JCI insight

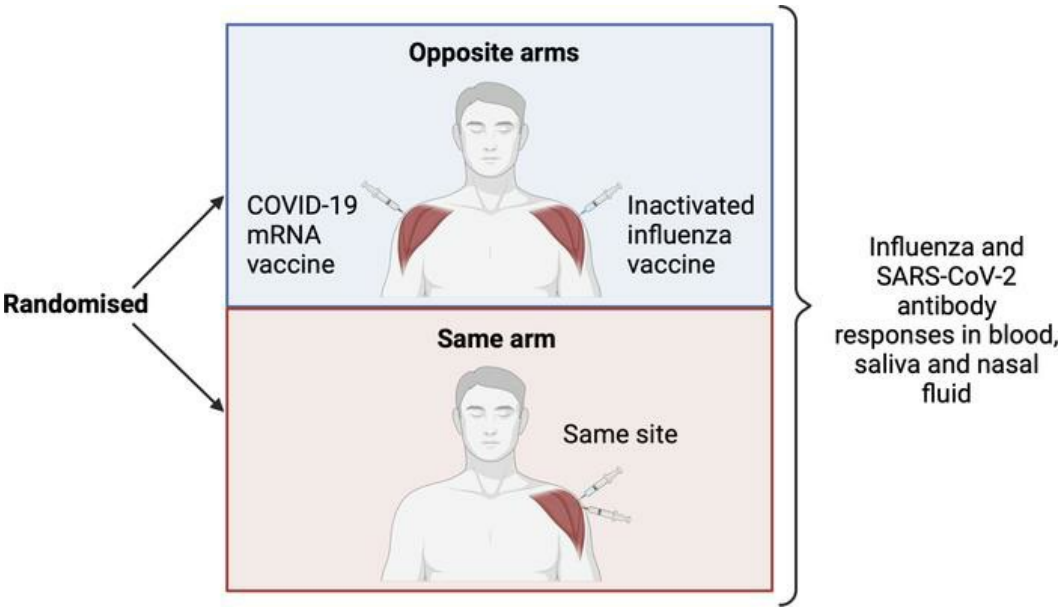
Randomised trial of same vs opposite arm co-administration of inactivated influenza and SARS-CoV-2 mRNA vaccines

Wen Shi Lee, … , Ian Barr, Stephen J. Kent

JCI Insight. 2025. https://doi.org/10.1172/jci.insight.187075.

Clinical Medicine In-Press Preview Clinical trials Vaccines

Graphical abstract



Randomized trial of same vs opposite arm co-administration of inactivated influenza and SARS-CoV-2 mRNA vaccines

Wen Shi Lee[1]*, Kevin J. Selva[1]*, Jennifer Audsley[2]*, Helen E. Kent[1], Arnold Reynaldi[3], Timothy E. Schlub[4], Deborah Cromer[3], David S. Khoury[3], Heidi Peck[5], Malet Aban[5], Mai Ngoc Vu[1], Ming Z. M. Zheng[1], Amy W. Chung[1], Marios Koutsakos[1], Hyon-Xhi Tan[1], Adam K. Wheatley[1], Jennifer A. Juno[1], Steven Rockman[1,6], Miles P. Davenport[3], Ian Barr[5], Stephen J. Kent[1,7]

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

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

5 WHO Collaborating Centre for Reference and Research on Influenza, Royal Melbourne Hospital, at the Peter Doherty Institute for Infection and Immunity, Melbourne, Victoria, Australia

6 Vaccine Innovation Unit, CSL Seqirus Ltd, Parkville, Victoria, Australia

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

*WSL, KJS and JA contributed equally to this work and are co–first authors

Address correspondence to: Stephen J. Kent, Peter Doherty Institute for Infection and Immunity, University of Melbourne, Melbourne, Victoria, 3000, Australia. Phone: 61.3.8344.9939; Email: skent@unimelb.edu.au

Abstract

Background

The immunogenicity of current influenza vaccines need improvement. Inactivated influenza and COVID-19 mRNA vaccines can be co-administered but randomized controlled trial data is lacking on whether the two vaccines are more immunogenic if given in the same or opposite arms. Murine studies suggest mRNA vaccines can adjuvant influenza vaccines when co-formulated and delivered together.

Methods

We randomly assigned 56 adults to receive the Afluria quadrivalent inactivated influenza and Moderna monovalent SARS-CoV-2 XBB.1.5 mRNA vaccines, either in opposite arms or both in the same arm at the same site. The primary endpoint was the difference in median combined serum haemagglutination inhibition titre to the H1, H3 and B-Vic vaccine influenza strains after vaccination.

Results

We found no significant difference in haemagglutination inhibition antibody levels between the groups ($p = 0.30$), with the same arm group having a 1.26-fold higher titre than the opposite arm group. There was no difference in analyses of antibodies to individual influenza strains, nor in nasal or saliva antibody levels. While both binding and neutralising antibody titres against SARS-CoV-2 were not significantly different between groups post-vaccination, there was a higher fold-change in BA.5 and ancestral strain neutralising antibodies in the opposite arm group.

Conclusion

Influenza vaccination is equivalently immunogenic if given in same or opposite arms as the SARS-CoV-2 vaccine, but it may be preferable to administer the SARS-CoV-2 vaccine at a different site to influenza vaccines.

Trial registration

Australian New Zealand Clinical Trials Registry ACTRN12624000445572

Funding

Australian National Health and Medical Research Council and Medical Research Future Fund.

Introduction

There is a need to safely improve the immunogenicity and efficacy of the standard unadjuvanted inactivated influenza vaccine (1, 2). Use of the MF59 adjuvant or a higher dose improves efficacy, but the effect is modest and generally only recommended for older or immunocompromised subjects since such vaccines are more expensive and reactogenic (3, 4). mRNA lipid nanoparticle vaccines for SARS-CoV-2 induce high levels of protective immune responses (5). The ionizable lipid component of the lipid nanoparticle is a potent adjuvant of mRNA vaccines (6). Indeed, in the context of primary immunisation, ionizable lipids have a robust adjuvant effect on protein vaccines in animal studies (7).

The antigenic drift of both Influenza and SARS-CoV-2 viruses have led to regular reformulation of both vaccines to incorporate more recently circulating strains. Influenza vaccines are recommended annually, and regular SARS-CoV-2 boosters are also commonly recommended (8). Since both unadjuvanted influenza vaccines and SARS-CoV-2 mRNA lipid nanoparticle boosters can be given annually, it may be practical and lead to higher community uptake if both vaccinations are given at the same medical visit. The question then arises if the two vaccines should be given in the same or opposite arms. Most jurisdictions advise that either the same or opposite arms can be used since there is little data on which to recommend one strategy over the other (9).

