investigator/institution should maintain the trial documents as specified in Essential Documents for the Conduct of a Clinical Trial (see 8.) and as required

by the applicable regulatory requirement(s). The investigator/institution should take measures to prevent accidental or premature destruction of these documents.

1.9.5 Essential documents should be retained until at least 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents should be retained for a longer period however if required by the applicable regulatory requirements or by an agreement with the sponsor. It is the responsibility of the sponsor to inform the investigator/institution as to when these documents no longer need to be retained (see 5.5.12).

1.9.6 The financial aspects of the trial should be documented in an agreement between the sponsor and the investigator/institution.

1.9.7 Upon request of the monitor, auditor, IRB/IEC, or regulatory authority, the investigator/ institution should make available for direct access all requested trial-related records.

1.10 Progress Reports

1.10.1 The investigator should submit written summaries of the trial status to the IRB/IEC annually, or more frequently, if requested by the IRB/IEC.

1.10.2 1.10.2 The investigator should promptly provide written reports to the sponsor, the IRB/IEC (see 3.3.8) and, where applicable, the institution on any changes significantly affecting the conduct of the trial, and/or increasing the risk to subjects.

1.11 Safety Reporting

1.11.1 All serious adverse events (SAES) should be reported immediately to the sponsor except for those SAEs that the protocol or other document (e.g., Investigator's Brochure) identifies as not needing immediate reporting. The immediate reports should be followed promptly by detailed, written reports. The immediate and follow-up reports should identify subjects by unique code numbers assigned to the trial subjects rather than by the subjects' names, personal identification numbers, and/or addresses. The investigator should also comply with the applicable regulatory requirement(s) related to the reporting of unexpected serious adverse drug reactions to the regulatory authority(ies) and the IRB/IEC.

1.11.2 Adverse events and/or laboratory abnormalities identified in the protocol as critical to safety evaluations should be reported to the sponsor according to the reporting requirements and within the time periods specified by the sponsor in the protocol.

1.11.3 For reported deaths, the investigator should supply the sponsor and the IRB/IEC with any additional requested information (e.g., autopsy reports and terminal medical reports).

1.12 Premature Termination or Suspension of a Trial

If the trial is prematurely terminated or suspended for any reason, the investigator/institution should promptly inform he trial subjects, should assure appropriate therapy and follow-up for the subjects, and, where required by the applicable regulatory requirement(s), should inform the regulatory authority(ies). In addition:

1.12.1 If the investigator terminates or suspends a trial without prior agreement of the sponsor, the investigator should inform the institution where applicable, and the investigator/institution should promptly inform the sponsor and the IRB/IEC, and should provide the sponsor and the IRB/IEC a detailed written explanation of the termination or suspension.

1.12.2 If the sponsor terminates or suspends a trial (see 5.21), the investigator should promptly inform the institution where applicable and the investigator/institution should promptly inform the IRB/IEC and provide the IRB/IEC a detailed written explanation of the termination or suspension.

1.12.3 If the IRB/IEC terminates or suspends its approval/favourable opinion of a trial (see 3.1.2 and 3.3.9), the investigator should inform the institution where applicable and the investigator/institution should promptly notify the sponsor and provide the sponsor with a detailed written explanation of the termination or suspension.

1.13 Final Report(s) by Investigator

Upon completion of the trial, the investigator, where applicable, should inform the institution; the investigator/institution should provide the IRB/IEC with a summary of the trial's outcome, and the regulatory authority(ies) with any reports required.

2. SPONSOR

2.1 Quality Assurance and Quality Controls

2.1.1 The sponsor is responsible for implementing and maintaining quality assurance and quality control systems with written SOPs to ensure that trials are conducted and data are generated, documented (recorded), and reported in compliance with the protocol, GCP, and the applicable regulatory requirement(s).

2.1.2 The sponsor is responsible for securing agreement from all involved parties to ensure direct access (see 1.21) to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by domestic and foreign regulatory authorities. 5.1.3 Quality control should be

applied to each stage of data handling to ensure that all data are reliable and have been processed correctly.

