

PROTOCOL

RCH HREC/protocol no: 62586

NCT04327206

BCG vaccination to Reduce the impact of COVID-19 in healthcare workers (BRACE) Trial

Version 10.1, 10 December 2020

CONFIDENTIAL

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Statement of Compliance

This clinical trial will be conducted in compliance with all stipulation of this protocol, the conditions of the ethics committees approvals, and the Integrated Addendum to ICH E6 (R1): Guideline for Good Clinical Practice E6 (R2), dated 9 November 2016.

In Australia, this trial will also be conducted in compliance with with the NHMRC National Statement on ethical Conduct in Human Research (2007 and all updates), the Integrated Addendum to ICH E6 (R1): Guideline for Good Clinical Practice E6 (R2), dated 9 November 2016 annotated with TGA comments and the NHMRC guidance Safety monitoring and reporting in clinical trials involving therapeutic goods (EH59, 2016).

This clinical trial is not sponsored by any pharmaceutical company or other commercial entity.

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PROTOCOL SYNOPSIS

TITLE	<i>BCG vaccination to Reduce the impact of COVID-19 in healthcare workers (BRACE) Trial</i>
TRIAL DESCRIPTION	<p>Phase III, two group, multicentre, randomised placebo controlled trial in up to 7244 healthcare workers to determine if BCG vaccine reduces incidence and the severity of COVID-19 disease during the 2020 SARS-CoV-2 pandemic. The trial includes a pre-planned meta-analysis with data from the 2834 participants recruited in first stage of this study which followed the same protocol but where participants were randomised between BCG and no BCG at the time of receiving a flu vaccination, with a total sample size of 10078.</p> <p>Randomisation and immunisation will occur at each participating site. Participants will be randomised to receive BCG vaccine or 0.9% NaCl placebo. Participants will be followed-up for 12 months with notification from a smartphone application (up to daily when ill) or via phone calls, electronic messages, home visits and surveys to identify and detail suspected COVID-19 infection. Additional information on severe disease will be obtained from hospital medical records and/or government databases. Blood samples will be collected prior to randomisation and at 3 and 6, months and in a sub-set of participants at 9 and 12 months to determine SARS-CoV-2 exposure. Where required swab/blood samples will be taken at illness episodes to assess SARS-CoV-2 infection.</p>
OBJECTIVES	<p>Primary objectives</p> <ol style="list-style-type: none"> 1. To determine if BCG vaccination (Intervention) compared with placebo (Comparator) <u>reduces the incidence of COVID-19 disease</u> (Outcome) measured over the 6 months following randomisation (Time) in healthcare workers exposed to SARS-CoV-2 (Participants). 2. To determine if BCG vaccination (Intervention) compared with placebo (Comparator) <u>reduces the incidence of severe COVID-19 disease</u> (with <u>COVID 19 related</u> non-hospitalised severe disease, hospitalisation or death) (Outcome) measured over the 6 months following randomisation (Time) in healthcare workers exposed to SARS-CoV-2 (Participants). <p>SECONDARY OBJECTIVES</p> <ol style="list-style-type: none"> 3. To determine if BCG vaccination (Intervention) compared with placebo (Comparator) <u>reduces the incidence of COVID-19 disease</u> (Outcome) measured over the 12 months following randomisation (Time) in healthcare workers exposed to SARS-CoV-2 (Participants). 4. To determine if BCG vaccination (Intervention) compared with placebo (Comparator) <u>reduces the incidence of severe COVID-19 disease</u> (non-hospitalised severe disease, hospitalisation or death)

(Outcome) measured over the 12 months following randomisation (Time) in healthcare workers exposed to SARS-CoV-2 (Participants).

5. To determine if BCG vaccination (Intervention) compared with placebo (Comparator) prolongs the time to first SARS-CoV-2-proven respiratory illness (Outcome) measured over 6 and 12 months following randomisation (Time) in healthcare workers exposed to SARS-CoV-2 (Participants).
6. To determine if BCG vaccination (Intervention) compared with placebo (Comparator) reduces the severity of COVID-19 disease (Outcome) measured over 6 and 12 months following randomisation (Time) in healthcare workers exposed to SARS-CoV-2 (Participants).
7. To determine if BCG vaccination (Intervention) compared with placebo (Comparator) reduces the rate and severity of illness (fever or at least one sign or symptom of respiratory disease) measured over 6 and 12 months following randomisation (Time) in healthcare workers (Participants).
8. To determine if BCG vaccination (Intervention) compared with placebo (Comparator) reduces absenteeism (days off work) in healthcare workers (Participants).
9. To evaluate the safety of BCG vaccination in adult healthcare workers.

Planned exploratory analyses

10. To determine in a subgroup of adults with recurrent cold sores whether BCG vaccination compared with placebo reduces herpes simplex recurrences (such as cold sores).
11. To determine the BCG vaccination induced changes in the immune system that are associated with protection of adult healthcare workers from non-tuberculous infectious diseases including COVID-19.
12. To determine and compare changes in the immune system induced by vaccination of adult healthcare workers.
13. To identify factors (e.g. age, sex, chronic conditions such as diabetes and cardiovascular disease, smoking, asthma, prior BCG vaccination, genetics, other vaccinations including COVID-19-specific vaccines, latent TB, immunological/molecular factors) that influence adult immune responses, infection and COVID-19 risk.

<p>OUTCOMES AND OUTCOME MEASURES</p>	<p>Primary outcomes:</p> <ol style="list-style-type: none"> 1. Number of participants with COVID-19 disease defined as fever or at least one sign or symptom of respiratory disease including cough, sore throat, shortness of breath, respiratory distress/failure (using self-reported questionnaire), plus a positive SARS-Cov-2 test (PCR or serology) over the 6 months following randomisation. 2. Number of participants with <u>COVID-19 positive test</u> plus <ol style="list-style-type: none"> 1. Dead (as a consequence of COVID-19 disease) <u>OR</u> <ol style="list-style-type: none"> 2. <u>Hospitalised</u> (including mechanical ventilation and death) <u>OR</u> <ol style="list-style-type: none"> 3. <u>Non-hospitalised severe disease</u>, defined as Non-ambulant¹ for ≥ 3 consecutive days OR Unable to work² for ≥ 3 consecutive days <p>¹ “pretty much confined to bed (meaning finding it very difficult to do any normal daily activities”</p> <p>² “I do not feel physically well enough to go to work”</p> <p>Secondary outcomes: All assessed at 6 and 12 months following randomisation unless otherwise indicated.</p> <ul style="list-style-type: none"> - The following outcomes are for both COVID-19 disease and fever or respiratory illness: Number of participants with: COVID-19 disease, days unable to work, days confined to bed, of days with symptoms, pneumonia, need for oxygen therapy, admission to critical care, need for mechanical ventilation - Number of episodes of COVID-19 disease, fever or respiratory illness - Time to first symptom of COVID-19, fever or respiratory illness - Number of deaths - Number of days of unplanned absenteeism - Type and severity of local and systemic adverse event over the 3 months following randomisation - Planned exploratory analyses : Number of participants with, episodes of and time to first recurrence of herpes simplex recurrence, immunological studies
<p>TRIAL POPULATION</p>	<p>7244 adult healthcare workers from Brazil, Europe and Australia (Victoria, Western Australia, South Australia and New South Wales) will be involved in the study, plus 2834 recruited in the earlier stage of this study. Key exclusion criteria are having BCG vaccine contraindication, previously had a SARS-CoV-2 positive test result and prior involvement in this trial at an alternate study site. Participants will be randomised at 1:1 ratio giving approximately 5039 per group.</p>
<p>DESCRIPTION OF SITES</p>	<p>Multiple sites will enrol healthcare workers in Brazil, Europe and Australia.</p>

ENROLLING PARTICIPANTS	<p>Australian sites involved in this study include the RCH VIC, Monash Health VIC, Epworth Healthcare VIC, Perth Children’s Hospital WA, Fiona Stanley Hospital WA, Sir Charles Gairdner Hospital WA, the Royal Adelaide Hospital SA, Women’s and Children’s Hospital Adelaide SA, The Children’s Hospital at Westmead NSW, Westmead Hospital NSW, Prince of Wales Hospital NSW, St Vincent’s Hospital NSW and Sydney Children’s Hospital, Randwick NSW. Recruitment and follow-up may occur on site or at centrally identified locations overseen by Site Investigators.</p> <p>In Brazil, the study will be carried out in two cities, Campo Grande-MS and Rio de Janeiro-RJ. In Campo Grande, the Faculty of Medicine of UFMS, State Regional Hospital of Mato Grosso do Sul, Municipal Health Units, CASSEMS Hospital and Santa Casa Hospital will participate. In Rio de Janeiro the Centro de Referência Professor Hélio Fraga (CRPHF) da Escola Nacional de Saúde Pública Sergio Arouca (ENSP), FIOCRUZ and Municipal Health Office of Rio de Janeiro.</p> <p>Additional sites are not yet completely confirmed but will include various sites in Brazil, the Netherlands, Spain and the United Kingdom.</p>
DESCRIPTION OF INTERVENTIONS	<p>BCG vaccination group: BCG Denmark, 0.1 mL injected intradermal over the distal insertion of the deltoid muscle onto the humerus</p> <p>Control group: 0.1 ml of 0.9% NaCl injected intradermal over the distal insertion of the deltoid muscle onto the humerus</p>
TRIAL DURATION	2.5 years
PARTICIPANT DURATION	13.5 months from randomisation to final follow-ups

GLOSSARY OF ABBREVIATIONS

ABBREVIATION	TERM
AE	Adverse Event
AR	Adverse Reaction
BCG	Bacillus Calmette–Guérin vaccine
BRF	Biobank Registration Form (MCRI)
COVID-19	coronavirus disease 19
CRF / eCRF	Case Report Form / electronic Case Report Form
CPI	Chief Principal Investigator
DSMB	Data Safety Monitoring Board
ED	Emergency Department
GCP	Good Clinical Practice
HCW	Health Care Worker/s
HREC	Human Research Ethics Committee
ICH	International Conference on Harmonisation
ITT	Intention To Treat
MERS	Middle East respiratory syndrome
MCRI	Murdoch Children’s Research Institute
NHMRC	National Health and Medical Research Council
NSE	Non-specific effects
NSW	New South Wales
PPE	Personal Protective Equipment
PI	Principal Investigator
QC	Quality Control
RGO	Research Governance Office
RCH	Royal Children’s Hospital (Melbourne)
RPI	Region Principal Investigator
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAR	Serious Adverse Reaction
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
SOP	Standard Operating Procedure
SSI	Significant Safety Issue
SPI	Site Principal Investigator
SUSAR	Suspected Unexpected Serious Adverse Reaction
TB	Tuberculosis
TGA	Therapeutic Goods Administration
UAR	Unexpected Adverse Reaction
USM	Urgent Safety Measure

We use the following terminology with regards to the term ‘investigators’:

- **Chief Principal-Investigator** – is used to describe the **overall trial level** Investigator for this multi-site trial: Prof Nigel Curtis of MCRI in Australia (Overall Sponsor)
- **Region Principal Investigator** – is used to describe **the region-level Investigator** (i.e. the Region Principal Investigator) responsible for an area including multiple sites in this multi-site trial.
- **Site Principal Investigator** – is used to describe **the site-level** Investigator at a participating site in a multi-site trial.

For some trial sites, one investigator fulfils the role of both Region Principal Investigator and Site Principal Investigator.

INVESTIGATOR AGREEMENT

I have read the protocol entitled “BCG vaccination to Reduce the impact of COVID-19 in healthcare workers BRACE) Trial”.

By signing this protocol, I agree to conduct the clinical trial, after approval by a Human Research Ethics Committee or Institutional Review Board (as appropriate), in accordance with the protocol, the principles of the Declaration of Helsinki and the good clinical practice guidelines [Integrated Addendum to ICH E6 (R1): Guideline for Good Clinical Practice E6 (R2), dated 9 November 2016].

Changes to the protocol will only be implemented after written approval is received from the applicable Human Research Ethics Committee or Institutional Review Board (as appropriate), with the exception of medical emergencies.

I will ensure that study staff fully understand and follow the protocol and evidence of their training is documented.

Name	Role	Signature and date
Prof Nigel Curtis	Chief Principal Investigator	
Prof Marc Bonten	Region Principal Investigator for The Netherlands and Spain	
Prof Peter Richmond	Region Principal Investigator for Western Australia	
Prof David Lynn	Region Principal Investigator for South Australia	
A/Prof Nicholas Wood	Region Principal Investigator for New South Wales, Australia	
Prof John Campbell	Region Principal Investigator for United Kingdom	
Prof Julio Croda	Region Principal Investigator for Mato Grosso do Sul, Brazil	
Prof Margareth Dalcolmo	Region Principal Investigator for Rio de Janeiro, Brazil	

1. ADMINISTRATIVE INFORMATION

1.1. Trial registration

This trial is registered on [ClinicalTrials.gov](https://clinicaltrials.gov), NCT04327206.

1.2. Overall Sponsor

Trial Sponsor	MCRI
Chief Principal Investigator Contact name	Nigel Curtis
Address	Royal Children's Hospital, 50 Flemington Road

On behalf of the Sponsor, MCRI, the Chief Principal Investigator leading the trial will undertake and/or oversee those Sponsor responsibilities delegated by the Sponsor.

1.3. Expected duration of study

The recruitment and IP administration period is expected to take place from March 2020 to March 2021. The individual's follow-up will be 13.5 months from randomisation.

1.4. Stakeholder involvement

Stakeholder
Melbourne Children's Trials Centre (MCTC)
Royal Children's Hospital (RCH)
Hospital directors and staff where participants (staff) will be recruited
Hospitals whose staff will be included as sites
Department of Health (for each state)
Melbourne Academic Centre for Health (MACH)
Royal Children's Hospital Immunisation Service
Australian Health Research Alliance (AHRA)

2. INTRODUCTION AND BACKGROUND

2.1. Trial rationale and aim

In recent months severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2) has emerged as a novel human pathogen. With no pre-existing immunity against this virus, susceptibility among humans is presumed to be universal. Healthcare workers are at the frontline of novel infectious disease outbreaks such as this. Due to their contact with patients and production of aerosols during some medical procedures they have greater exposure and potentially risk of contracting newly emerged human pathogens. Current strategies to

protect healthcare workers rely on the use (and sustained supply) of personal protective equipment. Healthcare worker absenteeism due to infection with the outbreak pathogen or illness cause by another disease with similar symptoms, compounds the pressure already placed on the healthcare system.

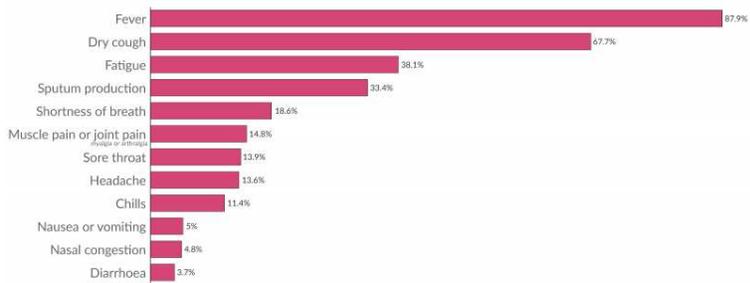
Prophylactic interventions to protect against emerging pathogens are needed, particularly for healthcare workers. The tuberculosis (TB) vaccine, Bacillus Calmette-Guérin (BCG) has beneficial off-target effects and has been shown to protect against non-TB infections¹. This is proposed to result from BCG mediated boosting of early immune responses. As such, BCG vaccination represents a potential prophylactic intervention to provide protection against emerging pathogens such as SARS-CoV-2.

The aim of this trial is to determine whether in healthcare workers, BCG can reduce the incidence and severity of illness caused by the novel coronavirus, SARS-CoV-2.

2.2. Background

Since the emergence of coronavirus disease 19 (COVID-19) in China in December 2019, there have been over 18,000,000 cases disease and greater than 690,000 deaths caused by the disease globally² (as of August 2020). The causative agent of COVID-19 a novel coronavirus, severe acute respiratory syndrome-coronavirus 2 (SARS-CoV-2), has already spread to 108 countries (including over 200 cases in Australia) and it is predicted that up to 60% of the global population could become infected³. Following from SARS in 2002⁴ and Middle East respiratory syndrome (MERS) in 2012⁵, SARS-CoV-2 is the third coronavirus to make the jump from animals to humans and emerge as a serious human pathogen in less than 20 years.

In approximately 80% of cases COVID-19 results in mild to moderate disease with symptoms similar to common respiratory diseases such as influenza-like illnesses, with fever in the majority (87.9%) of cases, followed by dry cough (67.7%), fatigue (38.1%), sputum production (33.4%)⁶. In 14% of cases, SARS-CoV-2 causes severe disease requiring oxygen supplementation and/or mechanical ventilation, with a further 6% being critical cases that have respiratory failure, septic shock and/or organ failure.



Data source: World Health Organization (2020). Report of the WHO-China Joint Mission on Coronavirus Disease 2019 (COVID-19). Symptoms in fewer than 1% are not shown. OurWorldinData.org - Research and data to make progress against the world's largest problems. Licensed under CC-BY by the authors.

There are worldwide efforts to reduce the peak of SARS-CoV-2 infection, in order to have enough hospital resources. However, with no vaccines or preventative interventions available to protect against COVID-19 disease, current strategies rely on conventional control measures including travel restrictions, quarantines and increased hygiene practices. The overlap of COVID-19 symptoms with common respiratory diseases makes screening for SARS-CoV-2 infection difficult with diagnosis relying on microbiological confirmation of SARS-CoV-2 infection. Moreover, healthcare workers with these common respiratory symptoms are advised to be tested for SARS-CoV-2 infection prior to return to work. The loss of these healthcare workers with non-COVID-19 respiratory infections due to quarantine requirements places further pressure on the healthcare system during this critical time.

BCG, a vaccine given to over 120 million infants annually to protect against TB, represents a potential prophylactic intervention for the prevention of COVID-19 disease. In addition to protecting against TB, BCG has beneficial off target (also termed 'heterologous' or 'non-specific') effects that protect against unrelated infections in children and adults⁷⁻¹¹.

The beneficial off-target effects of BCG vaccination have been most extensively studied in children. A world health organisation (WHO)-commissioned meta-analysis of 12 studies in high mortality settings concluded that BCG vaccination reduces all-cause mortality in children under 5-years of age by 30-53%⁸. This protection is evident within days of vaccination is proposed to be attributable to reduced deaths from infections other than TB, particularly respiratory tract infections and sepsis. Two large cohort studies in children similarly found that BCG reduces non-TB infections. The first, a 25-year retrospective study of over 150,000 children from 33 countries reported that BCG-vaccinated children had an up to 37% lower risk of acute lower respiratory tract infections¹². The second, a study of paediatric hospitalisations in Spain, found that BCG-vaccinated children had a 41% lower risk of serious respiratory infection and 53% lower risk of sepsis not related to TB¹³.