Two recent observational studies suggested minimal differences in either influenza or SARS-CoV-2 specific antibody levels elicited with same or opposite arm immunisation (10, 11). However, those studies were not randomised and the selection of subjects could

introduce potential confounders. In one study, most subjects preferred opposite arm vaccination, and the time intervals of sampling were wide (10). Furthermore, the precise location of both vaccines given in the same arm immunisation was unclear. As noted above, murine studies show an ionizable lipid can adjuvant an influenza protein vaccine if given in the same location (7). This raises the hypothesis that the ionizable lipid within the SARS-CoV-2 mRNA lipid nanoparticle vaccine could act as an effective adjuvant to the influenza vaccine if given at the same location in the same arm. If effective, a same site vaccination strategy could enhance the effectiveness of the unadjuvanted influenza vaccine without the need for a more costly adjuvanted or high dose vaccine.

We conducted a randomised controlled trial of administering the unadjuvanted 2024 quadrivalent inactivated influenza vaccine (Afluria) on the same day as the XBB.1.5 monovalent SARS-CoV-2 mRNA booster (Spikevax) either at the same site in the same arm, or in opposite arms. The primary outcome was the serum haemagglutination inhibition titres to influenza strains within the vaccine.

Results

Clinical trial

Fifty-six adults were recruited between March 8th and April 9th 2024 and followed for 28 days. One subject randomised to the same arm allocation received the vaccines in opposite arms and was therefore excluded from analysis (Figure 1). Subjects were stratified for age, sex and receipt of 2023 influenza immunisation. Demographics show no major differences between the two treatment groups (Table 1). All subjects completed the study. The vaccines had an expected safety profile, with the same arm immunisation group having 67 total adverse events reported, compared to 52 for the opposite arm group (Table 2). The total cohort reported 119 adverse events, with 50/55 (91%) of subjects reporting at least one local reaction and 29/55 (52.7%) reporting at least one systemic reaction. All adverse events were Grade One or Two, except for a single report of pain at injection site that was Grade 3 (prevented daily routine). That subject was randomised to receive the vaccine in opposite arms and the Grade 3 reaction occurred in the non-dominant (SARS-CoV-2 vaccine) arm. There was no overall significant difference in the proportion of subjects reporting local nor systemic reactions between the same arm and opposite arm vaccine groups ($P = 0.35$ and $P = 0.79$, respectively, Fisher exact test).

Although local pain was reported in almost all subjects, the same arm group had a larger number of local swelling and redness events reported compared to the opposite arm (a total of 9 events reported by 6 subjects vs 2 events reported by one subject, respectively). This was likely related to both vaccines being given at the same site. In the opposite arm vaccination group, the proportion of subjects reporting at least one local reaction (pain, redness and/or swelling) was significantly higher ($P = 0.006$, Fisher exact test) in the non-

dominant arm (SARS-CoV-2 vaccine) compared to the dominant arm (influenza vaccine), as expected since local reactions are more common with the SARS-CoV-2 mRNA vaccine (Table 2).

Influenza antibody levels

Our hypothesis was that immunizing at the same site could boost influenza immunity through provision of an adjuvant by the lipid nanoparticle vaccine at the same site. The primary endpoint was the fold change in combined geometric mean of serum haemagglutination inhibition (HI) titres to the 3 circulating influenza strains within the vaccine (H1, H3 and B-Vic strains) 28 days after vaccination, in the same arm group compared to the opposite arm group. We found nearly identical levels of HI antibodies in the same arm and opposite arm groups, with no significant differences (1.26-fold higher [95% CI = 0.78 - 1.43] in same arm group; P = 0.30; Figure 2A). There was a 3.2-fold increase in HI antibodies to these 3 strains in both the same and opposite arm groups with no significant difference between the groups (P = 0.82; Figure 2B). Analysis of HI levels over time (Figure 2C) and to individual influenza vaccine strains (Figure 2D), including the B-Yam strain in the vaccine, also showed no differences between the groups.

Although the influenza vaccine-strain antibody immunity was not different between the arms, it was possible that immunity may have been broader in one or the other arms. We therefore analysed whether immunity to non-vaccine strains was preferentially boosted in either the same or opposite arm group by using a bead-based multiplex array to compare total binding antibody levels (Supplemental Figure 1). This analysis confirmed

the lack of difference in antibody responses to the vaccine strain HAs and showed no difference in antibody levels to 6 additional non-vaccine H1 and H3 proteins circulating in previous years (Figure 2E; Supplemental Figures 2, A, C and E). Prior to vaccination, there was a general hierarchy of responses towards older strains in both arms (Supplemental Figure 2, A, C and E). Unsurprisingly, the vaccine induced minimal responses to H5 proteins, with no differences between the arms to the recently circulating 2.3.4.4b clades (Texas 2024; S Carolina 2021) (Figure 2E; Supplemental Figure 3, A, D and G).

SARS-CoV-2 antibody levels

Our primary hypothesis was that the COVID-19 mRNA vaccine could boost the influenza vaccine response, but influenza vaccination could instead affect the response against SARS-CoV-2. We used a live virus assay to evaluate plasma neutralising antibody responses to the vaccine strain (XBB.1.5), a more recent circulating strain (JN.1), and older strains included in previous vaccines (Ancestral and BA.5) (Figure 3A). Our pre-specified secondary outcome was the fold-rise from day 0 to day 28 of the neutralising antibody titre to XBB.1.5 and other strains comparing between the same arm and opposite arm groups. We found a significantly higher fold-rise in antibody titres in the opposite arm group only for BA.5 and Ancestral strain (P = 0.01 and 0.02 respectively; Figure 3B). However, when looking at the absolute neutralisation titres, which are the predictors of protection, there were no significant differences in the day 28 neutralising antibody titres to XBB.1.5 and other variants (Figure 3B). The opposite arm group started with slightly lower day 0 neutralising antibody titres against BA.5, contributing to the

higher fold-rise following vaccination, though the baseline titres were not significantly different between groups for any of the strains tested (Supplemental Figure 4, A-D).

A time course of the rise of XBB.1.5 neutralizing antibody response is shown in Figure 3C, with the rise in XBB.1.5 neutralising antibodies being detectable by day 6, indicating rapid recall of pre-existing memory responses. We confirmed the neutralizing antibody results using a bead-based multiplex Elisa assay and found higher fold-increase in total plasma IgG antibody responses from day 0 to day 28 in the opposite arm group to both the XBB.1.5 and JN.1 spike proteins (Figure 3D). However, like the absolute neutralising antibody titres, total IgG titres against XBB.1.5 and JN.1 spike proteins were not significantly different at baseline nor at day 28 post-vaccination (Supplemental Figure 2, B, D and F).

Mucosal antibody levels

Mucosal immunity forms a protective barrier against infection by respiratory viruses such as influenza and SARS-CoV-2. We have previously shown that while intramuscular vaccinations were poor at inducing local mucosal IgA antibodies, they could elevate IgG antibodies in mucosal secretions, likely via transudation from blood (12). Here, we investigated if mucosal IgG antibodies against influenza and SARS-CoV-2 in saliva and nasal fluid would differ following administration to different immunization sites.

While influenza-specific antibodies in saliva and nasal fluid did increase following vaccination, there was no significant differences in antibody levels between the same and opposite arm groups across all influenza strains tested (Figure 4, A and C;

Supplemental Figure 5 and 6). As observed in blood, antibody responses to H3 Thailand 2022 following vaccination showed largest fold increases in saliva (5.6- vs 3.7- fold) and nasal fluid (2.9- vs 2.8-fold; same vs opposite arms; Figure 4, A and C). No mucosal IgG antibodies against H5 proteins were induced (Supplemental Figure 3, B, C, E, F, H and I).