2.1.3 Agreements, made by the sponsor with the investigator/institution and any other parties involved with the clinical trial, should be in writing, as part of the protocol or in a separate agreement.

2.2 Contract Research Organization (CRO)

2.2.1 A sponsor may transfer any or all of the sponsor's trial-related duties and functions to a CRO, but the ultimate responsibility for the quality and integrity of the trial data always resides with the sponsor. The CRO should implement quality assurance and quality control.

2.2.2 Any trial-related duty and function that is transferred to and assumed by a CRO should be specified in writing.

2.2.3 Any trial-related duties and functions not specifically transferred to and assumed by a CRO are retained by the sponsor.

2.2.4 All references to a sponsor in this guideline also apply to a CRO to the extent that a CRO has assumed the trial related duties and functions of a sponsor.

2.3 Medical Expertise

The sponsor should designate appropriately qualified medical personnel who will be readily available to advise on trial related medical questions or problems. If necessary, outside consultant(s) may be appointed for this purpose.

2.4 Trial Design

2.4.1 The sponsor should utilize qualified individuals (e.g. biostatisticians, clinical pharmacologists, and physicians) as appropriate, throughout all stages of the trial process, from designing the protocol and CRFs and planning the analyses to analyzing and preparing interim and final clinical trial reports.

2.4.2 For further guidance: Clinical Trial Protocol and Protocol Amendment(s) (see 6.), the ICH Guideline for Structure and Content of Clinical Study Reports, and other appropriate ICH guidance on trial design, protocol and conduct.

2.5 Trial Management Data Handling, and Record Keeping

2.5.1 The sponsor should utilize appropriately qualified individuals to supervise the overall conduct of the trial, to handle the data, to verify the data, to conduct the statistical analyses, and to prepare the trial reports.

2.5.2 The sponsor may consider establishing an independent data-monitoring committee (IDMC) to assess the progress of a clinical trial, including the safety data and the critical efficacy endpoints at intervals, and to recommend to the sponsor whether to continue, modify, or stop a trial. The IDMC should have written operating procedures and maintain written records of all its meetings.

2.5.3 When using electronic trial data handling and/or remote electronic trial data systems, the sponsor should:
a) Ensure and document that the electronic data processing system(s) conforms to the sponsor's established requirements for completeness, accuracy, reliability, and consistent intended performance (i.e. validation).
b) Maintains SOPs for using these systems. c) Ensure that the systems are designed to permit data changes in such a way that the data changes are documented and that there is no deletion of entered data (i.e. maintain an audit trail, data trail, edit trail).
d) Maintain security system that prevents unauthorized access to the data.
e) Maintain a list of the individuals who are authorized to make data changes (see 4.1.5 and 4.9.3).
f) Maintain adequate backup of the data.
g) Safeguard the blinding, if any (e.g. maintain the blinding during data entry and processing).

2.5.4 If data are transformed during processing, it should always be possible to compare the original data and observations with the processed data.

2.5.5 The sponsor should use an unambiguous subject identification code (see 1.58) that allows identification of all the data reported for each subject.

2.5.6 The sponsor, or other owners of the data, should retain all of the sponsor-specific essential documents pertaining to the trial (see 8. Essential Documents for the Conduct of a Clinical Trial).

2.5.7 The sponsor should retain all sponsor-specific essential documents in conformance with the applicable regulatory requirement(s) of the country (ies) where the product is approved, and/or where the sponsor intends to apply for approval(s).

2.5.8 If the sponsor discontinues the clinical development of an investigational product (i.e. for any or all indications, routes of administration, or dosage forms), the sponsor should maintain all sponsor specific essential documents for at least 2 years after formal discontinuation or in conformance with the applicable regulatory requirement(s).

2.5.9 If the sponsor discontinues the clinical development of an investigational product the sponsor should notify all the trial investigators/institutions and all the regulatory authorities.

2.5.10 Any transfer of ownership of the data should be reported to the appropriate authority(ies), as required by the applicable regulatory requirement(s).

