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Randomised controlled trial reveals no benefit to a 3-month delay in COVID-19 mRNA booster vaccine

Wen Shi Lee, … , Kevin J. Selva, Stephen J. Kent

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Clinical Medicine In-Press Preview COVID-19 Vaccines

BACKGROUND. There is uncertainty around the timing of booster vaccination against COVID-19 in highly vaccinated populations during the present endemic phase of COVID-19. Studies focused on primary vaccination have previously suggested improved immunity after delaying immunisation.

METHODS. We conducted a randomised controlled trial (Nov 2022 – Aug 2023) and assigned 52 fully vaccinated adults to an immediate or a 3-month delayed bivalent Spikevax mRNA booster vaccine. Follow-up visits were completed for 48 participants (n = 24 per arm), with saliva and plasma samples collected following each visit.

RESULTS. The rise in neutralising antibody responses to ancestral and Omicron strains were almost identical between the immediate and delayed vaccination arms. Analyses of plasma and salivary antibody responses (IgG, IgA), plasma antibody-dependent phagocytic activity, and the decay kinetics of antibody responses were similar between the 2 arms. Symptomatic and asymptomatic SARS-CoV-2 infection occurred in 49% (21/49) participants over the median 11.5 months of follow up and were also similar between the 2 arms.

CONCLUSIONS. Our data suggests no benefit from delaying COVID-19 mRNA booster vaccination in pre-immune populations during the present endemic phase of COVID-19

TRIAL REGISTRATION. Australian New Zealand Clinical Trials […]

Randomised controlled trial reveals no benefit to a 3-month delay in COVID-19 mRNA booster vaccine

Wen Shi Lee*[1], Jennifer Audsley*[2], Mai-Chi Trieu[1,3], Arnold Reynaldi[4], L Carissa Aurelia[1], Palak H Mehta[1], Joanne Patterson[5], Helen E Kent[1], Julie Nguyen[1], Thakshila Amarasena[1], Robyn Esterbauer[1], Ebene R Haycroft[1], Pradhipa Ramanathan[1], Miles P Davenport[4], Timothy E Schlub[6], Joseph Sasadeusz[2,5], Adam K Wheatley[1], Amy W Chung[1], Jennifer A Juno[†1], Kevin J Selva[†1], Stephen J Kent[†1,7].

Authorship note: *WSL and *JA contributed equally to this work and are co-first authors.

[†]JAJ, [†]KJS and [†]SJK are co-senior authors.

Address correspondence to:
Stephen J Kent, Peter Doherty Institute for Infection and Immunity, University of Melbourne, 792 Elizabeth Street, Melbourne, Victoria, 3000, Australia. Phone: +61 3 8344 9939. Email: skent@unimelb.edu.au
Kevin J Selva, Peter Doherty Institute for Infection and Immunity, University of Melbourne, 792 Elizabeth Street, Melbourne, Victoria, 3000, Australia. Phone: +61 3 9035 8640. Email: kevin.selva@unimelb.edu.au
Jennifer A Juno, Peter Doherty Institute for Infection and Immunity, University of Melbourne, 792 Elizabeth Street, Melbourne, Victoria, 3000, Australia. Phone: + 61 3 8344 3791. Email: jennifer.juno@unimelb.edu.au

Affiliations

1 Department of Microbiology and Immunology, Peter Doherty Institute for Infection and Immunity, University of Melbourne, Melbourne, Victoria, Australia.

2 Department of Infectious Diseases, Peter Doherty Institute for Infection and Immunity, University of Melbourne and Royal Melbourne Hospital, Melbourne, Victoria, Australia.

3 Influenza Centre, Department of Clinical Science, University of Bergen, Bergen, Norway.

4 Kirby Institute, University of New South Wales, Kensington, New South Wales, Australia.

5 Victorian Infectious Diseases Service, Royal Melbourne Hospital, Melbourne, VIC, Australia.

6 Sydney School of Public Health, Faculty of Medicine and Health, University of Sydney, Sydney, New South Wales, Australia.

7 Melbourne Sexual Health Centre and Department of Infectious Diseases, Alfred Hospital and Central Clinical School, Monash University, Melbourne, Victoria, Australia.

Conflicts-of-interest statement

The full conflict-of-interest statement is available in the Supplemental Materials.

40-word summary

Delaying uptake of the bivalent Spikevax mRNA booster by 3 months did not significantly improve neutralisation and IgG antibody responses against both ancestral and Omicron strains, suggesting no substantial benefit from delaying COVID-19 mRNA booster vaccination in pre-immune populations.

Abstract

Background

There is uncertainty around the timing of booster vaccination against COVID-19 in highly vaccinated populations during the present endemic phase of COVID-19. Studies focused on primary vaccination have previously suggested improved immunity after delaying immunisation.

Methods

We conducted a randomised controlled trial (Nov 2022 – Aug 2023) and assigned 52 fully vaccinated adults to an immediate or a 3-month delayed bivalent Spikevax mRNA booster vaccine. Follow-up visits were completed for 48 participants (n = 24 per arm), with saliva and plasma samples collected following each visit.

Results

The rise in neutralising antibody responses to ancestral and Omicron strains were almost identical between the immediate and delayed vaccination arms. Analyses of plasma and salivary antibody responses (IgG, IgA), plasma antibody-dependent phagocytic activity, and the decay kinetics of antibody responses were similar between the 2 arms. Symptomatic and asymptomatic SARS-CoV-2 infection occurred in 49% (21/49) participants over the median 11.5 months of follow up and were also similar between the 2 arms.

Conclusions

Our data suggests no benefit from delaying COVID-19 mRNA booster vaccination in pre-immune populations during the present endemic phase of COVID-19

Trial registration

Australian New Zealand Clinical Trials Registry number 12622000411741.

Funding

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Introduction

Ancestral spike-based COVID-19 vaccines have reduced effectiveness at preventing symptomatic Omicron variant infections due to progressive neutralising antibody escape (1). As such, COVID-19 vaccines have been serially updated to include Omicron spike variants. Bivalent COVID-19 mRNA vaccines (BA.1 first approved in Australia late 2022, followed by BA.5) are superior to ancestral monovalent vaccines at boosting Omicron neutralising antibodies (2-4), and preventing hospitalisation and severe disease (1, 5). Despite this, the bivalent mRNA boosters have shown only modest efficacy against infection with more recent XBB subvariants (6).

The durability of immunity afforded by bivalent booster vaccines and optimal timing of receiving boosters following last vaccination or infection remains unclear. Guidance on the timing of repeated boosters varies widely. Early studies found a longer interval between the first and second vaccine doses (8-16 weeks) elicited higher binding and neutralising antibody titres compared to the standard 3-4 week interval (7-9), possibly due to improved maturation of antibody and B cell responses (10, 11). An extended interval between vaccination and infection also enhanced neutralising antibody titres (12). However, a third mRNA vaccine dose equalised this response, resulting in similar neutralising antibody titres in individuals who had short or long intervals between the first two doses (13).

