PROTOCOL

A randomised controlled trial to assess the immunogenicity, safety and reactogenicity of a bivalent mRNA Moderna COVID-19 vaccine or a protein-based Novavax COVID-19 vaccine given as a fourth dose in healthy adults in Australia

Protocol Number: 91108

Protocol Version 8.0 and date:

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Document history:

Version Number and Date	Summary of changes
V8.0 9 January 2025	Extend follow-up to 30 months
V7.0 28 June 2024	Extend follow-up to 24 months
V6.0 21 February 2024	Extend follow-up to 18 months
V5.0 31 May 2023	Additional recruitment strategies added.
V4.0 27 April 2023	Change in recruitment, group numbers and product accountability sections
V3.0 14 March 2023	Change in Day 28 visit window and additional details for Moderna BA.4-5
V2.0 19 January 2023	Amendments including feedback from sponsorship committee and PATH
V1.2 23 November 2022	Revisions based on ethics review
V1.1, 27 October 2022	Revisions based on peer review and other minor changes
V1.0, 24 October 2022	Incorporated input from investigators

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).

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

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PROTOCOL SYNOPSIS

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<tr><td>TITLE</td><td colspan="2">A randomised controlled trial to assess the immunogenicity, safety and reactogenicity of a bivalent mRNA Moderna COVID-19 vaccine or a protein-based Novavax COVID-19 vaccine given as a fourth dose in healthy adults in Australia.</td></tr>
<tr><td>TRIAL DESCRIPTION</td><td colspan="2">This clinical trial will be a blinded*, two-arm randomised study to determine the safety, reactogenicity and immunogenicity of a fourth dose of SARS-CoV-2 vaccines in Australia in adults 18 years or older who have received their third dose of COVID-19 vaccine at least six months previously. A separate non-randomised control arm will be enrolled for comparison.</td></tr>
<tr><td></td><td>Group</td><td>Vaccine given</td></tr>
<tr><td></td><td>Group A – (Moderna)
225 participants</td><td>Bivalent Moderna (mRNA-1273.214) (Ancestral SARS-CoV-2 25µg + Omicron Variant (B.1.1.529) 25µg).

Replaced by Bivalent Moderna (mRNA-1273.222) (Ancestral SARS-CoV-2 25µg + Omicron Variant (BA.4-5) 25µg) when introduced.</td></tr>
<tr><td></td><td>Group B – (Novavax)
225 participants</td><td>Novavax (5µg of SARS-CoV-2 spike protein adjuvanted with 50µg of Matrix-M)</td></tr>
<tr><td></td><td>**Group C – (Control group)
150 participants</td><td>No vaccine given</td></tr>
<tr><td colspan="3">*Participants and reactogenicity assessors will be blinded for seven days as vaccine details will be uploaded to the Australian Immunisation Register seven days post-vaccination.
**Control group will not be randomised and will be recruited into proportional predefined age bands (18-29 years, 30-49 years, 50-59 years).</td></tr>
<tr><td>JUSTIFICATION</td><td colspan="2">• Investigating a fourth dose will allow us to explore whether additional boosters are needed in a setting with high rates of prior infection.
• We will include a non-randomised control group aged under 60 years who will not receive a fourth dose to investigate whether three doses are adequate for this age group and what additional benefit a fourth dose provides in terms of cellular immunity as a proxy for moderate/severe disease.</td></tr>
</table>

	• The design will allow comparison of immune responses following mRNA and protein-based vaccine boosters, including the breadth of immunity against different (emerging) variants. • We will follow-up participants for 30 months (i.e., at least 36 months following the third dose for the control arm) to determine the duration of immune responses. • We will document breakthrough infections to explore potential differences between groups with regards to number of infections and severity.
OBJECTIVES	Primary objectives – • To assess and compare the immune response in two randomised vaccine groups (groups A and B) at 28 days post-vaccination measured as binding antibodies (IgG ELISA – Wuhan and/or Omicron). • To assess the rate and severity of reactogenicity within one-week post-second booster for the two vaccine groups (groups A and B) (Timepoint – daily, for seven days post-vaccination). Secondary objectives - • To assess and compare the immune response in groups A and B at 28 days post-vaccination measured as functional antibodies (sVNT and nAb) and cell-mediated immunity (CMI). • To assess and compare the immune response in groups A and B at 6, 12, 18, 24 and 30 months post-vaccination measured as binding antibodies (IgG ELISA), functional antibodies (sVNT and nAb), and CMI. • To describe binding and functional antibodies in group C compared with groups A and B at baseline, 6, 12, 18, 24 and 30 months. • To assess whether a fourth COVID-19 vaccine dose provides an immunological benefit over a third dose by comparing CMI at 6, 12, 18, 24 and 30 months in group C with groups A and B. • To evaluate the safety of fourth booster dose regimens. Exploratory objective - • To describe the number and severity of breakthrough cases in group C compared with groups A and B, up until 30 months. • To correlate virological markers (viral load and viral genome characteristics) and immunological markers (humoral antibody, CMI) among mild and severe breakthrough cases.

OUTCOMES AND OUTCOME MEASURES	<u>Reactogenicity</u> Reactogenicity will be measured using a structured questionnaire for seven days post-vaccination by recording the following parameters: • Local reaction - pain, tenderness, erythema/redness, hardness, swelling, warmth, itch. • Systemic – Nausea, vomiting, diarrhoea, headache, fatigue/malaise, myalgia, arthralgia, fever, enlarged lymph nodes. <u>Immunology endpoints</u> • *Binding antibody* – These will be evaluated using the commercial Euroimmun S1 IgG ELISA (Wuhan and Omicron [once available]) on serum collected at seven timepoints (baseline, 28 days, 6, 12, 18, 24 and 30 months) for the two vaccine groups and six timepoints (baseline, 6, 12, 18, 24 and 30 months) for the control group. The 28 days post-vaccination sample will be assayed within four weeks of collection for the two vaccine groups. • *Functional antibody* – All samples will be assayed using the GenScript cPass SARS-CoV-2 Neutralization Antibody detection kit for WT and Omicron variant RBD antigen or other key newly emerging variants. Samples will be tested at the same time points as for binding antibodies. • *Neutralizing antibody* - A fraction of samples (20%) will be assessed using a SARS-CoV-2 assay at the Peter Doherty Research Institute (PDI), Melbourne up to 12 months. Testing will be at the same time points as for binding antibodies for Wuhan strain and two Variants of Concern (VoC), including Omicron or other key newly emerging variants. • *Cellular immunity* - Cellular immunity will be assessed on 50% of participants per group of samples collected as follows: o *QuantiFERON Human IFN-γ* SARS-CoV-2 (Qiagen) will be performed using whole blood (Wuhan), at four timepoints for Groups A and B (baseline, 28 days, 6 months, and 12 months) and three timepoints for Group C (baseline, 6 months, and 12 months). o Assays performed on PBMCs will include IFN-γ Elispot, intracellular cytokine assays (flow cytometry) and multiplex cytokine assays (Wuhan and Omicron) at seven timepoints for Groups A and B (baseline, 28 days, 6 months, 12 months, 18 months, 24 months and 30 months) and six timepoints for Group C (baseline, 6 months, 12 months, 18 months, 24 months, and 30 months). <u>Breakthrough infections</u>

	• At baseline, previous infections (RAT or PCR positive or symptomatic with close contact) and vaccinations will be recorded. • Breakthrough infections will be recorded and tested for during the study, until 30 months post-enrolment. • Nasal swabs will be collected from all severe breakthrough cases and a subset of mild cases within three days of illness. A representative sample of positive samples will be processed for viral load and whole genome sequencing of SARS-CoV-2. • We will explore the differences in viral load and viral genome characteristics and immunological markers among mild and severe breakthrough cases.
TRIAL POPULATION	Participants will be adults 18 years or older who have received three doses of any COVID-19 vaccine six or more months before the study start for all three groups. There will be no upper age limit for groups A and B. Only those aged 18 to <60 years will be recruited to group C (controls), as knowledge and the benefit of additional boosters for this age group are not well established. Participants will be recruited from the Murdoch Children's Research Institute, the Royal Children's Hospital (RCH), the University of Melbourne, the Peter Doherty Research Institute, and, if necessary, the greater Melbourne area. We will initially target people working in the medical, science, and healthcare fields and then expand to the general population as needed. 450 participants will be recruited into either the bivalent Moderna, or Novavax group. 150 participants will be recruited into the control group. There will be a total of 600 participants. Procedures will be implemented to ensure participants of all ages (aged 18 years and above) are included and that there is an equitable age distribution (<50 and ≥50 years) in each vaccine group and for the bivalent Moderna vaccine transition for the randomised controlled trial.
DESCRIPTION OF SITES	This will predominantly be a single-site study with participants enrolled at RCH. In select cases participants may be enrolled at GP clinics or community centres.
DESCRIPTION OF INTERVENTIONS	The two interventions to be studied are as follows:
	Bivalent Moderna (mRNA-1273.214) (Ancestral SARS-CoV-2 25µg + Omicron Variant (B.1.1.529) 25µg). Replaced by Bivalent Moderna (mRNA-1273.222) (Ancestral SARS-CoV-2 25µg + Omicron Variant (BA.4-5) 25µg) when introduced.
	Novavax (5µg of SARS-CoV-2 spike protein adjuvanted with 50µg of Matrix-M)

TRIAL DURATION	The recruitment period will be approximately 4-6 months. Each participant in the vaccine groups (A&B) will have ten visits, including their immunisation visit, with a follow-up period of 30 months. Group C will have eight visits.
PARTICIPANT DURATION	Participant duration will be 30 months. The vaccine groups (A & B) will have ten visits (three telephone calls and seven in-person). Group C will have eight visits (two telephone calls and six in-person). The DSMB will review participant safety data.

GLOSSARY OF ABBREVIATIONS

ABBREVIATION	TERM
AE	Adverse Event
ANOVA	Analysis of Variance
AR	Adverse Reaction
ATAGI	Australian Technical Advisory Group on Immunisation
BRF	Biobank Registration Form (MCRI)
CEPI	Coalition for Epidemic Preparedness Innovations
CMI	Cell mediated immunity
CRF / eCRF	Case Report Form / electronic Case Report Form
DSMB	Data Safety Monitoring Board
GCP	Good Clinical Practice
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practices
GMC	Geometric mean concentration
HREC	Human Research Ethics Committee
IB	Investigator's Brochure
ICH	International Conference on Harmonisation
IM	Intramuscular
MCRI	Murdoch Children's Research Institute
PBMC	Peripheral blood mononuclear cells
PDI	Peter Doherty Research Institute
PI	Principal Investigator
QC	Quality Control
RGO	Research Governance Office
RCH	Royal Children's Hospital (Melbourne)
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAR	Serious Adverse Reaction
SoA	Schedule of Assessments

SOP	Standard Operating Procedure
SSI	Significant Safety Issue
SST	Serum separator tube
SUSAR	Suspected Unexpected Serious Adverse Reaction
TGA	Therapeutic Goods Administration
UAR	Unexpected Adverse Reaction
USM	Urgent Safety Measure
VoC	Variants of concern

INVESTIGATOR AGREEMENT

I have read the protocol entitled "A randomised controlled trial to assess the immunogenicity, safety and reactogenicity of a bivalent mRNA Moderna COVID-19 vaccine or a protein-based Novavax COVID-19 vaccine given as a fourth dose in healthy adults in Australia."