2.5.11 2.5. 11 The sponsor specific essential documents should be retained until at least 2 years after the last approval of a marketing application in an ICH region and until there are no pending or contemplated marketing applications in an ICH region or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents should be retained for a longer period however if required by the applicable regulatory requirement(s) or if needed by the sponsor.

2.5.12 The sponsor should inform the investigator(s)/institution(s) in writing of the need for record retention and should notify the investigator(s)/institution(s) in writing when the trial related records are no longer needed.

2.6 Investigator Selection

2.6.1 The sponsor is responsible for selecting the investigator(s)/institution(s). Each investigator should be qualified by training and experience and should have adequate resources (see 4.1, 4.2) to properly conduct the trial for which the investigator is selected. If organization of a coordinating committee and/or selection of coordinating investigator(s) are to be utilized in multicentre trials, their organization and/or selection is the sponsor's responsibility.

2.6.2 Before entering an agreement with an investigator/institution to conduct a trial, the sponsor should provide the investigator(s)/institution(s) with the protocol and an up-to date Investigator's Brochure, and should provide sufficient time for the investigator/institution to review the protocol and the information provided.

2.6.3 The sponsor should obtain the investigator's/institution's agreement:
(a) to conduct the trial in compliance with GCP, with the applicable regulatory requirement(s) (see 4.1.3), and with the protocol agreed to by the sponsor and given approval/favourable opinion by the IRB/IEC (see 4.5. 1);
(b) to comply with procedures for data recording/reporting;
(c) to permit monitoring, auditing and inspection (see 4.1.4) and
(d) to retain the trial related essential documents until the sponsor informs the investigator/institution these documents are no longer needed (see 4.9.4 and 5.5.12).

2.6.4 The sponsor and the investigator/institution should sign the protocol, or an alternative document to confirm this agreement.

2.7 Allocation of Responsibilities

Prior to initiating a trial, the sponsor should define, establish, and allocate all trial-related duties and functions.

2.8 Compensation to Subjects and Investigators

2.8.1 If required by the applicable regulatory requirement(s), the sponsor should provide insurance or should indemnify (legal and financial coverage) the investigator/the institution against claims arising from the trial, except for claims that arise from malpractice and/or negligence.

2.8.2 The sponsor's policies and procedures should address the costs of treatment of trial subjects in the event of trial-related injuries in accordance with the applicable regulatory requirement(s).

2.8.3 When trial subjects receive compensation, the method and manner of compensation should comply with applicable regulatory requirement(s).

2.9 Financing

The financial aspects of the trial should be documented in an agreement between the sponsor and the investigator/institution.

2.10 Notification/Submission to Regu1atory Authority(ies)

Before initiating the clinical trial(s), the sponsor (or the sponsor and the investigator, if required by the applicable regulatory requirement(s) should submit any required application(s) to the appropriate authority(ies) for review, acceptance, and/or permission (as required by the applicable regulatory requirement(s)) to begin the trial(s). Any notification/submission should be dated and contain sufficient information to identify the protocol.

2.11 Confirmation of Review by IRB/IEC

2.11.1 The sponsor should obtain from the investigator/institution:
a) The name and address of the investigator's/institution's IRB/IEC.
b) A statement obtained from the IRB/IEC that it is organized and operates according to GCP and the applicable laws and regulations.
c) Documented IRB/IEC approval/favourable opinion and, if requested by the sponsor, a current copy of protocol, written informed consent form(s) and any other written information to be provided to subjects, subject recruiting procedures, and any other documents that the IRB/IEC may have requested.

2.11.2 If the IRB/IEC conditions its approval/favourable opinion upon change(s) in any aspect of the trial, such as modification(s) of the protocol, written informed consent form and any other written information to be provided to subjects, and/or other procedures, the sponsor should obtain from the investigator/institution a copy of the modification(s) made and the date approval/favourable opinion was given by the IRB/IEC.

2.11.3 The sponsor should obtain from the investigator/institution documentation and dates of any IRB/IEC reapprovals/re-evaluations with favourable opinion, and of any withdrawals or suspensions of approval/favourable opinion.

