



# OPEN Immune responses after two inactivated COVID-19 vaccine doses, a heterologous third dose and subsequent boosting with bivalent mRNA in adults

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To evaluate immune response and safety of a bivalent mRNA booster (ancestral/BA.4/5) vaccine in individuals who had received inactivated COVID-19 vaccine with different heterologous boost regimens. A prospective open-label study of bivalent ancestral/Omicron BA.4/5 was conducted. Healthy participants (age > 18) who completed two doses of inactivated COVID-19 vaccine and received any of these booster vaccines (Ad26.COV2.S, ChAdOx1, or mRNA-based) at least 12 months prior were enrolled. Immunogenicity data (anti-Spike (S) IgG, neutralization antibody titer (NT<sub>50</sub>) against ancestral and XBB.1.5, and S-specific IFN- $\gamma$  T- cells) was obtained at baseline, Day 28 $\pm$ 7, and Day 90 $\pm$ 14. Of 190 participants enrolled; 57 received Ad26.COV2.S, 66 received ChAdOx1, and 67 received mRNA vaccine as the third dose, respectively. Following bivalent mRNA vaccination, anti-S IgG rose at Day 28, and declined at Day 90. In contrast, the NT<sub>50</sub> titers against ancestral peaked at Day90. The NT<sub>50</sub> against XBB.1.5 peaked at Day 28 with the highest fold rise in the mRNA vaccine subgroup (29.16 [19.55–43.49]), followed by the ChAdOx1 (20.94 [14.18–30.92]), and the Ad26.COV2.S subgroups (13.04 [8.61–19.74]). Geometric concentration of T-cells producing IFN- $\gamma$  rose comparably in all three subgroups. Bivalent mRNA ancestral/BA.4/5 vaccine enhanced humoral immunity against both ancestral and Omicron XBB1.5, and T-cell immunity in inactivated COVID-19 vaccine primed with different heterologous boost participants. The study was registered at WHO platform: Thai Clinical Trial Registry (TCTR20230811004).

Coronavirus disease-19 (COVID-19) vaccines are effective in preventing symptomatic infection, reduce morbidity and mortality<sup>1–3</sup>. Vaccine effectiveness varies among various vaccines with different platforms<sup>4,5</sup>. It has been shown that heterologous prime-boost vaccination induces stronger immune responses than homologous prime-boost strategy<sup>6,7</sup>. However, despite prompt vaccination campaign and early vaccine rollout, Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) has evolved over time, leading to numerous outbreaks since the start of the COVID-19 pandemic<sup>8–10</sup>. Consistent with the global epidemiology, the largest outbreak in Thailand occurred during the 5th wave from January to June 2022, driven by the Omicron variant<sup>11,12</sup>. Many vaccine companies have adapted their vaccine designs to improve vaccine efficacy against variants of concern. For instance, bivalent mRNA vaccines such as BNT162b2 ancestral/BA.4/5 and Spikevax ancestral/

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BA.4/5 became available in 2022 to better target Omicron variant. Authorities in many countries recommended boosting immune response with additional vaccine dose; however, data on optimal timing is still limited. In addition, data on heterologous boost with bivalent mRNA vaccines in individuals primed with inactivated COVID-19 vaccines, which were available in many countries in Asia initially, is scarce. Furthermore, presence of hybrid immunity also likely has an impact of boosting effect which further complicates appropriate vaccine recommendation<sup>6</sup>. This study aimed to evaluate the vaccine responses both humoral and T-cell response to a booster bivalent mRNA ancestral/BA.4/5 COVID-19 vaccine in those who have previously received two doses of inactivated COVID-19 vaccines (CoronaVac or BBIBP-CorV) followed by a third dose of any of the three different platforms (Ad26.COV2.s, ChAdOx1, or mRNA) at least one year prior.

## Methods

The study was approved by the Ethics Committee of the Faculty of Tropical Medicine, Mahidol University (MUTM 2023-028-01). The study was registered at WHO platform: Thai Clinical Trial Registry (TCTR20230811004). All participants provided written informed consent prior to enrollment. All study procedures involving participants and the handling and analysis of their specimens were conducted in accordance with institutional, national, and international guidelines and regulations, including the Declaration of Helsinki, ICH GCP. Written informed consent was obtained from all participants.

## Study design and study population

An open-label prospective study of bivalent Spikevax Ancestral/Omicron BA.4/5 was conducted at the Vaccine Trial Centre, Bangkok, Thailand from June to October 2023. Healthy adult participants (age > 18) who completed two doses of inactivated COVID-19 vaccine and received any of these booster vaccine (Ad26.COV2.S, ChAdOx1, or mRNA platform more than 12 months prior, were enrolled. Participants with recent (within four months) or current COVID-19 infection, confirmed or suspected immunodeficient state, acute polyneuropathy, contraindication to intramuscular injections or blood draws, major active psychiatric illness, recent COVID-19 vaccination within three months, recent vaccination (14 days for non-live vaccine and 28 days for live vaccine), recent immunoglobulins infusion within three months, or blood transfusion within four months were excluded.

Participants were allowed to have four vaccine doses before enrolment. All participants were administered intramuscular Spikevax bivalent Ancestral/Omicron BA.4/5.

## Data collection

There were three study visits for each participant: Day 0 (V1), Day 28 ± 7 (V2), and Day 90 ± 14 (V3). Demographic data, medical history, previous vaccination history, and previous COVID-19 infection history were collected at V1. Diary card used to record local and systemic reactogenicity and solicited safety data for the first 7 days post-injection (as shown in supplementary tables) and all vaccine safety data collection continued and reviewed at V2 and V3. For each visit, 20 mL blood samples were collected in EDTA tubes, processed within 2 h, and stored at -80 °C until analysis.