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 adopted by the TGA [Integrated Addendum to ICH E6 (R1): Guideline for Good Clinical Practice E6 (R2), dated 9 November 2016 annotated with TGA comments].

Changes to the protocol will only be implemented after written approval is received from the Human Research Ethics Committee, with the exception of medical emergencies.

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

Name	Role	Signature and date
Prof Kim Mulholland	Sponsor-investigator	
Dr Claire von Mollendorf	Co-Principal Investigator	

1. ADMINISTRATIVE INFORMATION

1.1. Trial registration

1.1.1. Trial registry

The trial will be registered on ClinicalTrials.gov prior to trial commencement.

1.2. Sponsor

On behalf of the Sponsor, MCRI, the Sponsor-Investigator leading the trial, will undertake and oversee those Sponsor responsibilities delegated by the Sponsor. The delegated Sponsor responsibilities are documented in the study file.

Trial Sponsor	MCRI
Contact name	Kathryn Bright – kathryn.bright@mcri.edu.au
Address	Flemington Road, Parkville
Sponsor-Investigator	Prof Kim Mulholland

1.3. Expected duration of study

The expected study duration will be two years, including preparation and reporting. The recruitment period is anticipated to be 4-6 months and all participants will be followed up for 12 months.

1.4. Contributorship

Name	Summary of contribution	Affiliation
Prof Kim Mulholland	Sponsor-investigator	MCRI
A/Prof Paul Licciardi	Investigator - Immunology	MCRI
Dr Claire von Mollendorf	Investigator – Medical/Epidemiology	MCRI
Prof Nigel Crawford	Investigator - Advisor/ Chair of ATAGI/ Immunization specialist	MCRI
Prof Kanta Subbarao	Investigator - Immunology	PDI
A/Prof Siddhartha Mahanty	Investigator – Infectious Disease Specialist	PDI
Dr Nadia Mazarakis	Investigator - Immunology	MCRI
Dr Lien Anh Ha Do	Investigator - Virology	MCRI
Dr Cattram Nguyen	Investigator - Statistics	MCRI
Dr Kerryn Moore	Investigator - Statistics	MCRI
Dr Eleanor Neal	Investigator – Data Management/Analysis	MCRI
Kathryn Bright	Investigator – Melbourne Project Manager	MCRI
Emma Watts	Investigator – Project Manager (Indonesia)	MCRI
Fran Justice	Investigator – Project Manager (Mongolia)	MCRI
Dr John Hart	Investigator – Study Doctor	MCRI
Sonja Elia	Investigator – Immunisation Nurse Practitioner	RCH
Dr Zheng Quan (Ryan) Toh	Investigator - Immunology	MCRI
Prof Andrew Davidson	Investigator – Medical	RCH

1.5. Stakeholder involvement

The Coalition for Epidemic Preparedness Innovations (CEPI) is a foundation that takes donations from public, private, philanthropic, and civil society organisations to finance independent research projects to develop vaccines against emerging infectious diseases. CEPI is providing funding for this study up until the 12-month blood sample. CEPI has been involved in discussing and developing the project.

2. INTRODUCTION AND BACKGROUND

2.1. Background

First and second booster doses of COVID-19 vaccines are now being widely used. Some countries are likely to consider regular boosters for certain population groups, raising questions regarding the necessity of booster doses and which vaccines may be both effective and acceptable for this role. As recommended by the WHO, vaccine booster introduction should be based on evidence and focus on reducing severe disease, hospitalisations, and death. There is some evidence that fourth doses are effective for this purpose, although with limited follow-up. While all the WHO Emergency Use Listing (EUL)-listed products effectively prevent severe and fatal COVID-19 in the short term, with most shown to be effective as boosters, the duration of protection is highly variable.

Monovalent ancestral strain boosters are being offered in several countries. Several studies during Omicron predominance, including from Israel (1-6) and Canada (7), have reported short-term effectiveness of a fourth dose. Five studies were conducted in individuals older than 60 years of age (3-7), and two included healthcare workers (HCWs) (1, 2). The follow-up in these studies was short and ranged from two to ten weeks after the fourth dose. All studies in the elderly showed a reduction in severe disease with a fourth dose compared to a third dose (3-7). One study described vaccine effectiveness against severe disease compared to unvaccinated individuals and found that vaccine effectiveness increased with each additional dose (7). The two HCW studies were conducted in Israel and compared third to fourth dose recipients (1, 2). The first study (1) enrolled HCWs who had received their third dose of Pfizer BNT162b2 at least four months prior. A total of 154 HCWs received a fourth dose of BNT162b2, and 120 received mRNA-1273 with two age-matched controls for each vaccine. Both vaccines induced binding antibodies and increased neutralizing antibody titres by 9-10-fold. Vaccine efficacy against any SARS-CoV-2 infection was low (30% for BNT162b2 and 11% for mRNA-1273) but higher for symptomatic disease (1). Early data from a second study demonstrated that a fourth vaccine dose reduced breakthrough infection rates from 20% to 6-12% compared to a third dose (2). In combination, these studies showed some potential benefits of using a fourth dose of mRNA vaccines in healthcare workers and the elderly or immunocompromised, but a key issue is the limited follow-up time. There is a lack of data on healthy individuals to show that an additional booster would reduce hospitalisations or deaths. Immunogenicity and reactogenicity data are also limited.

Nucleic acid vaccines, specifically mRNA vaccines, are excellent boosting vaccines, including as dose four boosters, at least during the short-term follow-up available. Protein vaccines have been late to the field with subsequent limited data but may have characteristics that support their use as repeated boosters, perhaps with year-to-year modifications. The protein vaccine developed by Novavax (Nuvaxovid) has shown adequate boosting of Pfizer and AstraZeneca-primed individuals in the UK, although boosting responses were lower than those following the Pfizer vaccine (8). The clinical relevance of this lower response is unknown, as is the comparative duration of immunity. Notably, protein vaccine reactogenicity is lower than mRNA vaccines, which may be an important advantage, especially for long-term programs, reducing vaccine hesitancy and improving vaccine uptake. In the UK

study, 80% efficacy was maintained over six months, and efficacy against the variants circulating was superior to other vaccines (8).

With the emergence of new variants of concern, there is a need to improve current vaccine formulations to address the evolution of the SARS-CoV-2 virus. New formulations include bivalent vaccines containing the ancestral SARS-CoV-2 strain and a variant of concern. The Moderna mRNA-1273.214 vaccine (25µg ancestral Wuhan-Hu-1 and 25µg omicron B.1.1.529 spike SARS-CoV-2 mRNA) demonstrated a superior neutralising antibody response against Omicron and non-inferior neutralising antibody response against ancestral SARS-CoV-2 compared to the monovalent Moderna vaccine (9). Similar data have been reported for the Pfizer BA.1 adapted bivalent vaccine. BA.4/5 adapted vaccines are also available from Moderna and Pfizer and licensed in the USA for individuals aged five years and older, and in Europe for individuals from 12 years as booster vaccines. Further adaptations to both the mRNA vaccines and protein vaccines, such as Novavax, are expected to be produced as required to respond to further mutations in SARS-CoV-2.

In Australia, national COVID-19 vaccinations were rolled out in February 2021, starting with healthcare workers, the elderly and high-risk groups. Very high vaccine coverage for the primary schedule was achieved for all age groups due to vaccine mandates. Booster vaccines were introduced in October 2021, again starting with healthcare workers. The Australian Technical Advisory Group on Immunisation (ATAGI) recommended a second COVID-19 vaccine booster dose on 25 March 2022 to increase vaccine protection before winter. The recommendation initially focused on high-risk groups to reduce severe disease, with subsequent expansion to all adults 30 years and over (https://www.health.gov.au/news/atagi-updated-recommendations-for-a-winter-dose-of-covid-19-vaccine). The booster recommendations were initially for Pfizer and Moderna monovalent vaccines and from 9th October 2022 included the Moderna bivalent vaccine.

Vaccine breakthrough infections are made more likely due to host immune factors such as waning immunity (e.g., low neutralising antibodies) and virological factors, particularly new variants such as Omicron, that evade the immune response. The role that different factors play in breakthrough infections and the immune boosting of natural infections against SARS-CoV-2 for different variants are still being defined.

2.2. Trial rationale and aim

Omicron subvariants have shown evidence of immune escape (10, 11) with lower neutralising antibody titres induced by vaccination and infection, requiring updated vaccines. Omicron-targeting mRNA bivalent vaccines have been introduced as boosters in many countries.

Available data show that COVID-19 vaccine effectiveness wanes considerably, although much more slowly against severe disease than infection (12). There is strong evidence that a third dose of WHO EUL vaccines provides additional protection against severe disease and these are now widely

recommended. However, the information is not yet available to determine whether a fourth dose is required, particularly in the healthy population. It is also unclear regarding the utility of additional boosters in the context of varying natural immunity, especially with high rates of previous infections. Lastly, it is not clear if mRNA bivalent vaccines are necessarily the best choice of booster for long-term repeat booster programs, particularly given their relatively high reactogenicity and rapid waning immunity. This project will compare the long-term immunogenicity of fourth doses of Moderna bivalent and Novavax boosters and a control group who do not receive a fourth dose.

The study-specific aims are to:

- Assess and compare the immune response in two randomised vaccine groups (Moderna bivalent and Novavax) at 28 days, 6-, 12, 18, 24 and 30-months post-vaccination, measured as binding antibodies.
- To assess whether a fourth COVID-19 vaccine dose provides an immunological benefit over a third dose by comparing CMI at 6, 12, 18, 24 and 30-months in a control group compared with the two vaccine groups.
- Assess the rate and severity of reactogenicity within one-week post-second booster for each group.
- Describe the frequency and severity of breakthrough infections in the vaccine and control groups up to 30 months post-vaccination.

2.3. Risk/Benefit assessment

2.3.1. Known potential risks

This study will provide safety data for Omicron-specific bivalent second booster regimens and the protein-based vaccines being studied. The safety profiles of both vaccines are described (Appendix 1 - Product information sheets).

Risks associated with sample taking

Participants may experience discomfort and/or localised bruising from venepuncture. There is a small risk of fainting. The total volume of blood drawn over 18 months will be up to 150ml, which is safe in otherwise healthy adults.

Allergic reactions

Allergic reactions to any of the constituents of the booster vaccine may occur. Allergic reactions range from mild to severe but are rare. Patients should be questioned regarding allergic reactions to any vaccines in the past. All participants will have previously received three doses of a COVID-19 vaccine.