We also observed that the fold-increase in mucosal IgG antibody responses to XBB.1.5 spike from day 0 to day 28 trended higher in the opposite arm group in both saliva (2.5- vs 3.7-folds) and nasal fluid (1.6 vs 2.5-folds; same vs opposite arms; Figure 4, B and D), however the mucosal responses were more variable and these differences were not significant. Total IgG binding titres against XBB.1.5 and JN.1 spike proteins were not significantly different in saliva or nasal fluid at all timepoints tested (Supplemental Figures 5 and 6).

Discussion

We hypothesized the ionizable lipid with the SARS-CoV-2 lipid nanoparticle mRNA vaccine could adjuvant the inactivated influenza vaccine if administered at the same site. However, our randomised controlled trial showed no difference (1.26 fold, $P = 0.30$) in HI titres to the H1, H3 and B-Vic influenza strains between the vaccine groups at day 28 after vaccination, the primary endpoint. This result was supported by multiple secondary and exploratory endpoints, including binding antibody titres to all influenza strains tested and at both day 6 and day 28 time points post-vaccination.

Why did this strategy fail when it had biological plausibility from published murine studies (7)? It may be that the two vaccines must be co-formulated in the same syringe for the

lipid nanoparticle to efficiently adjuvant the influenza protein. A recent preprint suggested coformulation of influenza protein and lipid nanoparticles was superior to separate delivery within one hour in mice (13). Although we attempted to administer the two vaccines to precisely the same site in the muscle in the same arm group, we cannot be sure this was effective. Future studies co-formulating the vaccines would be of interest. Another possible reason for the lack of benefit of this strategy was that we studied the recall of memory responses in a pre-immune population instead of looking at primary immunisation of a naïve population. The influenza vaccine in the population studied had overall modest immunogenicity (<3.17-fold median fold increase in HI), as compared to the 256 to 1024-fold-increases in HI observed in mice with primary immunisation (7). The people recruited in our study were somewhat older (median age 56) and had been given multiple prior influenza vaccinations. Younger populations with reduced exposure to influenza may respond more robustly to influenza vaccination (14). None-the-less, older populations are a more important target population to improve influenza vaccination. The translation of animal to human studies of adjuvants is fraught with negative results owing in part to species-specific adjuvant effects. The ionizable lipid used in mouse studies is different to that in the Moderna vaccine we studied (7). Recent work illustrates combination effects of adjuvants are more helpful and may translate to humans more effectively (15). Additionally, we cannot exclude a small effect could have been missed in our 55-subject trial, although any such small effect might be of minimal clinical significance. Larger trials of less diverse populations, or giving multiple booster vaccinations, could uncover small differences in the immunogenicity of co-administrations strategies. Although neutralizing antibodies are a generally accepted correlate of Influenza and SAR-CoV-2 immunity, cellular immunity is also of critical

importance and exploring T and B cell immunity could generate additional hypotheses for future studies. We note our randomised trial results are consistent with recently reported observational study (10, 11). We conclude there is no benefit to influenza immunity when influenza and SARS-CoV-2 vaccines are administered at the same site.

We did however observe a small detrimental effect on the fold-increase in SARS-CoV-2 antibody levels when both influenza and SARS-CoV-2 vaccines were administered at the same site. This effect was most prominent in binding antibody levels against XBB.1.5 and JN.1, but we also detected a similar detrimental effect for BA.5 and Ancestral strain neutralizing antibody titres. While this finding was unexpected, we caution that this difference was relatively modest in magnitude, absolute binding and neutralizing antibody levels at day 28 post-vaccination were not different between groups and we did not correct for multiple comparisons in these secondary or exploratory endpoints. As such, it requires confirmation in other studies. Possible reasons for this effect could be (a) slight differences in baseline titres between the randomised groups or (b) a detrimental effect on the lipid nanoparticle integrity in vivo where the vaccines comingle at the same site. We note that a negative effect on SARS-CoV-2 mRNA vaccine immunogenicity was not detected in observational studies where the vaccines were presumably given at separate sites in the same arm (10, 11). Although not directly studying same vs opposite arm vaccination, some observational studies have reported slightly lower SARS-CoV-2 antibody titres when COVID-19 and influenza vaccines were concurrently administered compared to receiving a COVID-19 vaccine alone, possibly indicating some interference between the two vaccines (16). There is a possibility of antigenic competition between the two vaccines. Since the influenza vaccine is protein

based it may be presented earlier than the mRNA vaccine that requires in translation of antigen. The potential immunological mechanisms behind changes in immunogenicity of COVID-19 vaccines when co-administered with influenza vaccines are still unknown and warrant further investigation. In vitro and animal studies examining the effect of co-formulating the two vaccines on the integrity and/or trafficking of the SARS-CoV-2 vaccine would also be of interest.

In conclusion, our randomised trial administering an influenza vaccine at the same site as a SARS-CoV-2 mRNA lipid nanoparticle vaccine did not improve influenza immunity and may have led to a small detrimental effect on SARS-CoV-2 immunity. Further studies are warranted but in the interim, when the 2 vaccines are administered on the same day, it may be preferable to give them in separate arms or at least some distance apart if given in the same arm.

Methods

Sex as a biological variable

Male and female participants were enrolled in this study, which was open to all sexes. Randomization included matching for sex.

Study design

The CANNON study was an open-label, randomised trial of administering both an Influenza and COVID-19 vaccine respectively on the same day in the same arm or in opposite arms. Healthy adults (18-65 years) who had received 2 or more prior doses of COVID-19 vaccines at least 4 months before recruitment were eligible. Exclusion criteria included prior COVID-19 infection within 4 months, immunosuppression, previous significant adverse events to Influenza or COVID-19 vaccines and prior anaphylaxis of any cause. Subjects were recruited in Melbourne, Australia and were randomised to receive the 2024 Seqirus/CSL quadrivalent unadjuvanted Influenza vaccine (Afluria) and Moderna Omicron XBB.1.5.-containing COVID-19 mRNA booster vaccine (Spikevax) administered intramuscularly in either the same arm or opposite arms. The same arm group received both vaccinations in the non-dominant arm at the same injection site using separate needles and syringes. The opposite arms group received the Moderna COVID-19 mRNA vaccine in the non-dominant arm and the Afluria Influenza vaccine in the dominant arm. Blood and saliva samples (SalivaBio; Salimetrics) were collected prior to vaccination and days 6 and 28 post-vaccination. Nasal fluid samples were collected prior to vaccination and day 28 post-vaccination by nasosorption (Nasorption FX-i; Mucosal Diagnostics) (17). Adverse event (AE) data were collected on day 6.