## Immunogenicity

IgG antibodies to full-length pre-fusion spike protein of SARS-CoV-2 (anti-S RBD IgG) levels were quantified using a validated enzyme-linked immunosorbent assay (Roche Diagnostics. Elecsys® Anti-SARS-CoV-2 S assay; 2023.) expressed in ELISA units (U)/mL and converted to binding antibody units (BAU)/mL using the WHO international standard correlation factor (0.97) (at the Central Lab, Hospital for Tropical Diseases, Faculty of Tropical Medicine, Mahidol University). Seropositivity for anti-S IgG was defined as >0.8 BAU/mL per WHO standard (Roche Diagnostics Elecsys® Anti-SARS-CoV-2 S assay). Vaccination-induced neutralizing antibody inhibition of SARS-CoV-2 was quantified by SARS-CoV-2 Pseudovirus neutralization assay (PNA) and defined as 50% neutralizing titer (NT<sub>50</sub>) against Pseudovirus bearing spike proteins of Ancestral strain, and Omicron XBB.1.5 from baseline, at 28 and 90 days (at the National Center for Genetic Engineering and Biotechnology (BIOTEC), Thailand). 60% of the collected blood samples at all time points were subjected for SARS-CoV-2 T-cell responses using an interferon-gamma release ELISA assay (Euroimmun, Lubeck, Germany) (Thammasat University Laboratory)<sup>6</sup>. All labs meet international standards by employing validated protocols and adhering to the applicable cGMP and biosafety guidelines.

## Data analysis

The study aimed to recruit approximately 200 participants with an equal number of participants in each subgroup based on the vaccine platform received at dose three i.e., Ad26.COV2.S, ChAdOx1, or mRNA. Data was analyzed according to these subgroups and further stratified by the presence of hybrid immunity. Participants who reported symptomatic COVID-19 infection before bivalent mRNA vaccine administration were defined as having hybrid immunity. Baseline demographics were reported using descriptive statistics. Safety data analyses included unsolicited adverse events (AEs) at Day 28, and serious adverse events as well as adverse events of special interests, which included immune-mediated medical condition and AEs associated with COVID-19 infection, throughout the entire study period. Per protocol population defined by all subjects who received bivalent mRNA vaccine and had no major protocol deviations that were determined to potentially interfere with the immunogenicity assessment of the study product was used for immunogenicity analysis. Anti-S IgG results were summarized with 95% Clopper-Pearson confidence interval (95%CI), geometric mean concentrations (GMC), and geometric mean fold rise (GMFR) from baseline. NT<sub>50</sub> results were summarized with 95%CI, geometric mean titer (GMT), GMFR from baseline against ancestral and Omicron XBB.1.5 SARS-CoV-2 pseudovirus. At baseline GMC and 95%CI was adjusted for age; At other visits, GMC/GMT and 95%CI

were adjusted for age and baseline log-antibody concentration/titer. Data was analyzed, and tables/figures were generated using SAS version 9.4 (SAS Institute Inc., Cary, NC, USA).