The potential benefit of longer delays between subsequent boosters in highly vaccinated populations in the endemic phase of COVID-19 is currently unclear, with a fine balance between the potential for improved immunity with a longer duration

between doses, the serial escape of Omicron strains leading to transient protective immunity, and vaccine fatigue within the population.

To determine whether there is an immunological benefit with a longer interval between last vaccination/infection and subsequent booster vaccination, we undertook an open-label randomised controlled trial administering the Moderna BA.1 bivalent mRNA booster (mRNA-1273.214) upon recruitment (immediate arm) or 3 months following recruitment (delayed arm). We found that antibody-mediated immunity to circulating variants was not improved by delaying the booster.

Results

Study design

We recruited 52 adults over the period 11/09/2022-30/01/2023, of whom 49 completed follow up (Figure 1) in this open-label RCT. The follow-up period was 11/09/2022-02/12/2023. Recruitment was stopped prior to reaching the pre-defined sample size in the immediate arm (n=29, compared with n=25 recruited), due to the withdrawal and replacement of the BA.1 bivalent vaccine with the BA.4/5 bivalent vaccine. Forty-eight subjects (24 randomised to the immediate vaccine arm and 24 to the 3-month delay arm) were analysed for immunologic outcomes since one subject tested positive for SARS-CoV-2 1 day after receiving the vaccine (Supplemental Table 1). The relative immunogenicity of the Moderna Spikevax BA.1 bivalent vaccine booster in Australia was unknown at study initiation and the primary outcome was achieving a plasma neutralising antibody titre of >1:100 to Omicron BA.1 two weeks after vaccination in the immediate arm. Key additional endpoints included comparisons of antibody responses in plasma and saliva (mean titre of SARS-CoV-2 antibodies) between the two arms (Supplemental Figure 1 and 2), safety analyses (number of self-reported adverse events collected at day 3 and day 7 post-vaccination) and breakthrough COVID-19 infections during the study. Subjects were evenly matched for age, gender, number of prior vaccinations and number of COVID-19 infections (Supplemental Table 1). Median time since last vaccination or COVID-19 infection at enrolment was similar between the groups at 8.0 and 10.5 months for the immediate and delayed arms respectively. The delayed arm was boosted a median of 3.1 months later than the immediate arm. Three subjects randomised to the delayed arm acquired COVID-19 while waiting for vaccination and as per protocol waited 4 months after infection for their booster vaccine – two of these subjects received the BA.4/5 bivalent Spikevax

vaccine since the BA.1 bivalent vaccine had been withdrawn in the interim. One additional subject in the delayed arm also received the BA.4/5 bivalent Spikevax vaccine. There were 102 vaccine adverse events reported (Supplemental Table 2), with no statistically significant difference in reporting between the 2 arms. None of the adverse events were serious, and all were consistent with reactions reported previously (2).

Bivalent vaccine boosts immune responses similarly in immediate and delayed arms

Neutralising antibodies are a key correlate of protective immunity against COVID-19 (14). Plasma neutralising titres to BA.1 (in the booster) and XBB.1.5 (a dominant circulating Omicron strain during the study) were relatively low prior to vaccination in both groups (median IC_{50} 219 and 269 for delayed and immediate arms for BA.1; 24 and 29 respectively for XBB.1.5 in a live virus neutralisation test, Figures 2, A-C, F-H) despite a median of 3 prior COVID-19 vaccinations and 69.4% having at least 1 prior self-reported COVID-19 infection previously. For the delayed arm, BA.1 and XBB.1.5 neutralising titres were similar from study recruitment (3 months pre-booster) to the day of vaccination (Day 0) (Figure 2, B and G). After receiving the booster, all immediate arm subjects achieved an Omicron BA.1 neutralisation titre of >1:100 by 2 weeks ($P \leqslant 0.0001$), meeting the study primary endpoint (Figure 2C). Neutralising titres at day 14 post-booster were almost identical between the immediate and delayed arms to both Omicron BA.1 and XBB.1.5 (Figure 2, D and I), reaching median IC_{50} titres of 1548 and 1583 for BA.1, and 313 and 356 for XBB.1.5 in the delayed and immediate arms respectively. At day 84 post-vaccination, neutralising titres decayed ~1.4-2.1 fold from day 14 but remained similar between both arms (Figure 2, E and J).

Neutralising activity against ancestral and XBB.1.5 strains across all sampled timepoints from both arms was also analysed using a surrogate bead-based Spike-ACE2 inhibition assay (Supplemental Figure 3, A-G). Percent inhibition against both ancestral and XBB.1.5 spikes peaked at Day 14 for both arms at similar levels and gradually decayed over time.

Since antibodies in the upper airways may be important in preventing SARS-CoV-2 infection (15), we measured neutralising antibody responses in saliva using an ELISA-based surrogate virus neutralisation test (16) (Supplemental Figures 3, H-J). Salivary neutralising antibodies to the ancestral strain were boosted in most subjects at Day 14 ($P \leqslant 0.001$) and were similar in both immediate and delayed arms (Figure 2, K-N).

Spike-specific T cells were recently implicated to be a predictor of protection against symptomatic infection in vaccinated children (17). In addition to quantifying the serological response to booster vaccination, we assessed the frequency of spike-specific CD4 and CD8 T cells at day 0 and day 7 post-vaccination in a subset of the cohort (Supplemental Figure 4A). Immunisation drove a significant expansion of spike-specific memory for both CD4 and CD8 T cells, as measured by production of IFNγ, IL-2 and/or TNF (Supplemental Figure 4, B and C; p=0.007 for CD4 Tmem, p=0.016 for CD8 Tmem). We did not detect any substantial spike-specific cTFH responses, likely due to poor cytokine production by cTFH relative to other T cell subsets (18) (Supplemental Figure 4B). Spike-specific T cell frequencies at day 7 were comparable between the immediate and delayed vaccination arms for both CD4 and CD8

populations (Supplemental Figure 4, D and E; Supplemental Table 3), consistent with the serological data.

