**BCG vaccination/revaccination for prevention of *Mycobacterium tuberculosis* infection in healthcare students**

**entering clinical training:**

**A randomised placebo controlled proof of principle trial**

**(PoP BCG trial)**

**Protocol Number: 1**

**Sponsor:**

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**STATEMENT OF COMPLIANCE**

The Principal Investigator (PI) will assure that no deviation from, or changes to the protocol will take place without prior agreement from the Institutional Review Board (IRB). The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the IRB for review and approval. Approval of both the protocol and the consent form will be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. All changes to the consent form will be IRB approved.

# PROTOCOL SUMMARY

## Synopsis

|  |  |
| --- | --- |
| Title | Bacillus Calmette Guerin (BCG) vaccination/revaccination for prevention of *Mycobacterium tuberculosis* infection in health care students entering clinical training: A randomised placebo controlled proof of principle trial |
| Study description | This is a proof of principle randomised trial of BCG versus placebo in medical and nursing students in one hospital in Bandung, Indonesia, to prevent conversion of an Interferon Gamma Release Assay (IGRA; reflecting new *M. tuberculosis* infection) over 12 months following the commencement of clinical training. 150 students with IGRA and HIV negative results will be randomised to BCG vaccination/revaccination or placebo. They will be followed for 12 months with repeat IGRA test at 3, 6, 9, and 12 months. They will be given a logbook to record potential exposures to *M. tuberculosis*. The study will inform the final design of a full-randomised trial powered to detect a difference in IGRA conversion between arms. An interlocking immunological study will assess whether BCG vaccination/revaccination induces trained immunity in the students, with an extra sample taken at one month and three months post vaccination (n=100).  |
| Objectives | Primary objective: To assess acceptability of the BCG vaccination/revaccination, adverse events, and completeness of follow up.Secondary objectives:1. To assess if there is an indication of a trend towa*rds protection b*y BCG against new IGRA test conversion.
2. To assess whether BCG vaccination/revaccination induces innate immune cell and cytokine changes consistent with trained immunity.
3. To define an epigenetic signature associated with BCG vaccination/revaccination.
4. To establish a bio-repository for further testing.
 |
| Endpoints | Primary endpoints: Acceptability of BCG vaccination/revaccination, adverse events, and completion of follow-up. Secondary endpoints:1. IGRA test conversion at any time point during the study; persistent IGRA conversion defined as at least two consecutive IGRA tests over follow-up.
2. Trained immunity: Cytokine production and gene-transcriptional changes in stimulated peripheral blood mononuclear cell (PBMCs).
3. Changes in DNA methylation of monocytes associated with BCG-revaccination.
 |
| Study population | Medical and nursing students studying at Universitas Padjadjaran entering their clinical training in Hasan Sadikin Hospital |
| Phase | Proof of principle trial |
| Sites/facilities enrolling participants | Faculties of Medicine and of Nursing Universitas Padjadjaran Bandung, Indonesia |
| Description of study intervention | The intervention will be BCG vaccination/revaccination and control group will receive placebo |
| Study duration | 18 months |
| Participant duration | 12 months |

## Schematic of Study Design

Students will be tested by IGRA and HIV rapid test. A questionnaire will be used to collect data such as demographics, BCG vaccination history, and any TB exposures in the community or occupational prior to study enrolment. Blood sampling for immunological study will be collected (n=200).

Approximately 150 students who with IGRA and HIV negative result will be asked to participate in the trial.

Prior to

Enrolment

Randomise

Visit 1

Perform baseline assessments.

Immunological samples, symptom check +/- clinical reviews for suspected TB,

chest x- ray.

Administer BCG vaccine or placebo injection

Day-0

Visit 2

Immunological samples (n=100)

Month-1

IGRA test, symptom check +/- clinical review for suspected TB (n=150)

Immunological samples (n=100)

Visit 3

Month-3

IGRA test, symptom check +/- clinical review for suspected TB (n=150)

Visit 4

Month-6

Visit 5

IGRA test, symptom check +/- clinical review for suspected TB (n=150)

Month-9

**Final Assessments**

IGRA test, symptom check +/- clinical review for suspected TB, questionnaires

Visit 6

Month-12

## Schedule of Activities (SOA)

|  |  |
| --- | --- |
|  | **Study period** |
|  | **Enrolment** | **Allocation** | **Post-allocation** |
| **Procedures** | t-1 | t0 | t1month | t3 months | t6 months | t9 months | t12 months |
| **Enrolment:** |  |  |  |  |  |  |  |
| Eligibility screen | x |  |  |  |  |  |  |
| Informed consent | x |  |  |  |  |  |  |
| Baseline IGRA test | x |  |  |  |  |  |  |
| HIV test | x |  |  |  |  |  |  |
| Symptom check +/- clinical review for suspected TB | x |  |  |  |  |  |  |
| Baseline characteristics | x |  |  |  |  |  |  |
| Blood collection | x |  |  |  |  |  |  |
| **Interventions:** |  |  |  |  |  |  |  |
| BCG |  | x |  |  |  |  |  |
| Placebo |  | x |  |  |  |  |  |
| **Assessments:** |  |  |  |  |  |  |  |
| Adverse events review and evaluation |  | x | x |  |  |  |  |
| Trained immunity sub-study (n=100) |  | x | x | x |  |  |  |
| IGRA test |  |  |  | x | x | x | x |
| Symptom check +/- clinical review for suspected TB |  |  |  | x | x | x | x |
| TB exposure |  |  |  | x | x | x | x |

*\*At t-1*: *plasma; whole blood for flowcytometry and mass cytometry; deoxyribonucleic acid (DNA) from whole blood. At t0, t1month and t3months: PBMC and monocyte isolation for ex-vivo cytokines and for storage (for future epigenetic transcriptomic gene-transcriptional and metabolic analysis, flow cytometry.*

