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**BRACE**  
**Statistical Analysis Plan**  
**for the Interim Efficacy Analysis**

**Document Version History**

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**LIST OF ABBREVIATIONS**

AE	Adverse Event
App	Application
AR	Adverse Reaction
BCG	Bacille Calmette-Guerin
BMI	Body Mass Index
BRACE	BCG vaccination to Reduce the impAct of COVID-19 in hEalthcare workers
CI	Confidence Interval
COVID-19	Coronavirus Disease of 2019
DSMB	Data Safety Monitoring Board
GST	Group Sequential Test
ICU	Intensive Care Unit
ITT	Intent-To-Treat
LOCF	Last Observation Carry Forward
PCR	Polymerase Chain Reaction
SAE	Serious Adverse Event
SARS-CoV-2	Severe Acute Respiratory Syndrome Coronavirus 2
SD	Standard Deviation
SE	Standard Error
MedDRA	Medical Dictionary for Regulatory Activities
WHO DD	World Health Organization Drug Dictionary

## 1. INTERIM ANALYSIS OBJECTIVES

As part of the interim monitoring for the BRACE Trial, there will be a single formal interim analysis of the efficacy data. This will compare the proportion of participants who have severe COVID-19 - by 6 months (primary outcome 2) between the BCG group and the control group. Given the low prevalence of COVID-19 in participants recruited in Stage 1 of the trial (due to absent/very low community transmission in Australia), the interim analysis will include only participants recruited in Stage 2 of the trial.

## 2. BACKGROUND/INTRODUCTION

### 2.1. GENERAL TRIAL INFORMATION

#### Trial design

BRACE is a phase III, two group, multicentre, randomised controlled trial in healthcare workers to determine if BCG vaccine reduces incidence and the severity of COVID-19 during the SARS-CoV-2 pandemic compared to placebo.

The primary objectives are:

1. To determine if BCG vaccination (Intervention) compared with placebo (Comparator) reduces the incidence of COVID-19 (Outcome) measured over the 6 months following randomisation (Time) in healthcare workers during the COVID-19 pandemic (Participants).
2. To determine if BCG vaccination (Intervention) compared with placebo (Comparator) reduces the incidence of severe COVID-19 (defined as COVID-19-related death, hospitalisation, or non-hospitalised severe disease (defined as Non-ambulant<sup>1</sup> for  $\geq 3$  consecutive days OR Unable to work<sup>2</sup> for  $\geq 3$  consecutive days)) (Outcome) measured over the 6 months following randomisation (Time) in healthcare workers during the COVID-19 pandemic (Participants).

<sup>1</sup>“pretty much confined to bed (meaning finding it very difficult to do any normal daily activities)”

<sup>2</sup>“I do not feel physically well enough to go to work”

Initially the trial it was designed to compare primary and secondary outcomes between BCG and no BCG which was given concurrently with the flu vaccination (Stage 1). The trial was then expanded and the design was revised to compare primary and secondary outcomes between BCG and a placebo (Stage 2). The comparison of BCG vs placebo in Stage 2 is the primary analysis of interest, however it is planned to combine the data from the two stages of the trial (detailed below) in a meta-analysis for secondary analyses of the primary and secondary outcomes.

Further details of two stages of the trial are provided below:

Stage	Dates	Sample Size	Intervention	Control	Blinding
Stage 1	30-Mar-2020 to 13-May-2020	2841	BCG + Flu	Flu	Unblinded
Stage 2	14-May-2020 to 01-Apr-2021	3988	BCG	Placebo	Blinded

#### Treatment groups

Participants were randomly allocated in a 1:1 ratio to the BCG vaccine group or to the control group. Randomisation was stratified by stage of the study (prior to or post the addition of the placebo vaccination), by study site, by age (<40 years; 40 to 59 years;  $\geq 60$  years) and by presence of comorbidity (any of diabetes, chronic condition, respiratory disease, cardiac condition, hypertension).

The BCG vaccine group received an adult dose of 0.1 mL of BCG vaccine SSI injected intradermally over the distal insertion of the deltoid muscle onto the humerus (approximately one third down the upper arm).