Decay kinetics of vaccine-induced antibodies

Beyond peak antibody titres following vaccination, an important parameter of vaccine-induced antibodies is how fast they decay, leaving subjects vulnerable to breakthrough infection (19). Differences in decay kinetics of various antibody parameters were analysed across the immediate and delayed vaccination arms. Here, we studied not only plasma neutralising antibody responses (Figure 3, A-C), but also total IgG and IgA in plasma (Figure 3, D-F, G-I) and saliva respectively (Figure 3, P-R, S-U). Furthermore, as Fc-effector functions have been implicated in assisting antibody-mediated immunity to SARS-CoV-2 (20, 21), we also examined Fc-gamma receptor 2a (FcγR2a) engagement and antibody-dependent cellular phagocytosis (ADCP) in plasma (Figure 3, J-O; Supplemental Figure 5, A-E). While the decay analyses focused on antibody responses to Omicron XBB.1.5 (Figure 3, A-U) as this was a major circulating strain during our study, we also examined total IgG and IgA, and FcγR2a binding responses in plasma (Supplemental Figure 6, 8, 9, 11, 13) and saliva (Supplemental Figure 7, 8, 10, 12, 13) respectively against ancestral, Omicron BA.1 and Omicron BA.5 strains.

Decay kinetics of plasma neutralising antibodies (Figure 3, A-C), as well as total IgG and IgA against XBB.1.5 spike in plasma (Figure 3, D-I) and saliva (Figure 3, P-U) respectively were very similar between the immediate and delayed arms out to 84 days post-booster. Of note, spike-specific salivary IgA responses were not induced by the vaccine, consistent with the known poor mucosal immunity induced by

213 intramuscular vaccines (Figure 3, S and T; Supplemental Figure 10) (22, 23). FcγR2a-
214 binding antibodies to spike in plasma were elicited by the vaccine and had a modestly
215 faster decay rate in the delayed arm ($t_{1/2}$ of 45 vs 88 days, $P \leqslant 0.05$; Figure 3, J-L;
216 Supplemental Figure 10). However, this difference diminished when we compared Fc-
217 effector responses of plasma antibodies using a cell-based phagocytosis assay
218 (ADCP) (Figure 3, M-O). Overall, our results suggest delaying vaccination in the
219 context of our study has no substantial benefit in terms of preserving long-term
220 antibody immunity.
221
222 We also modelled the time required for the various XBB.1.5 antibody responses to
223 decrease to pre-booster levels (Figure 3, C, F, I, L, O, R, U). Plasma neutralising titres
224 against XBB.1.5 took an average of 240 days to decay to baseline levels. Saliva IgG
225 took the longest time to decay (1225 days) while plasma IgA took the shortest time
226 (162 days).
227
228 **COVID-19 infections during the study**
229 Australia has experienced multiple waves of COVID-19, including during the current
230 study. Although not powered for efficacy, we documented symptomatic COVID-19
231 infections over the course of follow-up. We identified 14 symptomatic infections out to
232 a maximum follow up of 12.4 months (Supplemental Table 4). This included 2 subjects
233 who reported two symptomatic infections (one subject in each arm). The symptomatic
234 infections were evenly distributed between the immediate and delayed arms with
235 similar Kaplan-Meier lines (Figure 4A, Log-rank Mantel-Cox test; $P = 0.109$). The
236 apparent reduction in COVID-free survival in the delayed arm was due last subject in

follow-up acquiring COVID-19. All documented infections were mild in severity consistent with multiple prior vaccinations.

Analyses of serial immune responses following breakthrough COVID-19 has been informative regarding the recall of immunity that helps control infection (24-26). Little is known about serial salivary antibody responses following breakthrough COVID-19 with recent Omicron strains. We were able to obtain nasal swab samples for four subjects with breakthrough COVID-19 during the trial and found that three of four subjects acquired the XBF strain (viral sequencing was unsuccessful in the last nasal swab). We also obtained additional serial saliva and blood samples and analysed antibody responses (Figure 4, B-G; Supplemental Figure 14). We detected transient rises in XBB.1.5-specific total IgG and IgA, and FcγR2a binding responses in both plasma and saliva in 3 of the 4 subjects (Figure 4, B-G), confirming that breakthrough COVID-19 can boost mucosal immunity.

As asymptomatic SARS-CoV-2 infections are also common, we analysed non-vaccine elicited antibodies to the N protein. We identified 10 subjects without symptomatic COVID-19 during our study with a clear and sustained rise in N antibodies (>4-fold increase over previous sampling timepoint; Figure 4, H and I) and a rise in XBB.1.5 neutralisation titres. Combined cases of symptomatic and asymptomatic infection were evenly divided between the arms and similar over time (Figure 4J, Log-rank Mantel-Cox test; $P = 0.838$).

Discussion

Timing of SARS-CoV-2 booster vaccination is contentious in high vaccinated populations in the present endemic phase of COVID-19, with (i) waning immunity, (ii) changing escape profiles of new variants, and (iii) booster fatigue all factors to consider. We randomised healthy adults to receive an immediate or 3 month delayed COVID-19 booster. The booster improved antibody and T cell immunity in all subjects. We found no difference in booster-induced antibody-based immunity to either ancestral, vaccine (BA.1) or circulating strains of SARS-CoV-2 (XBB.1.5) between the immediate and delayed arms. Further, the decay kinetics of spike-specific antibodies over the subsequent 12 weeks were not improved in the delayed arm, suggesting no longer term benefit from delaying vaccination. Remarkably, over 40% of participants (21 of 49) completing the study had symptomatic or asymptomatic COVID-19 during the mean 11.5-month study follow-up but the rates of infection were similar in both arms. Taken together, our results suggest no substantial benefit in delaying booster vaccination to improve antibody-based immunity to SARS-CoV-2.

The changing landscape of SARS-CoV-2 Omicron variants is a major factor driving poor immunity and breakthrough COVID-19 infections. Levels of neutralising antibodies against Omicron XBB.1.5 (which was a common circulating strain during our study) were low pre-booster (median IC_{50} of 24 or 29 respectively, with 75% being <1:100). XBB.1.5 titres reached a median of 346 across the whole cohort 2 weeks after vaccination, consistent with a previous study showing BA.1 bivalent vaccines boosted neutralising titres against XBB.1.5 (27), despite the poor effectiveness afforded against symptomatic XBB.1.5 infection (6). XBB.1.5 titres waned to a median of 186 by 84 days and were estimated to return to the low pre-booster baseline levels

by an average of 240 days after receiving the booster. This illustrates the relatively short-lived effect current mRNA booster vaccines.

Although the BA.1 bivalent vaccine we studied has been superseded with a XBB.1.5 monovalent vaccine (28), recent dominant Omicron strains such as JN.1 have continued to escape neutralising antibody responses (29). Maintaining high levels of neutralising antibodies to circulating and emerging variants with the current process of updating vaccines is inefficient, resulting in increasing cases of COVID-19 breakthrough infections, as we observed. Nonetheless, delaying booster vaccination with the hope of improving the peak or durability of antibody immunity during the present endemic phase of COVID-19 does not work nor prevent COVID-19. There is a need for vaccines that elicit broader and more durable protective immunity against SARS-CoV-2.