The primary endpoint was the difference in post-vaccination haemagglutination-inhibition (HI) titre combined to 3 Influenza vaccine strains (H1N1, H3N2 and B-Victoria) at day 28 in the same arm group compared to the opposite arm group. Based on HI titre data from 114 subjects receiving vaccination in 2023, we estimated the post vaccination HI titre standard deviation is 0.37 on a $\log_{10}$ scale. Calculating a geometric mean across the three influenza strains, we estimated we had 80% statistical power to detect a 2-fold-change (a 0.3 $\log_{10}$ difference) in the same arm group compared to the opposite arm group, (with statistical significance of 0.05) using a minimum of 25 subjects in each group. We considered a 2-fold change in HI likely to be a meaningful increase in protective immunity. Dynamic (adaptive) randomisation with minimisation to promote balance in age, sex and 2023 influenza immunisation was used to allocate subjects to either interventional group. Age was stratified by 10-year intervals. This was 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. Key additional endpoints included Influenza antibody responses in plasma, saliva and nasal fluids, antibody responses to other influenza strains, SARS-CoV-2 antibody responses and self-reported adverse events collected at day 6.

Immunologic assays

A haemagglutination inhibition assay (HI) was conducted at the WHO Collaborating Centre for Reference and Research on Influenza as previously described (18) using the 4 vaccine influenza strains as listed in Supplemental Table 1. A bead-based multiplex array containing both influenza HA and SARS-CoV-2 spike proteins (as listed in Supplemental

Table 1) was conducted as previously described (12), to measure antibody responses in plasma, saliva and nasal fluid (details and validation in Supplemental Figure 1). A live virus SARS-CoV-2 neutralisation assay was conducted as previously described (19) using strains listed in Supplemental Table 1. Assays were performed in duplicate.

Statistical analyses

Antibody responses post-vaccination to influenza and SARS-CoV-2 at day 28 and the fold-change in the responses from day 0 to day 28 were compared between the same and opposite arm group. When titres were averaged across variants or individuals, geometric means were used. For SARS-CoV-2 neutralisation titres, many values were below the limit of detection, this was accounted for using a censored regression analysis (performed in R (v4.3.1) using the *censReg* functions). In all box and whiskers plots the central horizontal line indicates the median value, the box indicates the interquartile range, and the whiskers indicate the range of the data. Comparisons of antibody titres, fold changes, or mean titres (across variants) between groups were analysed using 2-tailed Mann-Whitney *U* tests with the *wilcox.test* function in R (v4.3.1). Spearman correlations were used to assess the relationship of IgG antibody responses across plasma, saliva and nasal fluid samples with the cor.test function in R. The fisher exact test was used to compare the proportion of subjects with local reaction in the same and different arm group. *P* values of 0.05 or less were considered significant. All reported p values are raw p values without adjusting for multiple comparisons.

Study approval

This study was approved by the University of Melbourne HREC (approval no. 28318). Written informed consent was obtained from all participants prior to enrolment in the study. This study was registered with the Australian New Zealand Clinical Trials Registry (ID ACTRN12624000445572).

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 Supporting Data Values file.

Acknowledgements

The authors acknowledge funding from the Australian National Health and Medical Research Council and the Australian Medical Research Future Fund. The funders played no role in the study. The authors thank the participants for their generous time and provision of samples. We thank Julie Nguyen, Thakshila Amarasena, Robyn Esterbauer and Ellie Reilly (University of Melbourne) for excellent technical support. We thank Julian Druce, Leon Caly and Thomas Tran at the Victorian Infectious Diseases Reference Laboratory (Melbourne, Australia) for isolating and distributing SARS-CoV-2 virus isolates.

Author contributions

SJK conceived and designed the study. HEK and SJK recruited the study participants. TES generated the random allocation sequence and assigned participants to the interventions. WSL, KJS, JA, HP, MA, and IB were responsible for the acquisition of data. KJS, WSL, JA, AR, MPD, and SJK performed the analyses and interpreted the results. SJK, KJS, WSL, AR, and JA wrote the first draft of the manuscript. All authors contributed intellectually to the work, critically revised the report and approved the final version. The order of co–first authors' names was assigned on the basis of their experimental and editorial contributions to this study.

Conflict of interest

AKW has served as a consultant for CSL Seqirus. SR is an employee of CSL Seqirus. All other authors declare no conflicts of interest.

References

1. DiazGranados CA, Denis M, and Plotkin S. Seasonal influenza vaccine efficacy and its determinants in children and non-elderly adults: a systematic review with meta-analyses of controlled trials. *Vaccine.* 2012;31(1):49-57.
2. Frutos AM, Price AM, Harker E, Reeves EL, Ahmad HM, Murugan V, et al. Interim Estimates of 2023-24 Seasonal Influenza Vaccine Effectiveness - United States. *MMWR Morb Mortal Wkly Rep.* 2024;73(8):168-74.
3. Yang J, Zhang J, Han T, Liu C, Li X, Yan L, et al. Effectiveness, immunogenicity, and safety of influenza vaccines with MF59 adjuvant in healthy people of different age groups: A systematic review and meta-analysis. *Medicine (Baltimore).* 2020;99(7):e19095.
4. Leibovici Weissman Y, Cooper L, Sternbach N, Ashkenazi-Hoffnung L, and Yahav D. Clinical efficacy and safety of high dose trivalent influenza vaccine in adults and immunosuppressed populations - A systematic review and meta-analysis. *J Infect.* 2021;83(4):444-51.
5. Khoury DS, Cromer D, Reynaldi A, Schlub TE, Wheatley AK, Juno JA, et al. Neutralizing antibody levels are highly predictive of immune protection from symptomatic SARS-CoV-2 infection. *Nat Med.* 2021;27(7):1205-11.
6. Verbeke R, Hogan MJ, Lore K, and Pardi N. Innate immune mechanisms of mRNA vaccines. *Immunity.* 2022;55(11):1993-2005.
7. Alameh MG, Tombacz I, Bettini E, Lederer K, Sittplangkoon C, Wilmore JR, et al. Lipid nanoparticles enhance the efficacy of mRNA and protein subunit vaccines by inducing robust T follicular helper cell and humoral responses. *Immunity.* 2021;54(12):2877-92 e7.
8. Black CL, Kriss JL, Razzaghi H, Patel SA, Santibanez TA, Meghani M, et al. Influenza, Updated COVID-19, and Respiratory Syncytial Virus Vaccination Coverage Among Adults - United States, Fall 2023. *MMWR Morb Mortal Wkly Rep.* 2023;72(51):1377-82.
9. Grohskopf LA, Blanton LH, Ferdinands JM, Chung JR, Broder KR, and Talbot HK. Prevention and Control of Seasonal Influenza with Vaccines: Recommendations of the Advisory Committee on Immunization Practices — United States, 2023–24 Influenza Season. . *MMWR Recomm Rep.* 2023;72:1-25.
10. Pattinson D, Jester P, Gu C, Guan L, Armbrust T, Petrie JG, et al. Ipsilateral and contralateral coadministration of influenza and COVID-19 vaccines produce similar antibody responses. *EBioMedicine.* 2024;103:105103.
11. Aydillo T, Balsera-Manzanero M, Rojo-Fernandez A, Escalera A, Salamanca-Rivera C, Pachon J, et al. Concomitant administration of seasonal influenza and COVID-19 mRNA vaccines. *Emerg Microbes Infect.* 2024;13(1):2292068.
12. Lee WS, Audsley J, Trieu MC, Reynaldi A, Aurelia LC, Mehta PH, et al. Randomised controlled trial reveals no benefit to a 3-month delay in COVID-19 mRNA booster vaccine. *J Clin Invest.* 2024;134(17):e181244.
13. Bettini E, Chudnovskiy A, Protti G, Nakadakari-Higa S, Ceglia S, Castano D, et al. Distinct components of nucleoside-modified messenger RNA vaccines cooperate to instruct efficient germinal center responses. *bioRxiv.* 2024;05.17.594726.