The control group in Stage 2 of the trial received 0.1 mL of 0.9% NaCl injected intradermal over the distal insertion of the deltoid muscle onto the humerus. The control group in stage 1 of the trial received the flu vaccine on the day of randomisation. In Stage 2 of the trial, the control group received a placebo in an effort to blind participants to their treatment group allocation (although the subsequent local reaction at the injection site with BCG vaccination prevents total blinding). Members of the trial team, except immunisers, are also blinded to the group allocation in stage 2 by hiding or removing the treatment group variable and all other variables related to BCG from the dataset and will be until the formal detailed statistical analysis plan for the final analysis has been confirmed, and made publicly available, all data cleaning/preparation is complete, and database is locked for analysis.

### **Trial population**

Participants are adult ( $\geq 18$  years) healthcare workers from Europe (the Netherlands, Spain and the United Kingdom), South America (Brazil) and Australia (Victoria, Western Australia, South Australia and New South Wales). Exclusion criteria are: BCG vaccine contraindication, previous positive SARS-CoV-2 test result and prior involvement in this trial.

### **Original sample size**

The sample size was calculated based on the two primary outcomes of: (1) the proportion of participants with COVID-19; and (2) the proportion of participants with severe COVID-19, by 6 months following randomisation. Since the trial aims to assess two primary outcomes, an adjustment for multiplicity was applied to maintain a global Type I error rate of 5% by splitting of this alpha.

When designing the trial, we anticipated that:

- i) 7244 healthcare workers recruited in Stage 2 would provide 80% power to detect a risk ratio of 0.67 (equivalent to a 1.3% absolute difference) in the BCG group compared to the control group for severe COVID-19 at 6 months (primary outcome 2), assuming 4% of subjects will be infected by severe COVID-19 by 6 months in the control group and allowing for 16% lost to follow-up by 6 months (2-sided alpha = 0.04).
- ii) 2016 healthcare workers would provide 95% power to detect an absolute difference of 10% in incidence of COVID-19 (primary outcome 1), assuming 55% of subjects will be infected by COVID-19 in the control group (2-sided alpha = 0.005).
- iii) In the pre-planned meta-analysis, 10,078 healthcare workers recruited in phase 1 and 2 would provide 90% power to detect a risk ratio of 0.67 (equivalent to an absolute risk difference of 1.3%) in the BCG group compared to the control group for severe COVID-19 at 6 months (primary outcome 2), assuming 4% of subjects will be infected by severe COVID-19 by 6 months in the control group and allowing for 20% lost to follow-up by 6 months (2-sided alpha = 0.04).

For full details refer to section 11.1 of the trial protocol.

## **2.2. DESCRIPTION AND SCHEDULE OF THE INTERIM ANALYSIS**

### **Interim Analysis: original plan**

We originally 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 in stage 1 of the trial we planned to recruit 1,668 participants per group which gave us 72% power to identify a reduction from an incidence of 4% in severe COVID-19 at 6 months in the control group to 2% in the intervention group. If the assumptions were correct, this would equate to 100 cases in total. We therefore planned a formal interim analysis of severe COVID-19 once there had been 100 cases of severe COVID-19.

This interim analysis of severe COVID-19 was to be performed on 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 Data Safety Monitoring Board (DSMB) was to also be given information on which participants belong to the first stage of the trial. For full details, refer to section 11.4 of the trial protocol. With 100 events, there would have been 72% power to detect a risk ratio of 0.5 in the incidence of severe COVID-19 at 6 months at the interim analysis based on a two-sided test of  $\alpha=0.005$ , which was thought to be a reasonable power for this interim analysis.

#### **Modifications to the Interim Analysis Plan: restriction on inclusion criteria**

Recruitment into the BRACE trial was early stopped on 01-04-2021, after 3,988 participants had been recruited into Stage 2, 6,286 overall (including 2,841 recruited in Stage 1). The main reason for stopping before reaching the calculated sample of 10,078 was the rollout of COVID-19-specific vaccines in healthcare workers around the world, which started in December 2020, and affects the ability of BRACE to determine the effectiveness of BCG vaccination. With 3,988 participants randomised into Stage 2 of the trial there would be 63.3% power to identify a reduction of 1/3 in the incidence of severe COVID-19 at the end of the trial, from an incidence of 4% at 6 months in the control group to 2.67% in the intervention group (the assumptions used in the sample size calculation for stage 2) based on a two-sided test with  $\alpha = 0.045$ , if no interim analysis were planned.

