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Humoral and cellular immune responses against SARS-CoV-2 variants of concern induced by heterologous CoronaVac/ChAdOx-1 versus homologous ChAdOx-1 vaccination in the elderly

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Abstract

Background: The concept of heterologous vaccination against SARS-CoV-2 infection has been adopted in Thailand with limited data on the induction of humoral and cellular immunity, particularly the CoronaVac/ChAdOx-1 (CoVac/ChAd) regimen in the elderly.

Objectives: In this study, the immune responses of the elderly induced by heterologous CoVac/ChAd and homologous ChAdOx-1 (ChAd/ChAd) vaccinations were demonstrated.

Methods: A prospective observational study involving healthy participants aged ≥ 60 years who received heterologous CoVac/ChAd or homologous ChAd/ChAd vaccination was conducted. Surrogate neutralizing antibody (NAb) and T-cell responses against the SARS-CoV-2 wild type (WT) and variants of concern were determined at pre and post vaccinations.

Results: At 4 and 12 weeks after heterologous or homologous vaccination, the NAb levels against WT, Alpha, Beta, and Delta variants between each group were not significantly different, except for significant lower NAb against the Beta variant in heterologous group at 12 weeks after vaccination. The NAb against the Omicron at 4 weeks post-vaccination were below the cutoff level for antibody detection in both groups. However, higher spike-specific CD4 T cell producing IFN-γ and TNF-α in the heterologous than the homologous vaccination were observed. Insignificant difference of cellular immune responses to spike-peptides of Alpha, Beta, and Delta variants and their WT homologues was demonstrated.

Conclusion: In the elderly, heterologous CoVac/ChAd vaccination could induce NAb response against the WT and non-Omicron variants not different from the homologous ChAd/ChAd vaccination. Both regimens could not give adequate NAb of the Omicron strain. The heterologous vaccination, however, induced higher spike-specific Th1 cell response.

Key words: COVID-19 vaccine, Heterologous vaccination, Homologous vaccination, Elderly, Immune response

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Introduction

The emergence of SARS-CoV-2 virus in December 2019 has become global health problem until now. It causes pandemic COVID-19 which results in high morbidity and mortality worldwide, particularly in the vulnerable group of population. Aging is shown to have significant relationships with symptomatic infection, severe manifestations, intensive care need, and death from COVID-19.[1] In U.S.A., elderly people aged ≥ 65 years have been infected with SARS-CoV-2 for only 11.5% of total cases of COVID-19,[2] but account for 74.3% of total deaths from this disease.[3] Therefore, the old people, patients with comorbidities, and healthcare workers (HCWs) are prioritized as the early groups of population for vaccination in many countries including Thailand. Unfortunately, study specified on immunity of the vaccinated elderly is limited.

Many vaccines have been evolved and authorized for emergency use in the hope of controlling this pandemic disease. In Thailand, CoronaVac (CoVac) and ChAdOx-1 (ChAd) vaccines have been initially approved for national use since March 2021. However, after development of variants of concern (VOCs), the effectiveness of the vaccines has decreased and new global waves of the disease have developed. In Thailand, the Alpha, Delta, and lately Omicron variants were responsible for the outbreaks during early 2021, mid 2021, and early 2022, respectively. During the Delta wave in Thailand in June 2021, there were evidences of breakthrough disease among fully vaccinated HCWs, particularly in homologous CoVac vaccines. This finding was supported by a Thai study which showed that the Delta had less susceptibility to CoVac vaccine-induced neutralizing antibody.[4] In addition, data from a systematic review showed that the homologous CoVac vaccine had effectiveness for prevention of Delta infection of 59%. In contrast, the homologous ChAd vaccine had better effectiveness for prevention of Delta infection of 80.1%.[5] Unfortunately, the effectiveness of the 1st dose of ChAd vaccine was unacceptably low of only 44%.[5] This might be the results of low neutralizing antibody (NAb) response to single dose of ChAd vaccination, especially against the VOCs.[6] Besides, it required 3 months apart from the 1st dose to complete the vaccine series which was too slow during Delta spreading. Moreover, there was shortage of ChAd vaccine supply from the manufacturer which resulted in limited number of the available vaccine in Thailand during that time. Therefore, to shorten the time between priming and boosting, raise immunity as quickly as possible, and distribute the available ChAd vaccine to as many people as possible for urgent response to Delta outbreak, Thai Ministry of Public Health had recommended the heterologous CoronaVac-ChAdOx-1 (CoVac/ChAd) regimen as one of the national vaccination programs since July 2021. This regimen consisted of priming with one dose of CoVac vaccine and inoculation 4 weeks later with ChAd vaccine. However, this strategy had been implemented with limited data on the immunogenicity of the vaccine, particularly against the VOCs in the elderly. Hence, it is very interesting to know the immunogenicity of this heterologous vaccination regimen, in comparison with homologous ChaAdOx-1 (ChAd/ChAd) vaccination, against the VOCs in the healthy elderly.

In the current study, we investigated the effectiveness of heterologous CoVac/ChAd and homologous ChAd/ChAd vaccination regimens in the induction of humoral and cellular immune responses against SARS-CoV-2 variants of concern in Thai elderly.