# INTRODUCTION

## Study rationale, background, and scientific basis

After the introduction of Interferon Gamma Release Assays (IGRA) for the diagnosis of *Mycobacterium tuberculosis* infection, evidence was found that Bacillus Calmette Guerin (BCG) protects people, not just from tuberculosis (TB) disease, but also from becoming infected with *M. tuberculosis* in the first place. The efficacy estimate from cross-sectional studies in TB case contacts is approximately 20% [1]. Efficacy estimates from longitudinal studies in Africa [2] and Asia (Verrall et al, accepted) in TB case contacts, suggested that protection from new *M. tuberculosis* infection may even be stronger (up to 50%). Furthermore, in a randomised placebo controlled trial that included BCG revaccination in adolescents in South Africa [3], BCG vaccine significantly reduced the rate of sustained IGRA conversion, a secondary endpoint, with an efficacy of 45.4% (P=0.03), while protection against any IGRA conversion after an initial 84 day washout period was estimated to be 20% (p=0.29) over 2 years. The South African study was relatively small, with on only 41 IGRA conversions in 312 participants across 4 sampling points in the BCG arm. However, overall, data from these studies suggest that BCG vaccine efficacy against *M. tuberculosis* infection, not just against disease, is worthy of further study. Furthermore, assessment of new vaccines’ ability to protect against *M. tuberculosis* infection may be an important part of the early evaluation of their efficacy in humans, which should ideally be done in both African and Asian populations.

Interestingly, in the South African study, the rate of upper respiratory tract infections was lower in the BCG group than in either the new vaccine (H4:IC31) group or the placebo group (2.1%, 9.4%, and 7.9%, respectively; P<0.001 for both comparisons). This suggests that BCG may protect against a range of pathogens through training of the innate immune system. Certain vaccines have been shown already to directly induce innate immune responses as part of their protective effect [4]. BCG can potentiate innate immune responses; a process termed ‘trained immunity’ [5]. In a series of studies over recent years we have shown that BCG vaccination/revaccination in adults leads to epigenetic and metabolic reprogramming of monocytes [6] and myeloid progenitor cells [7], translating in a stronger innate cellular response to various microbial and other stimuli [8]. This BCG-induced trained immunity was also found to protect mice against experimental infection with *Candida albicans* [8]and *M. tuberculosis* [7],andto reducevaccine viremia following vaccination with (live) yellow fever vaccine [6].

No study has addressed whether epigenetic reprogramming explains BCG-induced protection against *M. tuberculosis* infection in humans.

Recently, we have identified that healthcare students going into clinical training may well be an ideal population for studying protection against new *M. tuberculosis* infection. This is because they are transitioning from a relatively low transmission environment to a high transmission one and they can be evaluated prior to high *M. tuberculosis* exposure, enabling immune response after exposure to be compared with baseline pre-exposure. The students are able to be recruited in ‘batches’ at the beginning of their academic year, can document known exposures through log books, and are informed and motivated.

A new vaccine for TB is a stated priority in the WHO strategy to ‘End TB’. Basic understanding of BCG protection against *M. tuberculosis* infection will be crucial to the development and evaluation of new vaccines. Evidence of BCG protection in healthcare students going into their clinical training in settings like Indonesia would enable BCG to be considered as a preventive measure in such students and for any individuals who are entering a high *M. tuberculosis* transmission environment.

## Risk/Benefit assessment

### Known potential risks

BCG is the world’s most used vaccine and has been given safely at birth, in later years and as revaccination to large populations. In the trial by Nemes et al [3], there were no clinically significant differences between the BCG and placebo groups in the rates of serious adverse events. However, as known to occur, mild-moderate injection-site reactions were more common with BCG vaccination, occurring in almost all those who were vaccinated.

### Known potential benefits

A new vaccine for TB is a stated priority in the WHO strategy to ‘End TB’. Basic understanding of BCG protection against *M. tuberculosis* infection will be crucial to the development and evaluation of new vaccines. Evidence of BCG protection in healthcare students going into their clinical training in settings like Indonesia would enable BCG to be considered as a preventive measure in such students and for any individuals who are entering a high *M. tuberculosis* transmission environment.

# OBJECTIVES AND ENDPOINTS

## Objectives

Primary objectives:

To assess acceptability of the BCG vaccination/revaccination, adverse events, and completeness of follow up.

Secondary objectives:

1. To assess if there is an indication of a trend towa*rds protection b*y BCG against new *M. tuberculosis* infection (which is defined by IGRA test conversion).
2. To assess whether BCG vaccination/revaccination induces innate immune cell and cytokine changes consistent with trained immunity.
3. To define an epigenetic signature associated with BCG vaccination/revaccination
4. To establish a bio-repository for further testing

## Endpoints

Primary endpoints:

* 1. Acceptability of the intervention: the proportion of participants who consent and accepted to be given the intervention (BCG vaccine or placebo) over the total number of eligible participants in the study.
	2. Adverse events: the proportion of participants who experience any adverse event.
	3. Completeness of the study: the proportion of participants who complete follow-up, including all tests.

Secondary endpoints:

1. IGRA test conversion: the key endpoint will be cumulative IGRA test conversion, defined as IGRA test conversion at any time point during the study (at 3, 6, 9 or 12 months). IGRA test conversion will be defined as a change from a negative to a positive test plus a minimum 30% increase in TB1 minus Nil or TB2 minus Nil over the baseline value [9]. Secondarily, we will assess persistent IGRA conversion defined as at least two consecutive IGRA tests over follow-up. Exploratory analyses will consider various combinations of IGRA test results across the four follow-up points. A sensitivity analysis will re-analyse the data based on a definition of IGRA test conversion that simply requires a change from a negative to a positive test.
2. Induction of trained immunity: the primary readout for immune cell function will be cytokine production of Peripheral blood mononuclear cell (PBMCs) in response to BCG, *M. tuberculosis* and a range of unrelated microbial stimuli; the increase of ex-vivo monocyte-derived and lymphocyte-derived pro-inflammatory cytokine production capacity following BCG vaccination/revaccination will be used as an established marker of trained immunity. The secondary read outs will be epigenetic changes associated with BCG vaccination/revaccination and immunophenotype of innate cells.
3. Changes in DNA methylation of monocytes associated with BCG vaccination/revaccination: DNA methylation of monocytes will be measured by reduced-representation bisulphite (RRBS) at baseline and 3 months. This will be an exploratory analysis.