14. van der Heiden M, Shetty S, Bijvank E, Beckers L, Cevirgel A, van Sleen Y, et al. Multiple vaccine comparison in the same adults reveals vaccine-specific and age-related humoral response patterns: an open phase IV trial. *Nat Commun*. 2024;15(1):6603.
15. Reed SG, and Carter D. Make it a combo. *Sci Transl Med*. 2024;16(758):eadq5644.
16. Dulfer EA, Geckin B, Taks EJM, GeurtsvanKessel CH, Dijkstra H, van Emst L, et al. Timing and sequence of vaccination against COVID-19 and influenza (TACTIC): a single-blind, placebo-controlled randomized clinical trial. *Lancet Reg Health Eur.* 2023;29:100628.
17. Thwaites RS, Ito K, Chingono JMS, Coates M, Jarvis HC, Tunstall T, et al. Nasosorption as a Minimally Invasive Sampling Procedure: Mucosal Viral Load and Inflammation in Primary RSV Bronchiolitis. *J Infect Dis*. 2017;215(8):1240-4.
18. Vanderven HA, Wentworth DN, Han WM, Peck H, Barr IG, Davey RT, Jr., et al. Understanding the treatment benefit of hyperimmune anti-influenza intravenous immunoglobulin (Flu-IVIG) for severe human influenza. *JCI Insight*. 2023;8(14):e167464.
19. Lee WS, Tan HX, Reynaldi A, Esterbauer R, Koutsakos M, Nguyen J, et al. Durable reprogramming of neutralizing antibody responses following Omicron breakthrough infection. *Sci Adv.* 2023;9(29):eadg5301.

Figure legends

Figure 1: CONSORT flow diagram describing trial recruitment for the CANNON study.

Figure 2: Plasma antibody responses to influenza following co-administration of COVID-19 mRNA boosters and inactivated influenza vaccines in the same or opposite arms. (A and B) Geometric means of haemagglutination inhibition (HI) titres across three strains (H1N1, H3N2 and B-VIC) included in the influenza vaccine for both the same (n=27; red circles) and opposite arm (n=28; blue squares) groups. Box and whisker plots compare either **(A)** responses at day 28 alone, or **(B)** the fold change in geometric means of HI titres from day 0 to day 28 post-vaccination. Statistical significance was calculated between groups using the 2-tailed Mann-Whitney U test. (C) Line graphs depict the geometric mean HI titres for each group at days 0, 6 and 28 post-vaccination (averaged across individuals and the three vaccine strains H1N1, H3N2 and B-VIC). (D) Line graphs show the geometric mean HI titres for each vaccine strain (H1N1, H3N2, B-VIC, B-YAM), averaged across individuals. The same arm cohort is depicted in solid lines, while the opposite arm cohort is in dotted lines. (E) Fold change in plasma IgG antibody binding levels against recombinant HA proteins from different circulating influenza strains as measured by bead-based multiplex (final dilution 1:25600). Vaccine strains are indicated in bold. Statistical significance was calculated between groups using the 2-tailed Mann-Whitney U test. For box and whiskers plots, central lines indicate medians, boxes indicate 25th and 75th centiles, whiskers indicate range (minimum and maximum).

Figure 3: Neutralizing antibody responses to SARS-CoV-2 in plasma between the same or opposite arms groups. (A and B) Box and whisker plots show plasma neutralization activity (IC_{50}) in a live virus neutralization assay against the vaccine strain XBB.1.5 (in bold) and other SARS-CoV-2 strains (JN.1, BA.5 and ancestral) at either **(A)** 28 days alone, or **(B)** as a fold change of responses between day 0 to day 28 post-vaccination. **(C)** Line graphs illustrate the geometric mean plasma neutralizing titres against XBB.1.5 for each vaccine group at days 0, 6 and 28 post-vaccination (accounting for values below the detection limit). **(D)** Box and whisker plots depict the fold change in plasma IgG antibody binding levels to XBB.1.5 and JN.1 spike proteins as measured using a bead-based multiplex assay (final dilution 1:25600). The same arm group (n=27) is represented by red circles while the opposite arm group (n=28) is shown as blue squares. Box plots show the interquartile range (box), median (line), and minimum and maximum (whiskers). Experiments were performed in duplicate. Statistical significance was calculated between groups using the 2-tailed Mann-Whitney *U* test.

Figure 4: Salivary and nasal IgG antibody binding responses to influenza HA and SARS-CoV-2 spike following vaccination. Box and whisker plots illustrate the fold change in IgG antibody binding levels in saliva (final dilution 1:25) **(A and B)** or nasal fluid (final dilution 1:50) **(C and D)** against a panel of influenza HA proteins (11 different influenza strains) **(A and C)** or SARS-CoV-2 spike proteins (XBB.1.5 and JN.1) (**B and D)**, as measured using a bead-based multiplex assay. Vaccine strains are indicated in purple. Subjects received both vaccines either in the same arm (n=27; red circles) or opposite arms (n=28; blue squares). Box plots show the interquartile range (box), median (line),

and minimum and maximum (whiskers). Experiments were performed in duplicate. Statistical significance was calculated between groups using the 2-tailed Mann-Whitney *U* test.

Table 1: Participant demographics (n=55) on study entry

Characteristic	Total cohort (n=55)	Opposite arm vaccination (n=28)	Same arm vaccination (n=27)
Age (years)[A]	56.0 (28.0, 59.0)	56.0 (27.3, 59.0)	56.0 (29.0, 59.0)
Sex (M/F), %	36.4/63.6	32.1/67.9	40.7/59.3
Any prior COVID-19 infections (Y/N), %	78.2/21.8	76.0/24.0	62.5/37.5
Number of prior COVID-19 infections	1 (1, 3)	1 (1, 1)	1 (1, 3)
Number of previous COVID-19 vaccine doses	4 (3, 5)	4 (3, 5)	5 (4, 5)
Influenza vaccine received in 2023 (Y/N), %	78.2/21.8	75.0/25.0	81.5/18.5
Number of influenza vaccines received 2020-22	3 (1, 3)	2 (2, 3)	3 (1, 3)

[A] All data presented as median (minimum, maximum) unless otherwise stated.