Materials and Methods

For neutralizing antibody assay, the cPass SARS-CoV-2 neutralization antibody detection kit for WT and VOCs were purchased from GenScript Biotech, NJ, USA. For spike (S)-specific T cell response assay, PepTivator® SARS-CoV-2 Prot_S Complete (peptide pools 15 mer sequences with a 11 amino acids overlap covering the full length of WT spike proteins) were purchased from Miltenyi Biotech (Bergisch Gladbach, Germany). For determining T cell responses to S peptide of VOCs, PepTivator® SARS-CoV-2 Prot_S B.1.1.7 Mutation Pool, PepTivator® SARS-CoV-2 Prot_S B.1.1.7 WT Reference Pool, PepTivator® SARS-CoV-2 Prot_S B.1.351 Mutation Pool, PepTivator® SARS-CoV-2 Prot_S B.1.351 WT Reference Pool, PepTivator® SARS-CoV-2 Prot_S B.1.617.2 Mutation Pool and PepTivator® SARS-CoV-2 Prot_S B.1.617.2 WT Reference Pool were purchased from Miltenyi Biotech. For immunofluorescence staining, FITC-labeled anti-CD3 monoclonal antibody (mAb), PerCP-labeled anti-CD4 mAb, BV785-labeled anti-CD8 mAb, PECy7-conjugated anti-human IFN-γ mAb, PECy7-conjugated anti-TNF-α mAb, BV421-conjugated anti-Fas ligand (FasL) mAb, BV421-conjugated anti-IL-17A mAb, PE-conjugated anti-human cytokine (IL-4, IL-10, IL-17A) mAbs and fluorochrome-conjugated isotype matched control mAbs were purchased from BioLegend (San Diego, CA, USA). Brefeldin A and monensin were purchased from Sigma-Aldrich (Saint Louis, Missouri, USA). Saponin and paraformaldehyde were purchased from Riedel de Haen (Seelze, Germany).

Study design and participants

This is a prospective observational study done at Chiang Mai University Hospital, Chiang Mai, Thailand. Forty healthy volunteers, aged ≥ 60 years, who decided to receive either the heterologous regimen of CoronaVac and ChAdOx-1 vaccine (CoVac/ChAd) or the standard homologous 2 doses of ChAdOx-1 (ChAd/ChAd), were invited to participate in the study. The participants were enrolled during 23rd June and 5th August 2021. The durations between the 1st and 2nd dose of each regimen were 4 weeks and 12 weeks, respectively. We excluded subjects with history of infection with SARS-CoV-2, contact with COVID-19 patients within 2 weeks prior to enrollment, received other SARS-Cov-2 vaccines, received live attenuated non-COVID-19 vaccine in the past 28 days, received other inactivated or subunit vaccines in the past 14 days, immunocompromised state, receiving immunosuppressive drugs, uncontrolled underlying diseases (diabetes, cardiovascular disease, pulmonary disease, end-stage renal disease, cirrhosis) and history of allergy to any study vaccine components.

This study was approved by the Ethical Committee of the Faculty of Medicine, Chiang Mai University (Institutional Review Board (IRB) approval number: MED-2564-08247, dated 16 June 2021) and filed under Clinical Trials Registry (Study ID: TCTR20210822002, dated 22 August 2021). The written informed consent was obtained from each subject before entering the study.

Study procedures

All participants could make decision on their own whether to be vaccinated with homologous or heterologous regimen. The general collected data included demographic data (age, sex, body mass index), smoking status, underlying diseases and their medications, history of vaccinations, and COVID-19 exposure risk. Heparinized blood was taken immediately prior to 1st dose of vaccination and at 4 and 12 weeks after the 2nd dose of vaccination of each regimen. The ELISA-based surrogate virus neutralization test was used for measuring the level of NAb against the WT, Alpha, Beta, and Delta at pre-vaccination and at 4- and 12-week after 2nd dose of vaccination. NAb against the Omicron was measured only at 4-week after 2nd dose of vaccination.

For T cell responses, blood samples from 10 age- and sex-matched participants of each group after the 2nd dose of vaccination were tested.

Assay for Neutralizing antibody

To determine SARS-CoV-2 NAbs, plasma, positive and negative controls were diluted with sample dilution buffer and pre-incubated with the horseradish peroxidase (HRP) labeled RBD proteins of WT, Alpha B.1.1.7 (mutation site at N501Y), Beta B.1.351 (mutation site at E484K, K417N and N501Y), Delta B.1.617.2 (mutation site at L452R and T478K) or Omicron B.1.1.529 (mutation sites at G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H) at 37°C for 30 minutes. The mixture was then added to the capture plate, which was pre-coated with the human angiotensin-converting enzyme 2 (ACE2) protein and incubated at 37°C for 15 minutes. The unbound HRP labeled RBD proteins were removed by washing. TMB (3,3′,5,5′-Tetramethylbenzidine) substrate solution was added followed by the stop solution. The absorbance of the final solution was read at 450 nm with a microtiter plate reader. The % Inhibition was calculated from O.D. at 450 nm as follows: [1 – (O.D. value of sample/average O.D. value of negative control from the corresponding strain)] × 100. The 30% signal inhibition was used as cutoff for SARS-CoV-2 NAb detection according to the manufacturer (GenScript Biotech). The cutoff 30% means that the NAb is detected. It could not be interpreted as adequate immunity for protection against the SARS-CoV-2 infection or severity of disease.