# STUDY DESIGN

## Overall design

This is a proof of principle randomised trial of BCG vaccination/revaccination versus placebo in medical and nursing students following the commencement of clinical training in a tertiary referral hospital in Bandung, Indonesia.

This trial will enrol medical and nursing healthcare students at the commencement of their clinical training in teaching hospitals in Indonesia in January 2020.

At baseline, all consenting enrolled students will be tested by IGRA and rapid HIV test. A questionnaire will be used to collect data about demographics, clinical characteristics, and TB exposure. Previous BCG vaccination will be confirmed by checking for a BCG scar.

Approximately 150 students who are IGRA and HIV negative will be randomised to BCG vaccination or placebo, including those who have been vaccinated at birth previously (revaccination). They will be followed for 12 months with repeat IGRA test at 3, 6, 9 and 12 months. At the present time the TB control guidelines in Indonesia do not recommend preventive treatment for healthcare workers who have evidence of *M. tuberculosis* infection.

Specific samples for immunological studies will be taken as shown in the figure (section 1.3) from 100 students, to examine if BCG vaccination/revaccination in adults induces trained immunity, characterized by epigenetic reprogramming and increased cytokine production.

The students will be given an electronic ‘log book’ to record potential exposures. At each sampling point they will be asked about possible symptoms of TB and investigated, or not, accordingly.At the one year follow up data from logbooks will be verified against university placement records. We will also collect information on direct contact with a family member or friend who had been diagnosed with TB in the last year and use of personal protection during work in health care facilities, using a questionnaire.

It is expected a small number of participants might have active TB (approx. 1%). These individuals will be referred for free treatment to the National TB Control Programme.

## Scientific rationale for study design

The study is designed to inform the final design of a fully powered trial to detect an effect of BCG against new M. tuberculosis infection, and to provide preliminary data regarding the underlying molecular mechanisms for such an effect.

## End of study definition

The end of study is defined as the last day of the 12-month period of observation post-intervention. A participant is considered to have completed the study if he or she has completed all phase of the study including the last schedule procedure shown in the Schedule of Activities (SoA), section 1.3.

# STUDY POPULATION

We will recruit 150 medical and nursing students who had an IGRA and HIV negative result at baseline.

##  Inclusion Criteria

Participants meet all following criteria at the time of randomisation:

1. Medical or nursing students who start their clinical training at Hasan Sadikin Hospital
2. Age > 18 years on study day 0
3. Tested IGRA negative at screening
4. Tested HIV negative at screening
5. Completed the written informed consent

## Exclusion Criteria

1. Retraining nursing students (retraining to transform their nursing qualification into a degree)
2. A positive prior tuberculin skin test (TST) and/or IGRA
3. A history of treatment for TB disease or latent TB infection
4. A history or evidence of TB disease
5. For female students: currently pregnant or lactating/nursing; or positive urine pregnancy test during screening
6. History of autoimmune disease or immunosuppression or used immunosuppressive medication

## Strategies for recruitment and retention

Identification of eligible participants will occur when they graduate with their bachelor degrees and before they start their clinical training. A meeting will be organised with students during the pre-clinical programmes held by the Faculties of Medicine and of Nursing at which information about the study will be provided to the students who will be asked to participate.

With respect to retention, all participants will be provided with a participant’s card in which information on scheduled visits will be provided. All information about scheduled visits and visit reminders will be shared with the student’s coordinator. A transport fee will be provided for all participants for each scheduled visit. A snack and small gift of appreciation will be provided to participants following collection of a blood sample. The provision of a snack and gift to participants is a culturally appropriate token following a blood draw, injection, and questionnaire. Participants will be made aware of the purpose and benefit of the study. Unlike other high-risk groups for TB acquisition, medical and nursing students can act as effective agents for change through awareness, advocacy, and, critically by acting to implement changes at the front lines. They also have understanding and awareness of the importance of protection against *M. tuberculosis* infection and TB disease. Therefore, their enthusiastic involvement in this trial is likely.

# STUDY INTERVENTION

## Study intervention(s) administration

### Study intervention description

1. Study intervention: BCG vaccine is a freeze-dried vaccine, which contains live attenuated of *Mycobacterium bovis*. We will use BCG vaccine manufactured by PT Biofarma Indonesia derived from the Pasteur 1173P strain. Each ampoule (20 doses) of vaccine contains: Live attenuated Bacillus Calmette-Guerin 1.5 mg semi-dried basil (1.5-6 million culturable particles), monosodium glutamate 7.5 mg. Each 1 mL of diluent consists of: Sodium Chloride 9 mg, Water for injection adds 1 mL.
2. Control product (Placebo): normal saline.

### Dosing and administration

1. BCG vaccination: 1 adult dose (0.1 mL) of vaccine (which reconstituted by 4 mL diluent) is administered intradermal with a 22-gauge needle.
2. Saline volume will be provided and equivalent to the BCG injection (0.1ml). It will be administered on day 0 by intradermal injection with a 22-gauge needle.

## Preparation/handling/storage/accountability

### Acquisition and accountability

The study manager who is required to maintain accurate study vaccine accountability records will supply BCG vaccine and the normal saline placebo. Instructions and forms to be completed and kept for accountability will be provided to the study manager.

### Product storage and stability

BCG vaccine will be stored at temperatures between +2oC and +8oC in a secure location. Protected from light. The diluent will be kept at room temperature. The normal saline placebo will be stored at room temperature in the study clinic.

### Preparation

BCG will be prepared and administered as per the manufacture’s recommendations. BCG vaccine will be prepared by the study pharmacist from multi-dose vials dispensed according to the package insert using aseptic technique. Each vial of BCG vaccine will be reconstituted as specified in the package insert. Reconstituted vaccine will be kept at +4oC and +8oC for up to 4 hours. Exposure to light will be kept to a minimum. Any reconstituted vaccine not used within 4 hours will be discarded.