In adults, in a human challenge model, prior BCG vaccination reduced viraemia by over 70% and improved anti-viral immune responses to yellow fever vaccine virus¹⁴. Notably, yellow fever virus is a single-stranded, positive-sense RNA virus like SARS-CoV-2. Consistent with BCG mediated protection against infections, in two randomised control trials in adults, BCG vaccination reduced incidence of acute upper tract respiratory infections by 70-80%^{15,16}. Several studies have also shown that BCG can reduce symptoms in human papilloma virus infection and herpes simplex virus infection adults¹⁷.

A plethora of studies in animal models, have also shown that BCG protects against disease and mortality caused by a wide range of bacterial, fungal, protozoan and viral infections including infections with single-stranded, positive-sense RNA viruses¹⁸⁻²⁰.

The beneficial off-target effects of BCG are proposed to result from BCG induced changes in immune responses^{1,14,19}. In adults, BCG vaccination increases immune responses to unrelated pathogens, an effect that is sustained for at least a year after vaccination²¹. BCG vaccination also boosts antibody responses to several vaccines including influenza vaccine²²⁻²⁴. Thus, in addition to protecting against viral infections, BCG provides further protection by increasing the efficacy of other vaccinations.

Therefore, by boosting the immune system, BCG vaccination may provide early protection against new human pathogen thus reducing their spread and severity. This will be of particular benefit among healthcare workers and high-risk groups for whom contraction of the disease would have the greatest impact.

This trial will determine whether BCG vaccination reduces the incidence and severity of COVID-19 but also whether BCG vaccination reduces other respiratory illnesses in healthcare workers. In this case of COVID-19, where symptoms overlap with common respiratory diseases and diagnostic tests currently take several days, the prevention of non-CODVID-19 respiratory illnesses will also reduce the strain on the healthcare system caused by the outbreak. This is particularly important in Australia and other countries in the southern hemisphere as the outbreak peak is expected to occur during the winter influenza season.

The results of this trial will establish whether, in future novel disease outbreaks, BCG vaccination could be implemented as an early intervention to protect healthcare workers and high-risk groups.

2.3. Risk/Benefit assessment

2.3.1. Known potential risks

This study involves minimal risk to participants.

HCWs randomised to receive BCG vaccine will have known potential risks associated with BCG vaccination. These risks are slightly increased for HCWs who have previously had BCG vaccine (revaccination), compared to HCWs receiving BCG vaccine for the first time (vaccine naïve).

There are additional known minimal risks for all HCWs re: blood tests and respiratory swabs.

BCG vaccination

Expected (common) reactions to BCG vaccination²⁵:

- A small swelling, redness and tenderness (measuring 0.5-1.5 cm in diameter) at the injection site appears within 1-2 weeks at the injection site. The local lesion evolves into a small ulcer. The ulcer heals over several weeks to months, usually healing into a small flat scar.
- Slightly swollen lymph nodes in the axilla in up to 10% of recipients, and usually resolve spontaneously.

Revaccination is associated with an earlier, accelerated reaction which begins within 24–48 hours of vaccination with induration followed by pustule formation in 5–7 days and healing within 10–15 days²⁶

(<https://www1.health.gov.au/internet/main/publishing.nsf/Content/cda-cdi3701h.htm>)

Tuberculous skin lesions are more common in people over 15 years or with revaccination^{27,28}.

Uncommon side effects of BCG vaccination (up to 1 in 100)^{25,29-31}:

- Large ulcer, abscess at the injection site
- Keloid scar at injection site
- Swelling of lymph nodes in the armpit larger than 1 cm across

Rare side effects (up to 1 in 1000)

- Significant inflammation of lymph nodes in the axilla, sometimes with oozing ulcers, possibly abscess
- Infection with the bacteria from the vaccine can occur. The infection can spread throughout the body, including the bones (osteomyelitis)
- Allergic reaction or anaphylaxis (e.g.: redness of the face and neck, swelling of the face, throat or neck, skin rash, breathing difficulties and collapse)
- Fainting, seizures and convulsions (rare among patients receiving injections)

Very rare side effects (1–4 cases per million vaccinated people²⁵):

Disseminated BCG infection has been reported rarely after BCG vaccination, mainly in immunocompromised individuals (who are excluded from the trial).

Co-administration of vaccines:

As indicated in the Australian immunisation handbook, BCG vaccine can be given at the same time as, or at any time after, other inactivated vaccines thus there is no additional risk for co-administration of influenza and BCG vaccines²⁵.

*BCG vaccination in Europe (current recommendations)*³²

In Europe, recommendation for BCG vaccination varies among countries. In some, BCG is no longer recommended (e.g. Spain), whereas in others it is given routinely to all neonates (mainly Eastern Europe).³² In The Netherlands it is limited to the children of parents from countries with a high incidence of tuberculosis (>50/100,000 people) and is not routinely recommended for healthcare workers.³³ In the UK, routine BCG vaccination of adolescents was stopped in 2005, with subsequent efforts focusing on high-risk groups for tuberculosis (UK 'Green Book' chapter 32).

BCG vaccination in Australia (current recommendations)

BCG vaccination in Australia is limited to selected high risk groups and is not routinely recommended for most healthcare workers (HCW)²⁶. BCG vaccination is recommended for Aboriginal and Torres Strait Islander neonates in communities with a high incidence of TB; neonates and children 5 years of age and under who will be travelling or living in areas with a high prevalence of TB for extended periods; and neonates born to parents with leprosy. BCG should be considered in HCWs who may be at high risk of exposure to drug resistant cases. It is usually recommended that all individuals have a tuberculin skin test (TST) prior to BCG vaccination, except infants less than 6 months of age with no history of tuberculosis (TB) contact, and that BCG should not be given to an individual with a tuberculin reading of 5mm or more. Additionally, BCG revaccination is not recommended, regardless of TST reaction size²⁶.

BCG vaccination in Brazil (current recommendations)

In Brazil, BCG vaccination has been mandatory since 1976 in newborns. Revaccination in school-aged children (<6 years) was suspended in 2019. The REVAC trial evaluated adverse reactions resulting from BCG vaccination and revaccination in 71,347 Brazilian school-aged children. The authors concluded that the rate of adverse reactions associated with BCG revaccination is approximately twice the rate associated with vaccination, but this difference was not statistically significant. Similar results have been observed in previous studies that concluded that BCG revaccination is not associated with a higher rate of serious adverse events than primary BCG vaccination.^{43,44}

Current contraindications of BCG vaccination

- BCG is contraindicated in immunocompromised individuals due to the risk of disseminated BCG infection²⁶. This includes individuals immunocompromised by HIV infection, primary immunodeficiencies, corticosteroids or other immunosuppressive agents, and malignancies involving bone marrow or lymphoid systems.
- BCG is also contraindicated in individuals with any serious illness and those with generalised septic skin diseases and active skin conditions such as eczema, dermatitis and psoriasis near the site of vaccination²⁵.
- While BCG has not been shown to cause foetal damage the use of live vaccines is contraindicated in pregnancy²⁶.

- Individuals who have previously had tuberculosis or a large tuberculin (TST) reaction

In this study, HCWs will be excluded from the study if they are immunocompromised, have serious illness, skin disease at site of vaccination or are pregnant.

Global BCG recommendations and practices

The current World Health Organization (WHO) position is that BCG revaccination is not recommended for any person, as there is no evidence to support the role of BCG revaccination in protection against tuberculosis³⁴. A number of countries have previously included BCG revaccination as part of their national immunisation policies³⁵. In 1999, 30 countries in Europe and an additional 18 countries in the Middle East, South East Asia and the Western Pacific region reported using BCG revaccination. In several countries the national policy included BCG in infancy and again at school entry or leaving. In other countries, particularly in Eastern Europe, revaccination with BCG up to age five has been recommended. Some countries, such as Poland, recommended universal revaccination while others restrict revaccination to individuals without a BCG scar or those with a 'negative' TST. Criteria for TST negativity differs between countries^{36,37}. ***In countries where BCG revaccination has been part of national immunisation practice, passive surveillance has not reported any particular issues, nor any cases of disseminated BCG in immunocompetent individuals.***

Pre-vaccination screening

TST and interferon gamma release assay (IGRA) screening aims to identify individuals with latent tuberculosis infection (LTBI)³⁸. The diameter of induration following TST gives an indication of the likelihood of LTBI, however, positive results can also arise from previous BCG vaccination and exposure to environmental mycobacteria. This is in contrast to IGRA which are unaffected by previous BCG vaccination. A positive IGRA indicates either current or past infection with TB³⁸. Screening of individuals using TST prior to BCG vaccination is recommended in Australia and other countries on the grounds that it may prevent complications due to pre-existing immunity due to previous exposure to mycobacterial antigens²⁸. ***However, a large review of adverse effects of over 1.5 billion doses of BCG vaccine in adults and children showed that a positive TST did not increase the likelihood of complications from the BCG vaccine and did not predict the development of local skin reactions, abscesses or axillary lymphadenitis²⁷.***

Trials of BCG revaccination

Three large randomised controlled trials of BCG revaccination in children and adults in Malawi (n=54865), children in Guinea Bissau (n=2871) and adolescents in South Africa (n=990) did not show increased rates of serious adverse events among BCG revaccinated participants^{15,39,40}. Participants in the Malawi study did not undergo any pre-randomisation screening with tuberculin skin test (TST) or interferon gamma release assay (IGRA)³⁹. This study found a lower rate of leprosy amongst revaccinated participants but no difference in the rates of tuberculosis or death between the groups. Of the children in the Guinea Bissau study, 3 of 6 children with a measurable TST (1-14mm) had increased rates of large local reaction compared to controls (18/388). Two months after revaccination all had healed vaccination scars with no axillary node enlargement, fever or suppurative lymphadenitis⁴⁰.

Participants in the South African study all had a negative IGRA at enrolment¹⁵. Among BCG revaccinated adolescents 93% reported mild local injection site reactions including swelling, induration, discharge, erythema, scab and ulceration. This was compared to 25% in the placebo group. The rates of moderate injection site reactions were similar between the BCG (5%) and placebo (6%) groups. There was 1 severe and 7 serious adverse events in each of the BCG and control groups. The serious adverse events reported in the BCG arm were not attributed to BCG revaccination and included gastroenteritis, chest injury, thermal burn, intentional self-injury, suicide attempt and small intestinal obstruction. The rate of upper respiratory tract infections was also lower in the BCG revaccinated group compared to placebo (2.1% compared to 7.9%, $p < 0.001$).

Further studies looking at BCG revaccination in individuals with positive TST or IGRA do not show increased risk of significant adverse effects. A case-control study of 200 healthy nursing students in India included 28 participants with a positive IGRA who received BCG revaccination⁴¹. There were no serious side effects reported and no participants developed active tuberculosis during the follow-up study period. A randomised controlled trial of BCG revaccination in healthy adults with a positive TST ($>15\text{mm}$) with or without isoniazid pre-treatment ($n=82$) showed no difference in the rate of reactions between groups with only local injection site reactions (35-76%) and mild systemic adverse effects (19%) including headache, fever and nausea⁴². Among the 76% of participants who developed ulceration the median ulcer size was 5mm (IQR 4.0-6.0). Maximum ulcer diameter did not correlate with IGRA result prior to BCG vaccination in either group. There were no reports of regional lymphadenitis or serious morbidity.

Enhanced routine passive surveillance of BCG revaccinated school children in the BCG-REVAC trial in Brazil is available for 71718 individuals⁴³. There are only 33 reported adverse events of which 60% were local cutaneous reactions and 28% axillary lymphadenopathy without suppuration. There were no deaths, permanent injuries or disseminated infections reported. In a case series of 13 children who experienced adverse events following BCG revaccination in Brazil all developed local ulceration or abscess formation with complete recovery following antimycobacterial therapy⁴⁴. There were no cases of suppurative lymphadenitis or disseminated BCG. Further, an ongoing randomised trial in 150 participants in the US is giving repeat BCG (two vaccinations in the first year, then annually for 4 years) to adults aged 18-65 with type 1 diabetes to test if multiple BCG vaccinations can improve diabetic control and prevent complications⁴⁵. They have reported variable local reactions but no increased risk of lymphadenopathy or disseminated BCG (Denise Faustman, personal communication).

The data presented above supports the WHO position that while BCG revaccination is not recommended due to a lack of evidence of efficacy against tuberculosis the risk of administering BCG vaccine to persons with positive tuberculin reactions due to either prior BCG vaccination or to natural infection is minimal.

One aim of the present study is to document the safety of BCG vaccination (and revaccination) in healthcare workers. The decision not to perform pre-vaccination TST screening in the study is pragmatic in order to reduce barriers to participation for already busy and stretched healthcare workers during the current COVID-19 outbreak. While it does not align with current Australian vaccination guidelines it has been carefully considered upon systematic review of the literature presented above.

Risks related to Placebo injection

Having an injection can sometimes cause very minor pain from the needle or be uncomfortable. The 0.9% NaCl is an inert salt solution that will not cause any degree of local reaction. The placebo injection will be administered by a trained immunization nurse.

Risks related to blood sample collection

Having a blood test can sometimes cause some pain from the needle or be uncomfortable. Occasionally a small amount of bruising can occur on the skin where the blood was taken. Trained members of the study team will collect the blood samples from participants.

Risks related to respiratory swab collection

Having a respiratory swab can sometimes be uncomfortable. Trained members of the study team will collect the respiratory swabs from participants. Self-testing swab kits may be provided as required, with clear instructions to participants on safe self-swabbing technique.

2.3.2. Known potential benefits

In most places in the world, BCG is given to infants and children living in or travelling to TB endemic areas. In adults its efficacy is variable, and likely to have little effect in adults living in low prevalence settings (such as Australia, UK, Spain or the Netherlands) as their risk of TB is very low. BCG also protects against non-TB mycobacterial infections (e.g. leprosy, Buruli ulcer) but these are also rare in Australia and in Europe.

However, BCG also induce beneficial off target effects, and therefore BCG vaccination may reduce COVID-19 illness and other respiratory infections in study participants. In addition to the direct benefit this would give the participants by reducing disease, this would also benefit the healthcare facilities that they work at by reducing their need to be absent (symptom related quarantine or illness) and thus enabling them to continue working and supporting the healthcare system during this period of intense demand.

2.3.3. Assessment of potential risks and benefits

BCG vaccination has a well-established safety profile in healthy individuals. While there are known adverse reactions to BCG, serious adverse reactions are rare. BCG vaccination does also cause a scar in over 80% participants. Participants will be screened prior to BCG vaccination to ensure they have no known contraindications for BCG vaccination. Vaccination will be done by staff trained in intradermal injection to reduce the potential subcutaneous injection which can increase scarring. Blood tests and respiratory swabs will be done by trained staff. If necessary (e.g. insufficient testing capacity or personal protective equipment) participants may be asked to self-collect throat/nose swabs for later collection by study staff.

Given the minor risks of BCG vaccination, the potential benefits of BCG vaccination for the participants (by reducing COVID-19 and other respiratory infections), the healthcare system (by reducing absenteeism) during this current COVID-19 outbreak far outweigh them. In addition to this current outbreak, the findings of this study have major implications for future outbreak responses globally. If BCG vaccination is found to be effective at reducing COVID-19, BCG vaccination could be implemented as an early preventative intervention in future outbreaks to protect healthcare workers globally. BCG vaccine is cheap and already administered to infants in over 80% of countries worldwide, therefore implementation of

BCG vaccination campaigns during outbreaks is a feasible intervention to complement other preventative strategies.

We will be using BCG vaccine outside of its standard/recommended use, therefore, as per use of any intervention outside of standard regulations we will be assessing the reactogenicity and safety of BCG vaccination in vaccine naïve and previously vaccinated healthcare workers.

Risks will be continuously reviewed by continuously checking the literature and communicating with the other research group doing similar BCG trials. We have planned an interim analysis as well within our own cohort.

3 TRIAL OBJECTIVES AND OUTCOMES

3.1 Objectives

Two primary outcomes have been chosen for this study: occurrence of COVID-19 disease and occurrence of severe COVID-19 disease. Considering the number of unknown factors and the little knowledge of this new virus, we deemed it of clinical importance to have sufficient power to detect the potential effect of BCG vaccine compared to control for both outcomes (occurrence of any COVID-19 disease, as well as occurrence of severe COVID-19). Our hypothesis is that, compared to control, the BCG vaccine will reduce both the number of cases of COVID-19 (increase the number of asymptomatic SARS-CoV-2 infections) and the number of severe cases of COVID-19. In other words, we have the hypothesis that BCG vaccine would be able to shift the “severity of COVID-19” curve down, i.e. to generally reduce the severity of the symptoms in healthcare workers. Because of the potential for multiplicity testing, the method of controlling type I error is explained in the sample size section (11.1).

3.1.1 Primary objective

1. To determine if BCG vaccination (Intervention) compared with placebo (Comparator) reduces the incidence of COVID-19 disease (Outcome) measured over the 6 months following randomisation (Time) in healthcare workers exposed to SARS-CoV-2 (Participants).
2. To determine if BCG vaccination (Intervention) compared with placebo (Comparator) reduces the incidence of severe COVID-19 disease (with COVID 19 related death, hospitalisation, or non-hospitalised severe disease (defined as Non-ambulant¹ for ≥ 3 consecutive days OR Unable to work² for ≥ 3 consecutive days) (Outcome) measured over the 6 months following randomisation (Time) in healthcare workers exposed to SARS-CoV-2 (Participants).

¹ “pretty much confined to bed (meaning finding it very difficult to do any normal daily activities”

² “I do not feel physically well enough to go to work”

3.1.2 Secondary objectives

3. To determine if BCG vaccination (Intervention) compared with placebo (Comparator) reduces the incidence of COVID-19 disease (Outcome) measured over the 12 months following randomisation (Time) in healthcare workers exposed to SARS-CoV-2 (Participants).

4. To determine if BCG vaccination (Intervention) compared with placebo (Comparator) reduces the incidence of severe COVID-19 disease (non-hospitalised severe disease, hospitalisation or death) (Outcome) measured over the 12 months following randomisation (Time) in healthcare workers exposed to SARS-CoV-2 (Participants).
5. To determine if BCG vaccination (Intervention) compared with placebo (Comparator) prolongs the time to first SARS-CoV-2-proven respiratory illness (Outcome) measured over 6 and 12 months following randomisation (Time) in healthcare exposed to SARS-CoV-2 (Participants).
6. To determine if BCG vaccination (Intervention) compared with placebo (Comparator) reduces the severity of COVID-19 disease (Outcome) measured over 6 and 12 months following randomisation (Time) in healthcare workers exposed to SARS-CoV-2 (Participants).
7. To determine if BCG vaccination (Intervention) compared with placebo (Comparator) reduces the rate and severity of illness (fever or at least one sign or symptom of respiratory disease) measured over 6 and 12 months following randomisation (Time) in healthcare workers (Participants).
8. To determine if BCG vaccination (Intervention) compared with placebo (Comparator) reduces absenteeism (days off work) in healthcare workers (Participants).
9. To evaluate the safety of BCG vaccination in adult healthcare workers.