Reactogenicity

Although it is anticipated that participants might experience more reactogenicity if their booster vaccine differs from the vaccine used in their primary two-dose schedule, data from a first booster

study showed similar reactogenicity between homologous and heterologous booster schedules (13). There is limited data on reactogenicity from second booster dose studies (14).

2.3.2. Known potential benefits

Second booster doses of COVID-19 vaccines are currently recommended by ATAGI for adults over 30 years of age in Australia. The bivalent Moderna and Novavax vaccines are approved by the TGA for booster doses in adults 18 years and older. There may be no direct benefits for participants enrolling in this study apart from the opportunity to learn their antibody response following the second booster. Data on the best second booster, in terms of immunogenicity and reactogenicity, and based on the previous vaccines received are limited.

Although there are some data on the use of second booster doses and the immunogenicity of the new Omicron-specific vaccines, additional data is needed from settings with variable community SARS-CoV-2 transmission and epidemiology to advise on optimal dosing schedules, vaccine choices and duration of protection. There is limited data on protein-based vaccines as second boosters. This study will provide additional data for Australia and other countries that are introducing second boosters to design optimal vaccine strategies.

2.3.3. Assessment of potential risks and benefits

Moderna bivalent and Novavax vaccines given as a second booster are available to Australians ≥30 years of age and those 16-29 years with complex, chronic, or severe medical conditions that increase their risk of severe illness from COVID-19. This trial will examine the second booster doses in all adults 18 years and older. This study will use vaccines in line with those available in the Australian government vaccine program. In some studies, heterologous boosters have been associated with slightly increased reactogenicity. Potential benefits include superior immunogenicity, broader protection of heterologous boosting, and access to antibody response results. In addition, this study will provide additional data to inform future booster strategies. Fourth doses may be unnecessary in healthy young individuals with a low risk of severe disease or hospitalisation. However, data for this group are limited, and a reduction in breakthrough infections was observed in specific groups such as healthcare workers (2).

3. TRIAL OBJECTIVES AND OUTCOMES

3.1 Objectives

3.1.1 Primary objective

The primary objectives of the study are to:

- Assess and compare the immune response in two randomised vaccine groups (bivalent Moderna and Novavax) at 28 days post-vaccination measured as binding antibodies (IgG ELISA – Wuhan and/or Omicron) who have been boosted at least six months earlier.
- Assess the rate and severity of reactogenicity within one-week post-second booster for the Moderna and Novavax groups (Timepoint – daily, for seven days post-vaccination).

3.1.2 Secondary objectives

The secondary objectives are to:

- Assess and compare the immune response in two randomised vaccine groups (bivalent Moderna and Novavax) at 28 days post-vaccination measured as functional antibodies (sVNT and nAb) and cell-mediated immunity (CMI).
- Assess and compare the immune response in two randomised vaccine groups (bivalent Moderna and Novavax) at 6, 12, 18, 24 and 30-months post-vaccination measured as binding antibodies (IgG ELISA), functional antibodies (sVNT and nAb), and CMI.
- To describe binding and functional antibodies in the control group compared with the two vaccine groups (bivalent Moderna and Novavax) at baseline, 6, 12, 18, 24 and 30-months.
- To assess whether a fourth COVID-19 vaccine dose provides an immunological benefit over a third dose by comparing CMI at 6, 12, 18, 24 and 30-months in the control group compared with the two vaccine groups (bivalent Moderna and Novavax).
- To evaluate the safety of fourth booster dose regimens.

3.1.3 Exploratory objectives

- To describe the number and severity of breakthrough cases in the control group compared with the two vaccine groups (bivalent Moderna and Novavax) to 30 months post-vaccination.
- To correlate virological markers (viral load and viral genome characteristics) and immunological markers (humoral antibody, CMI) among mild and severe breakthrough cases to 30 months post-vaccination.

3.2 Outcomes

3.2.1 Primary outcomes

Immune response

Binding antibodies – These will be evaluated using the commercial Euroimmun S1 IgG ELISA (Wuhan and Omicron [once available]) on serum collected at seven timepoints (baseline, 28 days, 6 months, 12 months, 18 months, 24 months and 30 months) for the two vaccine groups and six timepoints (baseline, 6 months, 12 months, 18 months, 24 months and 30 months) for the control group. Assays will be completed within 4 weeks of sample collection for the day 28 visit.

Reactogenicity

Reactogenicity for vaccine groups (A&B) will be measured using the accepted standardised method to evaluate systemic and local side effects following vaccination using a structured questionnaire for seven days post-vaccination, as outlined in Table 1 below.

Table 1. Grading system for evaluating systemic and local side effects following vaccination (15, 16).

Local Reaction to Injectable Products [a]				
	Mild (Grade 1)	*Moderate (Grade 2)*	*Severe (Grade 3)*	*Potentially Life Threatening (Grade 4)*
Pain	Does not interfere with activity	Repeated use of nonnarcotic pain reliever >24 hours or interferes with activity	Any use of narcotic pain relief or prevention of daily activity	ER visit or hospitalization
Tenderness	Mild discomfort to touch	Discomfort with movement	Significant discomfort at rest	ER visit or hospitalization
Erythema/ redness [b]	2.5 – 5 cm	5.1 – 10 cm	>10 cm	Necrosis or exfoliative dermatitis
Swelling[c]	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
Hardness[c]	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
Axillary lymphadenopathy	Does not interfere with activity	Repeated use of nonnarcotic pain reliever >24 hours or interferes with activity	Any use of narcotic pain relief or prevention daily activity	ER visit or hospitalization
Warmth (usually reported with redness)	Absence		Presence	
Itch	Absence		Presence	

[a] *Grade 0 will be recorded for parameters if specific symptoms are absent.*

[b] *The measurement should be recorded as a continuous variable in addition to grading the measured local reaction at the greatest single diameter.*

[c] *Hardness and swelling should be evaluated and graded using the functional scale as well as the actual measurement.*

Systemic (General) [a]				
	Mild (Grade 1)	*Moderate (Grade 2)*	*Severe (Grade 3)*	*Potentially Life Threatening (Grade 4)*

Fever (°C): Oral or axillary temperature	38.0 – 38.4	38.5 – 38.9	39.0 – 40	>40
Nausea	No interference with activity	Some interference with activity	Prevents daily activity	ER visit or hospitalization
Vomiting	No interference with activity or 1 – 2 episodes/24 hours	Some interference with activity or >2 episodes/24 hours	Prevents daily activity, or requires outpatient IV hydration	ER visit or hospitalization for hypotensive shock
Diarrhoea	No interference with activity	Some interference with activity	Prevents daily activity, or requires outpatient IV hydration	ER visit or hospitalization for hypotensive shock
Headache	No interference with activity	Repeated use of nonnarcotic pain reliever >24 hours or some interference with activity	Significant; any use of narcotic pain relief or prevention of daily activity	ER visit or hospitalization
Fatigue/Malaise	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization
Myalgia (muscle pain)	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization
Arthralgia (joint pain)	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization
[a] Grade 0 will be recorded for parameters if specific symptoms are absent, or the temperature is <38 °C. In addition, appearance of the following potential signs of myocarditis/pericarditis will prompt referral for cardiology evaluation: Chest pain, dyspnoea, painful breathing, palpitations, or syncope.				

3.2.2 Secondary outcomes

Immunogenicity

- *Functional antibody* – All samples will be assayed using the GenScript cPass SARS-CoV-2 Neutralization Antibody detection kit for WT and Omicron variant RBD antigen or other key newly emerging variants. Samples will be tested on serum collected at seven timepoints (baseline, 28 days, 6 months, 12 months, 18 months, 24 months and 30 months) for the two vaccine groups and six timepoints for the control group.

- *Neutralizing antibody* - A fraction of samples (20%) will be assessed using a SARS-CoV-2 assay at the PDI, Melbourne up to 12 months. Testing will be at the same time points as for binding antibodies for Wuhan strain and two Variants of Concern (VoC), including Omicron or other key newly emerging variants.
- *Cellular immunity* - Cellular immunity will be assessed on 50% of participants per group of samples collected at seven timepoints for the two vaccine groups and six timepoints for the control group as follows:
    - *QuantiFERON Human IFN-γ* SARS-CoV-2 (Qiagen) will be performed using heparinised whole blood (Wuhan), only up to 12 months.
    - Peripheral blood mononuclear cells (PBMCs) will be isolated by density gradient centrifugation within 12 hours of collection and stored in liquid nitrogen at MCRI for all samples up to 30 months. Assays will include IFN-*γ* Elispot, intracellular cytokine assays (flow cytometry) and multiplex cytokine assays (Wuhan and Omicron).

Safety

All solicited AEs will be collected for 7 days, all unsolicited AEs will be collected for 28 days, and all medically attended AEs will be collected for 3 months. SAEs will be collected throughout the 12-month follow-up period.

3.2.3 Exploratory outcomes

- At baseline, previous infections (RAT or PCR positive or symptomatic with close contact) and vaccinations will be recorded.
- Breakthrough infections will be recorded and tested for during the study.
- Nasal swabs will be collected from all severe breakthrough cases and a subset of mild cases within three days of illness. A representative sample of positive samples will be processed for viral load and whole genome sequencing of SARS-CoV-2.
- We will explore the differences in viral load and viral genome characteristics and immunological markers among mild and severe breakthrough cases.

4 TRIAL DESIGN

4.1 Study design

This clinical trial will be a blinded, two-arm randomised study to determine the safety, reactogenicity and immunogenicity of a fourth dose of SARS-CoV-2 vaccines in Australia in adults 18 years or older who have received their third dose of COVID-19 vaccine, at least six months previously. A separate non-randomised control arm will be enrolled for comparison. The groups are described below. The trial intervention will be a single booster dose of vaccine: -

Group	Vaccine given
Group A – (Moderna) 225 participants	Bivalent Moderna (mRNA-1273.214) (Ancestral SARS-CoV-2 25µg + Omicron Variant (B.1.1.529) 25µg). Replaced by Bivalent Moderna (mRNA-1273.222) (Ancestral SARS-CoV-2 25µg + Omicron Variant (BA.4-5) 25µg) when introduced.
Group B – (Novavax) 225 participants	Novavax (5µg of SARS-CoV-2 spike protein adjuvanted with 50µg of Matrix-M)
Group C – (Control group) 150 participants	No vaccine given

We will aim to enrol approximately 225 individuals in each vaccine group and 150 individuals in the control group. This will ensure sufficient representation of participants aged < 50 and ≥50 years receiving the newer Moderna BA.4-5 vaccine.

The participants and those evaluating reactogenicity will be blinded to the vaccine allocation for the first seven days following vaccination until details are entered in the Australian Immunisation Register (AIR). After that, participants will be aware of their investigational product allocation. Immunologists and laboratory staff will remain blinded to the investigational product allocation.

4.2 Justification for dose

The trial will use standard doses of the bivalent Omicron-specific Moderna and Novavax vaccines in line with current TGA recommendations. The trial will transition to newer Omicron-specific vaccines according to availability in Australia.