## Measures to minimise bias: randomisation and blinding

Trial- group assignment will be concealed by an interactive web-response system. The assignment will be based in block randomisation in a 1:1 ratio to BCG vaccination/revaccination and placebo. Administration of the vaccine and placebo will be blinded (patient and administrator). To guarantee the blinding of those administering injections, syringe contents will be masked, injection volumes will be identical, and the person injecting the vaccine and placebo will be different from the one preparing the syringe (which will be done in different location). While those who are BCG vaccinated will be likely to have a significant local reaction, investigators will be blinded to this where possible – including those conducting the laboratory investigations.

## Study intervention compliance

As study intervention will be administered at day 0 only, adherence to the intervention will be assessed directly and research staff will fill a standard form.

# STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION/WITHDRAWAL

## Discontinuation of study intervention

As study intervention will only be given at Day 0, discontinuation from study intervention means discontinuation from the study, and remaining study procedures should not be completed as indicated by the study protocol.

## Participant discontinuation/withdrawal from the study

Participants are free to withdraw from participation in the study at any time upon request. An investigator may discontinue or withdraw a participant from the study for the following reasons:

1. Participant unable to receive the study intervention within 1 month
2. Participant unable to be tested by IGRA at baseline or follow up time

The reason for participant discontinuation or withdrawal from the study will be recorded on a specific form.

## Lost to follow-up

A participant will be considered potentially lost to follow up if he or she is not able to be found on the scheduled visit and is unable to be contacted by the study staff. The following actions will be taken under such circumstances:

1. The study staff will attempt to contact participant schedule a visit and counsel the participant on the importance activity in the study and ascertain if the participant wishes to and/or should continue in the study.
2. Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with participant (where possible, 3 telephone calls). Contact attempts will be documented in the participant’s record or study file.
3. Should the participant continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of “lost to follow up”.

# STUDY ASSESSMENTS AND PROCEDURES

## Recruitment assessment

At baseline, all consenting enrolled students will be tested by IGRA and rapid HIV test. A questionnaire will be used to collect data about demographics (age, gender, student type, housing, and ethnicity), clinical characteristics (BCG vaccination history, immunocompromised condition, smoking, alcohol consumption), TB exposure (previous work in a health care facility, previous training in health care facility, direct contact with TB patient at health care facility, direct contact with family or friend who had been diagnosed with TB). Previous BCG vaccination will be confirmed by checking for a BCG scar and medical records (if applicable).

## Follow-up assessments

The students will be given an electronic ‘log book’ to record potential exposures, such as to known TB patients or to known high-risk procedures such as bronchoscopy. At each sampling point they will be asked about possible symptoms of TB and investigated, or not, accordingly.We will also collect information on direct contact with a family member or friend who had been diagnosed with TB in the last year and use of personal protection during work in health care facilities, using a questionnaire. Those with positive results will be asked for a review to assess symptoms of active disease, referral to the TB clinic for diagnostic evaluation and referral for an appropriate free treatment by the National TB control programme. It is expected a small number of participants might have active TB (approx. 1%). These individuals, as per local policy, will be advised to take days off work until symptoms disappear (usually at least two weeks). They will be followed to check that they have received appropriate treatment.

## IGRA test interpretation

IGRA will be measured by QuantiFERON-TB Gold Plus (QFT-Plus) in the Immunology Laboratory, Faculty of Medicine Universitas Padjadjaran as per the manufacturer’s instructions. Blood sample will be collected, incubated at 370C for 24 hours, centrifuged and stored at 40C. The enzyme-linked immune sorbent assay (ELISA) will be performed manually in batches. The interferon gamma (IFN-ɣ) response level, measured in IU/ml, will be determined by measuring the amount of IFN-ɣ elaborated in response to the antigens ESAT-6 and CFP-10 that are associated with *M. tuberculosis* infection. The interpretation of QFT-Plus results is describing in table 1 [10].



For indeterminate results, a blood sample will be recollected and retested, and the result of the second sample will be recorded as the final result. IGRA conversion will be defined as a negative baseline test (IFN-ɣ <0.35 IU/ml) and a positive follow up test (IFN-ɣ >0.35 IU/ml). Other cut-off points for conversion used in previous studies as defined as baseline test IFN-ɣ <0.35 IU/ml and a follow up test IFN-ɣ >0.35 IU/ml, plus a 30% increase in IFN-ɣ over the baseline value [9].

## Safety and other assessments

The following procedures and evaluations will be done as part of the study to monitor safety.

1. Interviews: all interviews of participants will be conducted in private and in confidence. The purpose of the questionnaire will be explained to all students, they will be assured of the confidentiality of information, and they can opt out of answering any particular question if they wish.
2. IGRA test: trained and experienced research staff will do blood taking. All equipment and procedures used will meet all the required safety standards. Any adverse event will be recorded and evaluated.
3. HIV test: Provider-initiated testing and counselling (PITC) will be conducted in private and confidence. HIV testing and counselling will be done in order to enable decisions to be made for study eligibility. Trained and experienced research staff will do blood taking and counselling. All equipment and procedures used will meet all the required safety standards. Participants will be informed of their HIV test result in a sealed envelope, in person. If the HIV result is positive, referral to the HIV clinic will be made confidentiality.
4. BCG vaccination or placebo (saline injection): the process of administering the BCG vaccination or placebo involves a small amount of pain, minimal risk of infection and an in some cases a local reaction. Injection procedure will be done by trained and experienced research staff. All equipment and procedures used will meet all the required safety standards. Any adverse event will be recorded and evaluated.
5. Immunological samples: trained and experienced research staff will do blood taking. All equipment and procedures used will meet all the required safety standards.
6. Participants will be informed of their IGRA result in a sealed envelope, in person. Some of the screened participants in this study will have *M. tuberculosis* infection defined by a positive IGRA. Current WHO guidelines recommend treating those with TB Preventive Therapy (TPT) only in HIV-positive HCWs.
7. Diagnostic evaluation will be made for participants who have symptoms of TB disease; and if found positive and consistent with TB, they will be referred for treatment. A diagnosis of *M. tuberculosis* infection or TB disease could be disturbing emotionally to the participant and/or result in stigmatisation by family or friends. As part of the informed consent procedure, the participants will be made aware of all consequences of the test and diagnosis. All participants will be given assurance of the confidentiality of their results at all times, and the study staff will be trained to provide counselling and guidance to participants regarding their results and any treatment that they may require.