At the time recruitment was stopped, we were monitoring the occurrence of severe COVID-19 cases to be ready for the interim analysis, originally planned to occur once we reached 100 severe cases (refer to section 11.4 of the protocol). Like many other COVID-19 studies which have had to alter their plans for their interim analyses as a result of the rapidly changing landscape, the earlier than expected rollout of COVID-19-specific vaccines made us reconsider the rational and plan for the interim analysis.

Specifically, we revised the plan for the interim analysis of severe COVID-19:

- **To be restricted to participants recruited in Stage 2 of the trial.** Originally the interim analysis was going to include all randomised participants. However, participants recruited in Stage 1 of the trial were exclusively recruited in Australia. As there has been limited community SAR-CoV-2 exposure in Australia, there are very few episodes of COVID-19 in the participants recruited in Stage 1, and also (because of the low community transmission of COVID-19), few participants had COVID-19 swab testing when they had symptoms. For both of these reasons it was decided to restrict the interim analysis to participants recruited in Stage 2.
- **To Include exposure time before any dose of any COVID-19-specific vaccine only.** Given that it is unknown what effect a COVID-19-specific vaccine will have on the effectiveness of the BCG vaccination (or vice versa), participants will be censored at the time of their first COVID-19-specific vaccine.

#### **Modifications to the Interim Analysis Plan: alpha spending function**

The use of SARS-CoV-2 serology in the definition of COVID-19 outcomes (which will take time to process), the changing landscape of COVID-19, and the vaccine rollout (which will reduce the useable follow-up data in trial participants), also means that the importance of the interim analysis relative to the final analysis has increased. It has also meant that the interim analysis has been delayed. For both of these reasons, we decided to revise the stopping rule to be used in the interim analysis of severe COVID-19 to maximise the chance of identifying BCG as a successful intervention at the interim analysis.

We originally allocated  $\alpha=0.045$  to the primary outcome of severe COVID-19, taking a conservative approach of splitting the alpha between the interim and final analysis, spending 0.005 of the alpha at the interim analysis and saving 0.04 of the alpha for our final analysis. Instead we decided to change the stopping rule to use an alpha spending function, where the threshold to identify efficacy is based on the amount of data available at the time of the interim analysis.

The database lock for the interim analysis happened on the 30-04-2021, and has been followed by blinded data checking and cleaning. Up to the 30-04-2021, 82.3% of participants had received a COVID-19-specific vaccine, or had an episode of severe COVID-19, and/or had been followed for at least 6 months from randomisation . Using an alpha-

spending function based on the Pocock stopping rule, an interim conducted on 82.3% of the available information on 3988 participants, and an overall alpha of 0.045 for this outcome, results in a nominal alpha of 0.04 at the interim analysis, and 0.021 at the end of the study (calculated using a Group Sequential Test (GST) of Two Proportions in NQuery (PTT12-1). Thus, the threshold of 0.04 will be used as the stopping rule for the interim analysis. At this interim time point we will have 52.9% power to detect a risk ratio of 0.67 in the incidence of severe COVID-19 at 6 months. Under this spending function we will have 0.021 alpha left for the final analysis if the treatment comparison does not reach the threshold at the interim analysis.

### **Unblinding and Stopping Guidelines**

The results of the interim analysis for the primary outcome of severe COVID-19 will be presented to the DSMB (with the modifications as described in the next section). If the p-value for the treatment comparison is below the threshold of 0.04 then the DSMB will be advised to recommend that the trial be unblinded to the Chief Principal Investigator and Trial Steering Committee. 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 factors and information on the efficacy of BCG vaccination on the incidence of COVID-19 and their view of the clinical and public health importance of the results in the context of the current pandemic. Since the interim analysis will be done after recruitment to the trial has ceased, no modification to recruitment as a result of the interim analysis will occur. If the DSMB members recommend the interim results be unblinded then further analyses of the severe COVID-19 outcome will also be made available, as detailed in section 5.2.4 of this SAP.

Should the interim analysis results not reach the threshold of alpha 0.04 calculated using the GST method, the final analysis of the severe COVID-19 outcome will be based on a nominal alpha of 0.021.