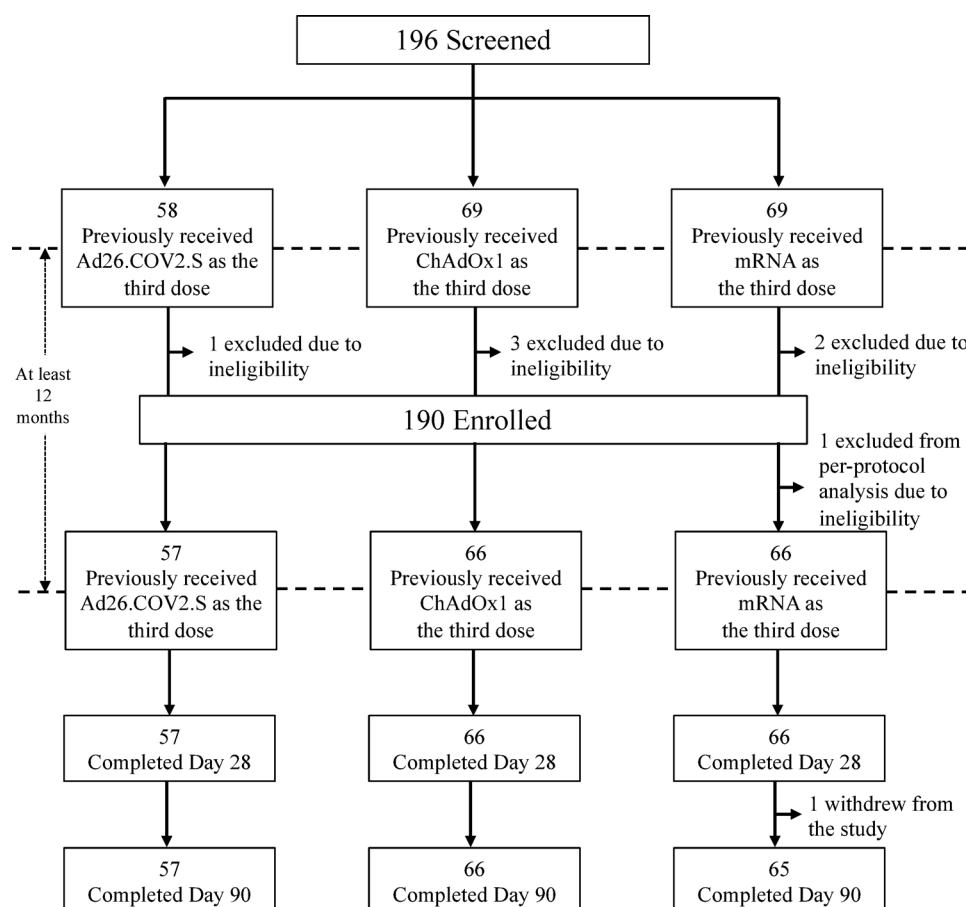
## Results

A total of 196 potential participants were screened. Of these, 58 received Ad26.COV2.S, 69 received ChAdOx1, and 69 received mRNA platform vaccine as their third dose (Fig. 1). An attempt was made to recruit an equal number of participants in the three subgroups. However, since Ad26.COV2.S was not widely available in Thailand, slightly fewer participants were screened for this group. Ultimately, six individuals were deemed ineligible and were not enrolled, resulting in a total of 190 participants included in the study. This consisted of 57, 66, and 67, in the Ad26.COV2.S, ChAdOx1, and mRNA vaccine subgroups, respectively (Fig. 1). All patients except one in the mRNA vaccine subgroup completed Day 90 study visit. Demographics of the study participants were summarized in Table 1. Higher proportion of female participants were enrolled across groups, 66.7% of participants in the ChAdOx1 subgroup received vaccine dose 4 before study enrolment, whereas 14% and 43.3% of Ad26.COV2.S and mRNA vaccine subgroups received dose 4 respectively. Almost all participants (77/81, 95.1%) who had four doses had mRNA vaccine as their fourth dose (Table 1). Nevertheless, mean time since last dose was similar across all three subgroups (15.1 to 17.2 months, Table 1). There were 52.6%, 54.5% and 51.5% participants in each of three subgroups reported history of COVID-19 infection (Table 1 and Supplementary Table 1). The mean time since last infection was  $11 \pm 4.2$ ,  $10.6 \pm 3.9$ , and  $12.3 \pm 2.9$  months, respectively. There was no reported symptomatic COVID-19 infection during the study period.

## Humoral immune response

GMC (95% CI) of anti-S IgG at baseline before bivalent mRNA vaccination was 5,091.43 (3,713.95–6,979.80), 5,617.86 (4,476.63–7,050.04), and 5,763.49 (4,615.08–7,197.68) BAU/mL, for the Ad26.COV2.S, ChAdOx1, and mRNA vaccine boosted subgroups, respectively (Fig. 2). Following bivalent (Ancestral/BA.4/5) mRNA vaccination, anti-S IgG rose by 6.62 (4.46–9.26), 7.81 (6.07–10.06), and 7.52 (5.81–9.73) folds at Day 28. The anti-S IgG concentration declined at Day 90 but was still higher than baseline by 3.36 (2.51–4.50), 4.04 (3.16–5.16), and 4.37 (3.37–5.67) folds, respectively (Table 2; Fig. 2).

$NT_{50}$  titers against ancestral strain increased at Day 28 and further increased at Day 90 to 8.86 (7.42–10.57), 8.18 (6.24–10.72), 10.98 (7.87–15.32) folds from baseline (Table 2; Fig. 2). Interestingly,  $NT_{50}$  titers against XBB.1.5 increased at Day 28 from baseline with the trend of highest fold rise in the mRNA vaccine subgroup



**Fig. 1.** Study participant flow chart.

Characteristics	Booster SARS-CoV-2 vaccines		
	Ad26.COV2.S (N = 57)	ChAdOx1 (N = 66)	mRNA platform (N = 66)
<b>Sex, n (%)</b>			
Male	22 (38.6%)	18 (27.3%)	26 (39.4%)
Female	35 (61.4%)	48 (72.7%)	40 (60.6%)
<b>Race, n (%)</b>			
Thai	57 (100%)	66 (100%)	66 (100%)
Other	0 (0%)	0 (0%)	0 (0%)
<b>Age (years)</b>			
Mean (Min–Max, SD)	45.3 (21–83, 14.0)	44.0 (23–73, 10.8)	38.7 (21–73, 12.7)
<b>BMI (kg/m<sup>2</sup>)</b>			
Mean (SD)	27.35 (5.64)	26.90 (5.35)	25.39 (5.70)
<b>COVID-19 vaccination history</b>			
3 doses	49 (86.0)	22 (33.3)	38 (56.7)
4 doses	8 (14.0) <sup>a</sup>	44 (66.7) <sup>b</sup>	29 (43.3) <sup>c</sup>
<b>Subgroups</b>			
Vaccine group			
N	27	30	32
Time since last dose (months), Mean (SD)	15.1 (4.1)	17.2 (3.8)	16.9 (2.5)
Hybrid group			
N	30	36	34
Time since last infection (months), Mean (SD)	11.0 (4.2)	10.6 (3.9)	12.3 (2.9)

**Table 1.** Subject characteristics. <sup>a</sup>All 8 participants received mRNA vaccine. <sup>b</sup>4(6.1%) received ChAdOx1 and 40(66.7%) received mRNA vaccine. <sup>c</sup>All 29 patients received mRNA vaccine.

(GMFR 29.16 (19.55–43.49) folds), followed by the ChAdOx1 (20.94 (14.18–30.92) folds) and the Ad26.COV2.S subgroups (13.04 (8.61–19.74) folds) (Table 2; Fig. 2). However, at Day 90, NT<sub>50</sub> titers declined from Day 28 though higher than the baseline values (Table 2; Fig. 2). P-values were not calculated, as this study was not designed for testing between groups. Overall, there was not a significant difference in anti-S IgG, NT<sub>50</sub> (Ancestral) and NT<sub>50</sub> (XBB.1.5) in participants who had received ChAdOx1 booster compared to participants previously received mRNA-based vaccine as third dose (Fig. 3). In addition, NT<sub>50</sub> (XBB.1.5) trended to be higher at Day 28 and Day 90 following bivalent mRNA vaccination (GMR 0.82 (0.63–1.06) and 0.79 (0.55–1.12), Fig. 4) in participants with reported prior symptomatic COVID-19 infection than those without. The importance of time since last infection in the hybrid subgroup was also explored. There did not appear to be a significant difference in humoral immune response between time since last infection of < 12 months vs. ≥ 12 months (Supplementary Tables 1–4). Although the GMR values suggested trend toward higher responses in the mRNA subgroup compared to vector-based subgroups, the study was not powered to test statistical significance between groups. Thus, these findings are presented as descriptive trends only.

### T cell response

Median IFN- $\gamma$  production from T-cells following Spike peptide stimulation was 1,851.51 (1,612.59–2,389.94) mIU/mL and rose to 5,277.24 (4,377.17–6,133.8) at Day 28 then declined to 3,501.51 (2,560.48–4,181.70) at Day 90. Median T-cell response appeared to be the highest in the group who received mRNA vaccine as third dose; however, the 95% CI was wide 2,714.69 to 7,007.90 mIU/mL (Fig. 5). Among participants with previous symptomatic infection, T-cell response did not appear to be different between time since last infection of < 12 months vs. ≥ 12 months (Supplementary Table 5).