## Adverse events and serious adverse events

### Definition of adverse events

An adverse event is defined as any untoward medical occurrence in a participant administered a pharmaceutical product and which dose not necessarily have a causal relationship with this investigational product. An adverse event can therefore be any unfavourable and unintended sign (including abnormal laboratory findings), symptom or disease temporally associated with the use of an investigational product, whether or not related to the investigational product.

### Definition of serious adverse events

A serious adverse event is defined as one that results in any of the following outcomes: death, a life-threatening adverse events, inpatient hospitalisation or prolongation of existing hospitalisation, a persistent or substantial incapacity or disruption in the ability to conduct normal life functions, a congenital anomaly or birth defect, or an adverse event that jeopardizes the patient and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Adverse events will be recorded at all available post-vaccination time points and for a minimum of 6 months after the last vaccination.

### Classification of an adverse event

#### Severity of event

The severity of an adverse event will be assessed on the basis of a toxicity table, as modified from a table published by the Division of AIDS of the National Institute of Allergy and Infectious Diseases for grading of the severity of adult and paediatric adverse events.

* **Mild** – Events require minimal or no treatment and do not interfere with the participant’s daily activities.
* **Moderate** – Events result in a low level of inconvenience or concern with the therapeutic measures. Moderate events may cause some interference with functioning.
* **Severe** – Events interrupt a participant’s usual daily activity and may require systemic drug therapy or other treatment. Severe events are usually potentially life-threatening or incapacitating. Of note, the term “severe” does not necessarily equate to “serious”.

#### Relationship to study intervention

All adverse events must have their relationship to study intervention assessed by the clinician who examines and evaluates the participant based on temporal relationship and his/her clinical judgment. The degree of certainty about causality will be graded using the categories below. In a clinical trial, the study product must always be suspect.

* **Related** – The adverse event is known to occur with the study intervention, there is a reasonable possibility that the study intervention caused the adverse event, or there is a temporal relationship between the study intervention and event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study intervention and the adverse event.
* **Not Related** – There is not a reasonable possibility that the administration of the study intervention caused the event, there is no temporal relationship between the study intervention and event onset, or an alternate etiology has been established.

### Time periods and frequency for event assessment and follow-up

The occurrence of an adverse event or serious adverse event may come to the attention of study personnel during study visits and interviews of a study participant presenting for medical care, or upon review by a study monitor.

All adverse events including local and systemic reactions not meeting the criteria for serious adverse events will be captured on the appropriate case report form (CRF). Information to be collected includes event description, time of onset, clinician’s assessment of severity, relationship to study product (assessed only by those with the training and authority to make a diagnosis), and time of resolution/stabilization of the event. All adverse events occurring while on study must be documented appropriately regardless of relationship. All adverse events will be followed to adequate resolution.

Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an adverse event. However, if the study participant’s condition deteriorates at any time during the study, it will be recorded as an adverse event.

Changes in the severity of an adverse event will be documented to allow an assessment of the duration of the event at each level of severity to be performed. Adverse events characterized as intermittent require documentation of onset and duration of each episode.

The study manager will record all reportable events with start dates occurring any time after informed consent is obtained until 7 (for non-serious adverse events) or 30 days (for serious adverse events) after the last day of study participation. At each study visit, the investigator will inquire about the occurrence of adverse events/serious adverse events since the last visit. Events will be followed for outcome information until resolution or stabilisation.

### Adverse event reporting

Adverse events will be reported on the adverse event CRF using a recognized medical term or diagnosis that accurately reflect the event. Adverse event evaluations will be reviewed by the PI or by a designated medically qualifier practitioner. Adverse event CRF pages are to be completed by designated study team. The onset and resolution dates of the event and action taken in response to the event will be documented. All adverse events will be followed until resolution is demonstrated. The resolution date will be recorded on the CRF as the last date on which the subject experienced the adverse event. If an adverse event resolution date is uncertain the PI or designee should estimate the completion date based on medical judgment and interview of the subject. Approximate dates of resolution from interviews may be taken as adverse event resolution dates. Information recorded on the CRF must be substantiated in the source documents. If an adverse event evolves into a condition that becomes “serious,” it will be designated as serious on the Adverse Event CRF and a Supplemental Serious Adverse Event Report (SAER) form will be completed.

The PI will be notified immediately of all adverse events that are very serious (Grade 4 or death) within 24 hours. Staff will contact the site PI to verify the situation, remind them of the procedures for diagnosis and management of a suspected outcome. These staff will continue to monitor the adverse event – as it is investigated and managed. The Data and Safety Monitoring Board (DSMB) will be notified immediately of all Grade 4 adverse events that the PI believes were not anticipated, and were possibly related to the study regimens, and any deaths that the PI believes could possibly be related to the study regimen. When the investigation has been finalized (or resolved), the PI will complete the adverse event final report.

### Serious adverse event reporting

The study clinician will immediately report to the sponsor any serious adverse events, whether or not considered study intervention related, including those listed in the protocol or investigator brochure and must include an assessment of whether there is a reasonable possibility that the study intervention caused the event. Study endpoints that are serious adverse event (e.g., all-cause mortality) must be reported in accordance with the protocol unless there is evidence suggesting a causal relationship between the study intervention and the event (e.g., death from anaphylaxis). In that case, the investigator must immediately report the event to the sponsor.

All serious adverse events will be followed until satisfactory resolution or until the site investigator deems the event to be chronic or the participant is stable. Other supporting documentation of the event may be requested by the study sponsor and should be provided as soon as possible.

### Reporting events to participants

Participants will be informed about adverse events and serious adverse events, and study-related results on an individual level by the study staff. Plans for detecting and managing incidental findings associated with study procedures will be explained during informed consent.