Table 2 Adverse events reported for the total cohort and by allocation

Reaction type	**Total cohort (n=55)**	**Opposite arm vaccination[A] (n=28)**			**Same arm vaccination (n=27)**
		Either arm	Non-dominant	Dominant	
≥1 local reaction, Y/N (%)[B]	50/5 (91,9)	24/4 (86/14)	22/6 (79/21)	11/17 (39/61)	26/1 (96/4)
Injection site pain, n	50	24	22	11	26
Redness, n	4	1	0	1	3
Swelling, n	7	1	0	1	6
≥1 systemic reaction Y/N (%)[B]	29/26 (53, 47)	14/14			15/12
Headache, n	16	8			8
Fatigue, n	22	10			12
muscle pain, n	10	3			7
joint pain, n	6	2			4
Any fever, n (%)	4/51	3/25			1/26
Total adverse events	**119**	**52**			**67**

[A] Local reaction in at least one arm

[B] Participants may report multiple symptoms in each reaction category

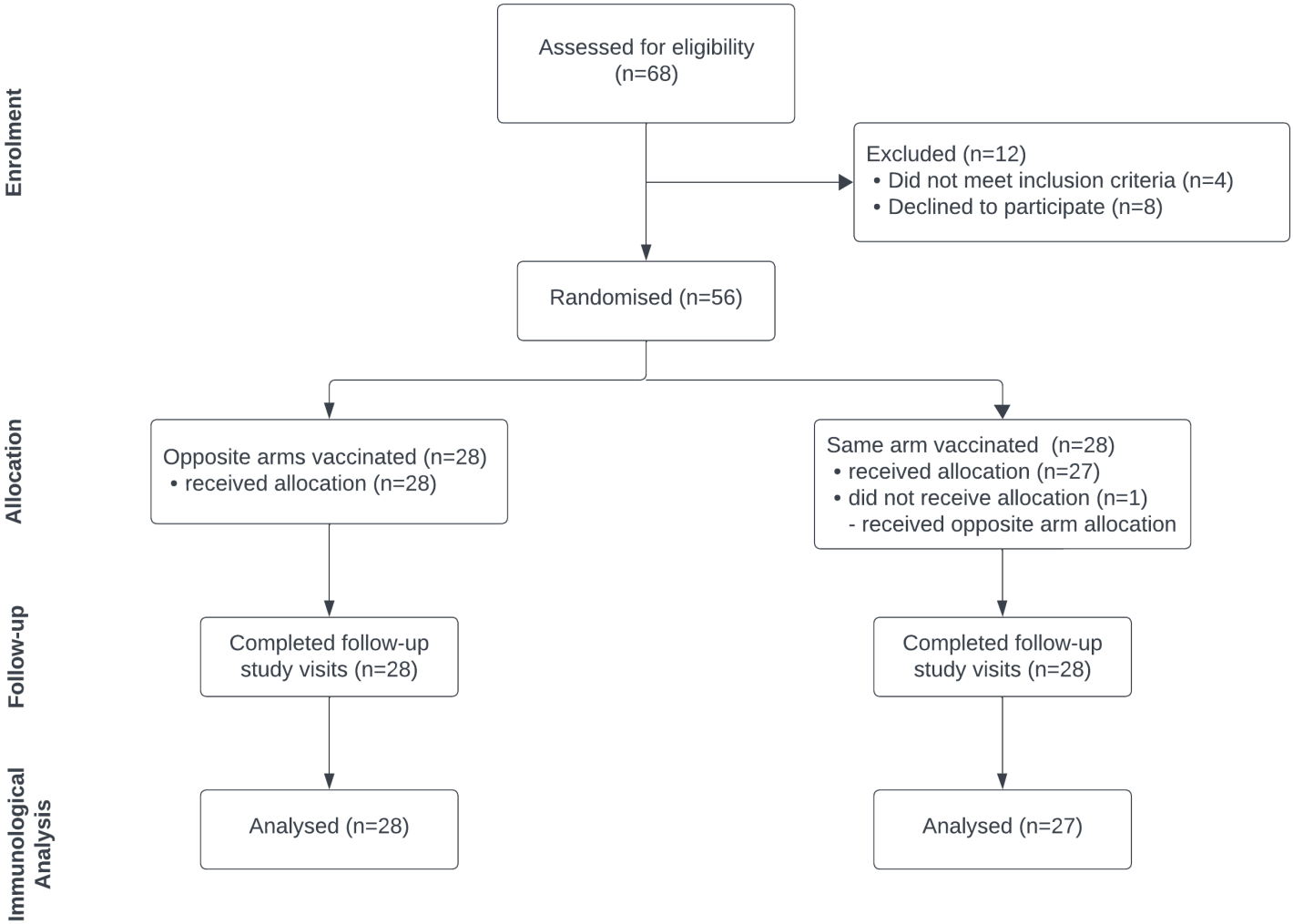


Figure 1: CONSORT flow diagram describing trial recruitment for the CANNON study.