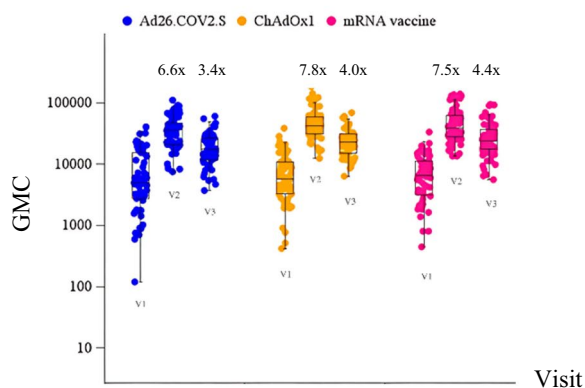
### Safety data

There were five severe adverse events reported from five patients during the first 28 days (Table 3). None was related to the bivalent mRNA vaccine and resolved within the follow-up period without sequelae. There were no fatal or potentially life-threatening events were reported, no serious adverse events or adverse events of special interests reported throughout the entire study period.

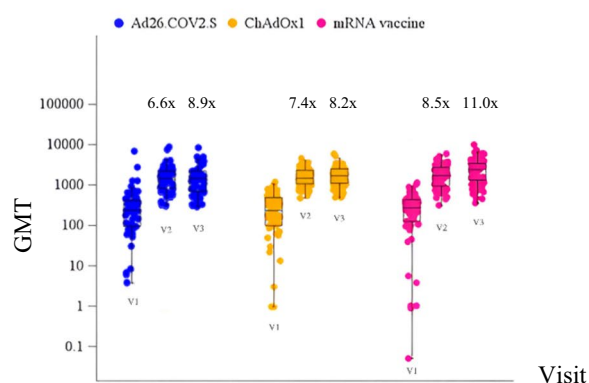
### Discussion

In this study, we reported that the bivalent mRNA vaccine enhanced both humoral and T-cell mediated immune responses in inactivated vaccine primed participants boosted with three different vaccines – Ad26.COV2.S, ChAdOx1, and mRNA vaccine, with the effect being more pronounced in the mRNA subgroup. We found that functional antibody against the ancestral strain continued to rise at three months post vaccination, unlike anti-S IgG which had already waned at three months. Additionally, we observed trend toward higher fold change in NT<sub>50</sub> against XBB.1.5 at Day 28 following the bivalent vaccination particularly those with a history of previous

## a. Anti-Spike IgG



	Ad26.COV2.S	ChAdOx1	mRNA platform
Day 1 (V1)	5,091.43 (3713.95 – 6,979.80)	5,617.86 (4,476.63 – 7,050.04)	5,763.49 (4,615.08 – 7,197.68)
Day 28 (V2)	33,706.93 (28,606.34 – 43,907.26)	43,883.76 (38,638.65 – 49,840.88)	43,338.20 (37,252.27 – 50,418.39)
Day 90 (V3)	17,102.53 (14,648.82 – 19,967.26)	22,692.14 (19,919.80 – 25,850.31)	25,057.14 (21,333.91 – 29,430.15)

b. NT<sub>50</sub> (Ancestral)

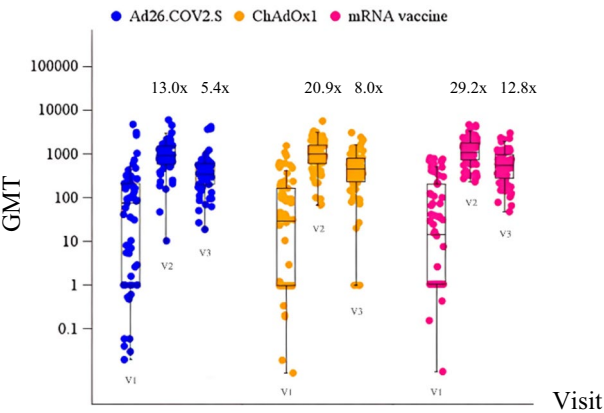
	Ad26.COV2.S	ChAdOx1	mRNA platform
Day 1 (V1)	193.36 (135.07 – 276.80)	189.78 (133.41 – 269.97)	165.51 (110.21 – 248.55)
Day 28 (V2)	1,315.93 (1,095.16 – 1,581.21)	1,531.59 (1,356.14 – 1,729.73)	1,629.64 (1,388.25 – 1,913.00)
Day 90 (V3)	1,527.31 (1,260.50 – 1,850.60)	1,694.09 (1,485.01 – 1,932.61)	2,078.37 (1,731.32 – 2,494.98)

**Fig. 2.** Geometric Mean Concentration (GMC) of 2a) Anti-Spike IgG and Geometric Mean Titer (GMT) of 50% Neutralization Titer (NT<sub>50</sub>) against 2b) Ancestral SARS-CoV-2 and 2c) XBB.1.5, per protocol analysis. Each dot represents an individual participant's data. The upper and lower ends of the whisker box indicate the 95% confidence interval (CI). Error bars represent the range. The mean values and 95% CIs are presented in the table below each plot. The mean fold rise values are also shown as numerical values with x. V1 - visit 1 or on day1, V2 - visit 2 or on day 28, and V3 - visit 3 or on day 90. \*p-values were not calculated, as this study was not designed for hypothesis testing between groups.

COVID-19 infection and waned by Day 90, suggesting immune imprinting effects despite initial high fold-rise at Day 28. Variance of T-cell response was wide but appeared to be stable overtime without significant waning at three months, especially those previously receiving monovalent mRNA vaccine.