## Unanticipated problems

### Definition of Unanticipated problems (UP)

The Office for Human Research Protections (OHRP) considers unanticipated problems involving risks to participants or others to include, in general, any incident, experience, or outcome that meets **all** of the following criteria:

* Unexpected in terms of nature, severity, or frequency given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the participant population being studied;
* Related or possibly related to participation in the research (“possibly related” means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
* Suggests that the research places participants or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

### Unanticipated problem reporting

The investigator will report unanticipated problems (UPs) to the reviewing IRB and to the lead PI. The UP report will include the following information:

* Protocol identifying information: protocol title and number, PI’s name, and the IRB project number;
* A detailed description of the event, incident, experience, or outcome;
* An explanation of the basis for determining that the event, incident, experience, or outcome represents an UP;
* A description of any changes to the protocol or other corrective actions that have been taken or are proposed in response to the UP.

To satisfy the requirement for prompt reporting, UPs will be reported using the following timeline:

* UPs will be reported to the IRB and to the study sponsor as soon as possible (within 24 hours) of the investigator becoming aware of the problem.
* All UPs should be reported to appropriate institutional officials (as required by an institution’s written reporting procedures), the supporting agency head (or designee), and the Office for Human Research Protections (OHRP) within 24 hours of the IRB’s receipt of the report of the problem from the investigator.

### Reporting unanticipated problems to participants

Participants will be informed about UPs if, in the opinion of the PI, they are considered to be relevant to them.

## Studies of trained immunity

### Stimulation assays

Before BCG vaccination/revaccination and at two different time points after vaccination (one month and three months) approximately 20 ml of blood will be drawn from which peripheral blood mononuclear cells (PBMCs) will be isolated by density centrifugation over Ficoll-Paque. Cells will be restimulated *ex vivo* with different stimuli:

* Control culture medium (RPMI)
* *Escherichia coli* LPS (10 ng/mL)
* *Mycobacterium tuberculosis* lysate 5 µg/mL
* *Candida albicans* 106 microorganisms/mL
* *Staphylococcus aureus* 106 microorganisms/mL

Then, cytokine production will be assessed with ELISA after 24h (IL-6, TNF-α, IL-1β) or 7 days (IFN-γ, IL-17, IL-22) incubation. The cytokine production at two different time points after vaccination will be compared with the response before vaccination. To look at the transcriptome, the cell lysates after stimulation will be stored in RNAprotect for RNAsequencing.

Leftover PBMCs will be stored in liquid nitrogen for future analysis. To unsure high viability upon recovery, cells will be frozen at a controlled rate of -1℃/minute using a CoolCell® freezing container to -80℃ before transferring them to liquid nitrogen.

**Circulating markers**

Plasma samples from enrolment, month 1 and month 3 will be stored for future analysis. A broad range of circulating inflammatory proteins will be measured to assess the inflammatory status before and after BCG vaccination.

**Cell sub populations and immunophenotyping: flow cytometry**

Unstimulated fixed whole blood samples from baseline, month 1 and month 3 will be cryopreserved for later analysis; 123 count eBeads will be included when samples are processed to allow calculation of absolute cell counts. Using multiparameter flow cytometry (up to 12 colours on a BD Fortessa), we will determine the expression of activation markers (reflecting recent activation *in vivo*) and chemokine receptors (reflecting ability to traffick to the lungs) by T cells, monocytes and natural killer cells.

### Stimulation assays: Mass cytometry

In addition, whole blood will be stimulated with various stimuli, including PBS, IFN-γ, LPS and *Mycobacterium tuberculosis* lysate, and the cells will be fixed after 15 minutes. For a subset of 30 participants, we will perform mass cytometry to assess cell-signalling pathways for all major immune cell subsets separately.

**Genomics**From each study participant, 4 mL of whole blood will be stored for DNA isolation. Genetic variation will be assessed by a genome-wide SNP array from Illumina.

**DNA methylation of monocytes**

Monocytes will be separated from 4mL of whole blood by immunomagnetic negative selection using the EasySep Direct™ isolation kit and magnet at baseline and 3 months. Monocytes will be frozen for later DNA extraction using the Qiagen MiniKit. After preparation, DNA methylation sites will be identified by RRBS-Sequencing.

# STATISTICAL CONSIDERATIONS

## Statistical hypotheses

As this a proof of principle trial, analysis will not focus on hypothesis testing around the primary endpoint for a larger trial (IGRA conversion) but will focus on acceptability, adverse events and completion of follow-up.

## Sample size determination

Recommendations for proof of principle trial (feasibility studies) propose that the analysis dataset comprises of a minimum of 30 participants for each arm in order to estimate parameters for future sample size calculations [11-13]. We anticipate that the total of loss to follow up will be no more than 20% and aim to recruit 75 participants in each arm, to account for potential missing data and to provide enough data for insights into IGRA test conversion and reversion necessary to design the larger study. The sample size for immunological studies is difficult to calculate. N=100 was determined by noting meaningful results have been obtained by our group in similar numbers of participants in our previous trained immunity studies [6].

## Populations for analyses

Statistical analyses will be on an intention-to-treat basis with participants being analysed according to their randomisation allocation.

## Statistical analyses

### General approach

The analyses will be descriptive in nature and provide initial estimates of key parameters needed to inform the design of a future randomised clinical trial (RCT). The quantitative measures will be used to address the research questions relating to the acceptability and the feasibility of conducting a definitive trial in the future. Data will be analysed at the end of the study when all data collection, entry, and validation are completed.

### Analysis of the primary endpoints

Acceptability, adverse events, and completion: The proportion of participants and 95% confidence intervals will be presented for each endpoint.

### Analysis of the secondary endpoints

IGRA test conversion: To estimate the cumulative IGRA test conversion, the number of participants who have IGRA test conversion at any time point during the study (at 3, 6, 9 or 12 months) will be used as the numerator and number total of participants included in the study will be used as the denominator. The cumulative IGRA test conversion will be presented with 95% confidence intervals. To estimate persistent IGRA conversion, the number of participants who have at least two consecutive IGRA tests over follow-up, or three consecutive IGRA tests over follow-up will be used as the numerator and the number total of participants included in the study will be used as the denominator.

Induction of trained immunity: We will compare participants of the intervention and control arms in terms of immune cell distribution and function at baseline, 1 month and 3 months after BCG vaccination/placebo.