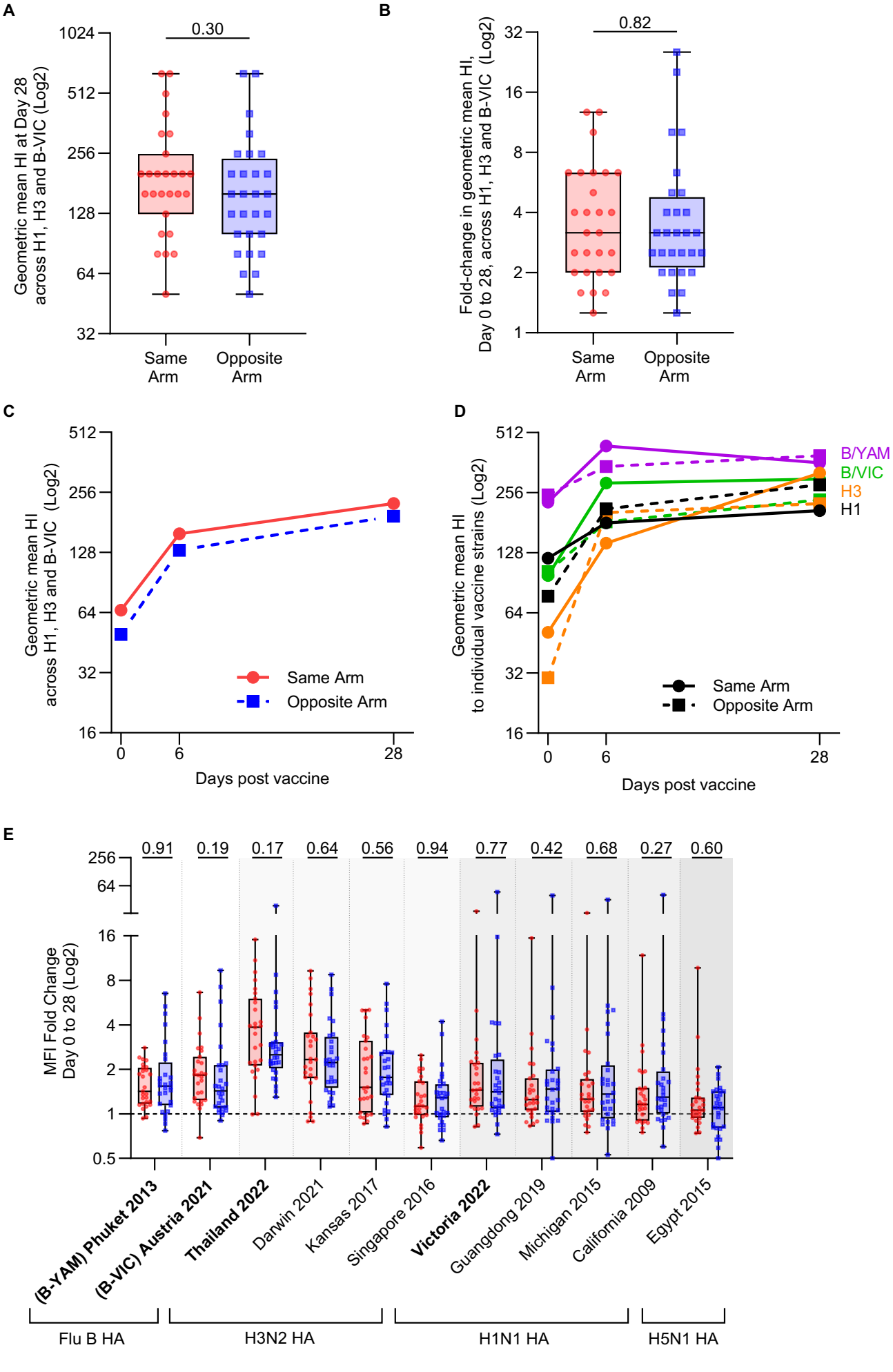

A
0.30
1024
512
256
128
64
32
Geometric mean HI at Day 28
across H1, H3 and B-VIC (Log2)
Same
Arm
Opposite
Arm
B
0.82
32
16
8
4
2
1
Fold-change in geometric mean HI,
Day 0 to 28, across H1, H3 and B-VIC (Log2)
Same
Arm
Opposite
Arm
C
512
256
128
64
32
16
Geometric mean HI
across H1, H3 and B-VIC (Log2)
Same Arm
Opposite Arm
0
6
28
Days post vaccine
D
512
256
128
64
32
16
Geometric mean HI
to individual vaccine strains (Log2)
B/YAM
B/VIC
H3
H1
Same Arm
Opposite Arm
0
6
28
Days post vaccine
E
0.91
0.19
0.17
0.64
0.56
0.94
0.77
0.42
0.68
0.27
0.60
256
64
16
8
4
2
1
0.5
MFI Fold Change
Day 0 to 28 (Log2)
(B-YAM) Phuket 2013
(B-VIC) Austria 2021
Thailand 2022
Darwin 2021
Kansas 2017
Singapore 2016
Victoria 2022
Guangdong 2019
Michigan 2015
California 2009
Egypt 2015
Flu B HA
H3N2 HA
H1N1 HA
H5N1 HA

Figure 2: Plasma antibody responses to influenza following co-administration of COVID-19 mRNA boosters and inactivated influenza vaccines in the same or opposite arms. (A and B) Geometric means of haemagglutination inhibition (HI) titres across three strains (H1N1, H3N2 and B-VIC) included in the influenza vaccine for both the same (n=27; red circles) and opposite arm (n=28; blue squares) groups. Box and whisker plots compare either **(A)** responses at day 28 alone, or **(B)** the fold change in geometric means of HI titres from day 0 to day 28 post-vaccination. Statistical significance was calculated between groups using the 2-tailed Mann-Whitney U test. **(C)** Line graphs depict the geometric means of HI titres across three vaccine strains (H1N1, H3N2 and B-VIC) at days 0, 6 and 28 post-vaccination. **(D)** Line graphs show the individual geometric mean HI titres for every vaccine strain (H1N1, H3N2, B-VIC, B-YAM). The same arm cohort is depicted in solid lines, while the opposite arm cohort is in dotted lines. Statistical significances were calculated between groups using a linear model (two-way ANOVA). **(E)** Fold change in plasma IgG antibody binding levels against recombinant HA proteins from different circulating influenza strains as measured by bead-based multiplex (final dilution 1:25600). Vaccine strains are indicated in bold. Box plots show the interquartile range (box), median (line), and minimum and maximum (whiskers). Experiments were performed in duplicate. Statistical significance was calculated between groups using the 2-tailed Mann-Whitney U test. *P* values of >0.05 were considered non-significant.