3.1.3 Planned exploratory analyses

10. To determine in a subgroup of adults with recurrent cold sores whether BCG vaccination compared with placebo reduces herpes simplex recurrences (such as cold sores).
11. To determine the BCG vaccination induced changes in the immune system that are associated with protection of adult healthcare workers from non-tuberculous infectious diseases including COVID-19.
12. To determine and compare changes in the immune system induced by vaccination of adult healthcare workers.
13. To identify factors (e.g. age, sex, chronic conditions such as diabetes and cardiovascular disease, smoking, asthma, prior BCG vaccination, genetics, other vaccinations including COVID-19-specific vaccines, latent TB, immunological/molecular factors) that influence adult immune responses, infection and COVID-19 risk.

3.2 Outcomes

OBJECTIVE	OUTCOME & OUTCOME MEASURE
Primary	
<p>1. To determine if BCG vaccination (Intervention) compared with placebo (Comparator) <u>reduces the incidence of COVID-19 disease</u> (Outcome) measured over the 6 months following randomisation (Time) in healthcare workers exposed to SARS-CoV-2 (Participants).</p>	<p>Number of participants with COVID-19 disease defined as</p> <p><u>Case definition</u></p> <p>"- positive SARS-CoV-2 test (PCR, antigen or serology), plus</p> <ul style="list-style-type: none"> - fever (using self-reported questionnaire), OR - at least one sign or symptom of respiratory disease including cough, sore throat, shortness of breath, respiratory distress/failure (using self-reported questionnaire)" <p>over the 6 months following randomisation</p>
<p>2. To determine if BCG vaccination (Intervention) compared with placebo (Comparator) <u>reduces the incidence of severe COVID-19 disease</u> (with COVID related hospitalisation, death, or non-hospitalised severe disease) (Outcome) measured over the 6 months following randomisation (Time) in healthcare workers exposed to SARS-CoV-2 (Participants).</p>	<p>Number of participants with severe COVID-19 disease with COVID related hospitalisation, death, or with non-hospitalised severe disease</p> <p>Case definition</p> <p>Non-ambulant¹ or ≥ 3 consecutive days OR Unable to work² for ≥ 3 consecutive days, or death (Outcome) measured over the 6 months following randomisation (Time) in healthcare workers exposed to SARS-CoV-2 (Participants).</p> <p>¹ "pretty much confined to bed (meaning finding it very difficult to do any normal daily activities"</p> <p>² "I do not feel physically well enough to go to work"</p> <p>(excludes stay at home exclusively for quarantine/workplace restrictions)</p>
Secondary	
<p>3. To determine if BCG vaccination (Intervention) compared with placebo (Comparator) <u>reduces the incidence of COVID-19 disease</u> (Outcome) measured over the 12 months following randomisation (Time) in healthcare workers exposed to SARS-CoV-2 (Participants).</p>	<p>Number of participants with COVID-19 disease as defined above over the 12 months following randomisation</p>
<p>4. To determine if BCG vaccination (Intervention) compared with placebo (Comparator) <u>reduces the incidence of severe COVID-19 disease</u> (COVID related hospitalisation, death, or non-hospitalised severe disease) (Outcome) measured over 6 and 12 months following randomisation (Time) in healthcare workers exposed to SARS-CoV-2 (Participants).</p>	<p>Number of participants with severe COVID-19 disease as defined above over the 12 months following randomisation</p>
<p>5. To determine if BCG vaccination (Intervention) compared with placebo (Comparator) <u>prolongs the time to first SARS-CoV-2-proven respiratory illness</u> (Outcome) measured over 6 and 12 months following randomisation (Time) in healthcare workers exposed to SARS-CoV-2 (Participants).</p>	<p>Time to first symptom of COVID-19 in a participant who subsequently meets the case definition over the 12 months following randomisation.</p>

<p>6. To determine if BCG vaccination (Intervention) compared with placebo (Comparator) <u>reduces the severity of COVID-19 disease</u> (Outcome) measured over 6 and 12 months following randomisation (Time) in healthcare workers exposed to SARS-CoV-2 (Participants).</p>	<p>All the following measures will be assessed, over the 12 months following randomisation</p> <p>Number of participants with COVID-19 disease as defined above</p> <p>Number of episodes of COVID-19 disease as defined above</p> <p>Number of participants with asymptomatic SARS-CoV-2 infection defined as</p> <ul style="list-style-type: none"> - Evidence of SARS-CoV-2 infection (by PCR or seroconversion) - Absence of respiratory illness (using self-reported questionnaire) - No evidence of exposure prior to randomisation (inclusion serology negative) <p>Number of days unable to work (using self-reported questionnaire) due to COVID-19 disease as defined above (excludes quarantine/workplace restrictions)</p> <p>Number of days confined to bed (using self-reported questionnaire) due to COVID-19 disease as defined above</p> <p>Number of days with symptoms in any episode of illness that meets the above the case definition for COVID-19 disease</p> <p>Number of pneumonia cases (abnormal chest X-ray) (using self-reported questionnaire and/or medical/hospital records) associated with a positive SARS-CoV-2 test</p> <p>Need for oxygen therapy (using self-reported questionnaire and/or medical/hospital records) associated with a positive SARS-CoV-2 test</p> <p>Number of admission to critical care and duration of stay (using self-reported questionnaire and/or medical/hospital records) associated with a positive SARS-CoV-2 test</p> <p>Need of mechanical ventilation and duration (using self-reported questionnaire and/or medical/hospital records) and a positive SARS-CoV-2 test</p> <p>Duration of hospitalisation due to COVID-19 (using self-reported questionnaire and/or medical/hospital records)</p> <p>Number of deaths (from death registry) associated with a positive SARS-CoV-2 test</p> <p>Data will be collected in self-reported participant questionnaires, medical/hospital records and/or government registries</p>
<p>7. To determine if BCG vaccination (Intervention) compared with placebo (Comparator) <u>reduces the rate and severity of illness</u> (fever or at least one sign or symptom of respiratory disease) measured over the 12 months following randomisation (Time) in healthcare workers (Participants).</p>	<p>All the following measures will be assessed, over the 12 months following randomisation</p> <p>For the following outcomes, fever or respiratory illness will be defined as:</p> <ul style="list-style-type: none"> - fever (using self-reported questionnaire), or - at least one sign or symptom of respiratory disease including cough, sore throat, shortness of breath, respiratory distress/failure, runny/blocked nose (using self-reported questionnaire)

	<p>Number of participants with fever or respiratory illness, as defined above</p> <p>Number of episodes of fever or respiratory illness, as defined above</p> <p>Number of days unable to work (using self-reported questionnaire) due to fever or respiratory illness, as defined above (excludes quarantine/workplace restrictions)</p> <p>Number of days confined to bed (using self-reported questionnaire) due to fever or respiratory illness, as defined above</p> <p>Number of days with symptoms in any episode of illness that meets the above the case definition fever or respiratory illness</p> <p>Number of pneumonia cases (abnormal chest X-ray) (using self-reported questionnaire and/or medical/hospital records)</p> <p>Need for oxygen therapy (using self-reported questionnaire and/or medical/hospital records)</p> <p>Number of admission to critical care (using self-reported questionnaire and/or medical/hospital records)</p> <p>Need of mechanical ventilation (using self-reported questionnaire and/or medical/hospital records)</p> <p>Number of deaths (from death registry)</p> <p>Duration of hospitalisation due to fever or respiratory illness (using self-reported questionnaire and/or medical/hospital records)</p> <p>This data will be collected in self-reported participant questionnaires, medical/hospital records and/or government registries</p>
8. To determine if BCG vaccination (Intervention) compared with placebo (Comparator) <u>reduces absenteeism</u> (days off work) in healthcare workers (Participants).	Number of days of unplanned absenteeism for any reason (using self-reported questionnaire) over the 12 months following randomisation
9. To evaluate the <u>safety of BCG vaccination</u> in healthcare workers.	<p>Type and severity of adverse events of interest over the 3 months following randomisation will be collected and graded using toxicity grading scale.</p> <p>Serious Adverse Events, over the 3 months following randomisation</p>
Exploratory analyses	
10. To determine in a subgroup of participants with recurrent cold sores whether BCG vaccination compared with placebo <u>reduces herpes simplex recurrence (such as cold sores)</u> .	<p>Number of participants with herpes simplex recurrence (self-reported e) over the 12 months following randomisation</p> <p>Number of episodes of herpes simplex recurrence (self-reported) over the 12 months following randomisation</p> <p>Time: to first of herpes simplex recurrence (self-reported) over the 12 months following randomisation</p>

<p>11. To determine the impact of BCG vaccination on the immune system that are associated with protection of adult healthcare workers from non-tuberculous infectious diseases including COVID-19.</p> <p>12. To determine and compare changes in the immune system induced by vaccination of adult healthcare workers.</p>	<p>The immune system will be assessed by several methods including:</p> <ul style="list-style-type: none"> - Cytokine levels in supernatants from whole blood stimulated with off-target pathogens (including BCG, <i>Staphylococcus aureus</i>, <i>Escherichia coli</i>) and Toll-like receptor (TLR) agonists, measured by multiplex - Cytokine production, activation and differentiation of immune cells (measured by flow cytometry) - Epigenetic modifications (e.g. histone methylation/acetylation and CpG methylation) measured by ChIP-Seq and/or microarray - Anti-vaccine and anti-pathogen (including SARS-CoV2) antibody levels measured by ELISA, multiplex or VirScan - RNA expression measured by qRT-PCR or RNA-Seq
<p>13. To identify factors (e.g. age, sex, chronic conditions such as diabetes and cardiovascular disease, smoking, asthma, prior BCG vaccination, genetics, other vaccinations including COVID-19-specific vaccines, latent TB, immunological/molecular factors) that influence adult immune responses, infection and COVID-19 responses.</p>	<p>Association of demographic factors, exposure, genetic factors (e.g. single nucleotide polymorphisms) and immune factors (e.g. cell numbers, circulating cytokines, anti-vaccine/anti-pathogen antibodies) with the function of the immune system as described above and COVID-19 prevalence or severity as defined above</p>

TRIAL DESIGN

3.3 Overall design

This is a phase III, two group, multicentre, randomised placebo controlled trial in up to 7244 frontline healthcare workers to determine if BCG vaccine reduces prevalence and the severity of COVID-19 disease during the 2020 SARS-CoV-2 pandemic. As part of this study we plan to combine the data from this study in a pre-planned meta-analysis with data from the 2834 participants recruited in the first stage of this study which followed the same protocol but where participants were randomised between BCG and no BCG at the time of receiving a flu vaccination, for a total sample size of 10078. Although we recognise that the first stage of this study was addressing a slightly different research question, we feel that it is important to combine data from the initial stage of study as they both provide estimates of the efficacy of the BCG vaccination, which is critical to provide adequate power to determine the efficacy of the BCG vaccination.

In Europe, healthcare workers from the Netherlands, the UK, Spain and possibly other countries will be recruited across multiple sites.

In Australia, participating sites are hospitals within Victoria, Western Australia, South Australia and New South Wales. Australian sites involved in this study include the RCH VIC, Monash Health VIC, Epworth Healthcare VIC, Perth Children’s Hospital WA, Fiona Stanley Hospital WA, Sir Charles Gairdner Hospital WA, Royal Adelaide Hospital SA, Women’s and Children’s Hospital Adelaide SA, The Children’s Hospital at Westmead NSW, Westmead Hospital NSW, Prince of Wales Hospital NSW, St Vincent’s Hospital Sydney NSW and Sydney Children’s Hospital, Randwick NSW.

In Brazil, there will be a participating sites in Mato Grosso do Sul State and Rio de Janeiro. In Mato Grosso do Sol the principal site will the Faculty of Medicine of the Federal University of Mato Gross do Dul (UFMS) with additional locations: Regional Hospital of Mato Grosso do

Sul, Hospital CASSEMS, Hospital Santa Casa and Municipal Health Units. In Rio de Janeiro the principal site will be the Centro de Referência Prof Hélio Fraga (CRPFH) da Escola Nacional de Saúde Pública Sérgio Arouca (ENSP), FIOCRUZ and Municipal Health Office of Rio de Janeiro. Other sites in Rio de Janeiro and Mato Grosso do Sul will be identified.

Recruitment may be held at participating sites or centrally identified locations with appropriate safety and privacy infrastructure.

Participants will be randomised to receive BCG or placebo.

During the initial stage of the study in Australia, randomisation and immunisation coincided with the annual staff influenza immunisation roll out at each hospital. During the first stage of the study, influenza vaccine occurred at the same time as randomisation and BCG vaccination or no BCG for 2834 health care workers. During the second stage in locations where the annual influenza vaccine is available, participants are asked to confirm they have received the influenza vaccination a minimum of 72 hours prior to randomisation.

The control group will receive a placebo injection of 0.9% NaCl. Most people vaccinated with the BCG vaccine develop a papule/blister at the injection site around two-weeks after vaccination. Due to this, even using a placebo, it is not possible to completely blind participants to their treatment group allocation. The outcomes (incidence of COVID-19 disease or admission to hospital for COVID-19 disease) are objective measures, it is however still plausible that participant's suspicion of their group allocation might bias the study results. This risk will be mitigated by using a placebo where an element of doubt over treatment allocation may persist even in the absence of scar formation. Members of the research team doing follow-up, data cleaning and analysis will be blinded to the group allocation (by the hiding of this variable and all other variables related to BCG from the dataset) until the formal detailed statistical analysis plan is confirmed and signed by all investigators and all data cleaning/preparation is complete.

Randomisation will be stratified for all factors that might influence the effectiveness of the intervention. For more details see section 6.

Follow-up for all participants will last 1-year. For each episode of fever with a respiratory symptom during the follow-up period, all participants complete a survey in a smartphone app, electronic message or by phone, and may have a home visit by members of the research team for sample collection (e.g. if the government ceases or limits COVID-19 testing; respiratory swab preferred, however blood sample will be taken if no swab testing kits are available). If necessary (e.g. insufficient testing capacity or personal protective equipment) participants may be asked to self-collect throat/nose swabs for later collection by study staff, or self-test a finger-prick blood sample and send a photo of the results to the study team.

3.4 Justification for dose

The dose and route of BCG administration are the standard accepted dosage for BCG vaccine when used to prevent TB. There is no justification to vary from this.

3.5 Trial population

4.3.1 Eligibility criteria

Participants will be assigned to a randomised trial treatment only if they meet all of the inclusion criteria and none of the exclusion criteria.

4.3.2 Inclusion criteria

- Over 18 years of age
- Healthcare worker
 - This is defined as anyone who works in a healthcare setting or has face to face contact with patients.
- Provide a signed and dated informed consent form
- Australian sites only: If annual influenza vaccination is available, receiving the flu vaccine is an eligibility requirement. The flu vaccine will be required a minimum of 3 days in advance of randomisation in the BRACE trial.
- Pre-randomisation blood collected

4.3.3 Exclusion criteria

- Has any BCG vaccine contraindication
 - Fever or generalised skin infection (where feasible, randomisation can be delayed until cleared)
 - Weakened resistance toward infections due to a disease in/of the immune system
 - Receiving medical treatment that affects the immune response or other immunosuppressive therapy in the last year.
 - These therapies include systemic corticosteroids (≥ 20 mg for ≥ 2 weeks), non-biological immunosuppressant (also known as 'DMARDS'), biological agents (such as monoclonal antibodies against tumour necrosis factor (TNF)-alpha).
 - People with congenital cellular immunodeficiencies, including specific deficiencies of the interferon-gamma pathway
 - People with malignancies involving bone marrow or lymphoid systems
 - People with any serious underlying illness (such as malignancy)
 - NB: People with cardiovascular disease, hypertension, diabetes, and/or chronic respiratory disease are eligible if not immunocompromised, and if they meet other eligibility criteria
 - Known or suspected HIV infection,¹¹ even if they are asymptomatic or have normal immune function.

- This is because of the risk of disseminated BCG infection^{12,13}
- People with active skin disease such as eczema, dermatitis or psoriasis at or near the site of vaccination
 - A different adjacent site on the upper arm can be chosen if necessary
- Pregnant
 - Although there is no evidence that BCG vaccination is harmful during pregnancy, it is a contra-indication to BCG vaccination. Therefore, we will exclude women who think they could be pregnant or are planning to become pregnant within the next month.
 - UK specific: Although there is no evidence that BCG vaccination is harmful during pregnancy, it is a contra-indication to BCG vaccination. Therefore, we will exclude women of childbearing potential (WOCBP) who think they could be pregnant. See section 8.2 for definition of WOCBP and Appendix 3 for UK specific pregnancy test requirements.
 - Spain specific: If the patient is female, and of childbearing potential, she must have a negative pregnancy test (provided by Sponsor) at the time of inclusion and practice a reliable method of birth control for 30 days after receiving the BCG vaccination. See Appendix 6 for Spain specific requirements
- Another live vaccine administered in the month prior to randomisation
- Require another live vaccine to be administered within the month following BCG randomisation
 - If the other live vaccine can be given on the same day, this exclusion criteria does not apply
- Known anaphylactic reaction to any of the ingredients present in the BCG vaccine
- Previous active TB disease
- Currently receiving long term (more than 1 month) treatment with isoniazid, rifampicin or quinolone as these antibiotics have activity against *Mycobacterium bovis*
- Previous adverse reaction to BCG vaccine (significant local reaction (abscess) or suppurative lymphadenitis)
- BCG vaccine given within the last year
- Have previously had a SARS-CoV-2 positive test result (positive PCR on a respiratory sample or a positive SARS-CoV-2 diagnostic antigen test approved by the local jurisdiction's public health policy)

- Already part of this trial, recruited at a different site/hospital.
- Participation in another COVID-19 prevention trial
- Have previously received a COVID-19-specific vaccine

4.4 Lifestyle considerations

Not applicable

4.5 Screen failures

Screen failures are defined as participants who consent to participate in the trial but who are found, during the screening procedures, to be ineligible to continue into the trial. They therefore do not receive the intervention / are not randomised.

4.6 Recruitment and Consent

Potential participants will receive information (via email, healthcare facilities notice board and/or website/social media etc) about the trial. This will include a short blurb about the study and a link to a website where they can read further information contact details for further questions. Potential participants will be able to evaluate their eligibility online via the REDCap public link and having met the eligibility criteria access the site specific participant information and consent form (PICF) prior to attending clinic.

Interested healthcare workers will be given the opportunity to talk with a member of the research team by phone or video conferencing if they have any questions (social distancing practices will still be applied wherever possible). The process will vary slightly between locations due to contextual adaptations, however, it will be built around the same core essentials:

- Providing accurate information regarding the trial through a combination of publicly available information and additional detailed explanation by trained study staff
- Eligibility screening for all participants. If participants are ineligible no identifying information will be collected
- Informed consent secured from all participants through signed (electronic or hard copy) PICFs. Consent will be voluntary and free from coercion.
- Study staff will confirm eligibility and consent with prospective participants.