4.3 Trial population

The trial population will be adults 18 years and older who have been boosted at least six months earlier with a third COVID-19 vaccine dose. There will be no upper age limit for groups A and B. Only those aged ≥ 18 - 60 years will be recruited to group C (controls), as this is the target age group for which the need for additional boosters is not well established. Additional booster doses are strongly recommended for individuals 60 years and over. Procedures will be implemented to ensure an even age distribution for Moderna product transition in each vaccine group.

4.4 Eligibility criteria

If informed consent is granted, study staff will ensure all the eligibility criteria are met. Potential participants must meet all the inclusion criteria and none of the exclusion criteria to be eligible.

4.4.1 Inclusion criteria

Each participant must meet all the following criteria to be enrolled in this trial:

1. Have received a third COVID-19 vaccine dose at least six months before the start of the study.

2. No confirmed SARS-CoV-2 infection by PCR or RAT within the last three months.
3. Willing and able to give written informed consent.
4. Aged 18 years or above.
5. Willing to complete the follow-up requirements of the study.

Note that subjects with comorbidities other than those listed below as contraindications will be included. Pregnant women or women who may become pregnant will be included.

4.4.2 Exclusion criteria

Potential participant meeting any of the criteria below will be excluded from this trial:

1. Currently receiving immunosuppressive medication or anti-cancer chemotherapy.
2. Known HIV infection.
3. Congenital immune deficiency syndrome.
4. Received immunoglobulin or other blood products in the three months prior to potential study booster vaccination.
5. Study staff and their relatives.
6. Have a history of a severe allergic reaction to any COVID-19 vaccines or have a medical exemption to receiving further COVID-19 vaccines.
7. Cannot read or understand English.

4.5 Lifestyle considerations

Not Applicable.

4.6 Screen failures

Screen failures are defined as participants who consent to participate in the trial but are found during screening procedures to be ineligible. They, therefore, do not receive the intervention and are not randomised. We anticipate that there will be very few screen failures due to the nature of the inclusion/exclusion criteria.

4.7 Recruitment and identification of potential participants

Prior to starting the study a comprehensive recruitment plan will be developed. The study will be advertised across the Melbourne Children's Campus (MCRI, Royal Children's Hospital, University of Melbourne), Royal Melbourne Hospital, the Doherty Research Institute, the Parkville precinct and, the broader Melbourne community. We will initially target people working in the medical, science, and healthcare fields and expand recruitment to the general public as needed. We will target specific study or age groups as needed as the study proceeds. Study information (via email, healthcare facilities notice board and/or website/social media, patient/participant registries and recruitment services etc.) will include an information sheet about the study and a link to the study website where they can evaluate their eligibility and obtain additional study details, such as the participant information sheet and consent form (PICF). The potential participant can register their interest and will be given the opportunity to talk with a research team member by phone or zoom if they have any further questions.

All potential participants will be recorded on a recruitment/enrolment log. No identifying information will be retained if the potential participant decides not to participate.

In addition, participants will also be recruited directly from interested general practices. Potentially eligible participants will be sent an email or SMS with the study details from their GP practice and be given the option to contact the COVID-19 booster study team if they want more information or wish to join the study. The COVID-19 booster study team will not have access to GP patient records. In select cases satellite clinics may be conducted at GP clinics with facilities to conduct vaccinations. Similarly at community clinics with access to medical facilities.

Participants from other relevant MCRI studies (for example the BRACE trial), who consented to be contacted for future research, will be contacted by the original study teams. The original study team will send the participants an email with the study details and give them the option to contact the COVID-19 booster study team if they want more information or wish to join the study. The COVID-19 booster study team will not have access to the participant details.

4.8 Consent

Informed consent is a process initiated prior to the agreement to participate in the trial and continues throughout study participation. Before obtaining informed consent, the vaccination's possible risks and benefits will be discussed with potential participants. Trial staff will go through the Informed Consent Form (ICF), which describes in plain language the trial interventions, the study procedures, and the risks and benefits of participation. The ICF will be HREC-approved, and the potential participant will be asked to read and review the ICF. The trial staff will explain the research study further and answer any questions.

Written documentation of informed consent is required prior to enrolment into the trial. The potential participant will sign and date the final page of the ICF. The trial staff will witness the consent, confirming that the trial has been fully explained and that the participant understands the rationale and processes for the trial. The potential participant will be informed that they are free to withdraw from the trial at any time. A copy of the ICF will be given to the potential participant.

4.9 Additional Consent

4.9.1 Extended Follow-Up Period: At or after the 12-month study visit, participants will be approached to provide consent for the continued collection of breakthrough infection information. This will involve monitoring any instances of breakthrough infections beyond the initial 12-month study period. The continuation of study procedures beyond the 18 month visit, including potential extensions up to 30 months, will be contingent upon the availability of future funding. Should additional funding be secured, the study intends to extend its duration to 30 months, incorporating blood collections at the 18, 24 and 30-month intervals. Participants will be asked to provide separate consent for these study procedures.

Extended Follow-up Period 2: Additional funding is available to collect a blood sample at 18 months and conduct some testing. The funding will cover the collection and biobanking of serum and PBMC samples (from CMI subset) and IgG testing. Further funding will be needed to complete the additional

immunological assays, including CMI assays. Funding applications for an NHMRC Ideas grant has been submitted. Participants will be asked to provide additional consent for the 18-month blood sample and for the continued collection of breakthrough information until 24 months. Participants will be informed prior to the 24-month visit whether this will occur in-person or via phone call. SMS messages will be sent to participants every 3 months to remind them to report any breakthrough infections.

Extended Follow-Up Period 3: We intend to extend the study to 30 months. The funding will cover the 24-month collection, biobanking of serum and PBMC samples (from CMI subset) and IgG testing. Further funding will be needed to complete the additional immunological assays, including CMI assays. A funding application for NIH has been submitted. Furthermore, funding for the additional 30-month visit is pending confirmation. If funding isn't secured participants will be informed and the scheduled 30-month appointments cancelled. We will ask participants to sign one consent form at the 24-month visit which includes the 30-month visit to avoid them having to sign another consent at 30-months again.

5 INTERVENTION

5.1 Treatment arms

Treatment Arms	Route	Dose
Group A: Bivalent Moderna (mRNA-1273.214) or Bivalent Moderna (mRNA-1273.222)	IM	Ancestral SARS-CoV-2 25µg + Omicron Variant (B.1.1.529) 25µg. Replaced by (Ancestral SARS-CoV-2 25µg + Omicron Variant (BA.4-5) 25µg) when introduced.
Group B: Novavax	IM	Novavax (5µg of SARS-CoV-2 spike protein adjuvanted with 50µg of Matrix-M)
Group C	-	No vaccine

5.2 Trial Intervention(s)

The bivalent Moderna (mRNA-1273.214 or mRNA-1273.222) COVID-19 Vaccine contains equal amounts of mRNAs (25µg of each mRNA sequence) that encode the prefusion stabilized spike glycoproteins of the ancestral SARS-CoV-2 (Wuhan-Hu-1) and the Omicron variant (B.1.1.529 [BA.1] or BA.4-5) with mRNAs encapsulated in lipid nanoparticles.

Novavax contains 5µg of SARS-CoV-2 spike protein and is adjuvanted with Matrix-M. Adjuvant Matrix-M contains, per 0.5 mL dose: Quillaja saponaria saponins fraction A (42.5 micrograms) and Quillaja saponaria saponins fraction C (7.5 micrograms).

5.2.1 Description of trial investigational products

5.2.1.1 Trial Products

Active substance	25µg of each mRNA sequence that encodes the prefusion stabilized spike glycoproteins of the ancestral SARS-CoV-2 (Wuhan-Hu-1) and the

	Omicron variant (B.1.1.529 [BA.1]). mRNAs are encapsulated in lipid nanoparticles.
Trade or Generic name	Moderna (mRNA-1273.214) or *Spikevax bivalent original/omicron®* (elasomeran/imelasomeran).
Dosage form	Liquid for injection – standard dose for boosting is 0.5ml containing 25µg of elasomeran (Wuhan-Hu-1) and 25µg of imelasomeran (Omicron variant, B.1.1.529 [BA.1]).
Route of administration	Intramuscular injection

Active substance	25µg of each mRNA sequence that encodes the prefusion stabilized spike glycoproteins of the ancestral SARS-CoV-2 (Wuhan-Hu-1) and the Omicron variant (BA.4-5). mRNAs are encapsulated in lipid nanoparticles.
Trade or Generic name	Moderna (mRNA-1273.222) or *Spikevax bivalent original/omicron BA.4-5 (elasomeran/davesomeran)*.
Dosage form	Liquid for injection – standard dose for boosting is 0.5ml containing 25µg of elasomeran (Wuhan-Hu-1) and 25µg of davesomeran (Omicron variant, BA.4-5).
Route of administration	Intramuscular injection

Active substance	SARS-CoV-2 rS (NVX-CoV2373) adjuvanted with Matrix-M
Trade or Generic name	*Nuvaxovid®*
Dosage form	Liquid for injection – standard dose for boosting is 0.5ml containing 5µg of SARS-CoV-2 spike protein and is adjuvanted with Matrix-M.
Route of administration	Intramuscular injection

5.2.2 Dosage

Bivalent Omicron-specific Moderna COVID-19 vaccine contains 25µg of ancestral SARS-CoV-2 (Wuhan-Hu-1) and 25µg of Omicron variant B.1.1.529 [BA.1] in 0.5ml or 25µg of ancestral SARS-CoV-2 (Wuhan-Hu-1) and 25µg of Omicron variant BA.4-5 in 0.5ml.

Novavax contains 5µg of SARS-CoV-2 spike protein in 0.5ml.

5.2.3 Dose modification

Dose modification is not permitted and must be given as per randomisation.

5.2.4 Storage, preparation, dispensing and administration of trial drug

All study vaccines will be stored in the pharmacy at RCH and will be stored in accordance with the manufacturers' recommendations. Vaccine accountability, storage, shipment, and handling will be in accordance with relevant SOPs and forms.

Storage

- The Moderna vaccine can be stored at -50ºC to -15ºC. It should not be stored or transported on dry ice or below -50ºC. Once thawed, it should not be re-frozen and may be stored refrigerated at 2 °C to 8 °C, protected from light for up to 30 days if not used (needle punctured). After removal from refrigerated conditions, an unopened vial's chemical and physical stability has been demonstrated for up to 24 hours at 8° to 25°C. Chemical and physical stability has been demonstrated for 19 hours at 2 to 25 ºC after first puncture (within the allowed use period of 30 days at 2°C to 8°C and 24 hours at 8°C to 25°C).
  Single use unopened pre-filled BA.4-5 syringes may be stored at 8°C to 25°C up to 24 hours after removal from refrigerated conditions. The prefilled syringe is for single use in one patient only. Any residue should be discarded.
- The unopened Novavax vaccine is stored refrigerated at 2°C to 8°C, protected from light. Do not freeze. It has been shown to be stable for up to 12 hours at 25°C when unopened. Storage at 25°C is not the recommended storage or shipping condition. Chemical and physical in-use stability has been demonstrated from the time of the first needle puncture to administration for 6 hours when opened at 2°C to 25°C.