Conversion of % inhibition of NAb to IU/ml of WHO international standard was performed by using the method described elsewhere.[7]

Assay for CD4 and CD8 T responses

Heparinized bloods were processed within 4 hours after collection. Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized blood by Ficoll-Hypque (IsoPrep) (Robbins Scientific Corporation, Sunnyvale, CA, USA) gradient centrifugation. The isolated PBMCs were stimulated with S peptides, consisting of SARS-CoV-2 Prot_S Complete (all functional domains of S protein of WT), Prot_S B.1.1.7 Mutation Pool, B.1.617.2 Mutation Pool, or Prot_S B.1.617.2 Mutation Pool (covering selectively the mutated regions in S protein of Alpha, Beta, and Delta variants, respectively), or Prot_S B.1.1.7 WT Reference Pool, Prot_S B.1.617.2 WT Reference Pool, or Prot_S B.1.617.2 WT Reference Pool (homologous peptides of the WT sequence of Alpha, Beta, and Delta variants mutation pool, respectively), according to manufacturer protocol. Briefly, PBMCs were stimulated with indicated peptide pools and incubated in 5% CO_2 incubator at 37°C for 2 hours. The protein releasing inhibitors, brefeldin A (1 µg/mL) and monensin (1 µM), were then added and continuously incubated for 4 hours. After incubation, cells were harvested and washed for 2 times. Cells were fixed with 4% paraformaldehyde for 15 minutes at room temperature and were washed 2 times. For Fc receptor blocking and permeabilization of cells, 0.1% saponin containing 10% human blood group AB serum was added and incubated at 4°C for 30 minutes. To determine spike-specific T cell response, cells were intracellularly stained with cocktail antibody for analysis of IFN-γ, TNF-α, IL-4, IL-10, IL-17A and FasL using fluorochrome conjugated specific mAbs. To identify T cell subset, the membrane surface markers including BV785-conjugated anti-CD8 mAb, PerCP-conjugated anti-CD4 mAb, and FITC-conjugated anti-CD3 mA were together stained with cocktail antibody. The expression of tested proteins in CD4 and CD8 T cells were measured by BD FACSCelesta™ flow cytometer BD Bioscience (San Jose, CA, USA) and analyzed with FlowJo software.

Statistical analysis

Results for numerical data were expressed as mean ± standard deviation (SD) or median, interquartile range (IQR). Results with proportion were expressed as frequencies and percentages. Independent sample t-tests and Mann-Whitney U Test were used to compare differences between the groups for parametric and non-parametric data, respectively. Fisher's exact test was used to compare the categorical data. The Wilcoxon signed-rank test was used for comparing the nonparametric data between before vaccinating and after vaccinating in each group. Statistical significance was accepted at the *p*-value < 0.05. All statistical analyses were performed using SPSS version 16 and GraphPad Prism software version 9.4.1 (GraphPad Software, San Diego, CA, USA).

Results

Forty volunteers, with 20 in each group of vaccination regimen, were initially recruited. One and 3 subjects in CoVac/ChAd and ChAd/ChAd group, respectively, were lost to follow-up at 4 weeks after 2nd dose of vaccination. Therefore, data from 19 and 17 individuals in the heterologous and homologous groups were analyzed. The mean ages of subjects in heterologous and homologous regimens were 69.9 ± 4.4 and 70.4 ± 4.5 years, respectively. Most of the participants in both groups were male. All demographic data were not statistically different (**Supplementary Table S1**).

Neutralizing antibody responses

The baseline NAb levels against WT, Alpha, Beta, and Delta variants in both heterologous CoVac/ChAd and homologous ChAd/ChAd groups were lower than the 30% NAb detection cutoff point (**Figure 1 and Table 1**). At 4 weeks after vaccination, the levels of NAb against the WT and Alpha, Beta, Delta variants increased significantly from baseline in both vaccination groups. When comparing between heterologous and homologous groups, the NAb levels against the WT, Alpha, Beta, and Delta were 84.8% vs 88.7%, 68.5% vs 77.9%, 47.5% vs 65.9%, and 75.4% vs 87.6%, respectively (**Figure 1 and Table 1**). Slightly lower trends of NAbs were observed in heterologous regimen, however, there were no statistical differences between the two groups. At the meantime, the number of subjects in each group who had NAb above the cutoff threshold of 30% in WT, Alpha, Beta, and Delta variants were 100% vs 94.1%, 100% vs 94.1%, 94.7% vs 94.1%, and 100% vs 94.1%, respectively, which were not significantly different (**Table 2**).

Table 1. Levels of % inhibition and WHO international standard unit (IU/ml) of neutralizing antibody against the wild type, Alpha, Beta, Delta, and Omicron variants of SARS-CoV-2 at before and at 4- and 12-week after receiving 2nd dose of each vaccination regimen.