The webpage text, PICFs (electronic or hard copy) and eligibility questionnaire will have prior approval of HREC before use.

In Australia, influenza vaccination 72 hours prior to randomisation is an inclusion criteria as outlined in 4.3.2. For sites outside Australia, the research team will provide recommendation to all participants not to have the influenza vaccine within the 72 hours prior or post randomisation.

For those who are eligible and provide informed consent, they will be asked to provide their contact details (including date of birth, healthcare card number (or equivalent), name and other identifying details) and a baseline questionnaire on participant characteristic (demographics and environmental information) into either REDCap database or hard-copy forms based on national privacy regulations.

Participants will be told when completing eligibility check (before consenting) that pregnancy, or planning to become pregnant within the next month is a contraindication to getting BCG. We will ask that if they are unsure to do a home pregnancy test, and on the day of randomisation we will have pregnancy tests available that they can use to take away self-test at the site before randomisation. In the UK and Spain completing a pregnancy tests will be eligibility requirement as outlined in Appendix 3 and 6. This will be done in a subtle way to limit the likelihood that other staff will be aware that they have requested a pregnancy test. We have structured it this way to allow people to test in the privacy of their homes rather than have a conversation with the researchers.

Because only 10,078 participants are to be recruited over multiple sites, it is possible that more staff will be interested in participating than can be included in the trial. Given there will likely be interested participants who complete e-consent (where relevant) but are not randomised (become sick, become ineligible, changed their mind) we will continue recruitment until we reach the required number of participants randomised (10,078 participants). Randomisation will cease on the day that 10,078 participants are randomised. On the consent form and other pre-information, interested participants will be informed that due to the limited numbers who can be included in the trial, despite consenting, we cannot guarantee they will be randomised.

Given the importance of finding an intervention that can be used early in future pandemics (before a disease-specific vaccine is available), we expect there will be significant interest from researchers to try and understand how BCG works to boost the immune system. To this end, we will include an optional consent for participants to indicate whether they are interested in being approached for other projects.

No identifying information will be provided to the hospital or recruiting sites regarding any staff who have consented to be part of the trial.

4.7 Pre-randomisation blood sample

To remain eligible for randomisation in BRACE a pre-randomisation bloods sample must be provided. This blood sample will be taken at enrolment but can be taken up to 24 hours prior to randomisation. This sample cannot be taken after administration of the intervention or placebo.

4.8 Re-consent

As required, participants will be contacted through REDCap and sent appropriate and relevant information for re-consent. Re-consent materials will contain contact details for the study team so that participants can ask questions. Participants will be asked if they agree to the changes by signing the re-consent in either electronic or hard copy format depending on country specific ethics requirements. All participant information for re-consent will be approved by HREC prior to use.

4 INTERVENTION

4.1 Treatment arms

Intervention group: BCG vaccine

Comparator group: 0.9% Saline

4.2 Trial Intervention(s)

4.2.1 Description of trial investigational products

4.2.1.1 BCG vaccine SSI

	Freeze-dried powder: Live attenuated bacteria of the type <i>Mycobacterium bovis</i> BCG (Bacillus Calmette-Guerin), Danish strain 1331 0.1 ml vaccine contains between 2 to 8 x 10 ⁵ colony forming units.
Active substance and excipients	Powder Excipient: Sodium glutamate Solvent for resuspension: magnesium sulphate heptahydrate, dipotassium phosphate, citric acid monohydrate, l-asparagine monohydrate, ferric ammonium citrate, glycerol 85%, and water for injections.
Trade or Generic name	BCG Vaccine SSI
Dosage form	Powder for Injection with solvent for resuspension
Route of administration	Intradermal

5.2.1.2 Placebo to match BCG vaccine SSI

Active substance and excipients	Sodium Chloride 0.9%.
Trade or Generic name	Sodium Chloride Injection BP or USP
Dosage form	Ampoule (10 mL)
Route of administration	Intradermal

4.2.2 Dosage

A single dose of BCG vaccine SSI or matched placebo will be given to all participants who are randomised. The adult dose is 0.1 mL (of BCG vaccine SSI or 0.9% NaCl) injected intradermally over the distal insertion of the deltoid muscle onto the humerus (approximately one third down the upper arm).

4.2.3 Dose modification

There are no allowable dose modifications

4.2.4 Storage and dispensing of BCG vaccine SSI

- Store between 2°C - 8°C
- Store in the original package in order to protect from light
- Do not freeze
- Do not use the vaccine after the expiry date which is stated on the carton as “EXP” and refers to the last day of the month listed
- Any unused vaccine at the end of the study, meaning vaccines unused after the last dosing of the last participant will be disposed of according to local regulations

Placebo – sodium chloride 0.9%

- Store less than 25°C
- Do not use after the expiry date which is stated on the carton and ampoule as “EXP” and refers to the last day of the month listed
- Any unused sodium chloride 0.9% at the end of the study, unused ampoules after the last dosing of the last participant will be disposed of according to local regulations

4.2.5 Preparation

BCG Vaccine SSI

BCG Vaccine SSI consists of a powder and solvent for suspension for injection ($2-8 \times 10^5$ CFU/0.1 mL dose).

Prior to reconstitution, the storage temperature of the BCG will be checked to ensure it the appropriate temperature has been maintained during storage, and transport (if applicable) unless the storage and transport has occurred in validated containers or conditions where the temperature is stable and the data readily available.

The rubber stopper must not be wiped with any antiseptic or detergent. In the eventuality of alcohol being used to swab the rubber stopper, it must be allowed to evaporate before the stopper is penetrated with the syringe needle. The BCG is re-suspended using the solvent provided according to the product directions then carefully inverted a few times to produce uniform resuspension of the lyophilised BCG. Study staff must not shake the vial. The study staff member who re-suspends the BCG will label the vial with the date, time of reconstitution and their initials.

To ensure a uniform suspension, and therefore dose, the vial will be gently swirled before drawing up each dose. When drawn up into the syringe the reconstituted vaccine should appear homogeneous, slightly opaque and colourless.

Each vial of BCG contains up to 10 adult doses. Study staff must NEVER administer the whole vial. Each vial can be kept for up to 4 hours after resuspension. During this time the vial is kept between 2-8°C. Each vial is discarded after 4 hours, or- when the vial is empty, whichever occurs first.

Sodium Chloride 0.9% placebo

During each recruitment session sodium chloride 0.9% will be decanted using aseptic technique into an empty sterile amber glass vial or prepared in 0.1 mL dosing syringes as per local vaccination practices. The study staff member who prepares the sodium chloride for injection will record the date, time of the preparation and their initials.

The prepared sodium chloride for placebo can be kept for up to 24 hours. During this time the placebo is kept between 2-25°C. All prepared syringes or vials unused at the end of a vaccination session will be discarded.

4.2.6 Administration of trial drug

The vaccine or placebo will only be administered by clinician members of the study team trained in the intradermal vaccination technique.

The vaccinator will follow the vaccination SOP and relevant site safety requirements.

Administration of the BCG vaccine or placebo will take place in locations set-up by the study team prioritising participant safety for example ensuring appropriate facilities for management of any potential adverse event are available (e.g anaphylactic reaction, extremely rare). There will be space to allow for privacy for the participant if required (e.g. upper left/right arm not accessible due to clothing).

As per standard practice, participants will be required to remain at the site for 20 minutes after vaccination, in case an allergic reaction should occur, wearing a sticker "I have received the BCG vaccine at [time of vaccination]" for both BCG and placebo recipients.

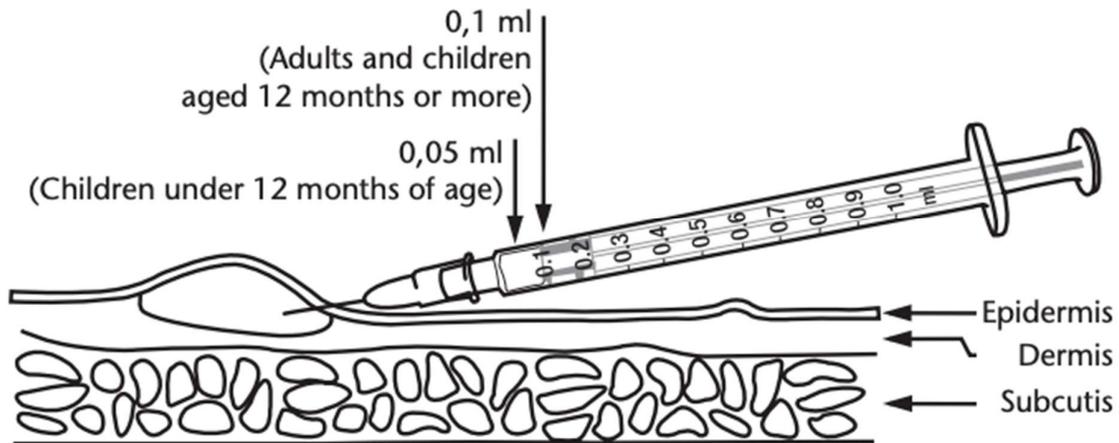
The time and date of resuspension of the vaccine vial, or placebo preparation, batch identifier, immunisation date/time, any issues with immunisation will be entered in the participants' study record in REDCap Vaccinators database.

Route/method of administration

The injection site should be clean and dry using non-alcohol based antiseptic. Alcohol antiseptics should not be used prior to administration. If alcohol is used to swab the skin, it must be allowed to evaporate before the vaccine or placebo is injected. The vaccine or placebo must be given strictly intradermally, approximately one third down the upper arm corresponding to the area of the distal insertion of the deltoid muscle, as follows:

- The skin is stretched between thumb and forefinger
- The needle should be almost parallel with the skin surface and slowly inserted (bevel upwards), approximately 2 mm into the superficial layers of the dermis. The needle should be visible through the epidermis during insertion

- The vaccine or placebo should be given slowly



- The mixed vaccine should be administered with a syringe of 1 ml graduated into hundredths of millilitre (1/100) fitted with a short bevel syringe needle (Preference to use 25G or 26G, accepted up to 30G).
- You should feel considerable resistance as you give the injection. If there is no resistance, the needle may be in the subcutaneous tissues.
- If the injection is not intradermal, withdraw the needle and repeat at a new site.
- A raised, blanched papule/bleb of about 7 mm diameter (looks like orange peel) at the needle point is a sign of correct injection
- The injection site is best left uncovered to facilitate healing
- Jet injectors or multiple puncture devices should not be used to administer the vaccine.
- A photo of the bleb (with measuring tape or 10 cent coin used as scale (or equivalent in local currency)) should be uploaded in REDcap form.

Over/under dosage or incorrect administration

Overdose increases the risk of suppurative lymphadenitis and may lead to excessive scar formation. Gross over dosage increases the risk of undesirable BCG complications. Deep injections increase the risk of lymphadenitis and abscess formation.

The clinician members of the research team who administers BCG or placebo as part of this trial will be required to document whether the vaccination was given 'perfectly' with appropriate bleb. Any variations will be documented, and standard procedures followed regarding the need for re-administration, notification to RPI (or delegate).

Complications

All BCG-related complications will be referred to the SPI for advice regarding management. In the very unlikely event a participant has a systemic infection of *Mycobacterium bovis* or persistent local infection following vaccination the SPI will provide advice to the local treating team regarding management, including antibiotic treatment choice. Any serious adverse event or adverse event of interest occurring during the administration of the IP or the 20 minutes post administration will be documented appropriately according to the safety monitoring and reporting section of this protocol.

4.2.7 Product accountability

A pharmacy in each region will act as the study central pharmacy and co-ordinate the storage, distribution and maintain accountability records of the BCG vaccine and placebo supply in that region as appropriate. The RCH pharmacy will act as the study central pharmacy for Australia. The UMC Utrecht pharmacy will be the study central pharmacy in mainland Europe. A UK based pharmacy will act as the central study pharmacy should any UK sites be included in the trial. The LAC/UFMS will act as the central pharmacy in Mato Grosso do Sul, Brazil and a central pharmacy in Rio De Janeiro will be identified. Trial accountability of IP including documentation of storage, dispensation and destruction (if required) will be maintained in the pharmacy files at each region/site as appropriate. A pharmacy summary/manual will outline the specific processes for each region in line with local processes and regulations.

Any reason for departure from the expected dispensing regimen will be recorded. At the end of the trial, there will be final reconciliation of trial drug received, dispensed, used and returned. Any discrepancies will be investigated, resolved and documented by the study team.

4.2.8 Excluded medications and treatments

BCG vaccination may be given on the same day of any inactivated or live vaccines. If not given on the same day a period of not less than 4 weeks must pass before giving another live vaccine (although there is no real data supporting this precaution). There must be an interval of at least 3 months before a vaccination in the same arm can take place. Inactivated vaccine (such as the diphtheria-tetanus-pertussis vaccine) can be given in the other arm at any time before, during, or after BCG vaccination if needed.

Participants should not take part in any other COVID-19 preventative intervention clinical trials during the 6 month follow-up period.

4.2.9 Discontinuation from trial intervention

The trial intervention is a once-off vaccination. Due to this there is no possibility to 'discontinue the trial intervention'. If a participant changes their mind between randomisation and vaccination, deciding that they do not want to have the vaccination (but are happy to continue in the study for the follow-up period) they will be included in the analysis as intention to treat.

5 RANDOMISATION AND BLINDING

Once consent has been obtained, and following baseline assessment, eligible participants will be recruited and randomised on the day of the enrolment via Redcap. Randomisation will be to intervention or placebo group with an allocation ratio of 1:1, using a web-based randomisation procedure. The randomisation schedule and web-based service will be provided by an independent statistician from the Clinical Epidemiology and Biostatistics Unit (CEBU) at the Murdoch Children's Research Institute. Randomisation will be in randomly permuted blocks of variable length (2, 4, or 6). Randomisation will be stratified by stage of the study (prior to or post the addition of the placebo vaccination), study site, by age (<40 years; 40 to 59 years; >=60 years) and by presence of comorbidity (any of diabetes, chronic respiratory disease, cardiac condition, hypertension). Stratification by age is necessary for data analysis because

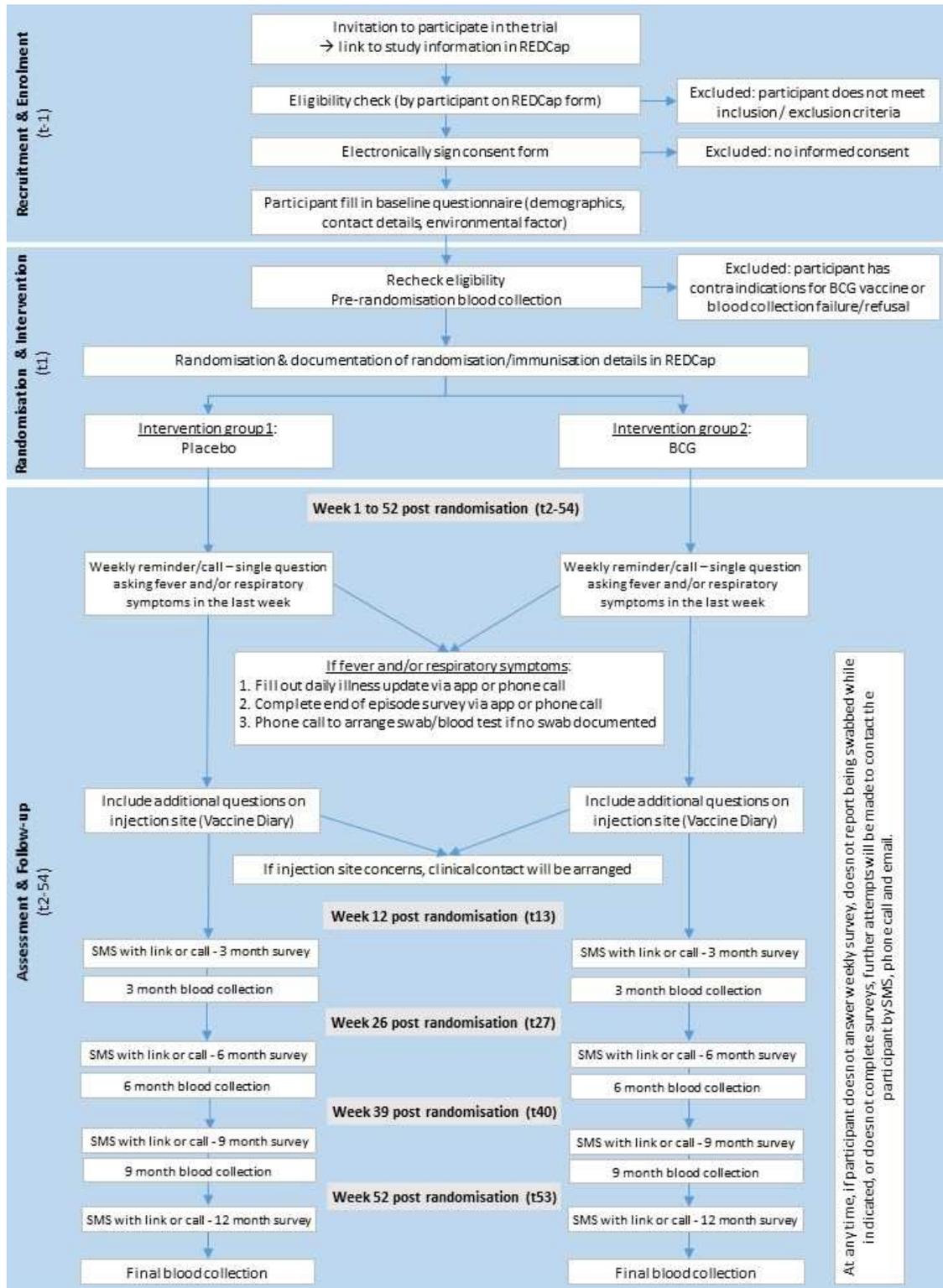
older ages are associated with a greater likelihood of developing severe COVID-19. Likewise, presence of comorbidity is associated with a greater risk of developing severe COVID-19. Each study site will have their own randomisation list stratified by study stage (where relevant), age and presence of comorbidity.

5.1 Concealment mechanism

The control group will receive a placebo of 0.9% NaCl. Most people vaccinated with the BCG vaccine develop a papule/blister at the injection site around two-weeks after vaccination. Due to this, even using a placebo, it is not completely possible to blind participants to their treatment group allocation. The outcomes (incidence of COVID-19 disease or admission to hospital for COVID-19 disease) are objective measures, it is however still plausible that participant's suspicion of their group allocation might bias the study results. This risk will be mitigated by using a placebo where an element of doubt over treatment allocation may persist even in the absence of scar formation. Members of the study team, except immunisers, will be blinded to the group allocation (by the removal of this variable and all other variables related to BCG from the dataset) until the formal detailed statistical analysis plan is confirmed and signed by all investigators and all data cleaning/preparation is complete.