Our study had limitations. First, our study had 24 subjects per arm analysed for antibody immunity owing to intercurrent COVID-19 infections and the updating of the bivalent vaccine. Although subject numbers were adequate for most analyses, our ability to detect small differences in peak or waning of antibodies between the 2 arms was less robust. However, the virtually identical levels of neutralising antibody responses, confirmed with multiple other analyses of antibody responses, suggests any real difference between immediate or delayed vaccination would be very small and of doubtful clinical significance. Second, there were many intercurrent asymptomatic and symptomatic SARS-CoV-2 infections, and presumably many more exposures to SARS-CoV-2 that did not lead to overt infections during our study. These COVID-19 breakthrough infections also modulate antibody responses (24-26), as

documented here in several cases (Figure 4, B-G; Supplemental Figure 14). While these infections and exposures could confound some of our antibody analyses, the infections were evenly distributed between the 2 arms and unavoidable given circulating SARS-CoV-2 levels during our study. Third, our subjects had an average of 3 prior vaccinations and an average time from prior vaccination or COVID-19 infection of 9.4 months. There might be scenarios with less prior vaccinations and/or COVID-19 infections, or different timing of booster vaccination that could reveal differences in immediate or delayed vaccination. Too short a time between a COVID-19 infection and a booster vaccine has been shown to be suboptimal (30). However, pre-booster neutralising antibodies to the circulating XBB.1.5 variant were low in our study and a significant proportion of our study population acquired SARS-CoV-2 infection during our trial. This suggests we studied a relevant population in efforts to improve immunity and protection from infection. Fourth, we studied a group of healthy adults who were less than 65 years old, while immunocompromised or elderly groups – key target groups for vaccination – may respond differently and have a larger benefit from more frequent booster vaccination (31). Lastly, our assays to date are largely focused on antibody immunity, while cellular immunity could theoretically be modulated to a greater degree by vaccination timing and potentially play an important role in long term immunity (32). Nevertheless, neutralising antibodies have emerged as a robust correlate of immunity to SARS-CoV-2 and guide most vaccine recommendations (14, 19).

In summary, this randomised controlled trial of highly vaccinated healthy adults during the present endemic phase of COVID-19 showed no benefit in the induction of

protective antibodies against SARS-CoV-2 by delaying booster vaccination 3 months. Regular booster SARS-CoV-2 vaccinations are supported by this study.

Methods

Sex as a biological variable

This study was open to all sexes, and male and female participants were recruited. Randomisation included matching for sex.

Study participants

Adults (18-65 years) who had received 2-3 doses of COVID-19 vaccines at least 4 months prior were eligible. Exclusion criteria included prior COVID-19 infection within 4 months, immunosuppression and previous significant adverse events to COVID-19 vaccines. A SARS-CoV-2 Omicron blood neutralising titre of >1:100 in >90% of participants was considered a successful outcome since this level is predicted to be reliably protective against the Omicron strain. Based on this, power calculations were carried out using G*Power version 3.1.9.7 using a one-tailed the Exact Generic Binomial Test. 29 participants in the immediate vaccine group was estimated to be required for a proportion of participants with a neutralisation titre of >1:100 greater than 90%. Dynamic (adaptive) randomisation with minimisation to promote balance in age, sex and timing of initial vaccines was used to allocate participants to either interventional group. Age was stratified by 10-year intervals and time since 2nd vaccine by monthly intervals, using equal weighting of covariate factors. This achieved using R: A language and environment for statistical computing, library Minirand, function Minirand using equal weighting of covariate factors and high probability of assignment = 0.90. Participants were recruited in Melbourne, Australia and were randomised to receive a Moderna BA.1 bivalent mRNA vaccine booster dose (0.5ml) administered intramuscularly upon enrolment (immediate arm) or three months later (delayed arm). Most participants received the Moderna BA.1 bivalent vaccine,

however during the study the Moderna BA.4/5 bivalent vaccine replaced the BA.1 formulation and three participants received the BA.4/5 vaccine. Participants were randomised into the two arms and matched for age (10-year intervals), sex (male, female, other) and timing of last COVID-19 vaccine dose (2 month intervals, from a minimum of 4 months). The study was open-labelled.

Serial blood plasma samples and saliva samples (SalivaBio, Salimetrics) were collected and stored at -80°C. Salivary samples from both delayed and immediate arms had comparable levels of total secretory IgA between respective timepoints (Supplemental Figure 1). Peripheral blood mononuclear cells (PBMC) were isolated from whole blood by Ficoll-Paque separation and cryopreserved in 10% DMSO/90% fetal calf serum (FCS).

Variant Spike multiplex bead assay

SARS-CoV-2 specific total IgG, IgA, and FcγR2a dimer (Bruce Wines, Burnet Institute) engagement in plasma (1:25600, 1:6400, 1:6400) and saliva (1:50, 1:50, 1:12.5) from the booster cohort were assessed using a customised multiplex bead-based array consisting of ancestral and Omicron spike trimers (BA.1, BA.5, XBB.1.5, Sino Biological) as previously described (26) (Supplemental Figure 2). SARS-CoV-2 nucleocapsid (N) protein was included to screen for asymptomatic infections. SIVgp120, H1Cal2009 (Sino Biological) and tetanus toxoid (MilliporeSigma) were included as controls. Briefly, spike-coupled beads were first incubated with samples overnight at 4°C, then washed and incubated with biotinylated detectors (isotype detection antibodies, MabTech; FcγR2a dimers) for 2 hours at room temperature (RT). After washing, beads were incubated with Streptavidin-R-Phycoerythrin

(ThermoFisher Scientific) for 2 hours at RT. Beads were washed again and read on the Intelliflex (Luminex). Assays were repeated in duplicates.

Virus neutralisation assay

Plasma live virus neutralisation assay with viability dye readout was performed against Omicron BA.1 and XBB.1.5 viruses as previously described (33). Infectivity of virus stocks was determined by titration on HAT-24 cells (a clone of transduced HEK293T cells stably expressing human ACE2 and TMPRSS2) (34). Virus stocks were titrated in quintuplicate in three independent experiments to obtain mean 50% infectious dose (ID_{50}) values.

To determine serum neutralization activity, heat-inactivated plasma samples were diluted 3-fold (1:20–1:43,740) in duplicate and incubated with SARS-CoV-2 virus at a final concentration of 2 × ID_{50} at 37ºC for 1 h. Next, 40,000 freshly trypsinized HAT-24 cells in DMEM with 5% FCS were added and incubated at 37ºC. “Cells only” and “Virus+Cells” controls were included to represent 0% and 100% infectivity respectively. After 48 h, 10 µL of alamarBlue Cell Viability Reagent (ThermoFisher) was added into each well and incubated at 37ºC for 1 h. The reaction was then stopped with 1% SDS and read on a FLUOstar Omega plate reader. The relative fluorescent units (RFU) measured were used to calculate %neutralization with the following formula: (“Sample” - “Virus+Cells”) ÷ (“Cells only” - “Virus+Cells”) × 100. IC_{50} values were determined using four-parameter non-linear regression in GraphPad Prism with curve fit constrained to have a minimum of 0% and a maximum of 100% neutralization.