		CoVac/ChAd			ChAd/ChAd		
		Before (n = 19)	4 weeks (n = 19)	12 weeks (n = 18)	Before (n = 17)	4 weeks (n = 17)	12 weeks (n = 17)
% Inhibition	Wild type	3.5 (-0.1, 7.0)	84.8* (70.1, 92.1)	76.8*,# (57.8, 87.8)	6.3 (3.7, 11.7)	88.7* (53.9, 93.5)	69.6*,# (41.3, 86.8)
	Alpha (B.1.1.7)	14.6 (12.9, 16.9)	68.5* (57.2, 78.7)	56.1*,# (38.7, 63.6)	12.6 (9.6, 15.1)	77.9* (44.7, 89.2)	51.7*,# (26.8, 73.4)
	Beta (B.1.351)	-11.8 (-14.6, -9.8)	47.5* (35.9, 62.1)	33.3*,# (18.7, 43.8)	15.7 (14.6, 16.8)	65.9* (40.4, 77.3)	43.7*,#,b (31.5, 67.1)
	Delta (B.1.617.2)	11.1 (8.9, 11.8)	75.4* (64.2, 85.1)	53.7*,# (40.6, 69.9)	14.5 (10.6, 15.7)	87.6* (59.4, 92.9)	63.3*,# (33.7, 83.2)
	Omicron (B.1.1.529)	N.D.	10.9 (1.2, 13.9)	N.D.	N.D.	7.8[a] (-0.4, 12.7)	N.D.
IU/ml	Wild type	2.0 (0.0, 5.0)	396.0* (162.0, 843.0)	247.5*,# (94.0, 515.3)	4.0 (2.0, 8.5)	562.0* (80.5, 1051.0)	158.0*,# (48.0, 515.0)
	Alpha (B.1.1.7)	11.0 (10.0, 13.0)	151.0* (91.0, 259.0)	88.0*,# (42.0, 120.8)	9.0 (6.5, 11.5)	246.0* (54.5, 602.0)	73.0*,# (24.5, 192.0)
	Beta (B.1.351)	0.0 (0.0,0.0)	61.0* (37.0, 112.0)	33.0*,# (14.8, 53.3)	12.0 (11.0, 13.0)	133.0* (45.5, 238.5)	52.0*,#,b (30.5, 141.0)
	Delta (B.1.617.2)	8.0 (6.0, 9.0)	214.0* (124.0, 406.0)	79.0*,# (45.5, 161.5)	11.0 (8.0, 12.0)	504.0* (102.5, 1015.0)	119.0*,# (34.5, 351.5)
	Omicron (B.1.1.529)	N.A.	8.0 (1.0, 10.0)	N.D.	N.D.	5.0[c] (1.0, 9.5)	N.D.

ChAd, ChAdOx-1; CoVac, CoronaVac. N.D., not determined; Data are presented as median (interquartile range, IQR); *p-value < 0.001 compared to before receiving vaccines; #p-value < 0.001 compared to 4 weeks after receiving vaccines using Wilcoxon test; [a]p-value = 0.496 and [c]p-value = 0.632 compared levels of % inhibition or IU/ml of neutralizing antibody against the Omicron between CoVac/ChAd and ChAd/ChAd groups using Mann-Whitney U test; [b]p-value < 0.05 compared levels of % inhibition or international unit of neutralizing antibody against each strain between CoVac/ChAd and ChAd/ChAd groups 12-week after receiving 2nd dose of vaccinations using Mann-Whitney U test.