## **3. POPULATIONS OF INTERIM ANALYSIS**

### **Intention-To-Treat (ITT) Population**

The interim analysis will be done following the ITT principle, analysing all randomised patients according to the study group to which they were randomised, irrespective of the intervention received.

## **4. DERIVATION OF DATA TO BE ANALYSED**

### **Definition of Baseline**

Baseline will be defined as the date of randomisation.

### **Primary Outcome Measure for the Interim Efficacy Analysis**

*Severe COVID-19 (primary outcome 2):*

The primary outcome for the interim analysis is the occurrence of severe COVID-19 by 6 months following randomisation. This will be defined as:

- 1) Death as a consequence of COVID-19, with a positive SARS-CoV-2 test
- 2) Hospitalised as a consequence of COVID-19, with a positive SARS-CoV-2 test
- 3) Non-hospitalised severe disease as a consequence of COVID-19, defined as non-ambulant<sup>1</sup> for  $\geq 3$  consecutive days or unable to work<sup>2</sup> for  $\geq 3$  consecutive days, with a positive SARS-CoV-2 test

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

<sup>2</sup> “I do not feel physically well enough to go to work”

As described above, the interim analysis will include only participants recruited in Stage 2 of the trial and will only include time at risk prior to receiving a COVID vaccine.

### Definition of Severe COVID-19 and SARS-CoV-2 Testing

Participants self-report fever, intermittent cough, persistence cough, shortness of breath or difficulty breathing, sore throat, runny/blocked nose, headache, muscle and/or joint ache, fatigue, nausea, vomiting and/or diarrhea, loss of taste and/or smell, being non-ambulant and absent from work, being hospitalised and testing for SARS-CoV-2. This is collected daily or weekly via a smartphone app (or in the case of Brazil via a weekly phone call). The information collected via the app/phone calls is subsequently confirmed through quarterly questionnaires.

When a participant reports an episode of illness with fever, cough, sore throat, shortness of breath, respiratory distress/failure or being non-ambulant or unable to work for  $\geq 3$  consecutive days (irrespective of symptoms), the participant is prompted to have a SARS-CoV-2 test. The participant is also required to complete an illness resolution form, once recovered from illness, documenting whether the SARS-CoV-2 test(s) was positive or negative. In circumstances where the participant does not recover from the illness this can also be entered by the study site coordinator or MCRI data team.

For the interim efficacy analysis, if an episode of illness occurs meeting the primary outcome 2 definition but the participant confirms that SARS-CoV-2 testing was not medically indicated (e.g. unable to work for  $\geq 3$  consecutive days due to sprained ankle), this will not be considered an episode of illness.

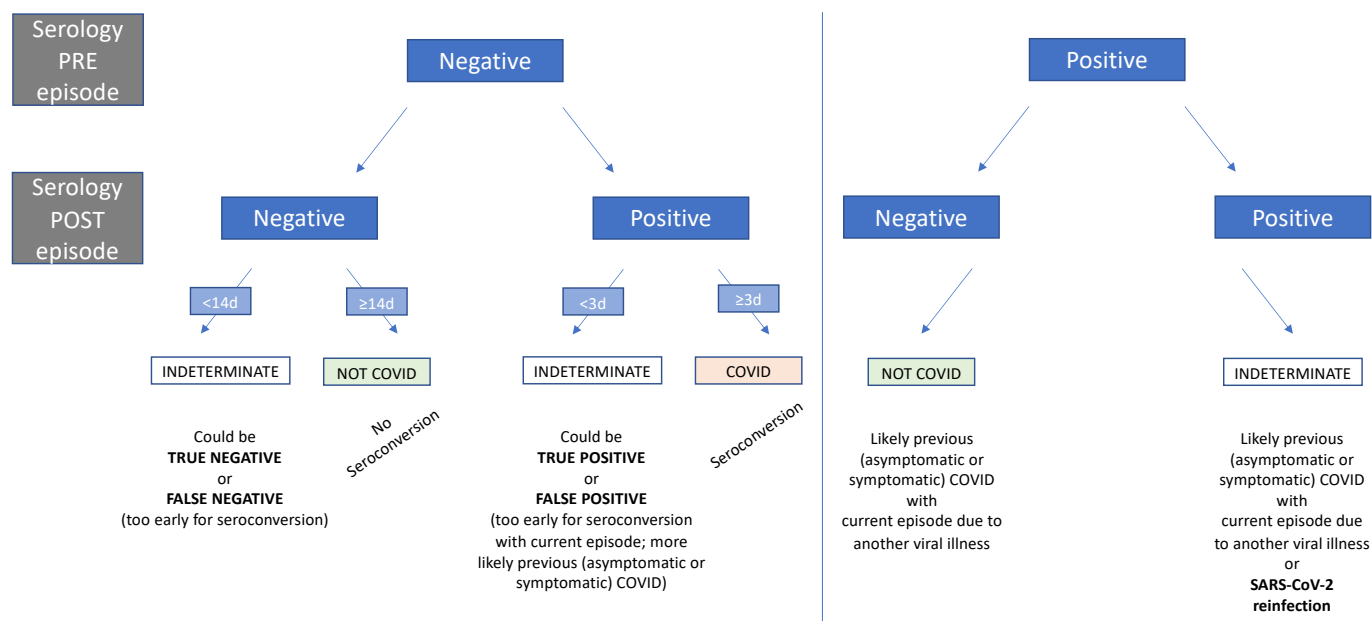
The date of occurrence for an episode of severe COVID-19 will be defined as the first date of any symptom onset for the episode. This is to account for any potential difference between groups in time to SARS-CoV-2 testing from symptom onset. Symptoms reported in the trial include fever, intermittent cough, persistence cough, shortness of breath or difficulty breathing, sore throat, runny/blocked nose, headache, muscle and/or joint ache, fatigue, nausea, vomiting and/or diarrhoea, and loss of taste and/or smell.