Surrogate virus neutralisation test (sVNT)

Neutralising activity of plasma (final dilutions 1:6400) were also assessed using an adapted surrogate spike-ACE2 inhibition assay (35) (Supplemental Figure 3, A-G). Briefly, ancestral or Omicron XBB.1.5 variant S1-coupled beads were incubated with diluted plasma overnight at 4°C. Avi-tagged biotinylated ACE2 (Nicholas Gherardin, University of Melbourne) was added and beads incubated for 1 hour at RT. After washing, beads were incubated with streptavidin-PE for 1 hour at RT, then R-Phycoerythrin Biotin-XX conjugate (ThermoFisher Scientific) was added incubated for another hour at RT. Beads were washed and read on the Intelliflex. Assays were repeated in duplicates. Saliva neutralising activity against the ancestral virus in saliva (final dilutions 1:2) and plasma (final dilutions 1:200) samples were measured using the sVNT kit (GenScript cPass) as per manufacturer's directions. Readings above the recommended 30% cut-off are positive for neutralising activity (Figure 3, H-J).

Bead-based THP-1 ADCP assay

A bead-based ADCP assay was performed as previously described (Supplemental Figure 5A) (36). Briefly, SARS-CoV-2 XBB.1.5 Spike trimer (Sino Biological) was biotinylated and coupled to 1 µM fluorescent NeutrAvidin Fluospheres (beads; Invitrogen) overnight at 4°C. Antigen-coated beads were washed and diluted in 1% BSA/PBS and incubated with plasma (final dilutions 1:1600; Supplemental Figure 5B) for 2 hours at 37°C in a 96-well U-bottom plate. THP-1 monocytes (100,000/well) were added to opsonised beads and incubated for 16 h under cell culture conditions. THP-1 monocytes were then fixed and acquired by flow cytometry on a BD LSR Fortessa with a high-throughput sampler. The data was analysed using FlowJo 10.9.0 (see Supplemental Figure 5A for gating strategy) and a phagocytosis score was calculated

as previously described using the formula: (% bead-positive cells x mean fluorescent intensity).

Spike-specific T cell Assays

Cryopreserved PBMC were thawed and rested for 4 hours in RPMI-1640 supplemented with 10% FCS and penicillin-streptomycin (RF10). $2x10^6$ PBMC were seeded per well in a 96-well U bottom plate and stimulated with 1ug/mL of a peptide pool covering the spike protein (PepTivator SARS-CoV-2 Prot_S Complete) or an equivalent volume of vehicle control (sterile H_2O). After 1 hour, Brefeldin A (Golgi Plug, BD Biosciences) was added to the cell culture. PBMC were cultured for a total of 16 hours before being washed with PBS. Cells were stained with live/dead (Invitrogen) for 3 minutes at room temperature and then incubated with the surface antibody cocktail for 30min at 4C. The surface antibody cocktail included: CD20 BV510, 2H7; CD3 BUV395, SK7; CD27 BUV737, L128; CXCR5 BB515, RF8B2 (all from BD Biosciences); CD4 BV605, RPA-T4; CD8 BV650, RPA-T8; and CD45RA PerCP-Cy5.5, HI100 (all from BioLegend). After fixation and permeabilization (BD CytoFix/CytoPerm) for 20 minutes at 4C, cells were incubated with the intracellular antibody cocktail (IFNγ APC, B27; TNF BV421, Mab11; IL-2 PE, MQ1-17H12; all from BioLegend). Cells were washed in Perm/Wash buffer, resuspended in PBS+1%FCS, and acquired on a BD LSR Fortessa.

Modelling

A piecewise model was used to estimate the growth and decay rate of various immune responses following vaccination. The model of the immune response y for subject i at time y_i can be written as:

$$y_i(t) = \begin{cases} Be^{gt}; \ t < T_{peak} \\ Be^{gT_{peak}} \times e^{-d(t-T_{peak})}; \ t \geq T_{peak}. \end{cases}$$

The model has 4 parameters; B, g, T_{peak}, and d. We assumed a constant baseline value B for the immune response pre vaccination. The immune response will grow at a rate of g until T_{peak}. From T_{peak}, the immune response will decay at a rate of d. For each subject, the parameters were taken from a normal distribution, with each parameter having its own mean (fixed effect). A diagonal random effect structure was used, where we assumed there was no correlation within the random effects. The model was fitted to the log-transformed data values, with a constant error model distributed around zero with a standard deviation σ. We also censored the data from below (left-censoring) if it was less than the threshold for detection. Model fitting was performed using Monolix2023R1.

Statistics

Statistical analysis was performed with GraphPad Prism 10.2.0 (GraphPad Software). Antibody responses between cohorts/timepoints/variants were presented as medians and compared using 2-tailed Mann-Whitney *U* tests, Kruskal-Wallis test followed by Dunn's test for multiple comparisons, Friedman test followed by Dunn's test for multiple comparisons or Wilcoxon matched-paired signed rank test where appropriate. *P* values ≤ 0.05 were considered significant.

Study Approval

The study was approved by Ethics Committees at the Royal Melbourne Hospital (Study number 2021/272) and University of Melbourne (Approvals 13793 and 23497). Written informed consent was obtained from all participants prior to enrolment. This

study was registered with the Australian New Zealand Clinical Trials Registry (anzctr.org.au, #12622000411741).

Data Availability

All the data and methods are presented in the manuscript or in the Supplemental Materials. All individual values for figures are available in the Supplemental Supporting Data Values file.

Author contributions

SJK conceived and designed the study. JS, JP and HEK recruited subjects. TES generated the random allocation sequence and assigned participants to the interventions. KJS, WSL, LCA, PHM, JA, MCT, JP, HEK, JN, TA, RE, ERH, PR, TES, JS, AWC, AKW, SJK and JAJ were responsible for the acquisition of data. KJS, WSL, JA, AR, MPD, JAJ and SJK performed the analyses and interpreted the results. SJK, KJS, WSL and JA wrote the first draft. All authors critically revised the report and approved the final version. The order of co-first authors was assigned based on their experimental and editorial contributions to this study.