The observed difference in kinetics of binding antibody and neutralizing antibody titers against ancestral SARS-CoV-2, i.e., NT<sub>50</sub> continued to increase at Day 90 while anti-S IgG peaked at Day 28 and waned at Day 90, was consistent with the existing literature and likely due to the natural B-cell maturation process<sup>13</sup> Moriyama et al. showed that while total antibody titers declining, neutralization potency per antibody to ancestral SARS-CoV-2 improved overtime, indicative of antibody response maturation<sup>14</sup>.

c. NT<sub>50</sub> (XBB.1.5)



	Ad26.COV2.S	ChAdOx1	mRNA platform
Day 1 (V1)	28.55 (14.47 – 56.33)	14.84 (7.79 – 28.26)	13.34 (6.96 – 25.56)
Day 28 (V2)	792.21 (624.32 – 1,005.24)	867.38 (699.29 – 1,075.88)	1,129.64 (951.74 – 1,340.79)
Day 90 (V3)	327.85 (263.70 – 407.62)	332.57 (228.95 – 483.09)	475.40 (383.26 – 589.70)

Fig. 2. (continued)

GMFR from Baseline	Ad26.COV2.S	ChAdOx1	mRNA platform
<b>Total</b>			
<b>Anti-Spike IgG</b>			
Day 28	6.62 (4.46–9.26), n = 57	7.81(6.07–10.06), n = 66	7.52 (5.81–9.73), n = 66
Day 90	3.36 (2.51–4.50), n = 57	4.04 (3.16–5.16), n = 66	4.37 (3.37–5.67), n = 65
<b>NT50 Ancestral</b>			
Day 28	6.55 (4.76–9.01), n = 57	7.40 (5.49–9.96), n = 66	8.45 (6.05–11.81), n = 66
Day 90	8.86 (7.42–10.57), n = 57	8.18 (6.24–10.72), n = 66	10.98 (7.87–15.32), n = 65
<b>NT50 XBB.1.5</b>			
Day 28	13.04 (8.61–19.74), n = 57	20.94 (14.18–30.92), n = 66	29.16 (19.55–43.49), n = 66
Day 90	5.4 (3.74–7.78), n = 57	8.03 (5.07–12.71), n = 66	12.77 (8.53–19.09), n = 65
<b>Hybrid group (N)</b>			
<b>Anti-Spike IgG</b>			
Day 28	5.60 (3.68–8.54), n = 30	7.14 (5.20–9.80), n = 36	5.85 (4.47–7.65), n = 34
Day 90	2.96 (2.01–4.38), n = 30	3.56 (2.65–4.77), n = 36	3.52 (2.74–4.52), n = 33
<b>NT50 Ancestral</b>			
Day 28	5.59 (3.67–8.51), n = 30	6.93 (4.75–10.12), n = 36	5.99 (4.40–8.17), n = 34
Day 90	6.40 (4.15–9.86), n = 30	7.73 (5.42–11.02), n = 36	8.19 (6.15–10.92), n = 33
<b>NT50 XBB.1.5</b>			
Day 28	13.42 (7.35–24.51), n = 30	18.91 (10.78–33.17), n = 36	32.49 (18.94–55.75), n = 34
Day 90	5.56 (3.09–10.01), n = 30	7.20 (3.80–13.64.80.64), n = 36	15.73 (9.20–26.89.20.89), n = 33

**Table 2.** Geometric mean fold rise (GMFR) from baseline of Anti-Spike IgG immune response against the Spike protein of SARS-CoV-2, and 50% neutralization titer (NT<sub>50</sub>) measured by pseudovirus neutralization assay (pNA) against SARS-CoV-2 ancestral and Omicron (XBB.1.5) strains in per protocol population and in subgroup of participants with hybrid immunity.

Substantial increase in NT<sub>50</sub> against XBB.1.5 compared to NT<sub>50</sub> against the ancestral strain following bivalent ancestral/BA.4/5 vaccination is intriguing. This rise is likely due to the absence of prior exposure to XBB.1.5 in most participants, as indicated by the very low baseline GMT (Fig. 2.3). Other study reported that bivalent (ancestral/BA.4/5) booster induced neutralizing antibodies against Omicron sub lineages but lowest to XBB.1<sup>15</sup>. Although we did not measure neutralizing antibodies against Omicron sub lineages in our study, it was possible that NT<sub>50</sub> titers could have been higher against earlier Omicron sub lineages such as BA.4-BA.5. It was reported



that inactivated vaccine can induce broadly neutralizing antibodies against SARS-CoV-2 variants which might partially explain NT<sub>50</sub> results in our study where all participants were primed with inactivated vaccines<sup>16</sup>.

Notably, our study observed a higher NT<sub>50</sub> titer against XBB.1.5 in participants with a history of symptomatic infection – most of whom were infected during the Omicron BA.1 outbreak, this suggests that Omicron-primed immunity may enhance the response to the Omicron-specific mRNA vaccine more effectively than previous exposure to the ancestral Spike epitope through vaccination alone. Recent work has shown that antibody responses to Omicron variants are dominated by pre-existing immunity resulting from prior exposure to ancestral strain, due to immune imprinting, rather than priming Omicron-specific naïve B cells<sup>17</sup>. In addition, repeated vaccination decreased breadth of the antibody response<sup>18</sup> as shown by relative reduction in the reactivity of the sera with the other variants compared to the ancestral strain, was observed after the second boosting vaccination against the ancestral strain. Longitudinal monitoring of anti-S IgG subclasses following three doses of mRNA monovalent vaccination showed that IgG4 became dominant with repeated vaccine doses. This was accompanied by reduced fragment crystallizable (Fc) gamma receptor-mediated effector functions such as antibody dependent cell phagocytosis<sup>19</sup>. Another study of a man who was hypervaccinated with over 200 vaccine doses within a period of 29 months revealed that hypervaccination increases the quantity, but not the quality of the adaptive immunity<sup>20</sup>. Together, it is conceivable that vaccination against new variants would enhance both quantity and quality of the adaptive immunity than repeated vaccination with the same antigenic epitope.