6 TRIAL VISITS AND PROCEDURES

6.1 TRIAL TIMELINE



6.2 Schedule of assessments

TIME POINT	TRIAL PERIOD									
	Pre-study	Inclusion & randomisation	Post-randomisation							
	<i>t</i> ₋₁	<i>t</i> ₁	<i>t</i> ₂₋₁₂	<i>t</i> ₁₃	<i>t</i> ₁₄₋₂₆	<i>t</i> ₂₇	<i>t</i> ₂₈₋₃₉	<i>t</i> ₄₀	<i>t</i> ₄₁₋₅₂	<i>t</i> ₅₃
RECRUITMENT:										
Eligibility screen	X									
Informed consent	X									
Contact details	X									
Allocation to intervention		X								
INTERVENTIONS:										
<i>BCG vaccine</i>		X (BCG group)								
<i>Saline injection</i>		X (Placebo group)								
ASSESSMENTS:										
<i>Baseline questionnaire</i>	X	X								
<i>Weekly survey</i>			X	X	X	X	X	X	X	X
<i>Instruction for swab testing</i> <i>(if indicated by weekly survey)</i>			(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)
<i>3-month survey</i>				X						
<i>6-month survey</i>						X				
<i>9-month survey</i>								X		
<i>12-month survey</i>										X
<i>Clinical advice on injection site *</i>			X	X						
<i>Blood collection**</i>		X		X		X		X#		X#
<i>Baseline SARS-CoV-2 Test ***</i>		X								

T=week (e.g. *t*₁=first week). A 42day window period is accepted for the periodic survey and the blood collection timepoints)

* In indicated Infectious Diseases clinician, or state-based organisation, as appropriate

** Optional consent for additional biological sample including blood sample when illness reported

*** Brazil only as outlined in Appendix 4

Sub-set of participants

6.3 Description of procedures

The procedures related to recruitment, consent, eligibility confirmation, randomisation and intervention are described in sections 4 and 5 of this protocol. The procedure for blood collection is described in the relevant SOP. Capture of applicable adverse events is described in section 8.

In Brazil only, a baseline respiratory swab will be collected as outlined in appendix 4.

After randomisation there are two key aspects of the 1-year follow-up period; questionnaires and sample collections for SARS-CoV-2 identification (respiratory swabs or blood samples). Participants will be asked to complete a questionnaire use the smartphone application (app) designed for the trial, electronic messages or via phone calls to report symptoms, access SARS-CoV-2 testing through the public health system and if needed self-collect a respiratory swab each time they have a febrile illness or a respiratory symptom,. Where app is utilised, participants will be trained on how to use the app on day of enrolment.

Questionnaires

Baseline

- Comorbidities: diabetes, cardiovascular disease, chronic respiratory disease, hypertension
- Risk factors: smoking, body mass index (calculated with weight and height)
- BCG/TB history: Prior BCG vaccination, ever positive TST
- Influenza immunisation: date of last influenza vaccine (if within influenza season)
- Exposure (presence of COVID-19 cases at workplace, average days working in hospital or other healthcare settings as appropriate)
- Other: Recurrent herpes infection (such as cold sores)

Regular (generally weekly) questionnaires on smartphone app, via phone call or via electronic messages

- Any symptoms of COVID-19: fever or at least one sign or symptom of respiratory disease such as sore throat, cough, shortness of breath, respiratory distress/failure (y/n)

For each episode of illness (via smartphone app, via phone call or via electronic messages)

- Which symptoms of COVID-19: fever, cough, shortness of breath and/or difficulty breathing, runny/blocked nose, sore throat, fatigue, muscle and/or joint ache, headache, nausea, vomiting and/or diarrhoea, loss of taste and smell
- Has a COVID-19 swab been taken? (if so what was the result)
- Date of/days since onset and cessation of symptoms
- Days absent from work (total number and number due to illness)
- ED presentations
- Hospital admission (oxygen, ICU admission, mechanical ventilation)
- Known test results
- If a swab has been taken for clinical purposes, who ordered it
- Impact on daily activities
- Days in bed
- Chest x-ray results

For local reaction to injection: the Vaccine Diary (daily diary for the two weeks following randomisation)

- Questionnaire collecting common reactions to the injection, including photograph of injection site

Periodic questionnaires (once every 3 months)

- Exposure (presence of COVID-19 cases at workplace, average days working in hospital or other healthcare settings as appropriate)
- Cold Sore recurrence
- Request for participants to confirm the main episodes of illness experienced in the prior 3 months.

- Screening, exposure or treatment of TB
- Detail on other vaccinations
- Hospitalisation (any)
- Information regarding participation in any other COVID-19 preventative intervention clinical trials
- Injection site evolution and side effects (photo of injection site)
- Treatment that could influence COVID-19 outcome

Additional questions for 3rd month questionnaire only:

- Record any non-serious adverse event of interest, including Injection site evolution and side effects (photo of injection site), with onset between randomisation and 3 months post randomisation
- If relevant: Influenza vaccine side effects
- Record any serious adverse event with onset between randomisation and 3 months post randomisation

Swabs

- Where a participant has had a swab sample assessed outside the indication of the study (e.g. with non-respiratory symptoms or asymptomatic) results will be collected via self-report in the 3 monthly questionnaires. All test results will also be obtain, where possible from centralised SARS-CoV-2 testing government database.

Where a participant has symptoms of febrile or respiratory illness (cough, sore throat, shortness of breath) and a swab sample for SARS-CoV-2 is not collected through standard pathways (for example due to swab shortage, or government decision to restrict screening to high-risk patients), a sample collection study visit may be done. A respiratory swab/s may be collected from the participant at home and linked with the relevant public health testing and reporting systems. If respiratory swab/s are done by participant self-collection (e.g. nasal/throat swabs) they will receive full instructions on how to take the samples, when to take them and how to correctly store them until a member of the research team collects them.

Blood samples

- At randomisation, a blood sample will be taken for later assessment of seroconversion (production of specific anti-SARS-CoV-2 antibodies). This will identify participants who had SARS-CoV-2 exposure and immunity prior to commencement of the study.
 - The baseline blood samples will be analysed in batch months after randomisation, so there will be no clinically actionable results. We will provide individualised results to participants via email after completion of the trial. This email will be sent to the applicable HRECs to review before being sent out to any participant.
 - In Brazil, IGRA testing will be completed on pre-randomisation blood samples as outlined in appendix 4.
- At 3 months and 6 months (+ 42 days) post randomisation, the study team will coordinate to collect study blood samples from participants and in a sub-set of

participants at 9 and 12 months (+ 42 days) post randomisation. This will identify participants who had an immune response to SARS-CoV-2 (surrogate marker of infection) during the study. This is needed to determine asymptomatic SARS-CoV-2 infections.

- Blood samples will also be taken for assessment of the immune system. This will be used to meet the planned exploratory analyses related to vaccine induced changes in the immune system. These blood samples will be taken at the same time as blood collection for serum or plasma samples (i.e. at randomisation and post randomisation)
- In the eventuality that it is unfeasible to collect swab samples to confirm SARS-CoV-2 infection at the time of febrile or respiratory illness episodes (or conduct a validated antigen test), seroconversion may be used to associate episodes of febrile or respiratory illness with SARS-CoV-2 infection. Therefore, for episodes of febrile or respiratory illness where a swab sample cannot be taken, 1 month after the onset of symptoms (expected peak post-infection antibody production), participants may be asked to come to the hospital to provide a blood sample. If rapid point-of care testing is available, these tests may be distributed to participants to self-test. Should these alternative methods of testing become required, an amendment will be submitted to HRECs to outline the process and submit any information for participants. This testing will not be conducted without further consultation with and approval from the HRECs, including providing the HRECs with details of the test and its efficacy.

For blood sample collection for serum/plasma plus analysis of the immune system, a venous blood sample (up to 10ml or up to 35ml depending on the study site) will be taken by a trained member of the study team and labelled with participant ID, date/time collected, study timepoint, year of birth (no identifying information). Samples will be transported to the site's designated laboratory for the trial. Samples will be processed for serum/plasma separation and analysis of the immune system and stored at -80°C or in liquid nitrogen for later assessment.

A self-collected dried blood spot may be requested from participants instead of a venous blood sample collection. These may be stored in a locked cabinet prior to elution and storage at -80°C. If blood samples are done by participant self-collection of dried blood spot, participants will receive full instructions on how to take the samples, when to take them and how to correctly store them until they are returned to the study site.

Data Retrieval

Data retrieval and linkage is further described in Section 9 of this Protocol.

The present study expects that it will acquire some research data from existing administrative and service data sources. In Victoria, for example, this would include obtaining details from the Victorian Department of Health and Human Services (VDHHS) who collects information about presentations to hospitals and emergency departments for medical care in Victoria. Similar processes will be followed in other Australian states. In mainland Europe and Brazil, participants will be required to consent to provide access to their medical records by study staff. In the UK self-reports from participants may be supplemented by tracking of participants using their NHS number or other relevant unique identifiers (provided by

participant), drawing on Hospital Episode Statistics and Office of National Statistics data to track health service use (admissions) and deaths.

6.4 Notes on specific trial visits

6.4.1 Unscheduled visit

If participants have any concerns related to side effects or the injection site evolution or scaring, they can call or email the study team for advice and if necessary, they will be seen by a clinician member of the study team or delegate. Reassurance, appropriate management or referral for medical care will be done according to best practice. Documentation of adverse event will be done as indicated in section 8.

6.5 Procedure discontinuation, participant withdrawals and losses to follow up

6.5.1 Discontinuation of blood collection - participant remains in trial for follow up

The Participants that decline further blood collection may still continue in all other aspects of the study.

6.5.2 Withdrawal of consent - participant withdraws from all trial participation

Participants are free to withdraw from the trial at any time upon their request. Withdrawing from the trial will not affect their access to standard treatment or their employment as their participation will not be shared with their employer.

For the safety of all participants withdrawing from the trial, reasonable efforts should be made to undertake protocol-specified safety evaluations.

A dedicated Case Report Form (CRF) page will be used to capture the date of participant withdrawal of consent, and the reason if offered.

6.5.3 Losses to follow-up

Due to the study taking place in healthcare workers during a pandemic, we expect that there may be periods that participants will ignore the smartphone app prompts, phone calls or electronic messages. This includes the eventuality that a participant has been admitted to hospital. The weekly smartphone app prompts will only ask whether the participant has had a fever or respiratory symptom since the last time they answered in the app (date provided). Alternatively where appropriate phone follow-up will be used (ie. Brazil). We deem this very unlikely to annoy participants excessively as they can ignore the notification or call if they are too busy (or withdraw). This will give the project the best chance of having a complete dataset to analyse as they can answer 'Yes' when they get the opportunity and fill in the associated questionnaire. Therefore, we will continue to send out weekly notifications or calls for the entire study regardless of whether they respond.

In Australia and Europe, if a participant does not answer 2 regular smartphone app prompts (2 consecutive weeks), further attempts will be made to contact them by electronic messages (maximal 3 attempts), phone call (maximal 3 attempts) and email (maximal 3 attempts). If there is still no response, and the participant is not found to have died on medical records, we will try to contact them later (when the workload is expected to have decreased).

In Brazil, if a participant does not answer 3 follow-ups phone contacts (phone call or electronic messages), a home visit may be carried out by study staff. If there is still no response, and the participant is not found to have died on medical records, we will try to contact them later (when the workload is expected to have decreased).

Where secondary contact provided, the study team will follow-up if unable to contact participants.

6.5.4 Replacements

Participants who have been randomised may NOT be replaced.

6.5.5 Trial Completion

A participant is considered to have completed the trial if he or she has completed all processes of the trial including the last visit or the last scheduled procedure shown in the Schedule of Assessments.

The end of the trial is defined as completion of the last visit or procedure shown in the Schedule of Assessments in the trial at all sites. At the end of the trial, the Sponsor-Investigator will ensure that all HRECs as well as all regulatory and funding bodies have been notified, if required.

This trial may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. If the trial is prematurely terminated or suspended, the Sponsor and Investigators will promptly inform trial participants, HRECs, the funding (where applicable) and regulatory bodies, providing the reason(s) for the termination or suspension.

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

- Determination of an unexpected, significant, or unacceptable risk to participants that meets the definition of a Significant Safety Issue (for the definition refer to Section 8.1).
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Demonstration of efficacy that would warrant stopping
- Determination that the primary endpoint has been met
- Determination of futility

In the case of concerns about safety, protocol compliance or data quality, the trial may resume once the concerns have been addressed to the satisfaction of the sponsor, HRECs, funding and/or regulatory bodies.

6.5.6 Continuation of therapy

As the treatment is 'once-off' there is no provision for continuation of therapy.

7 SAFETY MONITORING AND REPORTING

7.1 Definitions

Adverse Event (AE):

An AE is any untoward medical occurrence in a participant administered an investigational product and does not necessarily have a causal relationship with the study treatment. For this study, only certain adverse events are recorded, specifically serious adverse events as defined below, and non-serious adverse events of interest specified in section 8.2

Serious Adverse Event (SAE) :

Any serious adverse event (SAE) is an untoward medical occurrence that:

- Results in death; or
- Is life-threatening; or
- Requires hospitalisation or prolongation of existing hospitalisation;
 - Hospitalisation is to be considered an SAE only in the event of an overnight admission. Any elective hospitalisation does not constitute an SAE
- Results in persistent or significant disability/incapacity; or
- Is a congenital anomaly/birth defect

Note: Life-threatening refers to an event in which the participant was at risk of death at the time of the event. It does not refer to an event that hypothetically might have caused death if it were more severe.

Medical and scientific judgement should be exercised in deciding whether an adverse event should be classified as serious in other situations. **Important medical events** that are not immediately life-threatening or do not result in death or hospitalisation but may jeopardise the participant or may require intervention to prevent one of the other outcomes listed in this definition should also be considered serious.

For this study, all SAE will be collected for the period from randomisation to 3 months post randomisation.

Suspected Unexpected Serious Adverse Reaction (SUSAR):

A SUSAR is an AE that meets all of the following criteria:

- The AE is serious (as defined above; an SAE); and
- The SAE is suspected adverse reaction to the investigational product, meaning it is judged by either the reporting investigator or the sponsor as having a reasonable possibility of a causal relationship to a study vaccine (possibly, probably or definitely related), and
- The SAE is also unexpected: An unexpected serious adverse reaction is one for which the nature or severity of the reaction is not consistent with reference safety information (Which is comprised of the BCG vaccine Product Information and the *WHO information sheet: Observed rate of vaccine reactions Bacille Calmette Guerin Vaccine April 2012*).

Note that an event is instead considered 'expected' if it is listed in the Reference Safety Information and therefore cannot meet the definition of SUSAR.

Significant Safety Issue:

A significant safety issue is an issue that could adversely affect the safety of participants or materially impact on the continued ethical acceptability or conduct of the trial.

Comment: A significant safety issue is a new safety issue or validated signal considered by the Sponsor in relation to the study vaccines that requires urgent attention of stakeholders. This may be because of the seriousness and potential impact on the benefit-risk balance of the study vaccines, which could prompt regulatory action and/or changes to the overall conduct of the clinical trial, including the monitoring of safety and/or the administration of the study vaccines.

Urgent Safety Measure (USM):

A measure required to be taken in order to eliminate an immediate hazard to a participant's health or safety. Note: This is a type of significant safety issue that can be instigated by either the investigator or sponsor and can be implemented before seeking approval from HRECs or institutions.

7.2 Capturing and eliciting adverse event information

For the period of randomisation to 3 months post randomisation only, the following are non-serious adverse events of interest for this study:

- At injection site:
 - Reaction (pain, tenderness, redness, swelling) of grade 3 (severe) or 4 (potentially life threatening)
 - Abscess
 - Large ulcer (>1.5 cm diameter)
 - Keloid scar
 - Unusual local reaction
- Lymphadenopathy (in region of injection site)
- BCG osteitis/osteomyelitis
- Disseminated BCG infection (BCG-osis)
- Allergic reaction due to IP
- Fainting episode, seizures and convulsions following IP administration (recorded on the day of IP administration only)

Only these non-serious AE and all SAE, occurring between randomisation and 3 months post randomisation, will be recorded for this study. If applicable, for the remainder of the follow-up period, sites may additionally document participants' AE as required to meet reporting requirements of the applicable HREC/s and/or regulatory authority.

7.2.1 SAE capture

SAE are captured on the day of IP administration, as recorded by the site personnel. Information on any SAE since randomisation will be solicited from participants at the 3-month questionnaire. SAE may also be captured via participant notification, in the period

between randomisation and the 3-month questionnaire, such as through spontaneous contact by the participant via call or email, data entered in the Vaccine Diary or the study smartphone app (or equivalent, e.g. weekly phone calls). In cases where a participant does not respond to multiple attempts at contact, over several weeks, the participant's secondary contact will be contacted to confirm their status and record fatal SAE if applicable.

For this study, all SAE will be collected for the period from randomisation to 3 months post randomisation.

7.2.2 Non-Serious AE Capture

Non-serious AE of interest are captured:

- On the day of IP administration, recorded by the site personnel
- Within the Vaccine diary (which triggers an alert to the site personnel to contact the participant)
- Through the 3-month questionnaire (questions on injection site evolution)
- Through spontaneous contact (e.g. phone call, electronic message or email) from the participant to the site team.

7.3 Documentation of AEs

For the purposes of this study the investigator or delegate is responsible for recording the applicable Adverse Events, regardless of their relationship to study vaccines.

The documentation of each applicable AE on the REDCap CRF will include:

- A description of the AE
- The onset date, duration, date of resolution
- Severity
- Seriousness (SAE or not)
- Any action taken (e.g. treatment, follow-up tests)
- The outcome (recovery, death, continuing)
- The likelihood of the relationship of the AE to the trial treatment

All AEs will be followed to resolution or stabilisation, where possible.

7.4 Assessing the relatedness (causality) of a participant's AE

All non-serious AE of interest and SAE must have their relationship to the trial intervention assessed by the SPI (or delegate) who evaluates the AE based on temporal relationship and their clinical judgment. The degree of certainty about causality will be graded using the categories below.

The relationship of the event to the trial intervention will be assessed as follows:

Code	Causal Relationship	Description
1	Unrelated	The AE is clearly NOT related to the intervention
2	Unlikely	The AE is doubtfully related to the intervention
3	Possible	The AE may be related to the intervention
4	Probable	The AE is likely related to the intervention
5	Definite	The AE is clearly related to the intervention

7.5 Assessing the severity of a participant's AE

The SPI (or delegate) will be responsible for assessing the severity of an AE. The determination of severity for all AE should be made by the investigator based upon medical judgment and the severity categories of Grade 1 to 5 as defined in the first table below, with the following exceptions: injection site pain, redness, tenderness and swelling/induration are assigned severity grades using the specific toxicity grade specified in the second table below.