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Figures

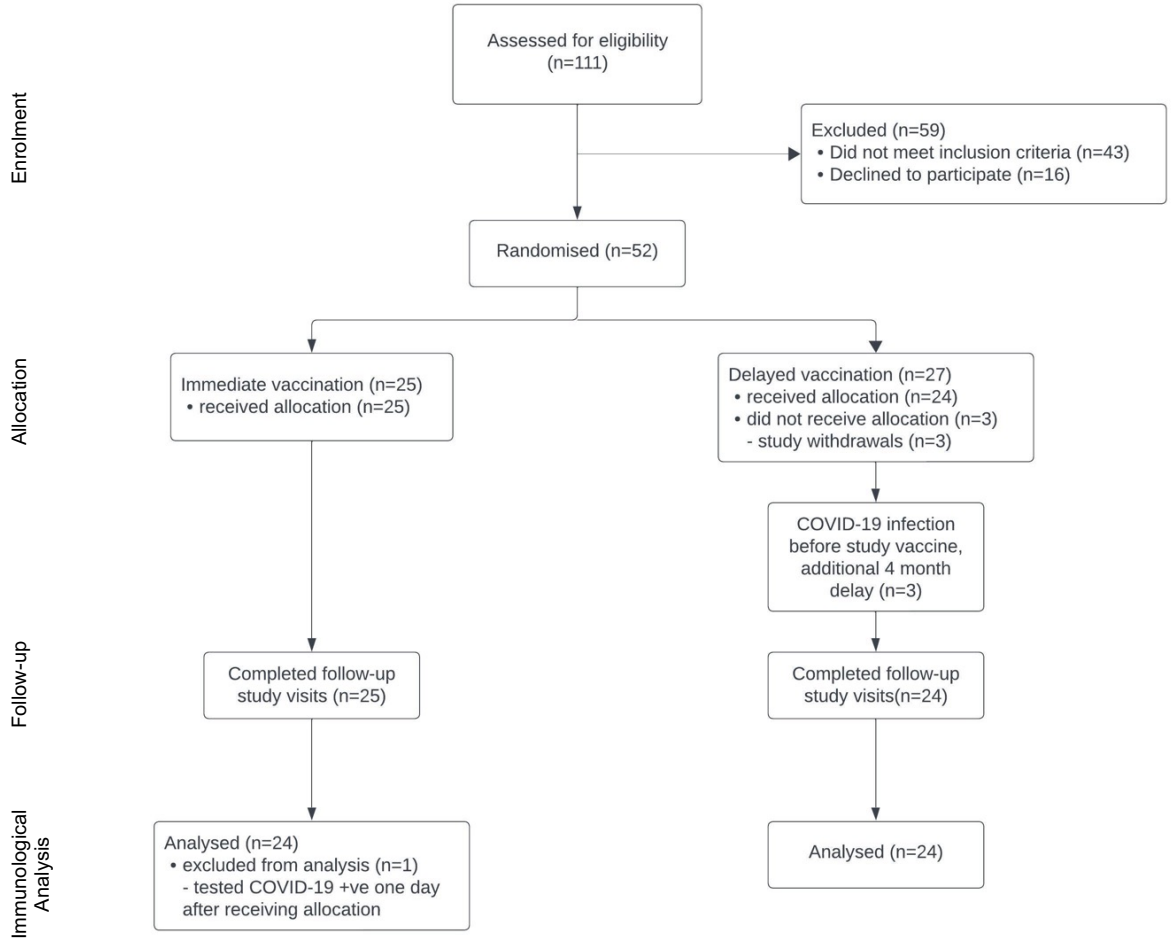

Figure 1: CONSORT flow diagram.