Preparation

All study vaccines will be prepared in accordance with the manufacturers' recommendations.

Administration

The unblinded vaccine administrator will inject the allocated vaccine intramuscularly into the left deltoid region or the right deltoid region if there is a reason to prefer this side, such as left-handedness. The vaccine administrator should ensure that an appropriate needle length is used, taking the participant's BMI into account.

5.2.5 Product accountability

The trial vaccines will be stored in the pharmacy at RCH. The RCH immunisation clinic pharmacist or designated vaccination nurse trained in administrating COVID-19 vaccines, will draw up the study vaccines each day based on confirmed study bookings. The study nurses will ensure that storage requirements are maintained. Study vaccines will be administered by unblinded vaccinators, who will maintain blinding such that those evaluating reactogenicity are blinded during the first-week post-vaccination when assessing symptoms.

The study clinic will maintain accurate records of the receipt of all trial vaccines, including dates of receipt. In addition, study nurses will keep accurate records regarding when and how much trial vaccine is dispensed and used for each participant in the trial. Reasons for departure from the expected dispensing regimen will be recorded. At the end of the trial, there will be final reconciliation of trial vaccine received, dispensed, consumed, and returned. The trial team will investigate, resolve, and document any discrepancies. Unused trial vaccines will be destroyed in compliance with applicable regulations. Detailed information regarding product accountability will be provided in an SOP.

5.2.6 Excluded medications and treatments

There are no excluded medications or treatments for enrolled participants.

Study participants will be asked to inform us of any non-study COVID-19 vaccine doses that they receive before their 30 month visit.

5.2.7 Concomitant therapy

Concomitant medications and vaccines taken by participants during the study will be documented as part of the safety review procedures.

From day 0 to 28, all concomitant medication will be recorded.

From 28 days until 30 months, only the following medications of interest will be recorded:

- Any vaccine.
- Any investigational products other than those given for this protocol.
- Immunosuppressive therapies.
- Therapies taken for suspected, or laboratory confirmed SARS-CoV-2 infection or COVID-19

The following information will be recorded on the CRF for all applicable medications:

- Trade name
- Indication
- Start and stop date
- Dosage
- Route of administration

Dietary supplements will not be recorded on the CRF.

5.2.8 Discontinuation from trial intervention

See Section 7.2.9.

6 RANDOMISATION AND BLINDING

The two vaccine groups will be randomised 1:1 to one of two interventions (bivalent Omicron-specific Moderna and Novavax) stratified by age (<50 and ≥50 years). An independent statistician from the Melbourne Children's Trial Centre at the Murdoch Children's Research Institute will provide a secure, password-protected web-based randomisation schedule. Blocked randomisation will be used with random blocks of permuted length.

50% of participants from each vaccine group will be included in the cell-mediated immunity (CMI) subgroup analysis. We will ensure that the age strata (<50 and ≥50 years) are equally represented in the CMI vaccine groups. As only 10-20 CMI samples can be processed per day, we will recruit the first 10-20 participants per day to participate in this substudy.

The control group will not be randomised and will be recruited from participants <60 years of age who indicate that they are not planning to receive a fourth vaccine dose at the time of recruitment. Controls will be recruited into proportional predefined age bands (18-29 years, 30-49 years, 50-59 years).

6.1 Concealment mechanism

Randomisation will be stratified according to age (<50 and ≥50 years). Fourth dose vaccine participants will be randomised in a 1:1 ratio for each of the two booster schedules using randomisation lists in REDCap (see table below).

Study staff involved in administering the vaccine will use REDCap to receive the information on the participant's randomisation (study ID) number and vaccine. Study staff will upload vaccination information to the AIR after one week. Participants will remain blinded to their vaccine type until day seven post-vaccination, when their vaccination history is uploaded to the AIR. Study staff involved in assessing reactogenicity and immunogenicity will be blinded to each participant's vaccine allocation, which will be hidden in REDCap for blinded study staff. Statisticians and analysts will remain blinded during the development of the SAP and will develop and finalise all codes using a dummy variable for treatment allocation.

The booster vaccines for all groups will be prepared daily by the RCH immunisation clinic pharmacist or designated vaccination nurse, delivered to the vaccination room, and stored in the vaccine fridge.

Group	Number	Age	Booster vaccine	Dose given
Group A	75	<50 years	Bivalent Moderna BA.1	50µg
	75	<50 years	Bivalent Moderna BA.4-5	50µg
	75	≥50 years	Bivalent Moderna BA.4-5	50µg
Group B	150	<50 years	Novavax	50µg
	75	≥50 years	Novavax	50µg
Group C	150	<60 years	No vaccine	-

6.2 Breaking of the trial blind

6.2.1 On trial

The study will only be blinded for the first seven days as vaccine details will be entered in the AIR after one week. In the unlikely event that it becomes necessary to unblind during this period, the PI will take that decision, and the subject and physicians caring for the subject will be made aware of the allocation.

7 TRIAL VISITS AND PROCEDURES

7.1 Schedule of assessments

Assessments/ activities*	Visit 1	Visit 2	Visit 3	Visit 4	Visit 5	Visit 6	Visit 7	Visit 8	Visit 9	Visit 10
Timing	Day 0	Day 1	Day 7	Day 28	3 months	6 months	12 months	18 months	24 months	30 months
Visit window			+ 3 days	- 3 days + 7 days	+/- 14 days	+/- 14 days	+/- 30 days	+/- 30 days	+/- 30 days	+/- 30 days
Group	A,B,C	A,B	A,B	A,B,C	A,B,C	A,B,C	A,B,C	A,B,C	A,B,C	A,B,C
Location of visits	At RCH	Phone call	Phone call	At RCH (A,B) Phone call (C)	Phone call	At RCH	At RCH	At RCH	At RCH	At RCH
Informed consent	X						X	X	X	
Eligibility confirmation	X									
Randomisation	X (A,B)									
Blood sampling	X			X (A,B)		X	X	X	X	X
Vaccination	X (A,B)									
Vital signs	X (A,B)									
Issue diary card, ruler, and thermometer	X (A,B)									
Collect diary card	X (daily) for vaccine groups (A&B)									
Documentation of Solicited AE	X	X	X							
Documentation of unsolicited AE	X	X	X	X						
Documentation of medically attended AE	X	X	X	X	X					
Documentation of SAE	Throughout study period									

Documentation of confirmed breakthrough SARS-COV-2 infection/ COVID-19	Throughout study period

*****Unless otherwise indicated, assessments/activities are valid for Groups A, B and C.

7.2 Description of procedures

7.2.1 Visit 1 (Day 0)

Vaccine groups (A&B)

Informed consent must be signed by both participant and the study doctor or nurse. Participant eligibility must be confirmed prior to randomisation and on the same date as the administration of the investigational product.

Eligibility must be confirmed by a study doctor or study nurse. Randomisation for vaccine groups must be completed by the vaccinator, who will document the participant's randomisation number on the recruitment/enrolment log.

Study staff will administer a Visit 1 questionnaire documenting demographic information, height and weight, history of previous breakthrough infections, dates and types of previous COVID-19 vaccines received any comorbidities and concomitant medication. A study doctor or nurse will record vital signs (temperature, pulse, and respiratory rate) before vaccination.

Before vaccination, 5mL of blood will be collected from all participants and processed according to the SOP. CMI participants will have an additional 25ml collected and processed for Quantiferon analysis and PBMC separation and storage.

Following randomisation, the unblinded vaccinator will administer the applicable vaccine to the participant. Study staff will give the participant a hard copy reactogenicity diary card or email them a REDCap survey form. Study staff will instruct the participant on completing the diary card for the day of vaccination and seven days following vaccination. Patients who complete a hard copy diary card will be asked to bring it to their next in-person visit.

Study staff will provide the participant with a thermometer and ruler and detailed instructions on how to use these for measuring temperature and potential erythema and induration. The study staff will provide the participant with a schedule of visit appointments and instruct the participant on how to contact the study staff in case of any SAE or symptoms suggestive of COVID-19. Study staff will inform participants that they can choose to have additional COVID-19 vaccine doses before their 30-month visit and should just inform the team if they have received a vaccine.

Study staff will keep the participant under observation for a minimum of 15 minutes post-vaccination and document any immediate adverse events. Participants with a history of anaphylaxis due to any cause should be observed for 30 minutes.

Control group (C)
Informed consent must be signed by both participant and the study doctor or nurse. Participant eligibility must be confirmed prior to enrolment by a study doctor or study nurse. Controls will be enrolled in three pre-defined proportional age bands (18-29 years, 30-49 years, and 50-59 years).

Study staff will conduct a Visit 1 questionnaire documenting demographic information, height and weight, history of previous breakthrough infections, dates and types of previous COVID-19 vaccines received any comorbidities and concomitant medication.

Following consent, 5mL of blood will be collected from all controls and processed according to the SOP. CMI participants will have an additional 25ml collected and processed for Quantiferon analysis and PBMC separation and storage.

The study staff will provide the participant with a schedule of visit appointments and instruct the participant on how to contact the study staff in case of any SAE or symptoms suggestive of COVID-19. Study staff will inform participants that if they change their mind and choose to have a fourth dose of vaccine before their 30 month visit, they should inform the study staff. They will still be able to continue in the study after they receive this additional dose.

7.2.2 Visit 2 (Day 1) – only vaccine groups (A&B)

Study staff will contact the participant by phone approximately 24 hours after vaccination to review and document adverse events.

7.2.3 Visit 3 (Day 7) – only vaccine groups (A&B)

Study staff will contact the participant by phone one week after vaccination. If participants have a hard copy diary card, study staff will ask participants to read the details over the phone or send a picture of their hard copy diary card (with the name obscured) for review. If the participant completes the REDCap diary card, the study staff will review it with the participant to confirm that all symptoms are captured. Study staff will also document any significant, unexpected, serious, or unusual adverse events on the AE CRF. Participants will be informed regarding their vaccine type if they wish to know, and vaccination information will be entered in the AIR.

7.2.4 Visit 4 (Day 28)

Vaccine groups (A&B)

An in-person visit will be conducted 28 days following vaccination. Participants with hard copy diary cards will be asked to bring the card to this visit for collection. All unsolicited adverse events will be documented. Study staff will document any new information regarding solicited adverse events with onset up to 7 days following vaccination, such as resolution. Information regarding unreported breakthrough infections following vaccination will be collected.
For all participants, 5mL of blood will be collected in an SST tube. For participants in the CMI subset, 5mL of blood for the Quantiferon test will be collected in a heparinised tube, and 20mL of blood for PBMC will be collected in a heparinised tube. Blood samples will be kept at room temperature and transferred to the laboratory within 3 hours of collection.

Control group (C)
Study staff will contact the participant by phone 28 days following enrolment and document any adverse events. Information regarding unreported breakthrough infections following enrolment will be collected. No blood will be taken from the control group at the 28-day visit.