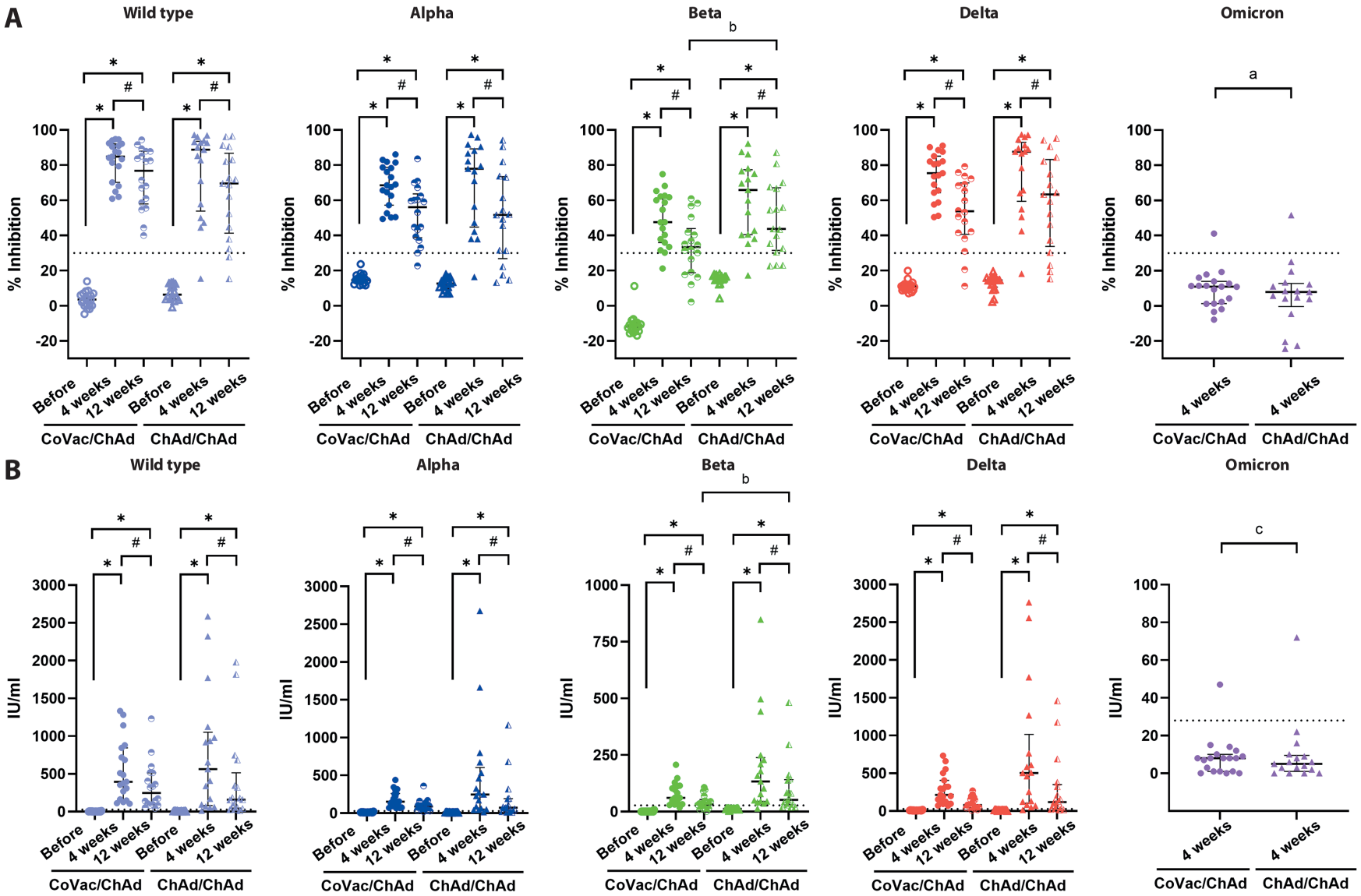


Figure 1. Levels of neutralizing antibody against SARA-CoV-2 wild type and variants of concern. Plasma was obtained from the healthy volunteers, aged ≥ 60 years before vaccination (before) and after 4 or 12 weeks of heterologous (CoVac/ChAd) or homologous (ChAd/ChAd) vaccination. (A) % inhibition or (B) WHO international standard unit (IU/ml) of neutralizing antibodies against wild type, Alpha, Beta, Delta, and Omicron variants are shown. The horizontal lines indicate 30% inhibition or 28 IU/ml threshold level of neutralizing antibody. Dot plots show as median with interquartile range which a dot represents each individual. *p-value < 0.001 compared to before receiving vaccines; #p-value < 0.001 compared to 4 weeks after receiving vaccines using Wilcoxon test; [a]p-value = 0.496 and [c]p-value = 0.632 compared levels of % inhibition or IU/ml of neutralizing antibody against the Omicron variants between CoVac/ChAd and ChAd/ChAd groups using Mann-Whitney U test; [b]p-value < 0.05 compared levels of % inhibition or IU/ml of neutralizing antibody against each strain between CoVac/ChAd and ChAd/ChAd groups at 4 week and 12 week after receiving 2nd dose using Mann-Whitney U test.

Table 2. The number of subjects who had levels of neutralizing antibody above 30% inhibition threshold

	CoVac/ChAd			ChAd/ChAd		
	Before (n = 19)	4 weeks (n = 19)	12 weeks (n = 18)	Before (n = 17)	4 weeks (n = 17)	12 weeks (n = 17)
Wild type	0 (0.0)	19 (100)*	18 (100)*	0 (0.0)	16 (94.1)*	15 (88.2)*
Alpha (B.1.1.7)	0 (0.0)	19 (100)*	16 (88.9)*	0 (0.0)	16 (94.1)*	13 (76.5)*
Beta (B.1.351)	0 (0.0)	18 (94.7)*	12 (66.7)*	0 (0.0)	16 (94.1)*	14 (82.4)*
Delta (B.1.617.2)	0 (0.0)	19 (100)*	16 (88.9)*	0 (0.0)	16 (94.1)*	14 (82.4)*
Omicron (B.1.1.529)	N.D.	1 (5.3)	N.D.	N.D.	1 (5.9)	N.D.

CoVac, CoronaVac; ChAd, ChAdOx-1; N.D., not determined, Data are presented as number (%); *p-value < 0.001 compared to before receiving vaccines using McNemar test; No significant difference in the proportion of subjects who had levels of neutralizing antibody above 30% inhibition threshold between 4 weeks and 12 weeks post-vaccination in both groups, No significant difference in the proportion of subjects who had levels of neutralizing antibody above 30% inhibition threshold between groups when compared at 4 weeks and 12 weeks post vaccination.