2.12 2.12 Information on Investigational Product(s)

2.12.1 When planning trials, the sponsor should ensure that sufficient safety and efficacy data from nonclinical studies and/or clinical trials are available to support human exposure by the route, at the dosages, for the duration, and in the trial population to be studied.

2.12.2 The sponsor should update the Investigator's Brochure as significant new information becomes available (see 7. Investigator's Brochure).

2.13 Manufacturing, Packaging, Labelling, and Coding Investigational Product(s)

2.13.1 The sponsor should ensure that the investigational product(s) (including active comparator(s) and placebo, if applicable) is characterized as appropriate to the stage of development of the product(s), is manufactured in accordance with any applicable GW, and is coded and labelled in a manner that protects the blinding, if applicable. In addition, the labelling should comply with applicable regulatory requirement(s).

2.13.2 The sponsor should determine, for the investigational product(s), acceptable storage temperatures, storage conditions (e.g. protection from light), storage times, reconstitution fluids and procedures, and devices for product infusion, if any. The sponsor should inform all involved parties (e.g. monitors, investigators, pharmacists, storage managers) of these determinations.

2.13.3 The investigational product(s) should be packaged to prevent contamination and unacceptable deterioration during transport and storage.

2.13.4 In blinded trials, the coding system for the investigational product(s) should include a mechanism that permits rapid identification of the product(s) in case of a medical emergency, but does not permit undetectable breaks of the blinding.

2.13.5 If significant formulation changes are made in the investigational or comparator product(s) during the course of clinical development the results of any additional studies of the formulated product(s) (e.g. stability, dissolution rate, bioavailability) needed to assess whether these changes would significantly alter the pharmacokinetic profile of the product should be available prior to the use of the new formulation in clinical trials.

2.14 Supplying and Handling Investigational Product(s)

2.14.1 The sponsor is responsible for supplying the investigator(s)/institution(s) with the investigational product(s).

2.14.2 The sponsor should not supply an investigator/institution with the investigational product(s) until the sponsor obtains all required documentation (e.g. approval/favourable opinion from IRB/IEC and regulatory authority(ies)).

2.14.3 The sponsor should ensure that written procedures include instructions that the investigator/institution should follow for the handling and storage of investigational product(s) for the trial and documentation thereof. The procedures should address adequate and safe receipt, handling, storage, dispensing, retrieval of unused product from subjects, and return of unused investigational product(s) to the sponsor (or alternative disposition if authorized by the sponsor and in compliance with the applicable regulatory requirement(s)).

2.14.4 The sponsor should:

a) Ensure timely delivery of investigational product(s) to the investigator(s).
b) Maintain records that document shipment receipt disposition, return, and destruction of the investigational product(s) (see 8. Essential Documents for the Conduct of a Clinical Trial).
c) Maintain a system for retrieving investigational products and documenting this retrieval (e.g. for deficient product recall, reclaim after trial completion, expired product reclaim).
d) Maintain a system for the disposition of unused investigational product(s) and for the documentation of this disposition.

2.14.5 The sponsor should:

a) Take steps to ensure that the investigational product(s) are stable over the period of use.
b) Maintain sufficient quantities of the investigational product(s) used in the trials to reconfirm specifications, should this become necessary, and maintain records of batch sample analyses and characteristics. To the extent stability permits, samples should be retained either until the analyses of the trial data are complete or as required by the applicable regulatory requirement(s), whichever represents the longer retention period.

2.15 Record Access

2.15.1

The sponsor should ensure that it is specified in the protocol or other written agreement that the investigator(s)/institution(s) provide direct access to source data/documents for trial-related monitoring, audits, IRB/IEC review, and regulatory inspection.

2.15.2
The sponsor should verify that each subject has consented, in writing, to direct access to his/her original medical records for trial-related monitoring, audit, IRB/IEC review, and regulatory inspection.

2.16 Safety Information

2.16.1

The sponsor is responsible for the ongoing safety evaluation of the investigational product(s).