Our immunogenicity data in individuals receiving heterologous prime-boost vaccines showed that despite time since last vaccine dose or last infection was longer than twelve months, there were still high levels of anti-S IgG, NT<sub>50</sub> titers, and S-specific T cell response before the bivalent booster (Figs. 2 and 4), unlike studies in countries where people received mRNA-based vaccine only<sup>21</sup>. This suggests that heterologous prime-boost strategy may allow a longer interval between boosters as there was a stronger immune response than homologous prime-boost strategy, consistent with other report<sup>22</sup>. The magnitude of the response observed in our study is comparable to that reported in other studies evaluating bivalent vaccine responses following heterologous prime-boost strategies with approximately one-year vaccine intervals<sup>23</sup> and homologous prime-boost strategies with shorter intervals, such as six months<sup>24</sup>. This suggests longer vaccine interval in the heterologous prime-boost strategy could be considered, although further research especially the data on vaccine effectiveness is needed.

Given that there is no clear correlate of protection against SARS-CoV-2, we cannot conclude based on our immunogenicity data if the bivalent mRNA booster in our setting where individuals were primed with inactivated vaccine would confer a strong vaccine effectiveness. In the US where most of the population had mRNA vaccines as primary series and again boosted with mRNA vaccines as 3rd or 4th dose, vaccine effectiveness following bivalent boosters against hospitalization or death waned from approximately 67.4% after 2 weeks to 38.4% after 20 weeks<sup>25</sup>. In contrast, the vaccine effectiveness of boosted BNT162b2 vaccine in individuals previously primed with two doses of Sinovac-CoronaVac offered high levels of protection against severe or fatal outcomes (97.9% [97.3–98.4]) after eight weeks<sup>4</sup>. Furthermore, another study in Hong Kong reported increased vaccine effectiveness against Omicron BA.4 infection in people receiving bivalent Ancestral/BA.4/5 vaccine as 4th dose following three doses of Sinovac-CoronaVac previously versus individuals who received homologous four doses of Sinovac-CoronaVac<sup>26</sup>. Thus, vaccine effectiveness may be different in countries where there was a great mix of vaccine platforms given to population from dose one to dose four.

Among different heterologous boosting regimens, the mRNA subgroup showed the highest immune response, while the Ad26.COV2.S subgroup elicited the lowest response to bivalent mRNA vaccination, though the difference did not show statistically significant. This outcome may be confounded by the lower proportion in the Ad26.COV2.S subgroup with four prior vaccine doses (14%) compared to the mRNA (43.3%), and the ChAdOx1 (66.7%) subgroups with this more individuals in the ChAdOx1 subgroup had received 4 doses, while Ad26.COV2.S subgroup had received mostly 3 doses prior to study dose. This imbalance may have influenced the observed immune responses and might influence the interpretation of subgroup comparisons.

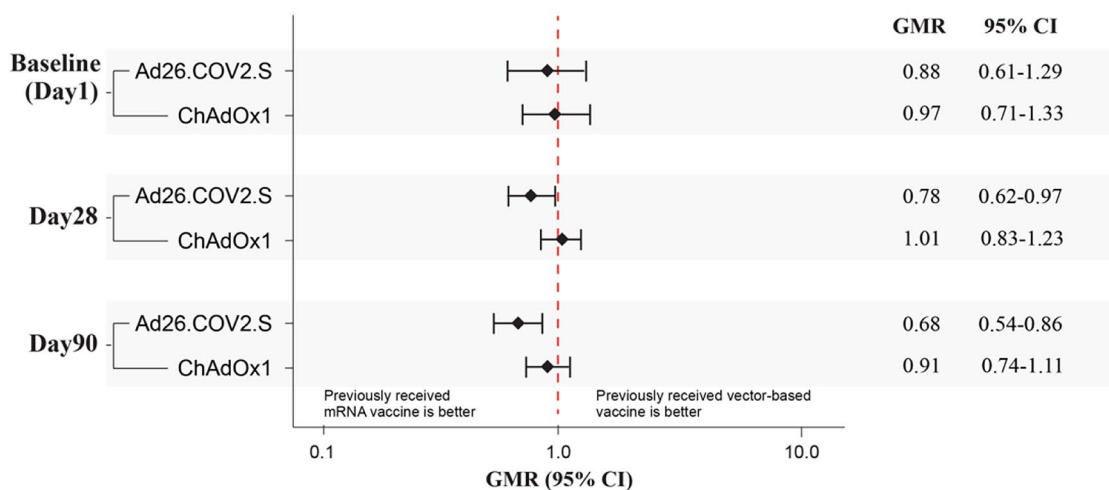
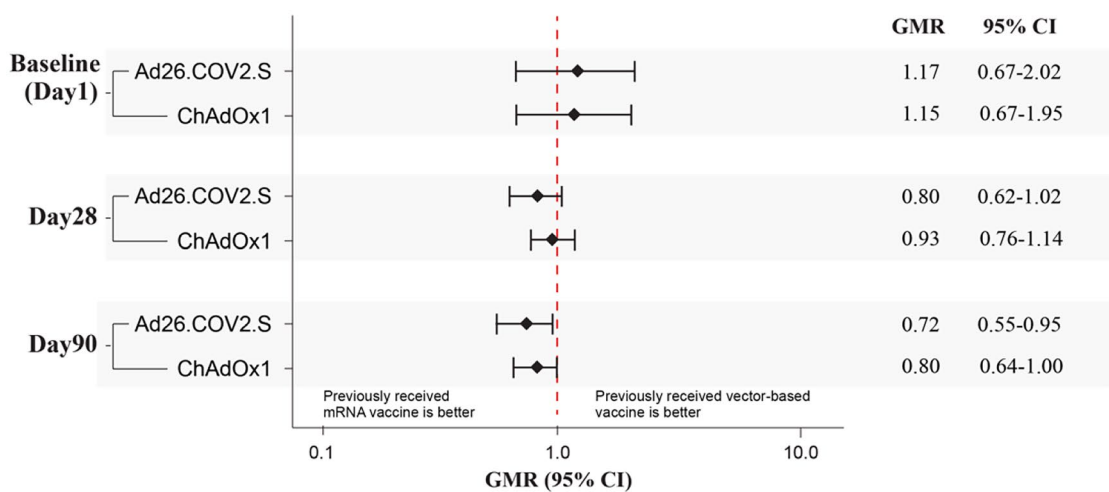
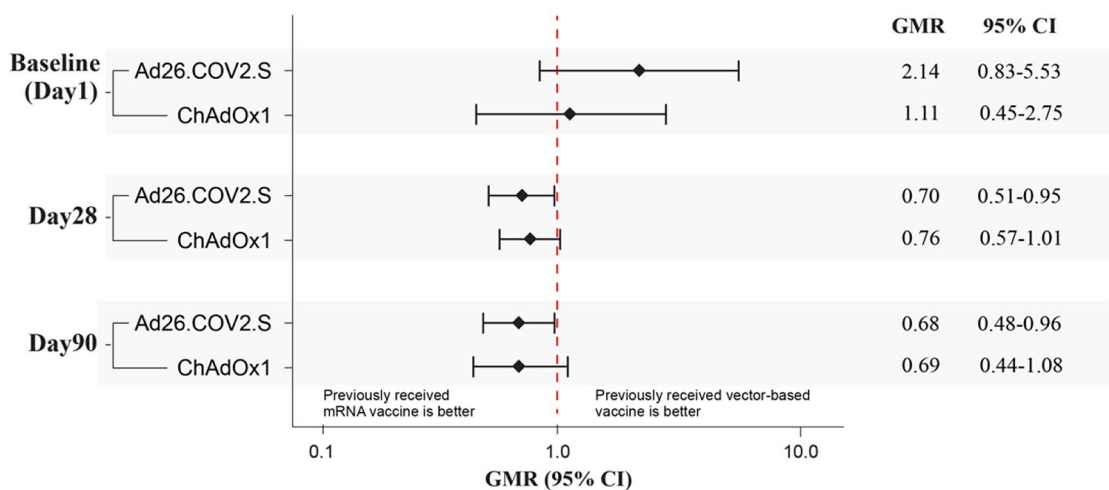
Similarly, our finding of T-cell response with insignificant waning at three months after booster is consistent with other reports<sup>27–29</sup>. In fact, our study showed that T-cell response was durable even more than 12 months since last vaccination or last infection as shown in the values of IFN-γ production from stimulated T-cells at baseline visit before bivalent mRNA vaccination. Nevertheless, this interpretation should be approached with caution, given the wide 95% CI observed in our study (Fig. 5). The wider 95% CI observed in the mRNA subgroup which reflects the greater variability in individual T-cell responses due to small sample size and/or duration of COVID-19 infections prior to receiving the bivalent mRNA vaccine [30].