7.2.5 Visit 5 (3 Months) – all groups (A,B,C)

Study staff will contact the participant by phone and document any medically attended AE or SAE.
Study staff will also document any new information regarding unsolicited adverse events with onset up to 28 days following vaccination, such as resolution. Information regarding unreported breakthrough infections since visit 4 will be collected.

7.2.6 Visit 6 (6 Months) – all groups (A,B,C)

For all participants, 5mL of blood will be collected in an SST tube. For participants in the CMI subset, 5mL of blood for the Quantiferon test will be collected in a heparinised tube, and 20mL of blood for PBMC will be collected in a heparinised tube. Blood samples will be kept at room temperature and transferred to the laboratory within 3 hours of collection.

7.2.7 Visit 7 (12 months) – all groups (A,B,C)

For all participants, 5mL of blood will be collected in an SST tube. For participants in the CMI subset, 5mL of blood for the Quantiferon test will be collected in a heparinised tube, and 20mL of blood for PBMC will be collected in a heparinised tube. Blood samples will be kept at room temperature and transferred to the laboratory within 3 hours of collection.
Control group participants will be able to receive a vaccine dose before visit 7 and continue in the study. Participants will receive a gift voucher and parking/public transport reimbursement at each in-person study visit. Available individual test results will be shared and discussed with interested participants by a study team member during their study visits.

7.2.8. Visit 8 (18 months) – all groups (A,B,C)

Prior to the 18 month visit, participants will be asked to attend the 18 month visit in-person for a blood draw. Further consent will be obtained prior to any additional blood sampling at 18 months.

All participants will have 5mL of blood collected in an SST tube. For participants in the CMI subset, 20mL of blood will be collected in a heparinised tube for PBMCs. Blood samples will be kept at room temperature and transferred to the laboratory within 3 hours of collection.

7.2.9 Visit 9 (24 months) – all groups (A,B,C)

All participants will be asked to consent and report on breakthrough infections up to 24 months post-vaccination. Prior to the 24 month visit, participants will be contacted to attend the 24 month visit in-person for a blood draw. Further consent will be obtained prior to any additional blood sampling at 24 months.

All participants will have 5mL of blood collected in an SST tube. For participants in the CMI subset, 20mL of blood will be collected in a heparinised tube for PBMCs. Blood samples will be kept at room temperature and transferred to the laboratory within 3 hours of collection.

7.2.10 Visit 10 (30 months) – all groups (A,B,C)

All participants will be asked to report on breakthrough infections up to 30 months post-vaccination. At the 24 month visit, participants will be asked to attend the 30 month visit in-person for a blood draw. Participants will be informed that the study continuation is dependent on funding being secured.

All participants will have 5mL of blood collected in an SST tube. For participants in the CMI subset, 20mL of blood will be collected in a heparinised tube for PBMCs. Blood samples will be kept at room temperature and transferred to the laboratory within 3 hours of collection.

Vaccine and control group participants will be able to receive a vaccine dose before visit 10 and continue in the study. Participants will receive a gift voucher and parking/public transport reimbursement at each in-person study visit. Available individual test results will be shared and discussed with interested participants by a study team member during their study visits.

Summary of blood schedule

Group	Day 0	Day 28	6 months	12 months	18 months	24 months	30 months
A (Moderna bivalent group) 225 participants (150 participants <50 and 75 participants ≥50 years)	Blood taken – IgG and CMI (75 for <50 and 37 for ≥50 years for CMI group)	Blood taken – IgG and CMI	Blood taken – IgG and CMI	Blood taken – IgG and CMI	Blood taken – IgG and CMI (CMI pending additional funding)	Blood taken – IgG and CMI (CMI pending additional funding)	Blood taken – IgG and CMI (CMI pending additional funding)
B (Novavax group) 225 participants (150 participants <50 and 75 participants ≥50 years)	Blood taken – IgG and CMI (75 for <50 and 37 for ≥50 years for CMI group)	Blood taken – IgG and CMI	Blood taken – IgG and CMI	Blood taken – IgG and CMI	Blood taken – IgG and CMI (CMI pending additional funding)	Blood taken – IgG and CMI (CMI pending additional funding)	Blood taken – IgG and CMI (CMI pending additional funding)
C (Control group) 150 participants (All below 60 years)	Blood taken – IgG and CMI (75 in CMI group)	No blood taken	Blood taken – IgG and CMI	Blood taken – IgG and CMI	Blood taken – IgG and CMI (CMI pending additional funding)	Blood taken – IgG and CMI (CMI pending additional funding)	Blood taken – IgG and CMI (CMI pending additional funding)

7.2.8 Unscheduled visits

To document breakthrough infections, enrolled participants will be requested to contact the Study Team if they develop symptoms of COVID-19. They will all be asked to take a RAT test and, if they test positive, may be asked to take a second nasal swab. The second swab will be stored for sequencing. To enrol those with severe disease and those with higher viral load, the second swab will be requested for the first 5-10 cases per month who present within three days of symptom onset, plus any severe cases. A representative sample of positive nasal swabs will be chosen for whole genome sequencing following

the completion of the 12 month visit and at the end of the study. All positive RAT tests and details regarding symptoms will be documented for all participants.

An optional blood sample (10ml, i.e., 5ml sodium heparin + 5 ml SST tube) will be collected if the participant consents 28 days after a positive RAT test (convalescent blood). The convalescent blood sample will determine whether breakthrough infections boost SARS-CoV-2 specific immune responses in previously vaccinated or control group individuals by comparing them to immune responses from previous blood samples. CMI will also be accessed. We will explore the association between immune protection and viral variants and identify potential viral signatures associated with severe breakthrough infections and/or immune escape. The participant will otherwise continue with scheduled study visits. The study team will maintain telephone contact with all participants who test positive for the course of their illness and fully document all telephone calls. With the participant's permission, the study team will seek clinical data from the health care providers or hospital(s) if the subject becomes clinically ill.

In-between study visits, participants will be sent 3 monthly SMS messages to remind them to report all breakthrough infections.

7.2.9 Withdrawal of consent - participant withdraws from all trial participation

A participant has the right to withdraw from the trial at any time and for any reason and is not obliged to give his or her reasons for doing so. The Investigator may withdraw the participant at any time in the interests of the participant's health and well-being. In addition, the participant may withdraw/be withdrawn for any of the following reasons:

- Administrative decision by the Investigator
- Ineligibility (either arising during the trial or retrospectively, having been overlooked at screening).
- Significant protocol deviation
- Participant non-compliance with study requirements

The reason for withdrawal will be recorded in the eCRF. If the participant has an AE at the time of withdrawal, appropriate follow-up visits or medical care will be arranged, with the agreement of the participant, until the AE has resolved, stabilised, or a non-trial-related causality has been assigned.
If a participant withdraws from the study, storage of samples and data collected before their withdrawal will still be used in the analysis unless the participant specifically requests otherwise.

7.2.10 Losses to follow-up

A participant will be considered lost to follow-up if he/she fails to return for two consecutive visits and is unable to be contacted by the trial staff. The following action must be taken if a participant fails to return to the clinic for a required trial visit:

- Trial staff will attempt to contact the participant within one week of the missed visit and reschedule the visit.

- Before a participant is deemed lost to follow-up, trial staff will make every effort to regain contact. Three telephone calls will be made, and two emails will be sent. All contact attempts will be documented in the participant's eCRF.
- If the participant continues to be unreachable, he/she will be considered to have withdrawn from the trial with a primary reason of loss to follow-up.

7.2.11 Replacements

Participants who have been randomised and enrolled may NOT be replaced.

7.2.12 Trial Closure

The end of the trial is the date of the last assay for primary and secondary outcomes conducted on the last sample collected.

8 SAFETY MONITORING AND REPORTING

8.1.1 Definitions for use in trials involving investigational medicinal products

Participant-specific adverse events

Adverse events must be assessed to determine each of the following:

1. Seriousness
2. Relatedness (i.e., causal relationship)
3. Expectedness

Adverse Event (AE): Any untoward medical occurrence in a patient or clinical trial participant administered a medicinal product and does not necessarily have a causal relationship with this treatment.

Adverse Reaction (AR): Any untoward and unintended response to an investigational medicinal product related to any dose administered.

Comment: All adverse events judged by the reporting investigator or the sponsor as having a reasonable possibility of a causal relationship to an investigational medicinal product would qualify as adverse reactions. The expression 'reasonable causal relationship' means to convey, in general, that there is evidence or argument to suggest a causal relationship.

Serious Adverse Event (SAE) / Serious Adverse Reaction (SAR): Any adverse event/adverse reaction that results in death, is life threatening, requires hospitalisation or prolongs existing hospitalisation, results in persistent or significant disability or incapacity or is a congenital anomaly or 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/reaction 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 require intervention to prevent one of the other outcomes listed in this definition should also be considered serious.

Suspected Unexpected Serious Adverse Reaction (SUSAR): An adverse reaction that is both serious and unexpected.

Safety issues (requiring expedited reporting)

The following definitions describe additional safety events that require expedited reporting to stakeholders, including the Sponsor, Investigators, HREC, local governance office and TGA:

Significant Safety Issue (SSI): A safety issue that could adversely affect the safety of participants or materially impact the trial's continued ethical acceptability or conduct.

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

Urgent Safety Measure (USM): A measure required to eliminate an immediate hazard to a participant's health or safety. Note: This type of SSI can be instigated by either the investigator or sponsor and can be implemented before seeking approval from HRECs or institutions.

8.2 Capturing and eliciting adverse event/reaction information

Participants will be asked to record local and systemic AEs for 7 days (and longer if symptoms persist at day seven, until resolution or stabilisation) following vaccination in the electronic or paper diary card (solicited AEs). Unsolicited AEs - all local and systemic AEs occurring in the 28 days following vaccination observed by the Investigator or reported by the participant, whether attributed to study medication, will be recorded in the AE section of the eCRF.

Serious adverse events (SAEs) will be followed up for the duration of the trial. Adverse events of special interest will be categorised as per CEPI guidelines (17).

Any unexpected, serious, or unusual AE following immunisation will be reported to SAEFVIC (vaccine safety surveillance service) through SAFEVAC: Integrated Vaccine Safety website that automatically forwards reports to the TGA (https://www.safevac.org.au/Home/Info/VIC). Adults who experience a significant adverse event reported to SAEFVIC (https://mvec.mcri.edu.au/saefvic/) should be referred to the Victorian Specialist Immunisation Services (VicSIS) for review and management of future COVID-19 vaccination.

8.3 Documentation of AEs

The AE will be described in the source documents (e.g., electronic or paper diary card) and captured on the eCRF and will include:

- A description of the AE
- The onset date, duration, date of resolution
- Severity (mild, moderate, or severe – how does the event impact the participant's daily life?)
- Seriousness (i.e., is it an SAE?)

- Any action taken (e.g., treatment, follow-up tests)
- The outcome (recovery, death, continuing, worsening)
- The likelihood of the relationship of the AE to the trial treatment (Unrelated, Possible, Probable, Definite)

Changes in the severity of an AE will be reported. AEs characterised as intermittent will be documented for each episode. All AEs will be followed to adequate resolution, where possible.