At 12th week after the 2nd dose of vaccination, the levels of NAb significantly dropped from the 4th week in each tested virus strain. When comparing between 2 vaccination groups, the levels of NAb were comparable, except for significant lower NAb against the Beta variant in heterologous group (**Figure 1 and Table 1**). The decrement of NAb from the 4th week between heterologous and homologous group against the WT, Alpha, Beta, and Delta were 9.7% vs 22.5%, 26.7% vs 33.6%, 35.2% vs 22.6%, and 28.8% vs 26.7%, respectively. There was no significant decrement rate between both groups in each tested strain (**Supplementary Table S2**). The number of participants who had NAb above the 30% threshold against the WT, Alpha, Beta, and Delta variant were 100% vs 88.2%, 88.9% vs 76.5%, 66.7% vs 82.4%, and 88.9% vs 82.4%, respectively (**Table 2**). It had a propensity for subjects to have NAb levels below the threshold in homologous group, except for the Beta strain.

Comparing among all VOCs, the Omicron had the lowest levels of NAb in both vaccination groups (**Figure 1**). At 4 weeks after heterologous or homologous vaccination only one subject in each group had NAb against the Omicron above 30% antibody detection threshold (**Table 2**).

Converting the % inhibition of NAb to IU/ml of WHO international standard was also performed as shown in **Figure 1 and Table 1**.

T cell responses to SARS-CoV-2 wild type spike peptides

To determine T cell responses upon vaccination, PBMCs at 4 weeks after heterologous and homologous vaccinated subjects were stimulated with peptide pooled of WT spike protein and determined the expression of IFN-γ, TNF-α, IL-4, IL-10, IL-17A and FasL of CD4 and CD8 T cells. The gating strategy is shown in **Supplementary Figure S1**. In comparison with homologous (ChAd/ChAd) vaccination, the heterologous (CoVac/ChAd) vaccination resulted in a significant higher frequency of CD4 T cell producing IFN-γ and TNF-α (**Figure 2**). However, there was no difference in CD4 T cell expressing IL-4, IL-10, IL-17A, and FasL (**Figure 2**). In contrast, there was no difference in number of CD8 T cell producing tested cytokines (IFN-γ, TNF-α, IL-4, IL-10 and IL-17A) and expressing FasL between heterologous and homologous groups (**Figure 3**).

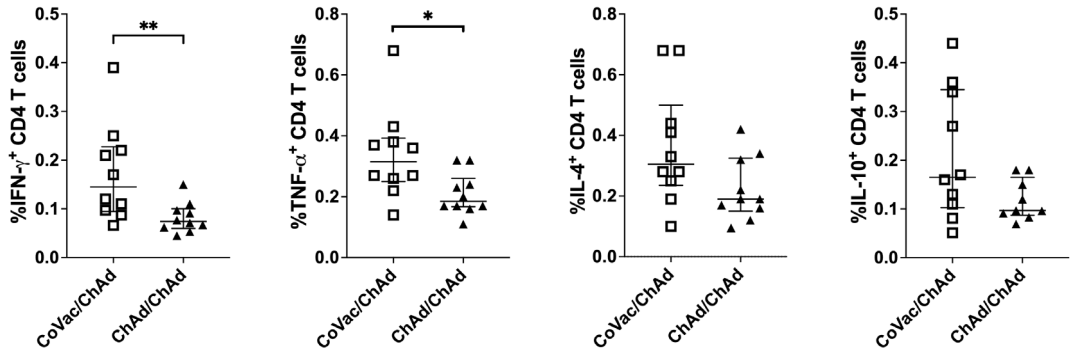
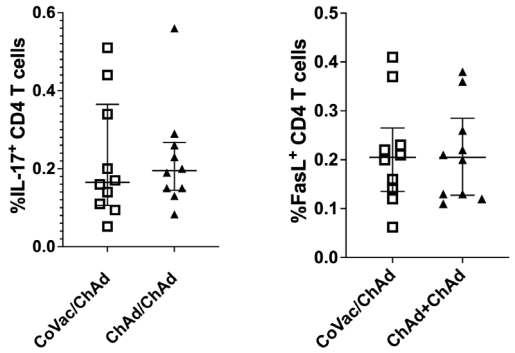


Figure 2. CD4 T cell responses to pooled peptides of SARS-CoV-2 wild type spike proteins. PBMCs (N = 10) were stimulated with pooled peptides of the wild-type spike protein (all functional domains) and analyzed for the molecules of interest using immunofluorescence staining and flow cytometry. The graphs indicate frequency of CD4 T cells expressing IFN-γ, TNF-α, IL-4, IL-10, IL-17A and FasL at 4 weeks after heterologous (CoVac/ChAd) or homologous (ChAd/ChAd) vaccination. Individual data at each condition are shown. Lines represent the median with the interquartile range. The Mann-Whitney U test was used for comparisons. *$p \leq 0.05$; **$p \leq 0.01$.