This study has several limitations that should be considered when interpreting the findings. The classification of hybrid immunity relied on a history of symptomatic COVID-19 infection, which may have led to misclassification if asymptomatic infections before enrollment were missed. As this was a single-center study with modest subgroup sizes. In addition, only a small number of participants received the booster less than 12 months after their last vaccination or infection, restricting our ability to evaluate the impact of interval length on immunogenicity. However, the study results are in line with the other publications as cited earlier.

## Conclusion

Boosted immune response with bivalent mRNA vaccine was shown in all subgroups who had different heterologous prime-boost regimens. Having previous COVID-19 infection improved NT<sub>50</sub> titers against XBB.1.5 but not anti-S IgG and NT<sub>50</sub> against ancestral following bivalent mRNA vaccination. T-cell response did not wane as fast as humoral immune response. Overall, The results provide immunological evidence that bivalent mRNA vaccines can enhance humoral and cellular responses in individuals previously primed with

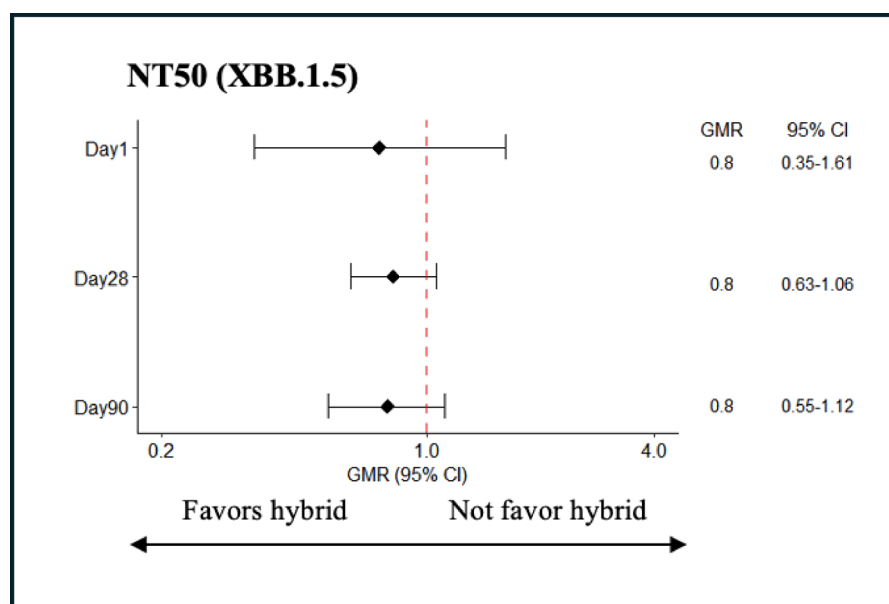
## a. Anti-S IgG

b. NT<sub>50</sub> (Ancestral)c. NT<sub>50</sub> (XBB.1.5)