An episode of illness will be considered to have an associated COVID-19 test if the episode has a respiratory swab polymerase chain reaction (PCR) test dated  $\leq 21$  days after the onset of symptoms or  $\leq 7$  days from the last day of symptoms. An episode of illness with a missing PCR test in this specified window, but with a positive PCR test done in the 3 days prior to the onset of symptoms will be also categorized as a severe COVID-19 event.

If an episode of illness is missing a respiratory swab PCR test within the testing window, serology data will be used if available to identify seroconversion. Seroconversion (e.g. the production of specific anti-SARS-CoV-2 antibodies) will be determined using the serology results prior to and after the onset of symptoms for a severe episode of illness. Blood samples are scheduled for collection at baseline, 3-months, and 6-months from randomisation. The figure below shows the algorithm for the interpretation of the serology results in those cases where an episode of illness is missing a respiratory swab PCR.



### Algorithm for interpretation of serology results



### COVID-19 specific vaccine

As described above, participants who receive a COVID-19-specific vaccine will be censored at the date of their first dose of vaccine for the primary analysis. Participants who have received COVID-19-specific vaccinations are still being followed up to identify 'breakthrough' COVID-19, by still being prompted to have a COVID-19 test subsequent to vaccination if they report an episode of illness with self-reported fever, cough, sore throat, shortness of breath or respiratory distress/failure or non-ambulant or unable to work for  $\geq 3$  consecutive days. This data will be used to conduct sensitivity analyses, including time following COVID-19-specific vaccination (sensitivity analysis *i*).

### Definition of censoring

For the primary analysis, a participant will be considered to have met the primary outcome 2 (at the time of first of symptoms of such an occurrence) if they:

- report a severe episode COVID-19 (death and/or hospitalised as a consequence of COVID-19 or non-hospitalised severe disease as a consequence of COVID-19), and
- have a positive respiratory swab PCR test for SARS-CoV-2 within the testing window or if blood samples taken before and after the episode show seroconversion to SARS-CoV-2