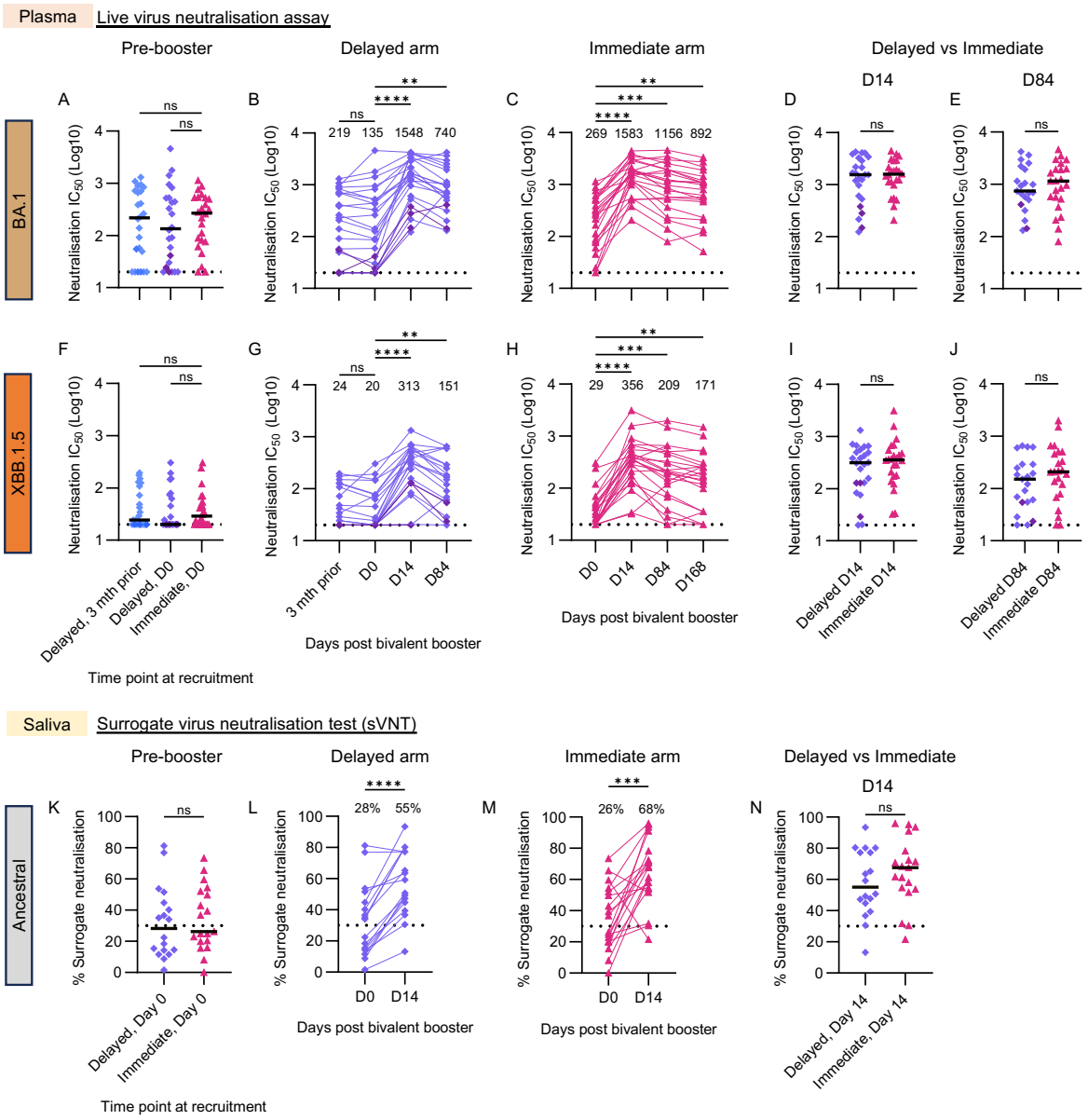

Figure 2: Neutralising antibodies following bivalent mRNA booster vaccination. Plasma neutralising activity was measured using a live virus neutralisation assay against SARS-CoV-2 Omicron BA.1 (**A-E**) and XBB.1.5 (**F-J**) variants. Pre-booster (**A and F**) and post-booster (Day 14; **D and I**) (Day 84; **E and J**) neutralising antibody responses were compared between the delayed (blue/purple diamond, n=24) and immediate arms (pink triangle, n=24) at the respective sampling timepoints. Line graphs describe the kinetics of plasma neutralisation activity of the delayed (**B and G**) and immediate (**C and H**) arms after receiving the bivalent booster. Numbers above

each timepoint describe the respective median neutralisation IC_{50} against each viral variant. Dotted lines depict the detection threshold for the assay (neutralisation IC_{50} =20). Dark purple diamonds and lines show the antibody responses of the 3 individuals who received the BA.5 bivalent booster in the delayed arm. Saliva neutralising activity against ancestral SARS-CoV-2 was measured using the surrogate virus neutralisation test (sVNT; Genscript). Pre-booster (**K**) and post-booster (Day 14; **N**) neutralising antibody responses are compared between the delayed (purple diamond, n=18) and immediate arms (pink triangle, n=19) respectively. Line graphs describe the change in saliva neutralisation activity following the bivalent booster (**L and M**). Numbers describe the % surrogate neutralisation observed at each timepoint. Dotted lines depict the sVNT cutoff for neutralising activity (30%). Statistical significance was calculated between cohorts and timepoints using the 2-tailed Mann-Whitney *U* test, or Kruskal-Wallis test followed by Dunn's test for multiple comparisons. Paired saliva analysis (D0 vs D14) was performed using Wilcoxon matched-paired signed rank test. Experiments were performed in duplicates. Graphs are displayed as median, and where significant, *P* values were reported (*$P \leqslant 0.05$; **$P \leqslant 0.01$; ***$P \leqslant 0.001$; ****$P \leqslant 0.0001$).

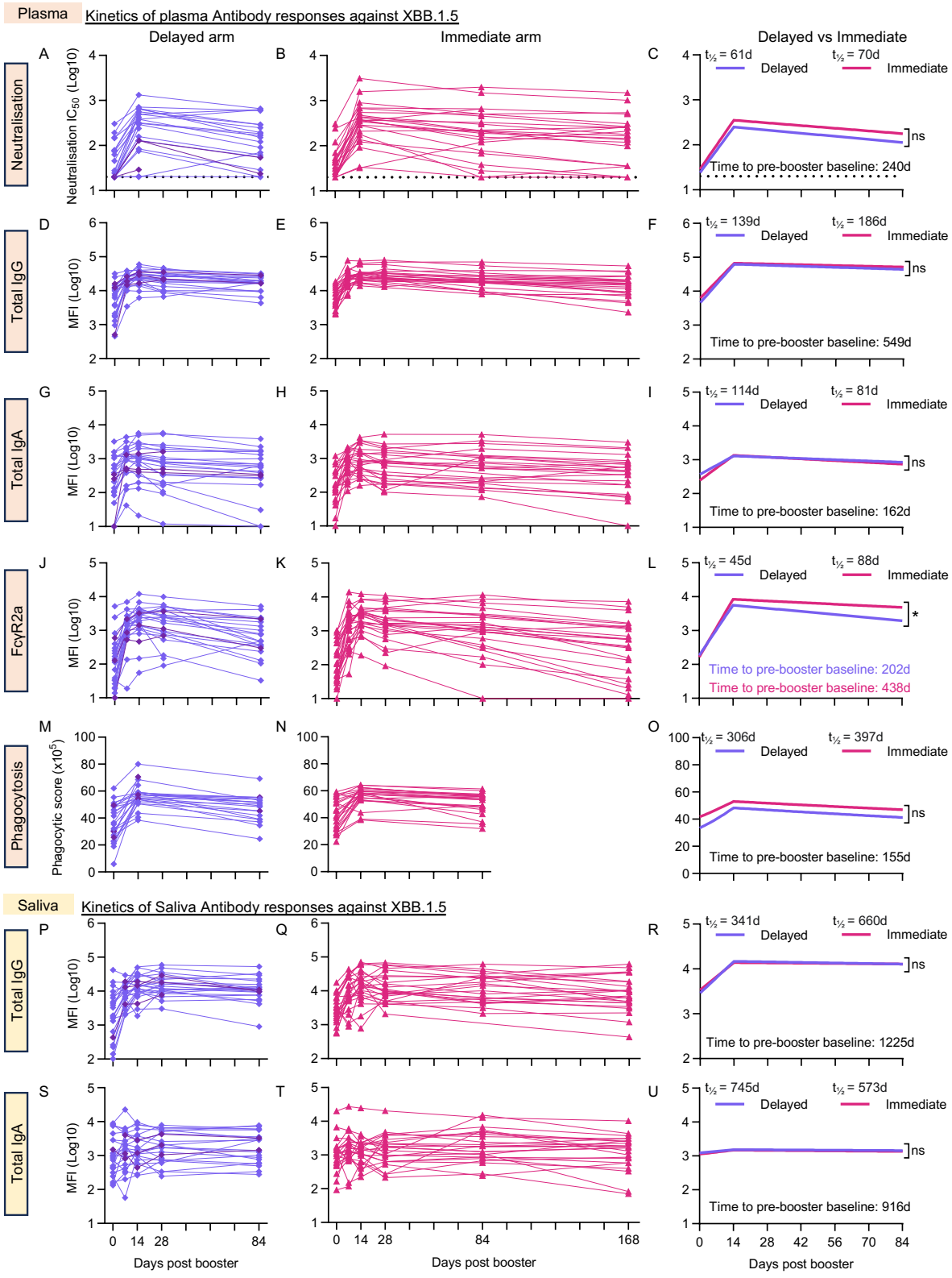

Figure 3: Antibody kinetics following bivalent mRNA booster vaccination. Kinetics of plasma (**A-O**) and saliva (**P-U**) antibody responses against SARS-CoV-2 variant Omicron XBB.1.5. Line graphs depict the plasma neutralisation responses in