Grade	Severity	Description
Grade 1	Mild	Asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated
Grade 2	Moderate	Moderate; minimal, local or non-invasive intervention indicated; limiting age appropriate instrumental activities of daily living (ADL)
Grade 3	Severe	Severe or medically significant but not immediately life-threatening; hospitalisation or prolongation of hospitalisation indicated; disabling; limiting self-care ADL
Grade 4	Life Threatening	Life-threatening consequences; urgent intervention indicated
Grade 5	Fatal	Death related to AE

Toxicity grading scale

Local reaction to vaccination are monitored using Vaccine diary completed by the participant up to 14 days after vaccination. A toxicity grading scale is used to categorise the reports (Food and Drug Administration 2007):

Local reaction	Grade 0 None	Grade 1 Mild	Grade 2 Moderate	Grade 3 Severe	Grade 4 Potentially life threatening
Pain	None	Does not interfere with activity	Repeated use of nonnarcotic pain reliever > 24 hours or interferes with activity	Any use of narcotic pain reliever or prevents daily activity	Emergency room visit or hospitalization
Redness	None	2.5 - 5 cm	5.1 - 10 cm	>10 cm	Necrosis or exfoliative dermatitis
Tenderness	None	Mild discomfort to touch	Discomfort with movement	Significant discomfort at rest	Emergency room visit or hospitalization
Swelling / induration	None	2.5 - 5 cm and does not interfere with activity	5.1 - 10 cm or interferes with activity	>10 cm or prevents daily activity	Necrosis
Itch	None	Itching localised to injection site that is relieved spontaneously or in <48 hours of treatment	Itching beyond the injection site that is not generalised OR Itching localised to injection site requiring ≥48 hours of treatment	Generalised itching causing inability to perform usual social & functional activities	Not applicable

Food and Drug Administration. (2007). "Guidance for Industry: toxicity grading scale for healthy adult and adolescent volunteers enrolled in preventive vaccine clinical"
Retrieved 08.04.2020, from <https://www.fda.gov/downloads/BiologicsBloodVaccines/GuidanceComplianceRegulatoryInformation/Guidances/Vaccines/ucm091977.pdf>.

7.6 Reporting of safety events

Site Principal Investigator Reporting Procedures:

The SPI (or delegate) is responsible for expedited reporting (within 24 hours of becoming aware of the event) to the Sponsor the following:

- USMs
- All SAEs (including SUSAR)

SAE reports should be submitted using the RedCap SAE form, or by alternative means specified in the Safety Reporting Plan.

At MCRI, the CPI (or delegate) will determine whether or not each SAE meets the definition of SUSAR and will notify all RPI in a timely manner.

The RPI and SPI will be notified of USM and other significant safety issues in a timely manner following MCRI first knowledge of the event/s.

In each country, USM, other significant safety issues, SUSARs and other SAE, will be reported to the applicable regulatory authorities and HRECs in accordance with the requirements.

Further details of event reporting responsibilities and processes are documented in the Safety Reporting Plan.

For safety reporting requirements specific to Brazil, refer to Appendix 4.

8 DATA AND INFORMATION MANAGEMENT

8.1 Overview

The Site Principal Investigator is responsible for storing essential trial documents relevant to data management and maintaining a site-specific record of the location(s) of the site's data management-related Essential Documents.

The Site Principal Investigator is responsible for maintaining adequate and accurate files of any relevant source documents that include observations or other data relating to participants at their site. Source data will be attributable, legible (including any changes or corrections), contemporaneous, original, accurate, complete, consistent, enduring and available. Changes to source data (hardcopy and electronic) must be traceable, must not obscure the original entry, and must be explained where this is necessary.

The Site Principal Investigator will also maintain accurate case report forms (CRFs) (i.e. the data collection forms) where applicable and be responsible for ensuring that the collected and reported data is accurate, legible, complete, entered in a timely manner and enduring. To maintain the integrity of the data, any changes to data (hardcopy and electronic) must be traceable, must not obscure the original entry, and must be explained where this is necessary.

Any person delegated to collect data, perform data entry or sign for data completeness will be recorded on the delegation log and will be trained to perform these trial-related duties and functions.

8.2 Data management

8.2.1 Data generation (source data)

In this study, the following types of data will be collected:

- personal identifying information (names, dates of birth, contact details; NHS number in UK, Medicare number in Australia, SUS CARD and CPF in Brazil)
- sensitive information including health data (medical history, participant eligibility, adverse reactions and other notes as appropriate)
- participant completed electronic questionnaires
- de-identified data from laboratory assays

Source document plan

Much of the data for this trial will be collected electronically directly from participants. There will be a limited number of source documents for this study recorded data from automated instruments, laboratory reports and the signed information and consent forms (in REDCap or hard copy). Each site participating in the trial will maintain a site-specific Source Document Plan that will document the source, i.e. original recording, for each data discrete item/ category of items collected for the study. This Source Document Plan, signed and dated by

the Site Principal Investigator, will be prepared prior to recruitment of the first participant and will be filed in the site's Investigator Site File.

8.2.2 Data capture methods and data use, storage, access and disclosure during the trial

Data collection methods

Data for this trial will be collected and entered using electronic database REDCap and a smartphone application developed for this trial. REDCap is a secure, web-based application for building and managing online surveys and databases. The trial smartphone application stores participant information directly in the REDCap database. In line with local privacy regulations, identifying or personal data may be maintained in complementary site level information management systems as required.

Use of the data

The data will be used for the analyses specified in the protocol and Statistical Analysis Plan.

Following the completion and analysis of the trial, the data will be retained long-term following the mandatory archive period for use in future research projects.

Storage and access

Hard copy data will be stored by collaborators in a locked cabinet in a secure location, accessible to the research team only.

Electronic data maintained on REDCap database will be securely stored in MCRI's 'network file servers, which are backed up nightly. Electronic or hard copy files containing private or confidential data will be stored only in locations accessible only by appropriate designated members of the research team.

REDCap is hosted on MCRI infrastructure and is subject to the same security and backup regimen as other systems (e.g. the network file servers). Data is backed up nightly to a local backup server, with a monthly backup taken to tape and stored offsite. REDCap maintains an audit trail of data create/update/delete events that is accessible to project users who are granted permission to view it. Access to REDCap will be provided via an MCRI user account or (for external collaborators) via a REDCap user account created by the MCRI system administrator. The permissions granted to each user within each REDCap project will be controlled by, and will be the responsibility of, the study team delegated this task by the Principal Investigator. REDCap has functionality that makes adding and removing users and managing user permissions straightforward. All data transmissions between users and the REDCap server are encrypted. The instructions for data entry to REDCap must be read and the training log signed prior to personnel commencing data entry on REDCap.

Authorised representatives of the sponsoring institution as well as representatives from the HREC, Research Governance Office and regulatory agencies may inspect all documents and records required to be maintained by the CPI for the participants in this trial. The trial site will permit access to such records.

Disclosure

The trial protocol, 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 unauthorised third party, without prior written approval of MCRI. Clinical information will not be released without written permission of the participant, except as necessary for monitoring by the HREC, Research Governance Office or regulatory agencies.

8.2.3 Data confidentiality

Data confidentiality

Participant confidentiality is strictly held in trust by the CPI, participating investigators, research staff, and the MCRI and their agents. This confidentiality is extended to cover testing of biological samples in addition to the clinical information relating to participating participants.

To preserve confidentiality and reduce the risk of identification during collection, analysis and storage of data and information, the following will be undertaken:

- (1) The number of private/confidential variables collected for each individual has been minimised. The data collected will be limited to that required to address the primary and secondary objectives
- (2) Participant data and samples will be identified through use of a unique participant study number assigned to the study participant (“re-identifiable”).

The CPI is responsible for the storage in REDCap of a master-file of identifiable data with the participant ID; access is managed by restricting user permissions to members of the research team and authorised persons.

- (3) Separation of the roles responsible for management of identifiers and those responsible for analysing content. The data will be analysed by members of the research team who will be provided with anonymised data identified only by the unique participant study ID.

8.2.4 Quality assurance

A REDCap data dictionary with range checks will be used to minimise data entry errors, such as out-of-range values. Data quality control checks (e.g. checking for invalid characters, invalid dates, data that is not consistent with data in other data fields) and data cleaning will be done by trained members of the research team on a regular basis. Any discrepancies will be reported to the CPI or delegate and addressed in a timely manner.

Quality control checks will be run by the data team, on a regular basis, who will highlight any queries to the CPI, RPI and SPI.

8.2.5 Archiving - Data and document retention

Upon completion of the study, data will be stored securely on MCRI server (restricted access) and/or locked in secure cabinet in MCRI laboratories (for hardcopy data) for at least 15 years after study completion, in accordance with the requirements of the Therapeutic Goods Administration and Health Privacy Principles and any other relevant regulatory authorities.

Prof Nigel Curtis (CPI) will be the custodian during the archive period, and members of the research team will have access to the stored data. If the CPI becomes unable to perform this

task all responsibilities of the custodian will fall to the sponsor (MCRI). At the end of the archival period, long-term retention of the data may occur.

Records should not be destroyed without the written consent of the Sponsor. The Sponsor will inform Site Principal Investigators when these documents no longer need to be retained.

8.2.6 Data sharing

Data sharing

De-identified data will be deposited on a recognised clinical trials data sharing repository and transferred to the Bill and Melinda Gates foundation.

Under the data sharing agreement with the Bill and Melinda Gates foundation the BRACE project will:

1. Register with and upload documents to clinicaltrials.gov
 - Study Protocol
 - Statistical Analysis Plan
 - Case Report Form Template(s)
 - Data Dictionary
 - Informed Consent Agreement Template
2. Share with the Gates Medical Research Institute the following documents;
 - Randomization Plan
 - Data Management Plan
 - Edit Check Specifications
 - Case Report Form Template (s) and Completion Guidelines
 - Data Transfer Agreements
 - All data generated by investigators funded by the Bill and Melinda Gates Foundation. This data will be de-identified.
3. All documents listed above will be available after being posted on a clinical trials data sharing repository and deidentified patient data related to the outcomes listed in the protocol will also be transferred to this repository.

Participant consent to the data sharing requirements is a mandatory requirement in updated Master PICF v8. Participants consented under earlier versions (prior to v7) of the PICF will be advised about the updated data sharing requirements and given an opportunity to opt-out via email of the data sharing arrangement. Data will not be shared, where participants have specifically requested their data not be shared.

Access to deidentified patient data in the data sharing repository and the Bill and Melinda Gates Foundation will be limited to ethically approved research and subject to the governance procedures of the data repository and the Bill and Melinda Gates Foundation

respectively. The governance procedures ensure the data are accessed for scientifically sound research.

After database lock, the following may be made available long-term for use by future researchers from a recognised research institution whose proposed use of the data has been ethically reviewed and approved by an independent committee and who accept MCRI's conditions, under a collaborator agreement, for accessing:

- Individual participant data that underlie the results reported in our articles after de-identification (text, tables, figures and appendices)
- Study protocol, Statistical Analysis Plan, PICF

8.2.7 Long-term custodianship (after archive period finished)

Prof Nigel Curtis will be the long-term custodian following the archive period. If he is unable to perform this task the responsibility of custodianship will fall to the sponsor (MCRI).

8.2.8 Data retrieval and linkage

The present study expects that it will acquire some research data from existing administrative and service data sources. In some instances, participant consent may allow retrieval of datasets without the need for linkage keys, as has usually been the case of other MCRI studies.

For datasets in Australia the study will work with organisations such as, the Centre for Victorian Data Linkage, the Australian Institute of Health and Welfare and the Population Health Research Network that supports data linkage and integration services within and between jurisdictions. For private sources such as pathologists, the study will establish appropriate initiatives.

Brazil, this data can be retrieved, if necessary, from the national government information systems, such as E-SUS, SIVEP-GRIPE, GAL and electronic medical records from SESAU / CG / MS (Municipal Health Secretariat of Campo Grande / MS, through unique identifiers of the participant (registration in the Individual Taxpayer Register - CPF and SUS CARD).

We anticipate data linkage and access will occur after the study recruitment period is complete, but the exact timing is yet to be determined. Working with both the capabilities of the data linkage services and through consultation with research studies that have extensive data linkage experience, this study will establish IT systems and SOPs to support data linkages processes that are efficient and minimise the risk of disclosure. These processes will use data linkage keys to separate the personally identifiable information needed for data linkage from the administrative and clinical data being sourced.

8.2.9 Sample management: Additional data management considerations

Data and information for biospecimens will be managed as above with the additional considerations.

Data collection: de-identified sample data may also be stored in OpenSpecimen (restricted access stored on secured servers at each study laboratory site), other site-specific electronic laboratory information management systems (LIMS, restricted access) or hard copy . Where biospecimen data is stored in OpenSpecimen or site-specific LIMS, data will be transferred to

the MCRI servers on a regular basis. Where biospecimen data is collected on hardcopy, data will be transcribed to REDCap on a regular basis.

8.2.10 Sample management: Specimen collection & storage.

Biospecimens will be processed, stored and data will be recorded at laboratory study sites. Samples will be identified using barcoded tubes or with the unique participant study ID, and year of birth. No identifying information will be stored on biospecimen labels. Biosamples will be stored securely at laboratory study sites in temperature-controlled freezers and liquid nitrogen tanks as appropriate for the sample type. Access to biosamples will be restricted to the study team. The samples will be used for the analyses specified in the protocol. Samples will be shipped from study sites to MCRI for long term storage. For tests that require equipment or technical expertise not available in Melbourne, select specimens may be sent to collaborating laboratories outside of Melbourne (interstate and/or overseas) for further testing. These samples may be shipped from MCRI or directly from study sites if they have not yet been shipped to MCRI. Shipment of samples to MCRI or collaborating laboratories doing testing will be done by International Air Transport Association (IATA) accredited staff with temperature control (e.g. ice pack, dry ice) as appropriate for the sample type.

The biosamples will be retained long-term according to the banking management detailed below. As per data, Prof Nigel Curtis (CPI) will be the custodian of the biosamples during the archive period.

8.2.11 Sample management: Specimen & Biobanking

All samples that are not used immediately for the laboratory assessments described in previous sections, may be cryopreserved for an indefinite period of time to enhance the possible benefit from this study, by providing a sample biobank that may be used for research related to immunology or infectious diseases, in the future. The biobank will be at MCRI laboratories (Infectious Diseases Group) in Melbourne, (please see Appendix 1 for Biobank Registration Form). The biobank will be registered with the Melbourne Children's Bioresource Centre (MCBC). Written informed permission (extended consent) for banking of specimens and future use for study objectives without further consent will be obtained from the participant. These samples may be used for additional research studies related to immunology or infectious diseases. For tests that require equipment or technical expertise not available in Melbourne, select specimens may be sent to collaborating laboratories outside of Melbourne (interstate and/or overseas) for further testing.

Databank is defined as: "A systematic collection of data, whether individually identifiable, re-identifiable or non-identifiable" (NHMRC National Statement on Ethical Conduct in Human Research)

Biobank is defined as: "... collections of human biological materials (biospecimens) linked to relevant personal and health information (which may include health records, family history, lifestyle and genetic information) and held specifically for use in health and medical research." (NHMRC Biobanks Information Paper 2010)

9 TRIAL OVERSIGHT

9.1 Governance structure

9.1.1 Trial Steering Committee (TSC)

The trial steering committee will be made up of representatives from the key stakeholders and the chief principal investigator along with independent content expert (s).

9.1.2 Independent Data and Safety Monitoring Board (DSMB)

An independent Data and Safety Monitoring Board (DSMB) will be convened three times during the study: at 3 and 9 months post initial recruitment, and once there have been 100 severe case of COVID-19 disease.

The DSMB at 3 and 9 months will monitor safety (including number of deaths and number of ICU admissions), data completeness, and the general study conduct.

A third DSMB is planned once there have been 100 cases of severe COVID-19 disease. This interim analysis will primarily be on a comparison of the number of cases of severe COVID-19 disease (primary outcome (2)) between the BGG group (irrespective of whether the participants received flu vaccine at randomisation) and the control group (irrespective of whether the participants received flu vaccine or placebo at randomisation), although data will also be presented on COVID-19 disease (primary outcome (1)) and also separately for those recruited prior to and post the introduction of the placebo to provide the DSMB with a complete picture. The DSMB will be given a stopping rule, but since the pandemic is rapidly evolving, the global situation should be considered with the context of any apparent differences. More information of this efficacy interim analysis is explained in section 11.4 of this current Protocol.

All the details of the DSMB analyses will be outlined in the DSMB charter.

The DSMB will be composed of individuals with the appropriate expertise, including at least three independent clinicians and/or biostatisticians who, collectively, have experience in the management of biostatistics and the conduct and monitoring of randomised controlled trials. Members of the DSMB will be independent of trial conduct. The DSMB will review data from each intervention group of the trial in a semi-blinded fashion. The DSMB will provide its input to the CPI.

9.1.3 Independent Safety Monitor

During the start of the recruitment period (until August 2020) an independent safety monitor will review a report of SAE and specified non-serious adverse events of interest on a weekly basis and report any concerns to the Sponsor-Investigator. For the remainder of the recruitment period, the monitor will review such reports monthly. This role will cease once recruitment is complete.

9.1.4 Quality Control and Quality Assurance

Both the Chief Principal Investigator and Site Investigators have responsibilities in relation to quality management.

The Chief Principal Investigator will ensure the development of procedures that identify, evaluate and control risk for all aspects of the study, e.g. study design, source data management, training, eligibility, informed consent and adverse event reporting. The Chief Principal Investigator will ensure the implementation of quality control (QC) procedures, which will include the data entry system and data QC checks. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution.

The Site Principal Investigator I will be responsible to ensure the verification that the clinical trial is conducted and data are generated and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, good clinical practice and applicable regulatory requirements. In some regions a subcontracted monitor may be engaged by MCRI as needed.

In the event of non-compliance that significantly affects human participant protection or reliability of results, the Chief Principal Investigator (or delegate) and/or Site Principal Investigator (or delegate) will perform a root cause analysis and corrective and preventative action plan (CAPA).

In addition, each clinical site will perform internal quality management of study conduct, data and biological specimen collection, documentation and completion. An individualised quality management plan will be developed to describe a site's quality management.

10 STATISTICAL METHODS

10.1 Sample Size Estimation

7244 healthcare workers will be enrolled in the trial outlined in this protocol, although data from this trial will be combined with the data from the 2834 participants enrolled into the first stage of this study which followed an identical protocol but where participants were randomised between BCG and no BCG which was given concurrently with the flu vaccination, resulting in a total sample size of 10078 participants.

Participants will be randomly allocated in a 1:1 ratio to BCG vaccine group (n=3622, plus 1417 from the first stage who received flu vaccine at time of randomisation), and to control (n=3622, plus 1417 from the first stage who received flu vaccine at time of randomisation and no 0.9% NaCl placebo). This sample size was calculated based on the two primary outcomes of (1) number of participants with COVID-19 disease and (2) number of participant with severe COVID-19 disease. Since the study aims to assess two primary outcomes, an adjustment for multiplicity will be applied to maintain a global Type I error rate of 5% by splitting of this alpha.