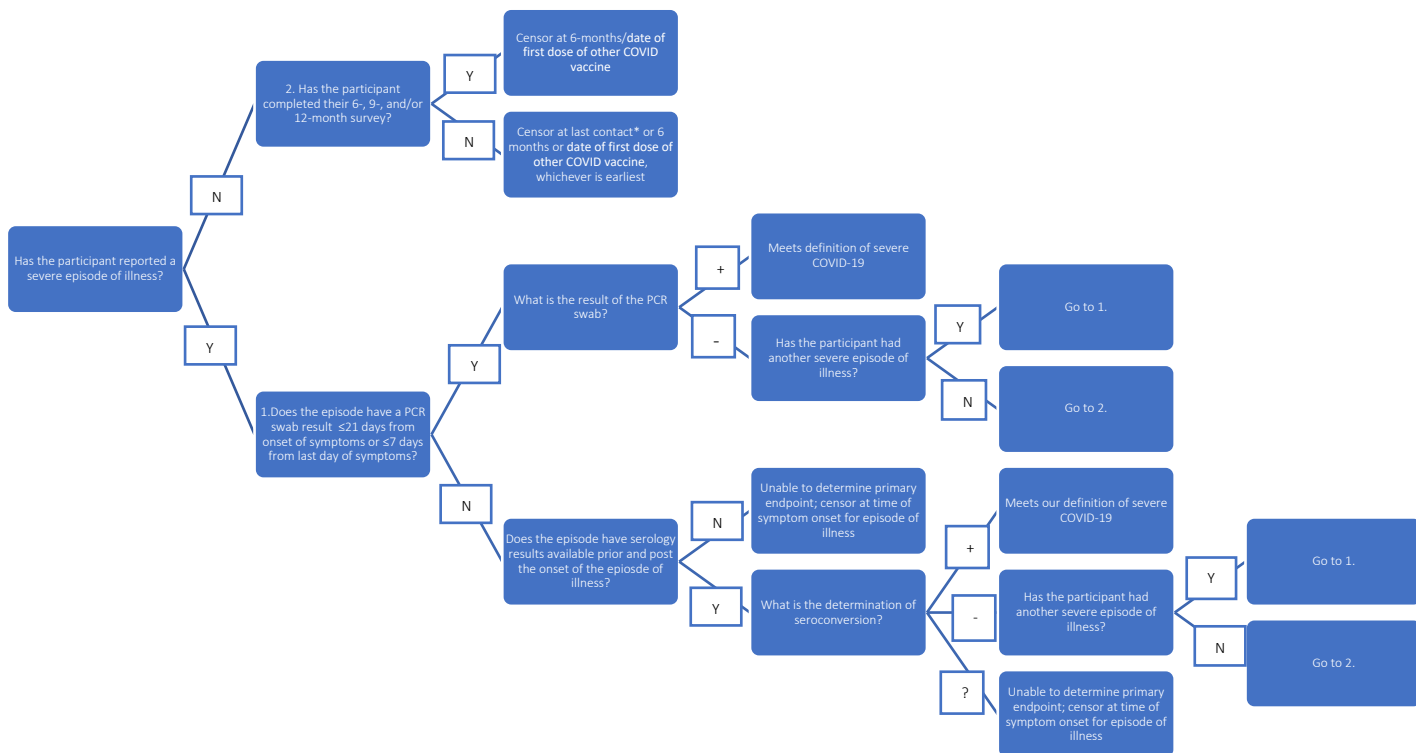
For all other participants, the following rules around censoring apply:

1. If a participant reports a severe episode of illness but is missing a swab PCR test within the testing window and serology data is unavailable or indeterminate, then it is not possible to determine the occurrence of primary outcome 2, and the participant will be censored at their first of such an occurrence.
2. For participants who have not had a severe episode of COVID-19 or have had severe episodes of illness(es) confirmed as negative by swab PCR or serology within 6 months of randomisation:
  - i) those who have a 6-month, 9-month and/or 12-month surveys will be censored at 6 months or date of first dose of a COVID-19-specific vaccine, whichever is earlier

- ii) those who do not have a 6-month, 9-month or 12-month survey will be censored at the last contact\* or 6 months or date of first dose of COVID-19-specific vaccine, whichever is earliest

\* last contact defined as latest of i) last App data/manual follow-up entry; ii) 3-month survey date; or iii) blood draw date (if serology used to determine seronegativity).