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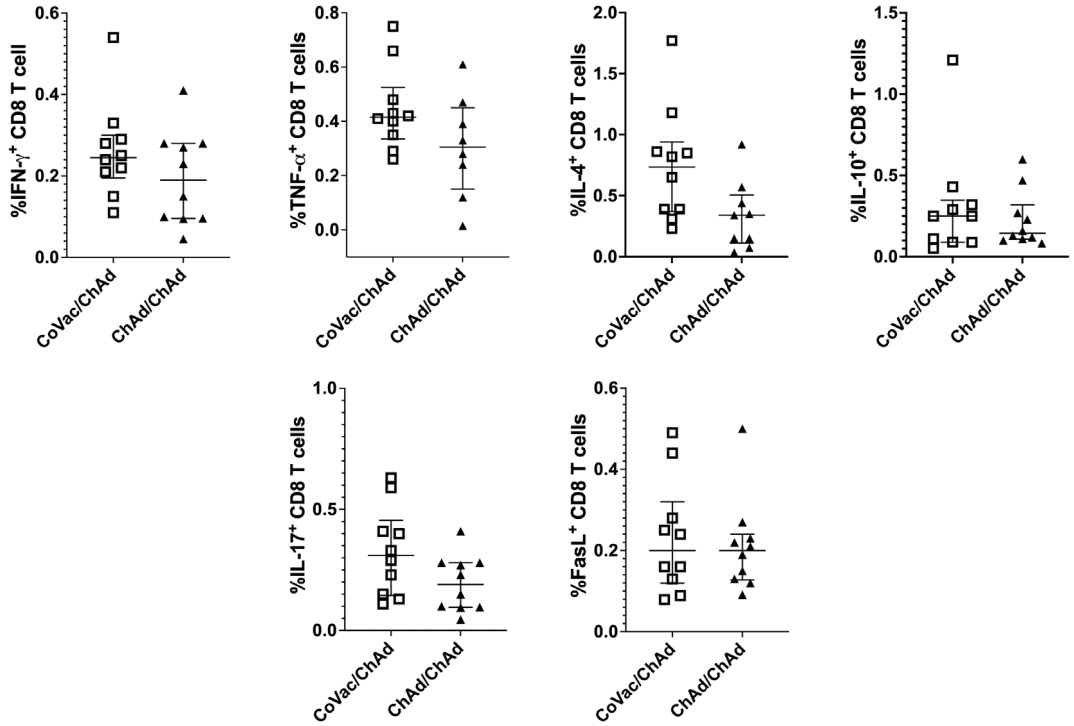

Figure 3. CD8 T cell responses to pooled peptides of SARS-CoV-2 wild type spike proteins. PBMCs (N = 10) were stimulated with pooled peptides of the wild-type spike protein (all functional domains) and analyzed for the molecules of interest using immunofluorescence staining and flow cytometry. The graphs indicate frequency of CD8 T cells expressing IFN-γ, TNF-α, IL-4, IL-10, IL-17A and FasL at 4 weeks after heterologous (CoVac/ChAd) or homologous (ChAd/ChAd) vaccination. Individual data at each condition are shown. Lines represent the median with the interquartile range. The Mann-Whitney U test was used for comparisons. No significant difference between heterologous and homologous vaccinations was observed.

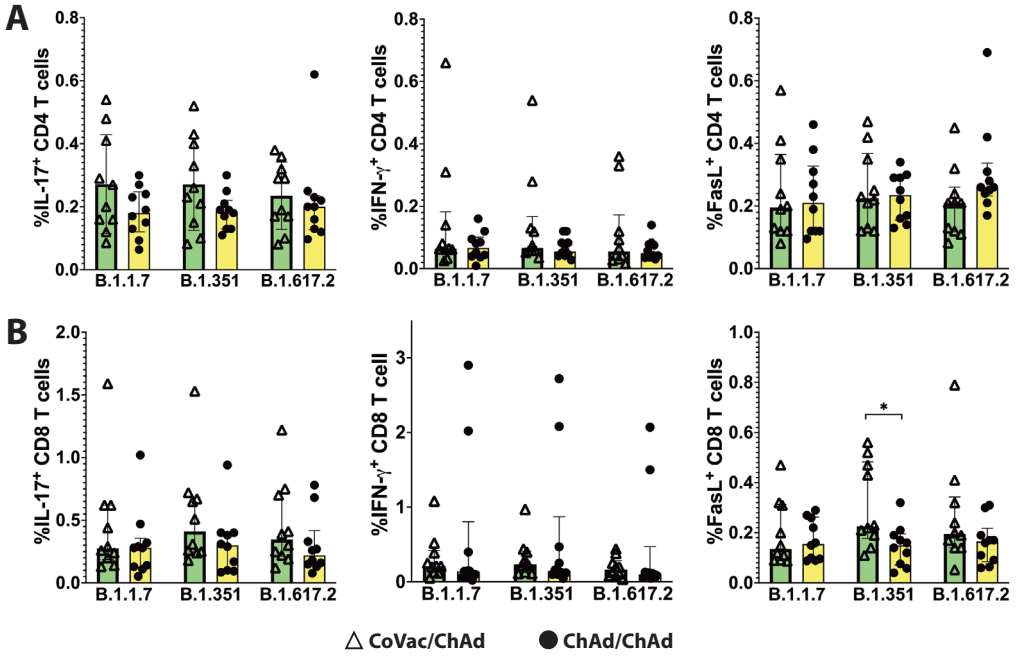

Figure 4. T cell responses to pooled peptides of SARS-CoV-2 variants of concern spike proteins. PBMCs (N = 10) at 4 weeks after heterologous (CoVac/ChAd) or homologous (ChAd/ChAd) vaccination were stimulated with spike peptide pools of B.1.1.7 (Alpha), B.1.351 (Beta), or B.1.617.2 (Delta) mutants and analyzed for the molecules of interest using immunofluorescence staining and flow cytometry. The graphs indicate frequency of CD4 T cells (A) and CD8 T cells (B) expressing IL-17A, IFN-γ, and FasL. Individual data at each condition are shown. Lines represent the median with the interquartile range. The Mann-Whitney U test was used for comparisons between heterologous and homologous vaccination. *, $p \leq 0.05$.