2.16.2

The sponsor should promptly notify all concerned investigator(s)/institution(s) and the regulatory authority (ies) of findings that could affect adversely the safety of subjects, impact the conduct of the trial, or alter the IRB/IEC's approval/favourable opinion to continue the trial.

2.17 Adverse Drug Reaction Reporting

2.17.1

The sponsor should expedite the reporting to all concerned investigator(s)/institutions(s), to the IRB(s)/IEC(s), where required, and to the regulatory authority(ies) of all adverse drug reactions (ADRS) that are both serious and unexpected.

2.17.2

Such expedited reports should comply with the applicable regulatory requirement(s) and with the ICH Guideline for Clinical Safety Data Management: Definitions and Standards for Expedited Reporting.

2.17.3

The sponsor should submit to the regulatory authority(ies) all safety updates and periodic reports, as required by applicable regulatory requirement(s).

2.18 Monitoring

2.18.1 Purpose

The purposes of trial monitoring are to verify that:

a) The rights and well-being of human subjects are protected.

b) The reported trial data are accurate, complete, and verifiable from source documents.

c) The conduct of the trial is in compliance with the currently approved protocol/ amendment(s), with GCP, and with the applicable regulatory requirement(s).

2.18.2 Selection and Qualifications of Monitors

a) Monitors should be appointed by the sponsor.

b) Monitors should be appropriately trained, and should have the scientific and/or clinical knowledge needed to monitor the trial adequately. Monitors qualifications should be documented.
c) Monitors should be thoroughly familiar with the investigational product(s), the protocol, written informed consent form and any other written information to be provided to subjects, the sponsors SOPS, GCP, and the applicable regulatory requirement(s).

2.18.3 Extent and Nature of Monitoring

The sponsor should ensure that the trials are adequately monitored. The sponsor should determine the appropriate extent and nature of monitoring. The determination of the extent and nature of monitoring should be based on considerations such as the objective, purpose, design, complexity, blinding, size, and endpoints of the trial. In general there is a need for on-site monitoring, before, during, and after the trial; however in exceptional circumstances the sponsor may determine that central monitoring in conjunction with procedures such as investigator's training and meetings, and extensive written guidance can assure appropriate conduct of the trial in accordance with GCP. Statistically controlled sampling may be an acceptable method for selecting the data to be verified.

2.18.4 Monitor's Responsibilities

The monitor(s) in accordance with the sponsor's requirements should ensure that the trial is conducted and documented properly by carrying out the following activities when relevant and necessary to the trial and the trial site:

a) Acting as the main line of communication between the sponsor and the investigator.
b) Verifying that the investigator has adequate qualifications and resources (see 4.1, 4.2, 5.6) and remain adequate throughout the trial period, that facilities, including laboratories, equipment, and staff, are adequate to safely and properly conduct the trial and remain adequate throughout the trial period.
c) Verifying, for the investigational product(s):
    i) That storage times and conditions are acceptable, and that supplies are sufficient throughout the trial.
    ii) That the investigational product(s) are supplied only to subjects who are eligible to receive it and at the protocol specified dose(s).
    iii) That subjects are provided with necessary instruction on properly using, handling, storing, and returning the investigational product(s).
    iv) That the receipt, use, and return of the investigational product(s) at the trial sites are controlled and documented adequately.
    v) That the disposition of unused investigational product(s) at the trial sites complies with applicable regulatory requirement(s) and is in accordance with the sponsor.
d) Verifying that the investigator follows the approved protocol and all approved amendment(s), if any.