675 the delayed (purple diamond, n=24; **A**) and immediate (pink triangle, n=24; **B**) arms
676 as previously described in Figure 2G-H. Line graphs also illustrate the rise and decay
677 of plasma total IgG (**D and E**), total IgA responses (**G and H**), Fc-gamma receptor 2a
678 binding (**J and K**) and antibody-dependent phagocytic activity **(M and N),** as well as
679 salivary total IgG (**P and Q**) and total IgA (**S and T**) responses in the delayed (purple
680 diamond, n=24; **D, G, J, M, P, S**) and immediate (pink triangle, n=24; **E, H, K, N, Q, T**)
681 arms respectively. Dark purple diamonds and lines show the antibody responses of
682 the 3 individuals who received the BA.5 bivalent booster in the delayed arm. Modelled
683 decay slopes (**C, F, I, L, O, R, U**) describe the half-life and time taken for the respective
684 antibody responses to return to pre-booster baseline levels. Statistical significance
685 was calculated between cohorts using the likelihood ratio test and where significant,
686 *P* values were reported (*$P \leqslant 0.05$). Experiments were performed in duplicates.
687

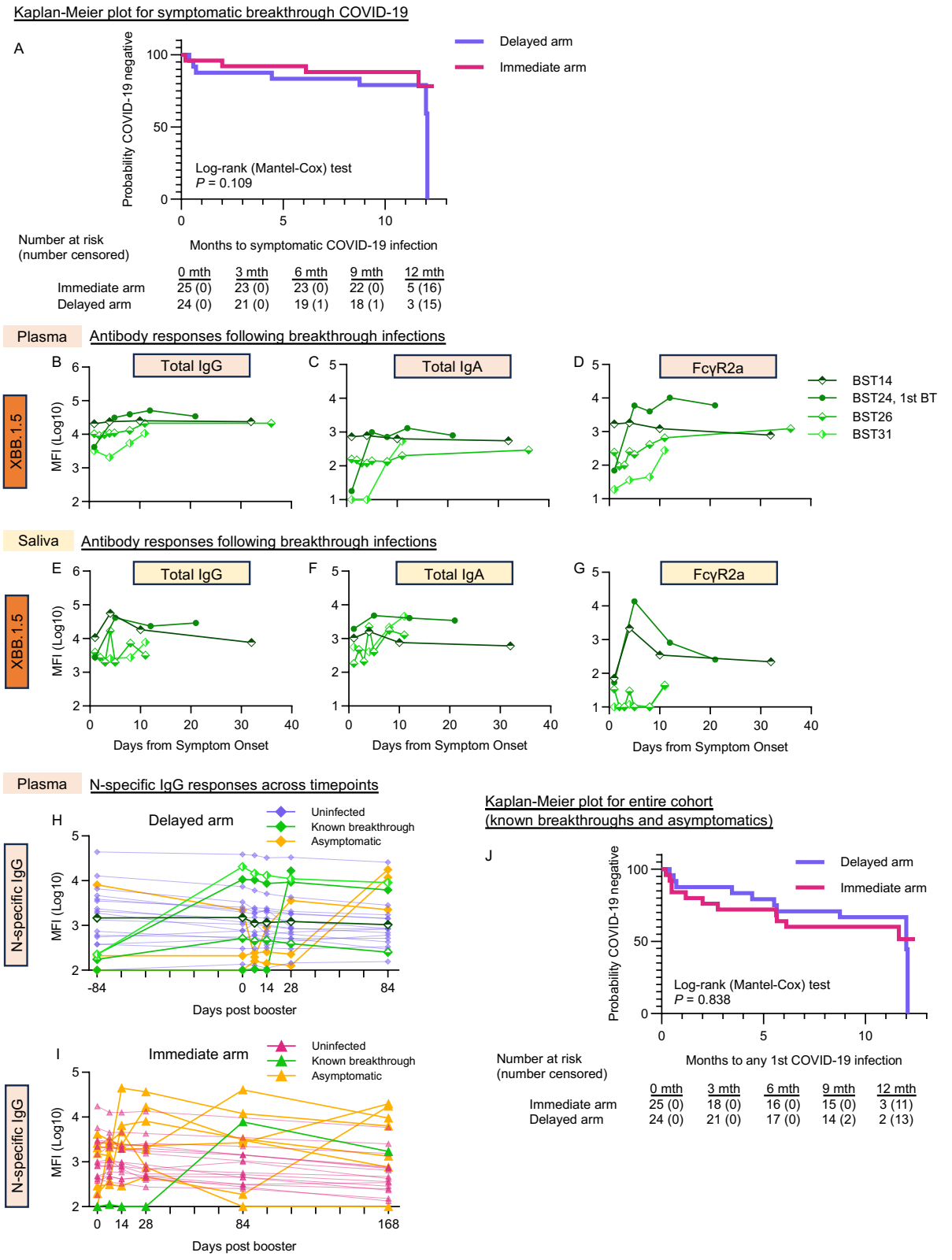

Figure 4: Breakthrough COVID-19. Kaplan-Meier probability of remaining symptomatic COVID-19 negative during the study in the delayed (purple) and immediate (pink) arms (**A**). Includes all first on-study COVID-19 symptomatic

infections (pre and post study vaccination, self-reported). Probability for the delayed arm reaches zero because the final three delayed arm subjects are positive/censored just after 12 months, while there are 5 final immediate arm participants remaining at risk. The numbers below the graph show the remaining number at risk (number censored) during the study at baseline (0 mth), month 3 (3 mth), month 6 (6 mth), month 9 (9 mth) and month 12 (12 mth). Statistical significance between survival curves were calculated via Log-rank Mantel-Cox test. Line graphs show the plasma (**B-D**) and salivary (**E-G**) antibody responses against Omicron XBB.1.5 from 4 representative individuals (green) with COVID-19 breakthrough infections (RATs positive). Total IgG (**B and E**), Fc-gamma receptor 2a binding (**C and F**), and total IgA responses (**D and G**) against Omicron XBB.1.5 are shown following their symptom onset. Line graphs also depict the kinetics of N-specific IgG for both the delayed (purple diamonds; **H**) and immediate arms (pink triangles; **I**) across sampling timepoints, highlighting Individuals with known symptomatic (RATs positive; green) and asymptomatic breakthrough infections (>4-fold rise in N-specific IgG from previous timepoint; yellow). Experiments were performed in duplicates. Kaplan-Meier probability of remaining COVID-19 negative during the study in the delayed (purple) and immediate (pink) arms (**J**). Includes all first on-study COVID-19 infections (pre and post study vaccination, self-reported and asymptomatic laboratory diagnosed). Probability for the delayed arm reaches zero because the final two delayed arm subjects are positive/censored just after 12 months, while there are 3 final immediate arm participants remaining at risk.