Trained immunity: We will measure proportional difference in the distribution, function (cytokine production) and epigenetic and gene-transcriptional signature of circulating immune cells before and after BCG vaccination as established marker of trained immunity.

**Flow cytometry**

Changes in the absolute cell count and phenotype of different innate immune cell populations from baseline to 1 month and baseline to 3 months will be compared between BCG and placebo recipients.

**DNA methylation of monocytes**

In this exploratory analysis methylation sites will be mapped to the GRCh38 build of the human genome using the Bismark aligner [14]. Differentially methylated sites between baseline and 3 month time points will be compared for BCG re-vaccinated and placebo recipients using the differential Methylation Analysis Package (DMAP). Proximal genes will be identified using the DMAP identgeneloc program and gene ontology will be explored using the GeneCards database ([www.genecards.org](http://www.genecards.org" \t "_blank)).

### Sub-group analyses

There are no planned sub-group analyses.

### Exploratory analyses

Exploratory analyses will consider various combinations of IGRA test results across the four follow-up points. A sensitivity analysis will re-analyse the data based on a definition of IGRA test conversion that simply requires a change from a negative to a positive test.

# SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

## Regulatory, ethical, and study oversight considerations

### Informed consent process

An information sheet, describing in detail the purpose of the study, the study procedures and risks, types of data collected and stored will be given to each participant. After reading this document, a field staff will explain the study, answer any questions that arise and potential participants will have sufficient opportunity to discuss the study and consider their participation. Written informed consent will then be required from each participant prior to starting study procedures. The information sheet and consent forms will be IRB approved. Participants may withdraw consent at any time during. A copy of the information sheet and signed informed consent form will be given to the participants for their records.

### Study discontinuation and closure

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to study participants, investigator, funding agency, and regulatory authorities. If the study is prematurely terminated or suspended, the PI will promptly inform study participants, the IRB, and sponsor and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension of this study include, but are not limited to:

* Demonstration of efficacy that would warrant stopping
* Insufficient compliance to protocol requirements
* Data that are not sufficiently complete and/or evaluable
* Determination that the primary endpoint has been met
* Determination of futility

 The study may resume once any concerns are addressed, and satisfy the sponsor, and IRB.

### Confidentiality and privacy

This proof of principle trial requires the collection of identifying details for follow up purposes. Each study participant will be given a unique study identification number, without any identifying details of the participants that will be used in databases and specimen collection. Analysis will be conducted on this de-identified data. Identifying details will be retained in a single password-protected database managed by the PI or database manager. This is retained to allow participants to withdraw consent in the future and to allow for investigators to contact participants if a clinically significant finding is made about a particular individual.

Participant confidentiality is strictly held in trust by the participating investigators and their study staff. The study documentation, data and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party, without prior written approval of the PIs. All databases will be password protected. Backup copies of the database will be stored on a hard drive and under lock and key. All specimens and documents will be stored under lock and key.

The study database will identify participants only by a study identification number. Identifying details will be kept in a separate database only accessed by the investigator and field staff for purposes of follow up.

### Future use of stored specimens and data

The investigator will keep the records relating to this study for a minimum of fifteen years after the conduct of this study. Any blood samples for further testing will be kept and used as soon as possible. Permission is not required for the destruction of records.

### Key roles and study governance

|  |
| --- |
| **Co Principal Investigators** |
| *Philip Hill, MBChB MPH, MD, FRACP, FNZCPHM, Professor* | *Lika Apriani, MD, MSc**Epidemiologist* |
| *University of Otago, New Zealand*  | *Universitas Padjadjaran Bandung, Indonesia* |
| *Address:* University of Otago Medical School, PO Box 56, Dunedin 9054, New Zealand | *Jalan Prof Eyckman No 38 Bandung 40161 Indonesia* |
| *Phone number: +64 21 279 7214* | *+62 22 204 4128* |
| *Email: philip.hill@otago.ac.nz* | *likaaji@gmail.com* *lika.apriani@postgrad.otago.ac.nz* |

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| **Other Investigators** | **Institution** | **Role** |
| Prof Reinout van Crevel | *Radboud University Nijmegen The Netherlands* | *Infectious disease specialist and clinical immunologist* |
| Prof Mihai Netea | *Radboud University Nijmegen The Netherlands* | *Immunologist* |
| Dr Bachti Alisjahbana | *Universitas Padjadjaran Bandung, Indonesia* | *Infectious disease specialist, Head of TB Research Group*  |
| Dr Sue McAllister | *University of Otago* | *Epidemiologist* |
| Assoc Prof Katrina Sharples | *University of Otago* | *Statistician* |
| Dr Ayesha Verrall | *University of Otago* | *Infectious disease specialist* |
| Dr James Ussher | *University of Otago* | *Immunologist* |

### Safety oversight

Safety oversight will be under the direction of a DSMB composed of individuals with clinical trial expertise. There will be 3 members including one or more clinicians, and one biostatistician with knowledge of and experience in the statistical methods to evaluate safety data from the trial. Members of the DSMB will be independent from the study conduct and free of conflict of interest, or measures should be in place to minimize perceived conflict of interest. The DSMB will meet to assess safety data on each arm of the study. The DMSB will operate under the rules of an approved charter that will be written and then reviewed at the organizational meeting of the DSMB. At this time, each data element that the DSMB needs to assess will be clearly defined. The DSMB will provide its input to the PI for action.

### Clinical monitoring

Clinical site monitoring is conducted to ensure that the rights and well-being of trial participants are protected, that the reported trial data are accurate, complete, and verifiable, and that the conduct of the trial is in compliance with the currently approved protocol/amendment(s), with International Conference on Harmonisation Good Clinical Practice (ICH GCP), and with applicable regulatory requirement(s).

* Dr Sue McAllister (SM) will perform two in person internal monitoring visits for this study.
* The study will be monitored regularly throughout the study period. A review will be done for data verification of endpoint and other key data variables.
* SM will provide copies of monitoring reports within 10 days of each visit.
* Details of clinical site monitoring are documented in a Clinical Monitoring Plan (CMP). The CMP describes in detail who will conduct the monitoring, at what frequency monitoring will be done, at what level of detail monitoring will be performed, and the distribution of monitoring reports.
* As this proof of principle trial, independent audits will not be conducted.