e) Verifying that written informed consent was obtained before each subject's participation in the trial.
f) Ensuring that the investigator receives the current Investigator's Brochure, all documents, and all trial supplies needed to conduct the trial properly and to comply with the applicable regulatory requirement(s).
g) Ensuring that the investigator and the investigator's trial staff are adequately informed about the trial.
h) Verifying that the investigator and the investigator's trial staff are performing the specified trial functions, in accordance with the protocol and any other written agreement between the sponsor and the investigator/institution, and have not delegated these functions to unauthorized individuals.
i) Verifying that the investigator is enrolling only eligible subjects.
j) Reporting the subject recruitment rate.
k) Verifying that source documents and other trial records are accurate, complete, kept up-to-date and maintained.
1) Verifying that the investigator provides all the required reports, notifications, applications, and submissions, and that these documents are accurate, complete, timely, legible, dated, and identify the trial.
m) Checking the accuracy and completeness of the CRF entries, source documents and other trial related records against each other. The monitor specifically should verify that:

i) The data required by the protocol are reported accurately on the CRFs and are consistent with the source documents.
ii) Any dose and/or therapy modifications are well documented for each of the trial subjects.
iii) Adverse events, concomitant medications and intercurrent illnesses are reported in accordance with the protocol on the CRFs.
iv) Missed Visits, tests that are not conducted, and examinations that are not performed are clearly reported as such on the CRFs.
v) All withdrawals and dropouts of enrolled subjects from the trial are reported and explained on the CRFs.

n) Informing the investigator of any CRF entry error, omission, or illegibility. The monitor should ensure that appropriate corrections, additions, or deletions are made, dated, explained (if necessary), and initialed by the investigator or by a member of the investigator's trial staff who is authorized to initial CRF changes for the investigator. This authorization should be documented.
o) Determining whether all adverse events (AEs) are appropriately reported within the time periods required by GCP, the protocol, the IRB/IEC, the sponsor, and the applicable regulatory requirement(s). p) Determining whether the investigator is maintaining the essential documents (see 8. Essential Documents for the Conduct of a Clinical Trial). q) Communicating deviations from the protocol, SOPS, GCP, and the applicable regulatory requirements to the investigator and taking appropriate action designed to prevent recurrence of the detected deviations.

2.18.5 Monitoring Procedures

The monitor(s) should follow the sponsor established written SOPs as well as those procedures that are specified by the sponsor for monitoring a specific trial.

2.18.6 Monitoring Report

a) The monitor should submit a written report to the sponsor after each trial-site visit or trial-related communication. b) Reports should include the date, site, name of the monitor, and name of the investigator or other individual(s) contacted.
c) Reports should include a summary of what the monitor reviewed and the monitor's statements concerning the significant findings/facts, deviations and deficiencies, conclusions, actions taken or to be taken and/or actions recommended to secure compliance.
d) The review and follow-up of the monitoring report with the sponsor should be documented by the sponsor's designated representative.

2.19 Audit

If or when sponsors perform audits, as part of implementing quality assurance, they should consider:

2.19.1 Purpose

The purpose of a sponsor's audit, which is independent of and separate from routine monitoring or quality control functions, should be to evaluate trial conduct and compliance with the protocol, SOPS, GCP, and the applicable regulatory requirements.

2.19.2 Selection and Qualification of Auditors

a) The sponsor should appoint individuals, who are independent of the clinical trials/systems, to conduct audits.
b) The sponsor should ensure that the auditors are qualified by training and experience to conduct audits properly. An auditor's qualifications should be documented.

2.19.3 Auditing Procedures

a) The sponsor should ensure that the auditing of clinical trials/systems is conducted in accordance with the sponsor's written procedures on what to audit how to audit, the frequency of audits, and the form and content of audit reports.
b) The sponsor's audit plan and procedures for a trial audit should be guided by the importance of the trial to submissions to regulatory authorities, the number of subjects in the trial, the type and complexity of the trial, the level of risks to the trial subjects, and any identified problem(s).
c) The observations and findings of the auditor(s) should be documented.
d) To preserve the independence and value of the audit function, the regulatory authority(ies) should not routinely request the audit reports. Regulatory

authority(ies) may seek access to an audit report on a case by case basis when evidence of serious GCP non-compliance exists, or in the course of legal proceedings.
e) When required by applicable law or regulation, the sponsor should provide an audit certificate.

2.20 Non-compliance

2.20.1 Noncompliance with the protocol, SOPS, GCP, and/or applicable regulatory requirement(s) by an investigator/institution, or by number(s) of the sponsor's staff should lead to prompt action by the sponsor to secure compliance.