For the primary outcome (1), the number of participants with COVID-19 disease: it is conservatively estimated that a proportion of 55% of subjects will be infected by COVID-19 disease in the placebo group; applying a 1:1 ratio for randomisation, a total sample size of n=2016 (1008 group) will provide 95% power with 2-tailed 0.005 significance level (10% of

the global significance level) for the Pearson chi-square test (with continuity correction) to detect an absolute difference of 10% between an incidence of COVID-19 disease of 45% in the BCG vaccine group and 55% in the placebo group.

For the primary outcome (2), the number of participants with severe COVID-19 disease at 6 months, we powered the study to identify a risk ratio of 0.67 in the BCG compared with the placebo group for severe COVID-19 disease at 6 months (which is much more realistic than a risk ratio of 0.5 as per the original sample size). Assuming that 4% of subjects will be infected by severe COVID-19 disease by 6 months in the control group, a total sample size of $n=6076$ (3038 per group) will provide 80% power with 2-tailed 0.04 significance level (80% of the global significance level) for the Pearson chi-square test to detect a risk ratio of 0.667, equivalent to an absolute difference of 1.3%. Note this calculation was conducted using an alpha of 0.04 to allow the remaining 0.01 to be spent on primary outcome (1) ($\alpha=0.005$) and the interim analysis ($\alpha=0.005$, see section 11.4 for details of the interim analysis). Allowing for a 16% loss to follow up, it is planned that the study will recruit 7244 healthcare workers.

In the pre-planned meta-analysis, we will have a sample size of 10,078 participants (7244+2834), or 8062 participants allowing for an overall 20% loss to follow up. For the combined analysis it is expected that the drop-out will be slightly higher (20% instead of 16%) because it also includes participants recruited prior to the introduction of the placebo, ie not placebo controlled. Again assuming that 4% of subjects will be infected by severe COVID-19 disease by 6 months in the control group, a total sample size of $n=8062$ (4031 per group) will provide 90% power with 2-tailed 0.04 significance level for the Pearson chi-square test to detect a risk ratio of 0.667, equivalent to an absolute difference of 1.3%. This will be a secondary analysis of the final study report.

10.2 Population to be analysed

The primary analysis of all outcome data will be an intention-to-treat (ITT) analysis including all randomised participants, regardless of whether they received trial drug.

10.2.1 Handling of missing data

For the primary analysis the imputation of missing data will only be considered if 10-20% of the primary outcome is missing and will be undertaken using multiple imputation (MI) models. Multiple imputation analysis will be performed on the ITT population. The frequency and patterns of missing data will be examined. Multiple imputation models will be conducted separately in the two treatment groups using chained equations applied to all outcomes, including baseline measures, as auxiliary variables. Fifty imputed datasets will be generated including all randomised subjects.

10.3 Methods of analysis

Data analysis for the study will be performed by CEBU at MCRI. Ms Francesca Orsini has been appointed for the trial.

Statistical analysis will follow standard methods for randomised trials and the primary analysis will be by intention to treat (ITT), including all randomised participants.

Categorical variables will be presented as the number and proportion in each category. Continuous variables will be presented as means and standard deviations (SDs), or medians and interquartile ranges for skewed data, and the range.

PRIMARY ANALYSIS. Comparison between the BCG and placebo groups in the proportions of participants with COVID-19 disease (primary outcome 1), as well as in the proportions of participants with severe COVID-19 (primary outcome 2), will be presented as the absolute risk difference (RD) as well as the risk ratio (RR) at 6 months and their 95% confidence interval (CI), obtained using a generalised linear model, with adjustment for the strata (defined by site, age and presence of comorbidity) used in the randomisation. The same analysis will be repeated on the same outcomes at 12 months. As secondary analyses the same models will be run to include also the following covariates: gender, number and type of comorbidities, whether already vaccinated for BCG in the past, and any other factor that may show imbalance between the groups at baseline.

A secondary analysis will be performed as above on the total 10078 participants, comparing all the participants who were randomised to BCG (irrespective of whether they received flu vaccine at randomisation) and those randomised to control (irrespective of whether they received flu vaccine or placebo at randomisation). Analysis will be as described above, but also adjusted for being in the initial stage of the study. As part of this analysis we will conduct an exploratory analysis of whether the treatment effect varies between the two stages of the study (prior to and post the introduction of the placebo) by including an interaction between treatment and study stage. Results will be interpreted with caution given that the study is underpowered for this comparison.

SECONDARY OUTCOMES. According to the nature of the secondary outcomes to be analysed (binary, continuous or categorical) the appropriate generalised linear model (GLM) will be used to estimate the effect of the BCG vaccine on the outcome of interest compared to the control group. All analyses will be adjusted for the stratification factors used in the randomisation (site, age and presence of comorbidity). As secondary analyses the same models will be run to include the following covariates: sex, number and type of comorbidities, whether already vaccinated for BCG in the past, and any other factor that may show imbalance between the groups at baseline.

Survival analysis techniques will be adopted to analyse time to event data.

A secondary analysis will be conducted on the total 10078 participants using the same methodology but also adjusted for being in the initial stage of the study. We will conduct an exploratory analysis of whether the treatment effect varies between the two stages of the study by including an interaction between treatment and study stage.

Sub-group analyses will be undertaken on outcomes of those who:

- Had previous BCG vaccine before enrolling into the trial
- Had a positive serology to SARS-CoV-2 when enrolling into the trial

The full details for each variable will be included in the Statistical Analysis Plan (SAP).

10.4 Interim Analyses

As part of the interim monitoring there will be a single formal interim analysis of the efficacy data. This interim analysis will primarily be on a comparison of the number of cases of severe COVID-19 disease (primary outcome (2)) between the BCG group (irrespective of whether the participants received flu vaccine at randomisation) and the control group (irrespective of whether the participants received flu vaccine or placebo at randomisation), although data will also be presented on COVID-19 disease (primary outcome (1)) and also separately for those recruited prior to and post the introduction of the placebo to provide the DSMB with a complete picture. The timing of the interim analysis will be event driven, and will be conducted using a time-to-event analysis, censoring participants who have not had the event at the time of their last follow-up. This data will be used to provide Kaplan-Meier estimates of the survival curve in the BCG and control groups, which will be used to estimate the proportion with severe COVID-19 disease at 6 months. These proportions will be used to compare the two groups.

We have allocated $\alpha=0.005$ to the interim analysis using the conservative approach of splitting the alpha allocated to primary outcome (2) between the interim and final analysis. Under the original sample size calculation, with 1668 per group and an incidence of 4% in severe COVID-19 disease at 6 months in the control group and 2% in the intervention group, this would equate to $67 + 33 = 100$ cases in total. We therefore plan to conduct a formal interim analysis of severe COVID-19 disease once there have been 100 cases of severe COVID-19 disease. This will include all randomised participants irrespective of whether they received flu vaccine at randomisation if randomised to BCG and irrespective of whether they received flu vaccine or placebo at randomisation if randomised to control.

This interim analysis of the severe COVID-19 disease will be performed on the all of the participants randomised up to the interim analysis time point, comparing all the participants who were randomised to BCG (irrespective of whether they received flu vaccine at randomisation) and those randomised to control (irrespective of whether they received flu vaccine or placebo at randomisation). The DSMB will also be given information on which participants belong to the first stage of the study.

With 100 events, we will have 72% power to detect a risk ratio of 0.5 in the incidence of severe COVID-19 disease at 6 months at the interim analysis based on a two-sided test of $\alpha=0.005$, which is a reasonable power for this interim analysis. The stopping rule for the interim analysis will therefore be $p<0.005$.

Given the dynamic nature of research in this field, the DSMB will be advised that this rule be used as a guideline rather than a formal rule, and should be interpreted in the context of external information and information on the efficacy of BCG vaccination on the incidence of COVID-19 disease (primary outcome (1)) which will also be presented in the DSMB report using the same methodology.

11 ETHICS AND DISSEMINATION

11.1 Research Ethics Approval & Local Governance Authorisation

This protocol and the informed consent document and any subsequent amendments will be reviewed and approved by the applicable human research ethics committee (HREC) prior to commencing the research at each site. A letter of protocol approval by HREC will be obtained prior to the commencement of the trial, as well as approval for other trial documents requiring HREC review.

11.2 Amendments to the protocol

This trial will be conducted in compliance with the current version of the protocol. Any change to the protocol document or Informed Consent Form that affects the scientific intent, trial design, participant safety, or may affect a participants willingness to continue participation in the trial is considered an amendment, and therefore will be written and filed as an amendment to this protocol and/or informed consent form. All such amendments will be submitted to the HREC, for approval prior to being implemented.

11.3 Protocol Deviations and Serious Breaches

All protocol deviations will be recorded in the participant record (source document) and on the CRF and must be reported to the CPI or delegate, who will assess for seriousness.

Those deviations deemed to affect to a significant degree rights of a trial participant or the reliability and robustness of the data generated in the clinical trial will be reported as serious breaches. Reporting will be done in a timely manner (the CPI or delegate to review and submit to the approving HRECs within 7 days, or as required).

Where non-compliance significantly affects human participant protection or reliability of results, a root cause analysis will be undertaken and a corrective and preventative action plan prepared.

Where protocol deviations or serious breaches identify protocol-related issues, the protocol will be reviewed and, where indicated, amended.

12 CONFIDENTIALITY

Participant confidentiality is strictly held in trust by the participating investigators, research staff, and the sponsoring institution and their agents. This confidentiality is extended to cover testing of biological samples in addition to the clinical information relating to participating participants.

The trial data and all other information generated will be held in strict confidence. No information concerning the trial or the data will be released to any unauthorised third party, without prior written approval of the sponsoring institution. Authorised representatives of the sponsoring institution may inspect all documents and records required to be maintained by the Investigator. The clinical trial sites will permit access to such records.

All laboratory specimens, evaluation forms, reports and other records that leave the site will be identified only by the Participant Identification Number (SID) to maintain participant confidentiality.

Clinical information will not be released without written permission of the participant, except as necessary for monitoring by HREC or regulatory agencies.

13 PARTICIPANT REIMBURSEMENT

In Australia and Europe, participants will not be reimbursed for their involvement.

As outlined in appendix 4 in Brazil in line with federal legislation, expenses resulting from participation in the study, such as transportation to the place where the vaccination will be carried out will be reimbursed. The amount will not be considered substantial and reimbursement system will be designed to reduce risk of reimbursement being considered compensation or inducement to participant in the trial.

14 FINANCIAL DISCLOSURE AND CONFLICTS OF INTEREST

This is an investigator-initiated study, and the funders will have no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript.. MCRI holds no commercial interest in the manufacture and trade of BCG.

15 DISSEMINATION AND TRANSLATION PLAN

The results of the trial will be reported to the participants after analysis is complete. The results of this trial will be submitted to peer reviewed journals, presented at conferences and may form part of student theses.

The Chief Principal Investigator holds primary responsibility for publication of the results of the trial.

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16.1 Appendix 1: Specimens for biobanking - completed biobank registration form

Document version & date	Version 1.1 24th Aug 2020
Name of the bank	BCG vaccine to prevent severe COVID-19 disease in healthcare workers (BRACE)
Custodian of the bank	Name: Prof Nigel Curtis
Purpose of the bank	To store data and samples collected in the 'BCG vaccination to Reduce the impact of COVID-19 in healthcare workers (BRACE)' trial so they can be used in future research related to infectious diseases and immunity.
Sample/data type(s) and where these will be accessed from and over what time period	<p><u>Data will be collected from</u></p> <p>Questionnaires, Medicare records and test results obtained as part of the research project 'BCG vaccination to Reduce the impact of COVID-19 in healthcare workers (BRACE)', by members of the research team.</p> <p>Blood and/or swab samples will be obtained via this research project also, and will be stored for an indefinite period of time.</p> <p>The samples/data may be sent overseas for future research related to infectious diseases, immunology, or vaccines.</p> <p><u>Data stored includes:</u></p> <ul style="list-style-type: none"> - Demographics (e.g. age, gender, date) - Environment (e.g. household members, exposure to SARS-CoV-2 positive people, role in the hospital, TB exposure, previous vaccinations) - Study outcome related data (e.g. SARS-CoV-2 test results, BCG and flu vaccine reactions, illnesses during study period, data generated from the laboratory analysis of samples collected) <p><u>Sample types stored:</u></p> <ul style="list-style-type: none"> - Swabs - Plasma - Serum - Peripheral blood samples - Granulocytes and whole blood - Nucleic acid <p>After data ceases to be collected directly from participants, data may be obtained/generated via access to their medical records, government data sets or as samples are analysed and the data is added back into the data/biobank.</p>
Sample/data identifiability	Clinical data in 'BCG vaccination to Reduce the impact of COVID-19 in healthcare workers (BRACE)' will be collected and stored

	<p>in a REDCap database; a secure password-encrypted online database, or similar electronic database hosted by MCRI.</p> <p>Data will be stored in re-identifiable format with the key held by the custodian or delegate. The REDCap database or comparable database will be hosted on the secure Murdoch Children’s Research Institute (MCRI) server and backed up regularly by MCRI Information Technology.</p> <p>Only members of the research team involved in data collection or data management will have access to the project’s REDCap database or similar electronic database.</p> <p>Samples will be stored (frozen) in re-identifiable format by using study ID number or tube barcodes.</p> <p>All data associated with sample storage location and tracking will be stored in a separate REDCap database or similar electronic database. Access to this database is limited to members of the research team working in data/sample management or sample processing.</p> <p>Laboratory generated data, any data collected outside of REDCap and data exported from REDCap or similar electronic database, will be stored in re-identifiable format by study ID. The data will be stored on the MCRI server in restricted folders on the Infectious Diseases group drive, as per MCRI policy.</p> <p>Samples/data stored in re-identifiable format can be linked by the custodian or delegate to participants’ identifiable information if it is ethically appropriate and required.</p>
<p>Criteria for Bank participants</p>	<p>Consenting to the project includes allowing the participants’ data and samples to be used as defined in the protocol.</p> <p>In addition there is an optional consent in the PICF for the storage of participants’ biospecimens and participants’ re-identifiable data for use in future research related to infectious diseases and immunity.</p> <p>Inclusion criteria for Bank participants</p> <ul style="list-style-type: none"> - Recruited participant in the research project ‘BCG vaccination to Reduce the impact of COVID-19 in healthcare workers (BRACE)’ - Provided informed consent for their data and samples to be stored for future ethically approved research (extended consent) related to infectious diseases and immunity.
<p>Access process for the obtaining samples/data</p>	<p>Researchers must discuss their research plan with a member of the research team of the project ‘BCG vaccination to Reduce the impact of COVID-19 in healthcare workers: (BRACE)’. The following will be taking into consideration:</p>

	<ul style="list-style-type: none"> - Scientifically justifiable hypothesis and aims - Study design is appropriate to achieve study aims - Inclusion/exclusion criteria for participants appropriate to answer question - If the research proposal is deemed to have merit, the researcher will complete a REDCap (or similar electronic database) access form detailing the proposed design, participants, data +/- samples that they would like access to. <p>This will be reviewed by the custodian (or delegate) of the data who will need to take into account the following, before approval is granted:</p> <ul style="list-style-type: none"> - Does the research plan involve research in the area of immunology or infectious diseases? If not, it is outside the scope of the data/biobank. To use the data one of the following will be required: <ul style="list-style-type: none"> o a new project approved by the RCH HREC and participants contacted for their consent o a new project approved by the RCH HREC and a waiver of consent granted - Is the planned analysis feasible with the data/samples available in the data/biobank? - Are there competing interests for the sample/data type in question? - Is another researcher already analysing the data in a similar way and would collaboration on the existing project be more appropriate? <p>The access form for access to the data/biobank will be kept on the REDCap database or similar electronic database.</p>
Sample and data input	Members of the research team working in data/sample management will input the data and samples to the data/biobank.
Location of the Bank	<p>Samples will be stored in the MCRI freezer farm or in the Infectious Disease Group's freezers, and may be distributed to other collaborating laboratories where they may also be stored.</p> <p>Data will be stored in a REDCap online database or similar electronic database, hosted on the secure Murdoch Children's Research Institute (MCRI) server, as well as in restricted electronic folders on the MCRI Infection and Immunity group drive.</p>

<p>Confidentiality/security of samples/data</p>	<p>Members of the research team of the project 'BCG vaccination to Reduce the impact of COVID-19 in healthcare workers (BRACE)' involved in data/sample collection or management will have open access to the bank data/samples.</p> <p>No identifying data will be provided to researchers using data/samples from the biobank. To re-identify data/samples, the custodian (or delegate) will have access to the key, but will not pass this information onto researchers unless approved by ethics, or as required by law.</p> <p>Data stored on REDCap database or similar electronic database will be password protected, and hosted on the secure MCRI server. This is backed up regularly by MCRI Information Technology.</p> <p>The Bank will be secure against unauthorised access and passwords will be changed at regular intervals (as per MCRI policy).</p> <p>The custodian (or delegate) will ensure removal of access to data once a project is finished or a researcher leaves the project.</p>
<p>Destruction of samples/data</p>	<p>Destruction of samples/data will occur upon participant request. This will be managed by the custodian (or delegate).</p>
<p>Modifications to Bank Protocol</p>	<p>If a change of purpose/data type/type of samples is to be considered, the custodian (or delegate) is required to submit to the HREC for approval and either contact the participants to obtain consent, or a waiver must have been granted.</p>

16.2 Appendix 2. Collection of stool samples from a subset of BRACE participants

Background and Rationale:

For reasons that are poorly understood, B and T cell responses to vaccination (including BCG vaccination) are highly variable between individuals and between different populations. While many host factors, such as genetics, can influence inter-individual variation in these responses, increasing evidence shows that the gut microbiota, a large and diverse group of microorganisms that colonise gastrointestinal tract (GIT), plays a key role in shaping immune responses to vaccination (reviewed Lynn & Pulendran, 2017). For instance, in human infants, the relative abundance of several bacterial species in the stool microbiota has been associated with vaccine-specific IgG and T cell proliferation responses (Huda et al., 2014). Similarly, the composition of the stool microbiota in infants from rural Ghana was correlated with responses to the oral rotavirus vaccine (Harris *et al.*, 2017). Interestingly, germ-free mice have also been found to have impaired antibody responses to immunization with the model antigen ovalbumin (Lamousé-Smith *et al.*, 2011) and to the non-adjuvanted influenza vaccine (Oh *et al.*, 2014). Moreover, one of the principal investigators involved in this trial has recently found that, in mice, dysregulation of the microbiota leads to significantly impaired B and T cells responses to five different adjuvanted and live vaccines (including BCG) that are routinely administered to infants worldwide (Lynn *et al.*, 2018). Restoring the commensal microbiota rescued impaired responses (Lynn *et al.*, 2018). These data strongly suggest that the composition of the gut microbiota plays an important role in specific immune responses to vaccination. Whether the gut microbiota also influences non-specific effects of vaccines is currently unknown.

