Specific and off-target immune responses following COVID-19 vaccination with ChAdOx1-S and BNT162b2 vaccines – an exploratory sub-study of the BRACE trial

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Supplementary Material

Index

[1. BRACE Trial Consortium Group 3](#_Toc143874813)

[2. Supplementary Methods 7](#_Toc143874814)

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# Supplementary Methods

**BRACE trial**

The BRACE trial (NCT04327206) is a phase 3 multicentre, randomised controlled trial (RCT) of BCG vaccination to reduce the impact of COVID-19 in healthcare workers (BRACE trial)(1, 2). The BRACE trial recruited participants in two stages. In Stage 1, between March and May 2020, healthcare workers were recruited from six healthcare centres in Australia in an open label design. Participants in Stage 1 were randomised 1:1 to receive BCG vaccination (a single intradermal dose of 0.1 ml of BCG-Denmark vaccine (AJ Vaccines; Danish strain 1331) or no BCG participants on the day of influenza vaccination. In Stage 2, between May 2020 to April 2021, healthcare workers were recruited from in 25 healthcare centres in Australia, Brazil, the Netherlands, Spain, and the United Kingdom in a double-blind design. Participants in stage 2 were randomised 1:1 to receive a single intradermal dose of 0.1 ml of BCG-Denmark vaccine (AJ Vaccines; Danish strain 1331) or saline placebo. Healthcare workers were eligible for inclusion if they worked in a healthcare setting or had face to face contact with patients. Exclusion criteria for the BRACE trial included: contraindications for BCG vaccination; receipt of BCG vaccine within the past year; a previous positive SARS-CoV-2 test or receipt of any COVID-19–specific vaccine; and involvement in another COVID-19 prevention trial. The full BRACE trial protocol is available at clincaltrials.gov (3).

*Data collection*

Participants were followed up for 12-months following randomisation. Participant data was collected using the REDCap platform (4). At baseline participants completed a detailed questionnaire to provide demographic (including gender), exposure and health data. Participants were asked weekly (using the custom-built Trial Symptom Tracker smartphone application by WeGuide, telephone call or text message) about episodes of illness, and daily during illness episodes (including symptoms and SARS-COV-2 test results). Participants also completed more detailed questionnaires every 3months during follow-up. Peripheral blood samples were collected at baseline (i.e. randomisation) and 3-monthly (i.e. 3, 6, 9 and 12 months post randomisation) and plasma was stored for anti–SARS-CoV-2 antibody testing. Participants in BCOS completed additional questionnaires to provide further details on COVID-19 vaccinations.

**Key Resources Table**

|  |  |  |
| --- | --- | --- |
| Reagent | Source | Catalogue # |
| Blood collection tubes |  |  |
| Vacutainer sodium heparin tubes | BD Biosciences | 367876 |
| Vacuette lithium heparin tubes | Greiner Bio-One | 456088 |
| Vacuette serum separator clot activator tubes | Greiner Bio-One | 455071 |
| Stimulants and media |  |  |
| RPMI 1640 + GlutaMAX, HEPES | Gibco | 72400-047 |
| BCG | Serum Statens Institut |  |
| BCG diluent | Serum Statens Institut |  |
| iVero | Victorian Infectious Diseases Reference Laboratory (VIDRL): Vero cell supernatants. |  |
| iSARS | VIDRL: hCoV-19/Australia/VIC01/2020 (VIC01, NCBI: MT007544.1) infected vero cell supernatant |  |
| *C. albicans,* 11145487 | Royal Children’s Hospital Melbourne (RCH) Microbiology Laboratory: Clinical isolate from children with invasive disease. Organism confirmed by VITEK. |  |
| *E. coli,* 12087149 | RCH Microbiology Laboratory: Clinical isolate from children with invasive disease. Organism confirmed by VITEK. |  |
| *S. aureus,* 11191224 | RCH Microbiology Laboratory: Clinical isolate from children with invasive disease. Organism confirmed by VITEK. |  |
| R848 | Invivo Gen | tlrl-r848 |
| Commercial assays |  |  |
| Bioplex Pro Human Cytokine Screening 48 Plex | BioRad | 12007283 |
| Cobas Elecsys Anti-SARS-CoV-2 assay | Roche | 9289267119 |

**Validation of antibodies**

Antibodies used in this study were validated by the company from which they were purchased or as previously described (5-7).

**Additional Biospecimen details**

Peripheral whole blood was collected from alive participants by venous blood collection at study visits. Samples were maintained at room temperature from the time of collection until stabilisation. Serum sample freezing was done a median of 1.68 (interquartile range (IQR) 1.28-2.20) hours after blood sample collection. Whole blood stimulation freezing was done a median of 2.97 (IQR 2.78-3.15) hours after blood sample collection.

Sample processing, any anomalies or errors in sample processing were documented in the biobank database. Prior to sample testing these were checked for errors that would impact the study, the aliquots selected for testing had no quality issues. Where sample transport was required, frozen sample aliquots were shipped on dry ice with temperature tracking. Sample aliquots with insufficient volume for testing were excluded from analysis and an alternative aliquot or pooled aliquots from the same participant and timepoint were used.

Researchers involved in sample processing, serology testing, whole blood stimulation and cytokine analysis (including quality control checks) were blinded as to which COVID-19 vaccine participants had received. For cytokine analysis, each participant’s pre and post vaccination samples were run on the same plate to ensure no impact of variability between plates/standards.

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