2.20.2 If the monitoring and/or auditing identify serious and/or persistent noncompliance on the part of an investigator/institution, the sponsor should terminate the investigator's/institutions participation in the trial. When an investigator's/institutions participation is terminated because of noncompliance, the sponsor should notify promptly the regulatory authority(ies).

2.21 Premature Termination or Suspension of a Trial

If a trial is prematurely terminated or suspended, the sponsor should promptly inform the investigators/institutions, and the regulatory authority(ies) of the termination or suspension and the reason(s) for the termination or suspension. The IRB/IEC should also be informed promptly and provided the reason(s) for the termination or suspension by the sponsor or by the investigator/institution, as specified by the applicable regulatory requirement(s).

2.22 Clinical Trial/Study Reports

Whether the trial is completed or prematurely terminated, the sponsor should ensure that the clinical trial reports are prepared and provided to the regulatory agency(ies) as required by the applicable regulatory requirement(s). The sponsor should also ensure that the clinical trial reports in marketing applications meet the standards of the ICH Guideline for Structure and Content of Clinical Study Reports. (NOTE: The ICH Guideline for Structure and Content of Clinical Study Reports specifies that abbreviated study reports may be acceptable in certain cases.)

15. APPENDIX C:

VACCINE SUPPLIES, PACKAGING AND ACCOUNTABILITY

1. Vaccine and/or other supplies

BIOFABRI will supply the following amounts of of study vaccines and/or other supplies, sufficient to administer the corresponding doses to all subjects as described in the present protocol.

- 18 vials of MTBVAC, 10 standard doses per vial, will be sent
- The corresponding MTBVAC diluent vials for reconstituting the vaccine
- The corresponding MTBVAC excipient solution vials (4.5 mL) for making the dilutions.

2. Secondary packaging

The vaccines will be packed in labelled boxes. The box label will contain, as a minimum, the following information: study number, lot number, instructions for vaccine administration, the statement "contains GMO" and any other relevant regulatory requirements.

3. Vaccine shipment
   from BIOFABRI to Centre Hospitalier Universitaire Vaudois (CHUV)

   Study vaccines will be sent from BIOFABRI TO Centre Hospitalier Universitaire Vaudois (CHUV) after the GMP and GCP releases which are documented and archived.

Shipments are made in tamper evident parcels containing:

- a copy of Certificate of Analysis if required by local regulatory authorities
- a Delivery Note describing: study and centre number, investigational site name and address, recipient, products name, quantity supplied, lot numbers, expiry dates and storage requirements
- 2 temperature control devices

All shipments shall be received and acknowledged by the appropriate recipient at Centre Hospitalier Universitaire Vaudois (CHUV).

The following documents should be completed and returned to BIOFABRI, S.L. on reception of vaccine shipment:

- Delivery Note

- Temperature control devices.

These documents and devices should then be returned to:

Attention of Dr Eugenia Puentes
Qualified Person
BIOFABRI , S.L
Fax : +34 986 345 201
E-mail: e.puentes@biofabri.es

Temperature recording charts will be obtained at BIOFABRI to check that cold chain has not been broken. Study vaccines will not be used at Centre Hospitalier Universitaire Vaudois (CHUV) until confirmation of suitability is provided by BIOFABRI. In case of any temperature deviation, the official approval for the use of vaccine must be obtained from BIOFABRI.

4. Vaccine accountability

At all times the figures on supplied, used and remaining vaccine doses should match. At the end of the study, it must be possible to reconcile delivery records with those of used and unused stocks. An explanation must be given of any discrepancies.

After approval from BIOFABRI, used and unused study vaccine vials/syringes should be destroyed at the study site using locally approved biosafety procedures and documentation unless otherwise described in the protocol. If no adequate biosafety procedures are available at the study site, the used and unused vaccine vials/syringes are to be returned to an appropriate BIOFABRI site for destruction.

5. Transfers of clinical vaccines or products from country medical department or dispatch centre to study sites or between sites

Not applicable.