Primary objective of exploratory sub-study:

In a subset of BRACE trial participants consenting for an optional stool sample collection at baseline, determine whether the composition or metagenome-encoded function of the stool microbiota is correlated with either specific or non-specific immune responses to the BCG vaccine.

Secondary objectives of exploratory sub-study:

- Assess whether the composition of the stool microbiota is associated with any of the other primary, secondary, or exploratory outcomes described in the study protocol.
- Characterise the composition of the stool microbiota in participants in the trial and investigate whether the composition of the microbiota is altered at 3 or 12 months later.
- Assess whether immunisation with BCG leads to an altered microbiota at 3 or 12 months compared to participants receiving the placebo.

Outcomes:

Microbiota composition, including identities and the relative abundance of the bacteria present and their encoded microbial genes.

Population:

BRACE trial participants consenting for an optional stool sample collection at baseline, 3 months and 12 months.

Study Duration:

As per the BRACE trial protocol – 2 years.

Participant Duration:

12 months from randomisation.

Sub-study Locations:

Optional inclusion for Australian sites.

Sub-study Principal Investigator:

Prof. David J. Lynn BA MSc PhD

EMBL Australia Group Leader, Precision Medicine Theme, South Australian Health & Medical Research Institute, Adelaide, SA 5001.

Professor, College of Medicine & Public Health, Flinders University, Bedford Park, South Australia.

Email: david.lynn@sahmri.com

Potential risks and benefits:**Known potential risks:**

This sub-study involves minimal risk to participants. Appropriate collection containers will be provided to participants to facilitate stool sample collection, storage and transport. A small stool sample will be collected by the participants at home. The tube contains a reagent that stabilises DNA at room temperature for up to 14 days. Participants will return the sample via a pre-paid addressed envelope. There will be no financial cost to the participant.

Known potential benefits:

This sub-study is exploratory in nature and is not expected to provide any direct additional benefit to the participants. The findings of the study, however, may be of significant value for understanding the role of the microbiota in influencing responses to vaccination.

Sub-study design:**Consent:**

An additional option has been added to the BRACE online consent form to allow participants to optionally consent for a stool sample collection at baseline, 3 months and 12 months. The BRACE participant information and consent form (PICF) has been also modified to explain to participants the process for collecting stool samples and why they are being collected. If a participant declines to consent for stool sample collection this will not affect their participation in the BRACE trial (assuming all other inclusion and exclusion criteria are met).

Sample collection process:

Participants consenting for a stool sample collection will be provided with a collection pack at existing study visits at baseline, 3 months, and 12 months. The provided pack will contain: Instruction sheet, gloves, pathology stool pot, stool specimen collector tube and spoon set, protective plastic carrying tube, specimen bag, labels for identification of samples and pre-paid addressed envelope (for return postage). Participants will take the collection pack home with them and follow the following instructions to collect and return the stool sample.

Collection instructions:

1. Wash hands thorough and apply gloves.
2. Collect stool sample into the pathology stool pot within 1-3 days of study appointment.
Note: Method of collecting the stool sample must prevent stool from falling into toilet water to avoid sample contamination.
3. Unscrew the stool specimen collector tube cap and use the spoon to scoop two spoonsful of stool (approximately 2 gram or 2mL in volume) from the sample.
4. Place the sample in the stool specimen collector tube.
5. Tighten the cap and shake to mix the contents thoroughly (invert 10 times) to create a suspension. Note: Some stool material may be difficult to re-suspend. As long as the material is suspended, the sample is stabilized. Foaming/ frothing during shaking is normal.
6. Dispose of gloves, unused stool material and the pathology stool pot and wash hands thoroughly.
7. Place stool specimen collector tube into the protective plastic carrying tube.

8. Place carrying tube into specimen carrier bag.
9. Place the sealed specimen bag containing the sample into the provided postage-paid reply envelop and post within 7 days of sample collection.
10. Samples will be returned to the nearest BRACE site laboratory for storage at -80C.

What we will do with the sample:

Briefly, samples will be collected at home by the study participants into Zymo fecal collection tubes which contain a reagent to stabilise DNA at ambient temperature. Samples were returned by mail within 2 weeks and stored at -80°C until processed. DNA will be extracted from pelleted samples using the appropriate DNA Isolation kit. We will perform 16S rRNA sequencing and/or metagenomic sequencing to profile the composition of the microbiota in the sample and the metagenome encoded by the microbiota. qPCR will be utilised to quantify bacterial load and quantify specific bacterial populations. We will then determine whether the composition or metagenome-encoded function of the stool microbiota is correlated with either specific or non-specific immune responses to the BCG vaccine. We will also assess whether the composition of the stool microbiota is associated with any of the other primary, secondary, or exploratory outcomes described in the study protocol. Furthermore, we will characterise the composition of the stool microbiota in participants in the trial and investigate whether the composition of the microbiota is altered at 3 or 12 months later. We will assess whether immunisation with BCG leads to an altered microbiota at 3 or 12 months compared to participants receiving the placebo.

References:

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16.3 Appendix 3 UK Specific Requirements

In the UK, the Competent Authority (MHRA) required the following two UK specific requirements:

1. In the UK, a negative pregnancy test is required for all WOCBP to confirm eligibility for the trial.
2. In the UK, the responsibility to break the treatment code in emergency situations resides solely with the UK Principle Investigator and will not be delayed by requiring other study staff in Australia such as the Chief Investigator or medical monitor to be involved in the decision to un-blind. The study code will only be broken for valid medical or safety reasons e.g. in the case of a severe adverse event where it is necessary for a treating physician (Requester) to know which intervention the participant has received, in order to manage the participant's condition appropriately.

The Requester contacts the local Principal investigator (PI), or delegate, to discuss the pros and cons of breaking the code. If the consensus is to break the code, the Requester contacts the holder of the code break list. In the UK, this has been delegated to the UK based Data Manager who will provide the Requester with the information on allocated group on direction from the PI. On receipt of the allocation details the Requester deals with the participant's medical emergency as appropriate. Should this code-breaking protocol be activated, the Chief Investigator will be alerted at the earliest opportunity, and within 2 working days at the latest.

Woman of Child Bearing Potential:

For the purpose of this document, a woman is considered of childbearing potential (WOCBP), i.e. fertile, following menarche and until becoming post-menopausal unless permanently sterile. Permanent sterilisation methods include hysterectomy, bilateral salpingectomy and bilateral oophorectomy. A postmenopausal state is defined as no menses for 12 months without an alternative medical cause. A high follicle stimulating hormone (FSH) level in the postmenopausal range may be used to confirm a post-menopausal state in women not using hormonal contraception or hormonal replacement therapy. However in the absence of 12 months of amenorrhea, a single FSH measurement is insufficient.

16.4 Appendix 4 Brazil Specific Requirements

SARS-CoV-2 Screening test

Due to public interest in determining the extent of asymptomatic SARS-CoV-2 infection in healthcare workers in Brazil, the Brazilian investigators will use the BRACE participants to estimate this prevalence rate. Therefore after enrolment a baseline respiratory swab will be collected by the study nurse. The swab samples will be analysed by PCR for detection of SARS-CoV-2 and participants advised when results are confirmed. Participants who return a positive SARS-CoV-2 result on the baseline swab will remain in the trial. In Mato Grosso do Sul, the samples will be analysed in batch months after randomisation, so there will be no clinically actionable results. Results will be shared with participants approximately 3 months after randomisation, for participant who return a positive SARS-CoV-2 result, the site will be required to report the participant's positive SARS-CoV-2 results to the applicable health agencies. They will be told that they will not be informed of their result before then. In Rio de Janeiro, due to high transmission rates, samples will be tested immediately and reported to participants. PCR tests will be conducted by the study lab team and the results reported to health agencies by a system called e-SUS VS, which constitutes a database of several diseases, including COVID-19, which is mandatory.

IGRA

At randomisation, blood for IGRA will be taken for later assessment of seroconversion (production of specific anti-SARS-CoV-2 antibodies and IGRA TB). Therefore the initial blood sample in Brazil will be 35ml. This will identify participants who had TB exposure prior to commencement of the study. IGRA results will not exclude participants at consent & randomisation stage. Results will be shared with participants approximately 3 months after randomisation. A study doctor will follow-up with participants with positive IGRA to offer further assessment and treatment through government service provision.

Participant reimbursement

In Brazil, Resolution No. 466 of December 12, 2012 outlines the guidelines and regulatory standards for research involving humans in Brazil. This resolution outlines the requirement to provide reimbursement to participants and their companions, when necessary, such as transportation. In line with this requirement, participants in Brazil will receive reimbursement for relevant transportation costs for participation in the BRACE trial.

Safety Reporting

In Brazil, the RPI/s and SPI/s must comply with the safety reporting requirements of CEP/CONEP (defined in Circular Letter number 13). The HREC/s must be notified of all SAEs through the Brazil Platform (Notification), after the end of the event. The following timelines will be met for this study:

1. 30 days in case of fatal SAE occurring in a participant of the site in the jurisdiction of the HREC
2. 7 days in case of an SAE with a causal relationship with the investigational product, in a participant of the site in the jurisdiction of the HREC (Casual relationship means that the SAE is judged by either the reporting investigator or the sponsor as having a reasonable possibility of a causal relationship to a study vaccine).
3. 6 months for other SAE.

The RPI (or delegate) will notify SUSAR in Brazil to all investigators in their region, as appropriate. The RPI (or delegate) will report significant safety issues (including USM) to SPI in their region, the regulatory authority and applicable HREC/s in accordance with the requirements. The RPI (or delegate) will provide periodic reports of SAE (from Brazil trial sites) to the applicable regulatory authorities and/or HREC/s, as appropriate.

16.5 Appendix 5 The Netherlands Specific Requirements

The following changes will apply for the performance of the protocol in the Netherlands:

Statement of Compliance

This clinical trial will be conducted in compliance with all stipulations of this protocol, the conditions of the ethics committee approval, the NHMRC National Statement on ethical Conduct in Human Research (2007 and all updates), the Integrated Addendum to ICH E6 (R1): Guideline for Good Clinical Practice E6 (R2), dated 9 November 2016 annotated with TGA comments and the NHMRC guidance Safety monitoring and reporting in clinical trials involving therapeutic goods (EH59, 2016), and General Data Protection Regulation (GDPR) , as well as local laws and regulations, such as the Wet medisch-wetenschappelijk onderzoek met mensen (WMO).

2. Recruitment and consent

In Europe, due to ethics regulations, an electronic PICF will not be used and no information on eligibility nor any contact information will be collected prior to the informed consent process being finalized. When participants are interested in the study, they can verify their eligibility with the criteria listed on the website. Then, they will be shown a list of participating centers and advised to contact one of the centers directly to make an appointment for the first study visit. At this visit, the informed consent procedure will be completed in a face to face setting where the the PICF will be read and signed by both participant and investigator. Exact date of birth will not be collected in the eCRF for the study due to GDPR constraints; instead, year of birth (or 01-01-yyyy) will be used in the eCRF. Medicare card number is not applicable in Europe.

3. Data capture methods and data use, storage, access and disclosure during the trial

Archiving will be in compliance with NFU requirements: study data, source documents and the Study File will be kept for 25 years.

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4. COVID-19 testing will be performed via the national testing policy and therefore, the General Practitioner will be notified of the results by the organisation that performs the testing: GGD or the hospital that performs the test.

5. Sharing of contact information

In order to send out the 3, 6, 9, and 12 month questionnaires, the participant's email address will be collected in the RedCAP database. No other identifying information will be stored in the database for EU participants.

6. BCG vaccination is not expected to cause an exacerbation of the immune response with adverse consequences, because of 3 main arguments: • By activating anti-viral mechanisms, BCG decreases virus load and systemic inflammation (Arts et al, Cell Host Microbe 2018). Influenza pathophysiology is the same so if BCG had adverse effects, this would have been known for a long time. • Information is available on individuals vaccinated with BCG last year and no COVID19 complications were observed in this group.

16.6 Appendix 6 Spain Specific Requirements

The following changes will apply for the performance of the protocol in Spain:

1. Statement of Compliance

This clinical trial will be conducted in compliance with all stipulations of this protocol, the conditions of the ethics committee approval, the Integrated Addendum to ICH E6 (R1): Guideline for Good Clinical Practice E6 (R2), dated 9 November 2016 and General Data Protection Regulation (GDPR) , as well as local laws and regulations

2. Inclusion criteria

According to recommendations of the competent authority AEMPS (Agencia Española del Medicamento y Productos Sanitarios) If the patient is female, and of childbearing potential, she must have a negative pregnancy test (provided by Sponsor) at the time of inclusion and practice a reliable method of birth control for 30 days after receiving the BCG vaccination. - Woman of Childbearing Potential is defined as a premenopausal female who is capable of becoming pregnant.

3. Recruitment and consent

In Europe, due to ethics regulations, an electronic PICF will not be used and no information on eligibility nor any contact information will be collected prior to the informed consent process being finalized. When participants are interested in the study, they can verify their eligibility with the criteria listed on the website. Then, they will be shown a list of participating centers and advised to contact one of the centers directly to make an appointment for the first study visit. At this visit, the informed consent procedure will be completed in a face to face setting where the PICF will be read and signed by both participant and investigator.

Exact date of birth will not be collected in the eCRF for the study due to GDPR constraints; instead, year of birth (or 01-01-yyyy) will be used in the eCRF. Medicare card number is not applicable in Europe.

4. Data capture methods and data use, storage, access and disclosure during the trial

Archiving will be in compliance with NFU requirements: study data, source documents and the Study File will be kept for 25 years.

5. Sharing of contact information

In order to send out the 3, 6, 9, and 12 month questionnaires, the participant's email address will be collected in the RedCAP database. No other identifying information will be stored in the database for EU participants.

16.7 Appendix 7 Optional Biological sample collection during episodes of illness

Assessment of immune responses during episodes of illness will provide crucial insights into the mechanisms by which BCG may protect against COVID-19. BCG is proposed to protect against unrelated infections by boosting the innate immune response¹ which can directly protect against infections and also shape the adaptive immune response²⁻³. Biological samples collected after infection provide meaningful insight into the long-lasting effects of the infection and immune memory. However, they do not provide information about the early immune response to infection that can promote early clearance, may impact disease severity and may define the long-lasting memory response. It is this part of the immune response where BCG vaccination may play a crucial role in protection against COVID-19 as well as non-COVID-19 respiratory infections.

Objectives of exploratory sub-study

The additional collection of biological samples from BRACE participants during episodes of febrile or respiratory illness will contribute to the planned subgroup exploratory analyses of BRACE:

11. To determine the impact of BCG vaccination on the immune system that are associated with protection of adult healthcare workers from non-tuberculous infectious diseases including COVID-19.
13. To identify factors (e.g. age, sex, chronic conditions such as diabetes and cardiovascular disease, smoking, asthma, prior BCG vaccination, genetics, influenza vaccination, immunological/molecular factors) that influence adult immune responses and COVID-19 responses.

It will also contribute to the following additional exploratory objectives:

In a sub-set of BRACE trial participants who consent for an optional biological sample to be collected during episodes of fever or respiratory illness:

- To characterise the immune response to SARS-CoV-2 infection
- To compare immune responses during an episode of respiratory illness (COVID-19 or non-COVID-19 illness) in BCG-vaccinated and non-BCG vaccinated participants

Outcomes:

Immune system characterisation and molecular markers of disease in episodes of COVID-19 or non-COVID-19 respiratory or febrile illness from BCG-vaccinated and non-vaccinated participants.

Population: A sub-group of the BRACE trial participants who consent to an optional biological sample to be collected during episodes of fever or respiratory illness.

Study Duration:

As per the BRACE trial protocol – 2 years.

Participant Duration:

12 months from randomisation.

Sub-study Locations:

Optional inclusion for Australian sites.

Sub-study Principal Investigator:

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Potential risks and benefits:

Known potential risks:

This sub-study involves minimal risk to participants. Having a blood test can sometimes cause some pain from the needle or be uncomfortable. Occasionally a small amount of bruising can occur on the skin where the blood was taken. Trained members of the study team will collect the blood samples from participants. Having a respiratory swab can sometimes be uncomfortable. Trained members of the study team will collect the respiratory swabs from participants. Self-testing swab kits may be provided as required, with clear instructions to participants on safe self-swabbing technique.

Known potential benefits:

This sub-study is exploratory in nature and is not expected to provide any direct additional benefit to the participants. The findings of the study, however, may be of significant value for understanding the immune response to COVID-19, the off-target effects of BCG vaccination on responses to COVID-19 and other respiratory infections and determinants of disease severity.

Sub-study design:

Consent:

An additional option has been added to the BRACE online consent form to allow participants to optionally consent additional biological sample collection during an episode of illness. The BRACE participant information and consent form (PICF) has been also modified to explain to participants the process for collecting these additional blood samples and why they are being collected. If a participant declines to consent for additional biological sample collection during an episode of illness this will not affect their participation in the BRACE trial (assuming all other inclusion and exclusion criteria are met).

Sample collection process:

Participants consenting for additional biological sample collection during an episode of illness may be contacted by the study team during any episode of respiratory or febrile illness that occurs during their involvement in the BRACE trial (i.e. up to 12 months from randomisation). Sample collection would occur during and up to one month after resolution of an episode of illness with fever or respiratory symptoms. The collection of samples will be done at a study site (e.g. if they are inpatients or obtaining SARS-CoV-2 testing at a study site) or at the participant's home, depending on the location of the participant.

Samples to be collected are:

-a blood sample

and/or

- saliva/respiratory swab/s

All samples will be collected, processed and stored in accordance with the BRACE trial protocol section 7.3. We will aim to take these samples at the same time as any other clinical or research samples where possible to minimise the number of sample collections for each participant, minimise contact of research staff with infectious patients and to reduce the need for research staff to use vital personal protective equipment (PPE).

What we will do with the sample:

Samples will be processed for analysis of the immune system as detailed in BRACE trial protocol section 3.2. Where indicated, saliva/respiratory swab/s collected will be linked with the relevant public health testing and reporting systems as BRACE trial protocol section 7.3. In addition, samples will be included in the BRACE biobank if participants have also consented for their samples being placed in the BRACE biobank.

References

1. Novakovic B, Messina N, Curtis N. Chapter 6 - The Heterologous Effects of Bacillus Calmette-Guérin (BCG) Vaccine and Trained Innate Immunity. In: Faustman DL, editor. *The Value of BCG and TNF in Autoimmunity (Second Edition)*. Second edition. ed: Academic Press; 2018. p. 71-90.
2. Arts RJW, Moorlag S, Novakovic B, et al. BCG Vaccination Protects against Experimental Viral Infection in Humans through the Induction of Cytokines Associated with Trained Immunity. *Cell Host Microbe*. 2018;**23**:89-100 e5.
3. Kleinnijenhuis J, Quintin J, Preijers F, et al. Long-lasting effects of BCG vaccination on both heterologous Th1/Th17 responses and innate trained immunity. *J Innate Immun*. 2014;**6**:152-8.