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

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

Unrelated	Unlikely temporal relationship to study product. Alternate aetiology likely (clinical state, environmental or other interventions) and does not follow known typical or plausible pattern of response to study product
Possible	Reasonable temporal relationship to study product; or event not readily produced by clinical state, environmental or other interventions; or similar pattern of response to that seen with other vaccines
Probable	Reasonable temporal relationship to study product; and event not readily produced by clinical state, environment, or other interventions or known pattern of response seen with other vaccines
Definite	Reasonable temporal relationship to study product; and event not readily produced by clinical state, environment, or other interventions; and known pattern of response seen with other vaccines

8.5 Assessing the expectedness and severity of a participant's AE

An AE must be assessed to determine whether the event is or expected or unexpected in terms of the current known safety profile of the investigational medicinal product. The investigator will be responsible for determining whether an AE is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the trial intervention.

The severity of an Adverse Event will be graded/ assessed as follows:

Grade 0	None
Grade 1	Mild: Transient or mild discomfort (< 48 hours); No interference with activity; No medical intervention/therapy required
Grade 2	Moderate: Mild to moderate limitation in activity – some assistance may be needed; no or minimal medical intervention/therapy required
Grade 3	Severe: Marked limitation in activity, some assistance usually required; medical intervention/therapy required.
Grade 4	Potentially Life-threatening: Requires assessment in A&E or hospitalisation

8.6 Reporting of safety events

Sponsor-Investigator Reporting Procedures

The Sponsor-Investigator must assess and categorise the Expedited Safety Reports received from Investigators and report these to all Site Principal Investigators, the approving HREC and TGA in accordance with the NHMRC's 'Safety monitoring and reporting in clinical trials involving therapeutic goods' (November 2016) and any additional requirements of the approving HREC. All safety reports must clarify the impact of the safety event on participant safety, trial conduct and trial documentation. The Sponsor-Investigator is responsible for the following reporting to PIs, the HREC(s), the DSMB and TGA:

1. All SSIs that meet the definition of a USM **within 72 hours** of becoming aware of the issue.
2. All other SSIs **within 15 calendar days** of instigating or becoming aware of the issue
3. For SSIs leading to an amendment of trial documentation:
    a. Submit details of the SSI without undue delay and **no later than 15 calendar days** after becoming aware of the issue.
    b. Submit amendment to the HREC without undue delay.
4. For SSIs leading to a temporary halt or early termination of a trial for safety reasons:
    a. Communicate reasons, scope of halt, measures taken, and further actions planned without undue delay and **no later than 15 calendar days** of the decision to halt.
    b. For a temporary halt, notify the PIs, HREC and TGA when the trial restarts, including evidence that it is safe to do so.

The Sponsor will report SUSARs to the TGA through the SAFEVAC website as follows:

1. Fatal or life-threatening SUSARs immediately, but **no later than 7 calendar days** after being made aware of the issue (follow-up info within a further 8 calendar days)
2. All other SUSARs **no later than 15 calendar days** after being made aware of the issue

The Sponsor is responsible for providing the additional safety information to the approving HREC:

1. Provide an annual safety report, including a summary of the evolving safety profile of the trial

The Sponsor is also responsible for providing updated Product Information/Investigator's Brochure to Investigators.

9 DATA AND INFORMATION MANAGEMENT

The Principal Investigator is responsible for storing essential study 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 Principal Investigator is responsible for maintaining adequate and accurate source documents that include all key observations on all 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 collected by MCRI must be traceable

and explained in a note at relevant variables on the electronic case report form (eCRF). A site-specific Source Document Plan will be maintained to indicate the location(s) of source documents.

The Principal Investigator will maintain accurate data collection forms (known as case report forms - CRFs) and ensure that the collected and reported data is accurate, legible, complete, entered promptly, and enduring.

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

Data generated for this study will be handled according to the relevant standard operating procedures (SOPs) and Data Management Plan (DMP). Full details of all processes are provided in a separate study-level DMP.

9.1 Data management

9.1.1 Data generation (source data)

Source documents are all documents used by the investigator that relate to the participant's medical history, verify the existence of the participant, the inclusion and exclusion criteria, and all records covering the participant's participation in the study. They include but are not limited to laboratory reports, memoranda, vaccination records, hospital records, and participant files. The site principal investigator is responsible for maintaining source documents.

An electronic CRF (eCRF) will be completed for all recruited participants.

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

- Identifying personal information (contact details, dates of birth, home address and telephone contact and gender)
- Sensitive information, including health data (dates of COVID-19 vaccinations, medical history, previous COVID-19 vaccines, intervention vaccines).
- Reactogenicity information (pain, tenderness, erythema/redness, hardness, swelling, warmth, itch, fever, nausea/vomiting, diarrhoea, headache, fatigue/malaise, myalgia, arthralgia
- Adverse events and serious adverse events
- SARS-CoV-2 infections prior to enrolment and breakthrough infections
- Biological information (blood sample, immunologic assay results, including binding antibodies, functional antibodies, neutralizing antibodies, cellular immunity)

<u>Source Document Plan</u>

The source documents for this study include COVID-19 vaccination details, date of birth, and sex; questionnaires completed by the participant and researcher (MCRI's eCRF).

A Source Document Plan will be maintained to 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 Principal Investigator, will be prepared before the recruitment of the first participant and filed in the site's Investigator Site File on eFlorence.

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

Data collection methods

Data capture and entry will be electronic. Data for this study will be collected and entered using electronic data collection forms, completed by authorised study staff and participants where applicable.

The following licensed research data collection tools will be used:

- Study data will be collected and managed using REDCap electronic data capture tools hosted at MCRI. REDCap (Research Electronic Data Capture) is a secure, web-based software platform designed to support capture for research studies, providing 1) an intuitive interface for validated data capture; 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for data downloads to common statistical packages; and 4) procedures for data integration and interoperability with external sources. (18, 19)
- Data recorded will be linked by unique participant identifiers

Further details on the data variables are in the study DMP.

Use of the data

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

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

Storage and access

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

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 creation,

update, and deletion events accessible to project users who are granted permission to view it. Access to REDCap will be provided 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 before personnel commence data entry on REDCap.

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

Disclosure

The study protocol, documentation, data, and all other information generated will be confidential. No information concerning the study, or the data will be released to any unauthorised third party without the prior written approval of the sponsoring institution. Clinical information will not be released without the participant's written permission except as necessary for monitoring by the HREC, Research Governance Office, or regulatory agencies.

9.1.3 Data confidentiality

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

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

(1) The number of private/confidential variables collected for individual study participants has been minimised. The data collected will be limited to that required to address the primary and secondary objectives.

(2) Participant identifiers will be stored separately from the data collected, and documents with identifiers will be stored separately from participant data. Participant data and samples will be identified using a unique study number assigned to the participant (“re-identifiable”). The Principal Investigator is responsible for storing a master file of names and other identifiable data with the participant ID; access to this document will be restricted to the site study team. The master file will be stored securely and separately from study data in locked/ password-protected databases, with

passwords kept separately. Age and sex will be included in the data; however, it is unlikely to make specific participants or families identifiable as identifiers will be stored separately.

Separation of the roles responsible for the management of identifiers and those responsible for content analysis. The Principal Investigator will ensure access to the data is restricted, using REDCap's permission control functionality ("REDCap Users and Permissions").

9.1.4 Quality assurance

Data validity and quality assurance will be ensured by the following:

(1) Data collection and entry into the eCRF will be completed by authorised staff designated by the investigator. Appropriate training on eCRF completion will be conducted with the relevant investigator and all authorised staff before the study starts and any data is entered.

(2) All data will be entered in English. eCRFs are to be completed as soon as possible after each participant's visit. For information collected specifically for the study, data may be entered directly into the eCRF without duplicating the information across separate source documentation and eCRF. If data are unavailable or missing, this will be indicated on the eCRF. Changes or corrections made on eCRF will be tracked via an audit trail.

(3) Once the eCRFs have been entered, edit and consistency checks will be performed as outlined in the DMP. Data queries generated for logic and legality will be reviewed by authorised study staff for clarification. After resolving the queries, the responsible Data Manager will make the necessary updates to the database. An audit trail of all changes to the database will be maintained. Once all queries have been resolved, the database will be locked.

(4) The Principal Investigator must sign off on the eCRF data before the final database lock.

(5) An audit is a systematic and independent examination of trial-related activities and documents to determine whether the evaluated related activities were conducted. The data were recorded, analysed, and accurately reported according to the protocol, study SOPs, GCP, and the applicable regulatory requirement(s).

Authorised representatives of MCRI, its designee, a regulatory authority, or the IEC may visit the centre to perform audits or inspections. The investigator should contact MCRI or the designee immediately if a regulatory agency contacts them about an inspection at their centre. If an audit or inspection occurs, the site PI agrees to allow the auditor/inspector direct access to all relevant documents and allocate their staff's time to the auditor/inspector to discuss findings and relevant issues.

The statistician will be provided anonymised data, with participants identifiable only by unique participant study numbers/codes. The statistician will analyse the de-identified data.

9.1.5 Archiving - Data and document retention

Archiving

The Investigator will take measures to prevent accidental or premature destruction of these documents. For this study, essential documents, and data (including biological samples) will be retained for a minimum of 15 years post-study.

The central study site and laboratory for this study are:
New Vaccines Research Group, Infection & Immunity, Murdoch Children's Research Institute
Records will not be destroyed without the written consent of the Principal Investigator.

Destruction

After the archival period of 15 years, The Principal Investigator will take measures to ensure the secure destruction of hard copies of study data. Any hardcopy data will be disposed of via a confidential shredding process. Noting that simply deleting files does not destroy the information, electronic CRFs will be destroyed under guidance from MCRI IT to ensure the files are permanently deleted.
The electronic, anonymised database will not be destroyed.
At the end of the archival period, eCRFs will be disposed of via secure destruction methods. Electronic databases will be retained by MCRI and stored on the secure server.

9.1.6 Sample management: collection and storage

The biobanking team will manage, process, and store the samples as per section 3 of the protocol.

10 TRIAL OVERSIGHT

10.1 Governance structure

The Governance structure is outlined below.

10.1.1 Trial Management Group (TMG)

The Site Principal Investigator is responsible for supervising any individual or party to whom they have delegated tasks at the trial site. They must provide continuous supervision and documentation of their oversight. To meet this GCP requirement, a small group will be responsible for the day-to-day management of the trial and will include, at a minimum, the Site PI, project manager, data manager and an Immunology team representative. The group will review all aspects of the trial's conduct and progress closely, ensuring a forum for identifying and addressing issues. Meetings must have minutes with attendees listed, pertinent emails retained, and phone calls documented.

10.1.2 Trial Steering Committee (TSC)

A TSC will be established to provide expert advice and overall supervision and ensure that the trial meets the required standards. The TSC will meet at least annually, with more frequent meetings as needed, and will work to a Terms of Reference.