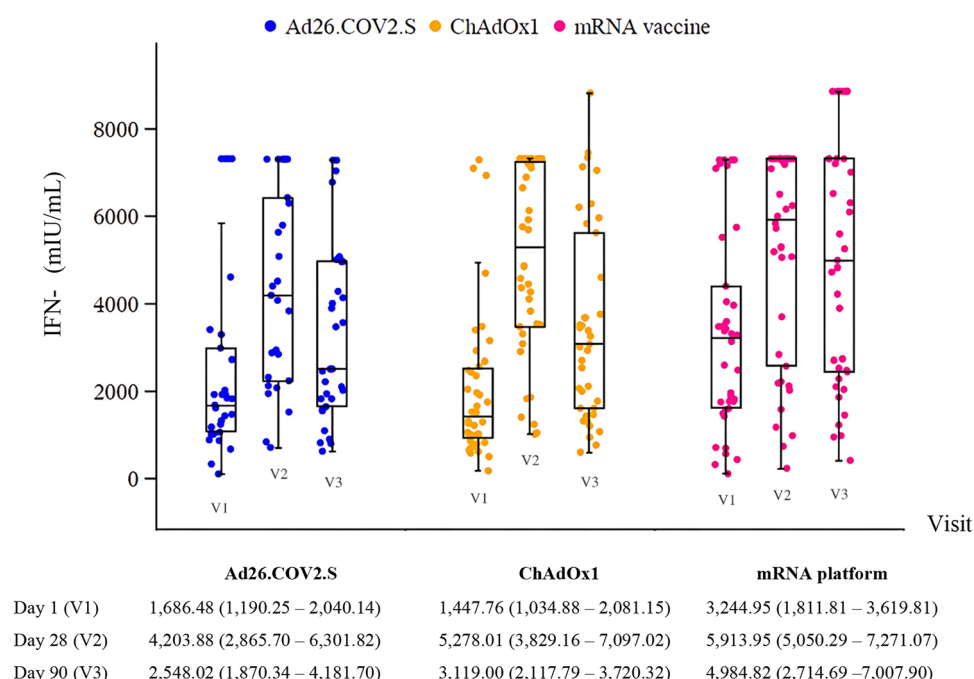
inactivated vaccines, including against Omicron XBB.1.5. These data may inform vaccine strategies targeting emerging variants of concern; however, clinical protection was not directly assessed in this study.



**Fig. 3.** Geometric Mean Ratio (GMR) comparing humoral immunogenicity (Anti-S IgG, 3a; NT<sub>50</sub> against ancestral, 3b; NT<sub>50</sub> against Omicron XBB.1.5, 3c) after boosting of bivalent mRNA COVID-19 vaccine between mRNA-based vs. Ad26.COV2.S vector-based or between mRNA-based vs. ChAdOx1 vector-based vaccine at dose 3, per-protocol analysis. A GMR value of less than 1.0 indicates that the previously received mRNA platform vaccine has a better response to the bivalent booster than the previously received vector-based vaccine.



**Fig. 4.** Geometric Mean Ratio (GMR) of NT<sub>50</sub> against XBB.1.5 after boosting of bivalent mRNA COVID-19 vaccine between participants with and without history of symptomatic COVID-19 infection (hybrid vs. no hybrid). A GMR value of less than 1.0 suggests a better immune response in the hybrid group.



**Fig. 5.** Median of IFN-γ production from SARS-CoV-2 specific T cells. Each dot represents an individual participant's data. The upper and lower ends of the whisker box indicate the 95% confidence interval (CI). Error bars represent the range. V1 - visit 1 or on day1, V2 - visit 2 or on day 28, and V3 - visit 3 or on day 90.

	Booster SARS-CoV-2 vaccines			
	All <sup>a</sup> (N = 190) n (%)	Ad26.COVS.2.S (N = 57) n (%)	ChAdOx1 (N = 66) n (%)	mRNA platform (N = 67) n (%)
With one or more AEs by severity*	29 (15.3%)	7 (12.3%)	12 (18.2%)	10 (14.9%)
Grade 1 (Mild)	15 (7.9%)	3 (5.3%)	6 (9.1%)	6 (9.0%)
Grade 2 (Moderate)	9 (4.7%)	1 (1.8%)	5 (7.6%)	3 (4.5%)
Grade 3 (Severe)	5 (2.6%)	3 (5.3%)	1 (1.5%)	1 (1.5%)
Grade 4 (Potentially life-threatening)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Grade 5 (Fatal)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
With no adverse event	161 (84.7%)	50 (87.7%)	54 (81.8%)	57 (85.1%)
With vaccine-related AEs by severity*				
Grade 1 (Mild)	7 (3.7%)	3 (5.3%)	1 (1.5%)	3 (4.5%)
Grade 2 (Moderate)	3 (1.6%)	0 (0%)	2 (3.0%)	1 (1.5%)
Grade 3 (Severe)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Grade 4 (Potentially life-threatening)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Grade 5 (Fatal)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
With one or more medically attended AEs**	10 (5.3%)	3 (5.3%)	4 (6.1%)	3 (4.5%)
With one or more AESIs	0 (0%)	0 (0%)	0 (0%)	0 (0%)

**Table 3.** Number of participants with adverse events onset during the first 28 days after vaccination by Severity, safety analysis Population. Note: \*Maximum severity grade was counted. \*\* None was vaccine-related.

<sup>a</sup> All participants received 1 booster dose of bivalent mRNA COVID-19 vaccine and these participants have previously received two doses of Sinovac or Sinopharm and booster doses of COVID-19 vaccines of any platforms comprised of: - received booster SARS-CoV-2 vaccines with the adenovirus vector vaccine Ad26.COVS.2.S or - received booster SARS-CoV-2 vaccines with the adenovirus vector vaccine ChAdOx1 or - received booster SARS-CoV-2 vaccines with the mRNA vaccine. Adverse event, AE; adverse events of special interest, AESI.

## Data availability

Data could be shared upon request to the corresponding author at punnee.pit@mahidol.ac.th.

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## Author contributions

SM study conception and design, data collection, data interpretation, manuscript drafting; SL- data management, data analysis, visualization, JC first manuscript draft writing, data analysis and interpretation; VL- data collection, data interpretation; AJ data collection, analysis, data validation and interpretation; SN data collection, data interpretation, validation; WP data collection; SK- data collection; NT- data collection; CD- data collection and validation; PP study conception and design, data interpretation, study guarantor, grant awardee. All authors critically reviewed and edited the manuscript.

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## Competing interests

The authors declare no competing interests.

## Ethical approval

This study was approved by the Mahidol University Institutional Research Ethics Board (MUTM 2023-028-01).

## Additional information

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