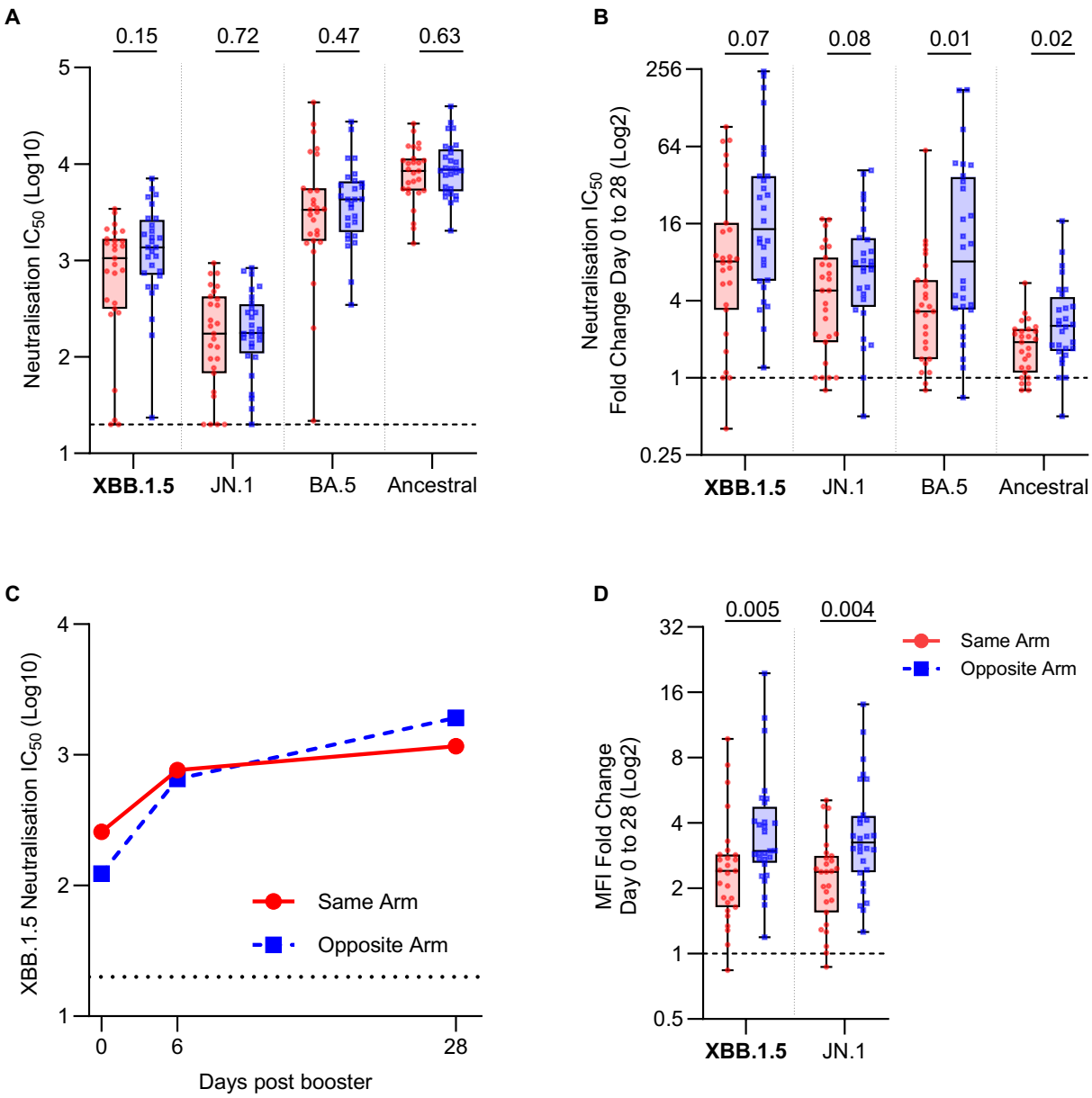


Figure 3: Neutralising antibody responses to SARS-CoV-2 in plasma between the same or opposite arms groups. (A and B) Box and whisker plots show plasma neutralisation activity (IC50) in a live virus neutralisation assay against the vaccine strain XBB.1.5 (in bold) and other SARS-CoV-2 strains (JN.1, BA.5 and ancestral) at either **(A)** 28 days alone, or **(B)** as a fold change of responses between day 0 to day 28 post-vaccination. **(C)** Line graphs illustrate the mean plasma neutralising titres against XBB.1.5 for each vaccine group at days 0, 6 and 28 post-vaccination. **(D)** Box and whisker plots depict the fold change in plasma IgG antibody binding levels to XBB.1.5 and JN.1 spike proteins as measured using a bead-based multiplex assay (final dilution 1:25600). The same arm group (n=27) is represented by red circles while the opposite arm group (n=28) is shown as blue squares. Box plots show the interquartile range (box), median (line), and minimum and maximum (whiskers). Experiments were performed in duplicate. Statistical significance was calculated between groups using the 2-tailed Mann-Whitney U test. *P* values of >0.05 were considered non-significant.

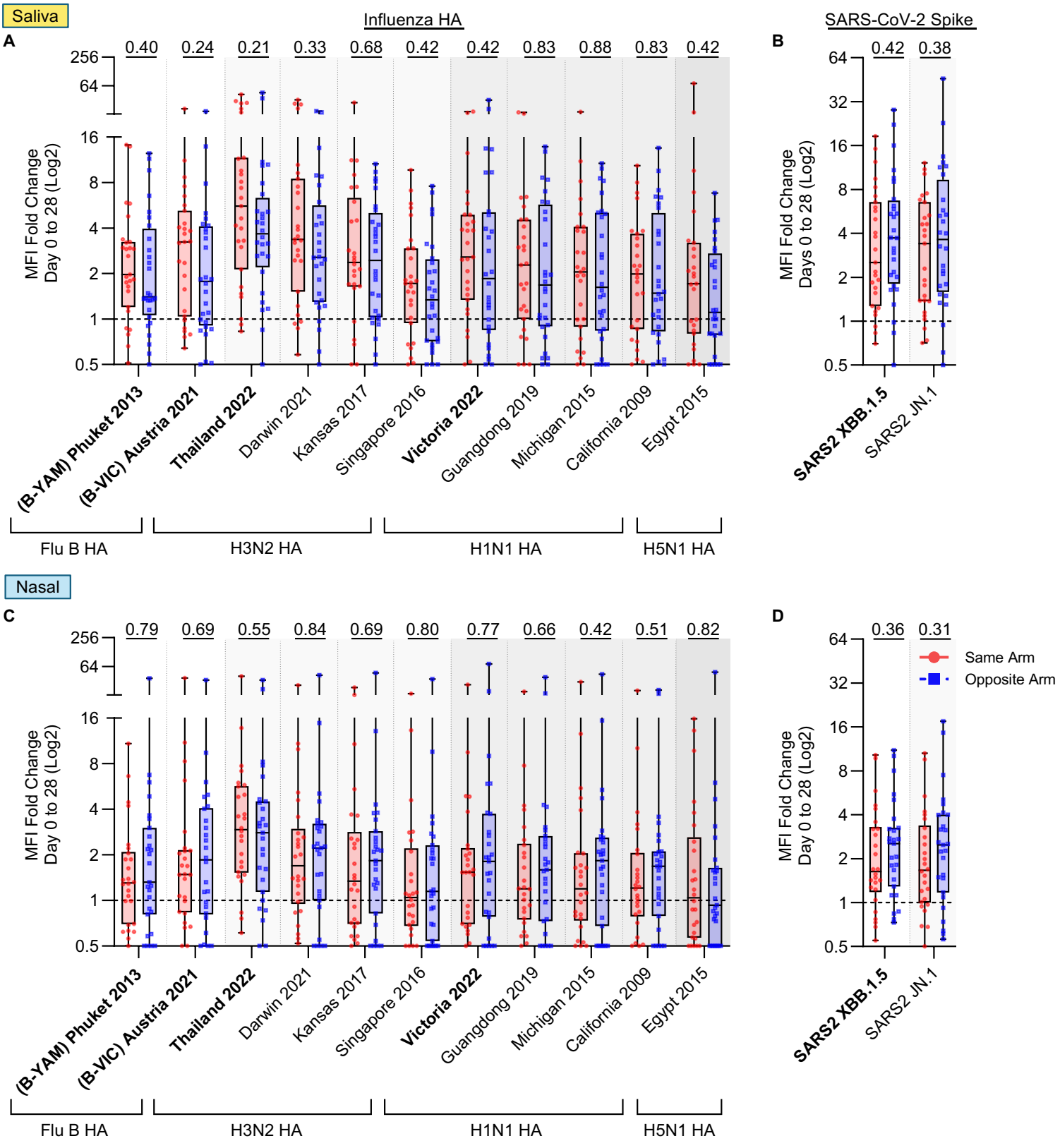


Figure 4: Salivary and nasal IgG antibody binding responses to influenza HA and SARS-CoV-2 spike following vaccination. Box and whisker plots illustrate the fold change in IgG antibody binding levels in saliva (final dilution 1:25) **(A and B)** or nasal fluid (final dilution 1:50) **(C and D)** against a panel of influenza HA proteins (11 different influenza strains) **(A and C)** or SARS-CoV-2 spike proteins (XBB.1.5 and JN.1) (**B and D)**, as measured using a bead-based multiplex assay. Vaccine strains are indicated in bold. Subjects received both vaccines either in the same arm (n=27; red circles) or opposite arms (n=28; blue squares). Box plots show the interquartile range (box), median (line), and minimum and maximum (whiskers). Experiments were performed in duplicate. Statistical significance was calculated between groups using the 2-tailed Mann-Whitney U test. *P* values of >0.05 were considered non-significant.