10.1.3 Safety Monitoring

Monitoring will be performed according to Good Clinical Practice (GCP) guidelines. Following written SOPs, the monitors will verify that the clinical trial is conducted, and that data are generated, documented, and reported in compliance with the protocol, GCP and the applicable regulatory requirements. The investigator will provide direct access to all trial-related source data/documents and reports for monitoring purposes.

Independent Data Safety Monitoring Board (DSMB)

Safety oversight will be under the direction of a DSMB. The DSMB will meet at least 6 monthly to review SAEs or AEs deemed possibly, probably, or definitively related to study interventions. The DSMB will make recommendations concerning the conduct, continuation, or modification of the study for safety reasons. The DSMB will meet virtually prior to the commencement of the trial and then again on at least two occasions to review the safety data. The DMSB will operate under the rules of an approved charter that will be written and reviewed at the organisational meeting of the DSMB. The DSMB will be chaired by an experienced vaccine trial specialist and will include a statistician and an adult physician as members. Members of the DSMB will be independent of trial conduct.

10.2 Site Monitoring

Trial site monitoring is conducted to ensure that the rights and well-being of trial participants are protected, that the reported trial data are accurate, complete, and verifiable, and that the conduct of the trial is in compliance with the currently approved protocol and amendment(s), good clinical practice, and applicable regulatory requirements.

Full details of trial site monitoring are documented in the Clinical Monitoring Plan (CMP). The CMP describes who will conduct the monitoring, at what frequency monitoring will be done, at what level of detail monitoring will be performed, and the distribution of monitoring reports.

Monitoring for this trial will be performed by one of the two external trial managers who are not the project manager for this study. The monitoring will take place on-site. The monitors will review 100% of original signed consent forms, trial eligibility data and data related to the primary outcome, safety and other key data variables, all withdrawals from the trial, and targeted review of other data, including investigational vaccine administration and accountability.

The investigational site will provide direct access to all trial-related sites, source data/documents, and reports for monitoring and auditing by the sponsor and inspection by local and regulatory authorities.

10.3 Quality Control and Quality Assurance

Both the Sponsor-Investigator and Site Investigator have responsibilities in relation to quality management.

The Sponsor-Investigator will develop SOPs that identify, evaluate and control risk for all aspects of the trial, e.g., data management, training, eligibility, informed consent, and adverse event reporting. The Sponsor-Investigator will implement quality control (QC) procedures, including the data entry system

and QC checks. Any missing data or data anomalies will be communicated to the study staff for clarification/resolution.

As outlined in the previous section (Site Monitoring), the trial monitors will verify that the clinical trial is conducted and data are generated, and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, good clinical practice, and applicable regulatory requirements.

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

In addition, the clinical site will perform internal quality management of trial conduct, data, and biological specimen collection, documentation, and completion.

The study trial master file, investigator site file documents and all other filing will be done on eFlorence.

11 STATISTICAL METHODS

11.1 Sample Size Estimation

Sample size calculations were performed using nQuery 8 software and methods described by Van Belle, et al. 1993 (20). Precision-based sample size calculations were performed based on the reactogenicity outcomes. Assuming the percentage of participants reporting local or systemic reactions is 50%, 100 participants will produce a confidence interval that is 9.8 percentage points from the observed percentage. For immunogenicity, sample size calculations were based on comparing day 28 post-booster geometric mean concentrations of IgG antibodies between vaccine arms. We assumed the coefficient of variation (standard deviation/mean) of the post-booster antibody concentrations would be 1.15 (based on data from Munro, et al. 2021 (8), but increased to be conservative). Even though previous studies demonstrated a geometric mean ratio (GMR) of up to 4 between the study vaccine groups, we considered a clinically important difference between groups to be a GMR of >1.4. Based on these assumptions the study will need to recruit 160 participants in each vaccine group overall to achieve 90% of power at two-sided 0.05 significance level. Allowing for ~20% loss to follow-up the sample size in each arm was expanded to 200. To ensure age representation (<50 and ≥50) in the second Moderna bivalent group (BA.4-5) numbers were increased in the younger age group.

We are confident that the sample size is sufficient as it is likely that the GMR difference will be higher than expected and the loss to follow-up lower than anticipated.

11.2 Statistical methods and the Estimand framework

Analysis for this trial will be planned using the estimand framework (21). Trial objectives will be translated into suitable estimands, with consideration of strategies for handling intercurrent events. Analysis methods (estimators) that align with the estimands will be selected. Comparisons will be made under the superiority framework.

Analyses will be performed using Stata software version 17 (22). Detailed methodology will be outlined in a separate Statistical Analysis Plan.

11.3 Methods of analysis - outcome variables and methods

11.3.1 Primary outcome

Reactogenicity following immunisations will be presented as the number and percentage of participants self-reporting local reactions (pain, tenderness, redness, hardness, swelling, warmth, itch at or near the injection site) and systemic reactions (fever, nausea, vomiting, diarrhoea, headache, fatigue/malaise, myalgia), and the severity of these reactions (mild, moderate, severe, life-threatening, or fatal). Reactogenicity data will be presented separately by group and time point. For reactogenicity proportions will be estimated with 95% Clopper-Pearson binomial confidence intervals.

For immunogenicity, antibody levels will be presented as geometric mean concentrations (GMC) with 95% confidence intervals. Changes from baseline will be expressed as geometric mean fold ratios (GMFR), calculated as the ratio of the post-booster GMC to the baseline GMC. GMCs will be compared between the two vaccine groups at 28 days. We will account for baseline levels, age, primary series and first booster vaccine in the analysis. We will use the Treatment Policy strategy for the analysis, meaning that each intercurrent event (i.e., breakthrough SARS-CoV-2 infection) is considered to be part of the treatment effect.

The binding IgG data using the Euroimmun S1 IgG ELISA kits will be reported as relative units/ml (RU/ml) per the manufacturer's instructions. A result of <8 RU/ml: negative (IgG Antibodies for SARS-CoV-2 are not detected), ≥8 to <11 RU/ml: borderline (IgG antibodies are indeterminate/equivocal with this sample, ≥11 RU/ml: positive (IgG antibodies for SARS-CoV-2 are detected). Conversion to binding antibody units (BAU/ml) will also be done using the WHO reference serum from NIBSC, UK. Data will be presented as geometric mean concentrations and 95% confidence intervals. The primary endpoint will be the GMR at Day 28 for binding antibody. Linear regression will be used to estimate the differences in log antibody concentrations, adjusted for stratifying variables (age group), duration between different doses, study day of blood-draw, and baseline levels. The GMR will be calculated as the antilogarithms of the mean difference (beta1) and it's 95% CI.

In addition to the group level assessments described above, for individual patients in the vaccine groups, seroresponse will be defined as:

- ≥4-fold rise in binding antibody concentrations at 28 days post-study vaccine from baseline among subjects with no pre-dose (<200 BAU/ml) detectable titres
- ≥ 2-fold rise in binding antibody concentrations at 28 days post-study vaccine among subjects with baseline titre of ≥200 BAU/ml pre-booster

11.3.2. Secondary outcomes

Binding antibodies - changes from baseline will be expressed as geometric mean fold ratios (GMFR), calculated as the ratio of the post-booster GMC to the baseline GMC. GMCs will be compared between the two vaccine groups and between the vaccine and control groups at 6, 12, 18, 24 and 30 months. We will account for age in the analysis.

Functional antibodies seven time points for vaccine groups [baseline, 1, 6, 12, 18, 24and 30 months] and six time points for controls [baseline, 6, 12, 18, 24 and 30 months]) – for the C-PASS assay (sVNT), data are reported as percentage (%) inhibition by neutralising antibodies using the manufacturer's instructions. A <30% inhibition is considered negative, while ≥30% inhibition is considered positive. Data will be presented as mean and standard deviation.

Neutralizing assay (20% subset of samples at all time points) up to 12 months – Data will be reported as endpoint titre calculated using the Reed/Muench method (23). A titre of 10 reflects undetectable neutralizing activity.

Cellular immunity: (on a 50% subset at seven time points for vaccine groups and six time points for controls)
Quantiferon COVID IFNγ release assay up to 12 months – Levels of IFNγ will be reported in IU/ml according to the manufacturer's instructions. Data will be reported as GMT and 95% confidence intervals.

IFNγ Elispot – The number of IFNγ-producing cells/million PBMCs will be reported using means and 95% confidence intervals.

Intracellular cytokine staining – The data will be reported as the frequency (%) of cytokine-expressing T cells (e.g., total T cells, CD4 T cells, CD8 T cells). Cytokines such as IFNγ (Th1) and IL-5 (Th2) will be measured as a minimum. This will be presented as means and 95% confidence intervals.

Multiplex cytokine assays – A 10-plex panel of cytokines will be measured in supernatants from the Elispot studies. Cytokine concentrations will be reported in pg/ml, and data will be presented as GMT and 95% confidence intervals.

11.3.3 Exploratory outcome

We will describe the number of confirmed mild, moderate, and severe breakthrough cases in each of the three groups.
Explore the association between immune protection and viral variants and identify potential viral signatures associated with severe breakthrough infections.

12 ETHICS AND DISSEMINATION

12.1 Research Ethics Approval & Local Governance Authorisation

This protocol, the informed consent document, and any subsequent amendments will be reviewed and approved by the human research ethics committee (HREC) before the research. A letter of protocol approval by HREC will be obtained before the commencement of the trial and approval for other trial documents requiring HREC review.

12.2 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 participant's 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 before implementation.

12.3 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 Site Principal Investigator, who will assess for seriousness.

Those deviations deemed to affect to a significant degree the 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 promptly (Site Principal Investigator is to report to the Sponsor-Investigator within 72 hours and to the Site RGO within seven days; Sponsor-Investigator is to review and submit to the approving HREC within seven days).

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

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

13 CONFIDENTIALITY

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

The trial protocol, documentation, data, and all other information generated will be strictly confidential. No information concerning the trial, or the data will be released to any unauthorised third party without the 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, including but not limited to medical records (office, clinic, or hospital) and pharmacy records for the participants in this trial. The clinical trial site 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 the participant's written permission except as necessary for monitoring by HREC or regulatory agencies.

Data will be shared with relevant government bodies.

14 PARTICIPANT REIMBURSEMENT

Study participants will be compensated for their time, the inconvenience of blood tests and procedures, and travel expenses (parking or public transport reimbursement).

15 FINANCIAL DISCLOSURE AND CONFLICTS OF INTEREST

No conflict of interests.

16 DISSEMINATION AND TRANSLATION PLAN

Participants will be given access to their personal antibody levels and the analysed data at each time point once it becomes available. The former will be on request at follow-up visits, while the latter and the overall study results will be provided by group email as the analysed antibody data becomes available.

After study completion, a major publication will be prepared for an international journal. The data will also be presented at appropriate international conferences and meetings.

17 ADDITIONAL CONSIDERATIONS

Not applicable.

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19 APPENDICES

APPENDIX 1 – Product information sheets

Moderna bivalent PI.pdf

Nuvaxovid_PIL.pdf

ModernaBA4_5_PI.pdf