### Quality assurance and quality control

Quality Control (QC): Scheduled operational checks will be done to verify that clinical data are generated, collected, handled, analysed, and reported according to protocol and standard operating procedures (SOPs). The study will be performed at a single centre.

SOPs will be developed and followed for the:

* Enrolment of participants
* Administer intervention
* Follow up of participants
* Laboratory procedures
* Management of data

Internal quality management of study conduct, data and biological specimen collection, documentation and completion will be done. QC procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the study site for clarification/resolution. Following written SOPs, the monitor will verify that the clinical trial is conducted and data are generated and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, ICH GCP, and applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP)).

The investigational site will provide direct access to trial related site, source data/documents, and reports for the purpose of monitoring.

### Data handling and record keeping

A database created in RedCap® will be designed by the established data management team. The data manager will monitor and review data collection, produce reports, manages retention of source documents, files and records. At the end of study, a copy of all datasets will be produced for the PI to keep. The data manager will ensure all processes are consistent with data management manual.

Data collection is the responsibility of the field and laboratory staff at the site under the supervision of the site PI. During the study, the investigator will maintain complete and accurate documentation for the study.

All source documents and laboratory reports will be reviewed by the PI and data entry staff, who will ensure that they accurate and complete.

Data Collection Forms (DCFs) will be administered by trained field staff in the clinic for all recruited participants. DCFs will be written in Bahasa Indonesia and include instructions to the interviewer. At the study centre, DCF responses will be coded according to a numerical coding dictionary for entry into the database.

A central, anonymised database will be designed, maintained, backed up and encrypted. DCFs will be entered into the database; electronically collected data will be transferred electronically into the database. A separate password protected database linking the study identification number to participant identifying details (name, address, telephone number) will be maintained by the field staff under the supervision of the site PI (Lika Apriani). The data will be entered, cleaned, verified, and checked for errors.

The following people will have access to records:

1. DCFs: field team, data management team, investigators
2. Database: data management team, investigators, statistician
3. Laboratory data: laboratory team, investigators

The study will have SOPs for quality management. Data will be evaluated for compliance with protocol and accuracy in relation to source documents. The study will be conducted in accordance with procedures identified in the protocol. Staff will be trained specifically for the study. SOPs will be used at all clinical and laboratory sites.

All study participants will be assigned a study identification number that will be on questionnaires and samples. A separate file will track participant names, contact details and study identification number and will be managed by the PI. Data will be collected on paper forms that will be designed and piloted prior to study commencement. These will be stored securely where recruitment occurs. All data will be entered into a secure database and verified as the study progresses. Laboratory studies will use the delinked data.

### Protocol deviations

A protocol deviation is any noncompliance with the clinical trial protocol, ICH GCP, or Manual of Procedures (MOP) requirements. The noncompliance may be either on the part of the participant, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with ICH GCP:

* 4.5 Compliance with Protocol, sections 4.5.1, 4.5.2, and 4.5.3
* 5.1 Quality Assurance and Quality Control, section 5.1.1
* 5.20 Noncompliance, sections 5.20.1, and 5.20.2.

It is the responsibility of the site investigator to use continuous vigilance to identify and report deviations within ten working days of identification of the protocol deviation, or within ten working days of the scheduled protocol-required activity. All deviations must be addressed in study source documents, reported to the sponsor. Protocol deviations must be sent to the reviewing IRB per their policies. The site investigator is responsible for knowing and adhering to the reviewing IRB requirements. Further details about the handling of protocol deviations will be included in the MOP.

### Publication and data sharing policy

The reporting of results to the community will be achieved through public lectures at the Universitas Padjadjaran and the University of Otago. To give maximum benefits and impact, the dissemination of the results to the Faculty of Medicine, Faculty of Nursing, and the students will be organised. In addition, local dissemination measures will include engagement with public health authorities. Timely publication of results in international scientific journals is planned and we anticipate publication journals such as the International Journal of Tuberculosis and Lung Disease and the Journal of Infectious Diseases, as well as oral presentations at conferences.

### Conflict of interest policy

The investigators have no known conflicts of interest. IGRA diagnostic kits will be provided by the manufacturer. Other costs of the study will be funded by the Centre for International Health (largely from the McAuley chair endowment fund for projects), Emerging Research First Grant from NZ Health Research Council (Grant number 19/614), and Radboud University Medical Centre Nijmegen The Netherlands.

## Abbreviations

|  |  |
| --- | --- |
| BCG | Bacillus Calmette Guerin |
| CMP | Clinical Monitoring Plan |
| DCF(s) | Data collection forms |
| DNA | Deoxyribo Nucleic Acid |
| DSMB | Data and Safety Monitoring Board |
| ELISA | Enzyme-linked immune Sorbent assay |
| GCP | Good Clinical Practice |
| GLP | Good Laboratory Practices  |
| GMP | Good Manufacturing Practices |
| HIV | Human Immunodeficiency Virus |
| ICH | International Conference on Harmonisation |
| IFN-ɣ | Interferon Gamma |
| IGRA | Interferon Gamma Release Assay |
| IRB | Institutional Review Board |
| MOP | Manual of Procedures |
| PBMCs | Peripheral Blood Mononuclear Cell |
| PI | Principal Investigator |
| PITC | Provider-Initiated Testing and Counselling (PITC) |
| QC | Quality Control |
| QFT-Plus | QuantiFERON-TB Gold Plus |
| RCT | Randomised Clinical Trial |
| RRBS | Reduced-Representation Bisulphite |
| SOA(s) | Schedule of Activities |
| SOP(s) | Standard Operating Procedures |
| TB | Tuberculosis |
| TPT | TB Preventive Treatment |
| TST | Tuberculin Skin Test |
| UP(s) | Unanticipated Problem(s) |

## Protocol amendment history

*The table below is intended to capture changes of IRB-approved versions of the protocol, including a description of the change and rationale. A Summary of Changes table for the current amendment is located in the Protocol Title Page.*

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| **Version** | **Date** | **Description of Change**  | **Brief Rationale** |
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