The flow diagram below details derivation of primary definition of primary outcome 2 censoring of data:



## 5. STATISTICAL METHODOLOGY

### 5.1. GENERAL METHODOLOGY

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

### 5.2. INTERIM ANALYSIS METHODOLOGY

The following data will be summarized:

#### 5.2.1 Baseline Characteristics

The following baseline characteristics will be described and presented by intervention group:

- Sex – Male/Female/Other/Declined/ Missing - N (%)
- Age, years - Mean (SD)
- BMI – < 18.5 / 18.5 to 24.9/ 25 to 29.9 / >30 / Missing - N (%)

- Department – Emergency / Intensive Care Unit or High Dependency Unit/ Operating Theatre/ General Ward / Pharmacy / Other/ Practice outside of hospital setting/ Missing- N (%)
- Role – Nurse of Midwife / Doctor/ pharmacist / Patient Service Assistant/ Clerical or Administrative staff/ Allied Health/ Other/ Missing N (%)
- Contact with patients, hours - <10 / 10-20 / >20 / Missing - N (%)
- Confirmed cases of COVID-19 within department – Yes / No / Missing - N (%)
- Smoking – Yes / No / Missing - N (%)
- Previous BCG vaccination -No / <1 year / 1-5 years / >5 years / Missing - N (%)
- Comorbidity Type- diabetes, cardiovascular and respiratory disease / diabetes and respiratory disease, cardiovascular and respiratory disease /diabetes and cardiovascular disease/respiratory disease, cardiovascular disease/ diabetes/ none indicated – N (%)

### 5.2.2 Safety

Adverse Events and Serious Adverse Events will be described and reported by intervention group.

### 5.2.3 Efficacy

#### Severe COVID-19– primary outcome (2)

The primary outcome of severe COVID-19 (primary outcome (2)) will be described overall by intervention group as absolute number of participants with severe COVID-19 prior to 6 months, which will then be broken down into these 3 categories, according to most severe event of the three (death > hospitalisation > non-hospitalised severe):

- Absolute number of participants with severe COVID-19 which resulted in death prior to 6 months
- Absolute number of participants with severe COVID-19 which resulted in hospitalisation prior to 6 months
- Absolute number of participants with non-hospitalised severe COVID-19 prior to 6 months

The numbers of participants whose follow-up data is censored for missing PCR or serology test, censored at or prior to 6 months with no report of any severe event, and censored for COVID-19 specific vaccine will also be reported by treatment group.

The primary outcome (severe COVID-19 by 6 months) will be compared between the BCG group and the placebo group recruited in Stage 2 using difference in proportion. This will be estimated using a time-to-event analysis. The analysis will adjust for stratification factors (site, age group and presence of comorbidity).

Specifically, the survival curve for each combination of strata and randomised group will be calculated using a flexible parametric survival model (Royston-Parmar model<sup>1</sup>) adjusting for stratification factors and randomised group. This will be done using the *stpm2* command in STATA, with the *meansurv* and *timevar* options specified. The average survival curve for each randomised group will be estimated as a weighted average of the corresponding stratum-specific survival curves, with weights proportional to the number of individuals in each stratum in the randomised group at baseline. The parameter of interest will be the (adjusted) point estimate for the difference in proportion with severe COVID-19 at 6 months between BCG and control group.

A two-sided bias-corrected 95% confidence interval (CI) for the difference in proportion (BCG – Control) will be calculated with bootstrap standard errors using the STATA bootstrap command. A bootstrap p-value will also be calculated. The bootstrapping will sample 1000 times (with replacement) and be stratified by stratification factors. Note because modelling will be used to estimate the difference in proportion, the results from this analysis will not correspond directly to the raw summaries which will be presented.

A Kaplan-Meier survival curve will also be presented by treatment arm for descriptive purposes.

#### 5.2.4 Sensitivity Analyses for primary outcome 2

If the DSMB members recommend the trial be unblinded or if they request them to help with their deliberations, the following sensitivity analyses will be performed:

- i) Including follow-up after first dose of any COVID-19-specific vaccine
- ii) Excluding severe COVID-19 cases occurring  $\leq 14$  days from date of randomisation
- iii) Including only severe COVID-19 cases detected with positive swab PCR test (ie no serology taken into account)
- iv) Analyses according to the actual vaccine received (BCG vs no BCG) rather than randomised group (as treated analysis)
- v) Censoring participants at the time of any subsequent vaccine (including flu vaccination)

The DSMB may request additional sensitivity analyses if they deem it would help their deliberations.


#### References

1. Royston P. Flexible Parametric Alternatives to the Cox Model: Update. *The Stata Journal* 2004; 4: 98-101. DOI: 10.1177/1536867x0100400112.


6. SIGNATURES PAGE

Signature of Principal Investigator:  Date 02-June-2021

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