T cell responses to SARS-CoV-2 variant spike peptides

T cell responses against S protein of Alpha, Beta and Delta variants were determined using variant specific S peptide pools as stimulants. As shown in Supplementary **Figure 4**, 4 weeks after vaccination, in responses to the tested variant-specific spike peptides (Alpha, Beta and Delta) stimulation, CD4 and CD8 T cell producing IFN-γ, IL-17 and FasL were not significantly different in comparison between heterologous and homologous vaccination regimens, except for the Beta variant which had higher FasL expressing CD8 T cell in heterologous group.

We also determined T cell responses to Alpha, Beta and Delta variants specific S peptide pools and their WT reference S peptide pool. No statistically differences in CD4 and CD8 T cell producing IFN-γ, IL-17 and FasL was observed in either heterologous and homologous vaccination regimens (**Supplementary Figure S2**).

Discussion

Several Covid-19 vaccine platforms have been developed and are available worldwide. Instead of homologous vaccination, heterologous vaccination has been introduced since early 2021. At the beginning, the idea of heterologous vaccination came from the report of rare but severe vaccine-induced immune thrombotic thrombocytopenia (VITT) side effect of ChAdOx-1 vaccine.[8] Therefore, in some western countries, the mRNA vaccine platform was recommended as 2nd vaccine in the population already received 1st dose of ChAdOx-1 vaccination.[9] Among the heterologous mixed and matched vaccinations, the regimen primed with ChAdOx-1 and boosted with any mRNA vaccines was the most popular. This mixed and matched vaccination had vaccine effectiveness of 88% in Denmark during outbreak of the Alpha variant.[10] Boosting with BNT162b2 following priming with ChAdOx-1 could induce over 70-fold increase in anti-RBD antibodies in comparison with the pre-boosting level.[11] In a non-inferiority trial, ChAd/BNT162b2 could induce geometric mean titer of anti-spike IgG of 9.2-fold higher than homologous ChAd/ChAd.[12] This heterologous regimen was safe and resulted in NAb response against the WT and VOCs better than homologous ChAdOx-1. This regimen also induced strong CMI response against VOCs (Alpha, Beta, Delta, and Gamma variants).[13,14] In addition, it was reported to induce better NAb response against the ancestral strain than homologous mRNA and ChAdOx-1 vaccine in relatively older subjects aged > 55 (56-75) years.[15] For the heterologous CoVac/ChAd vaccination, Cohen, et al. demonstrated that this regimen was safe and induced higher anti-RBD antibody level than the primary CoVac/CoVac series with the geometric mean ratio of 8.69.[16]

In Thailand, the heterologous CoVac/ChAd regimen was adopted for both non-ageing and ageing populations since July 2021. However, data on the immunogenicity of the vaccine against particularly the VOCs in the elderly was limited. In this study, we studied the immunologic response to the heterologous regimen which was priming with CoronaVac followed by boosting with ChAdOx-1, in comparison with the homologous ChAd/ChAd in the healthy elderly. We demonstrated that the NAb levels at 4-week post-vaccination with both regimens did not meet statistical

difference between each tested strain. The NAb response was the best in the WT than other variants. Our results were comparable to the study done by Yorsaeng, et al. who showed that the levels of SARS-CoV-2 anti-S antibody response to the heterologous CoVac/ChAd was comparable to the homologous ChAd/ChAd vaccination in the non-ageing participants.[17] Notably, in our study, the levels of NAb against Beta variant were lower than WT, Alpha, and Delta variants in both vaccine regimens. This is consistent with several studies indicated that, among the Alpha, Beta, and Delta variants, the vaccine-induced NAb titer was the lowest against the Beta strain.[18] The mechanism of NAb resistance of Beta variant was the result of mutation from E484K substitution.[19] The CoronaVac, in comparison to other vaccine platforms, induce the lowest NAb against the Beta variant.[18] The heterologous CoronaVac/ChAdOx-1 vaccine regimen, which priming with CoronaVac, thus resulted in lower level of NAb against Beta variant than the homologous ChAdOx-1/ChAdOx-1 vaccine regimen.

Evidences of declining in humoral immunity were found in various homologous vaccination regimens. Dropping of anti-S IgG levels between 1 month and 3 months following homologous vaccination with CoronaVac, ChAdOx-1, and BNT162b2 were reported to be 56.6%, 80.3% and 55.8%, respectively.[20,21] Levin, et al. showed that the anti-RBD IgG and NAb at 3 months were more than 3 times lower than the levels at 1 month following vaccination with homologous BNT162b2.[22] The higher the age, the lower antibody level over time was observed.[22] Waning of antibody responses after vaccinations was also observed in our study. The antibody dynamic data, comparing levels of NAb between 4th and 12th week in non-Omicron strains, showed that the waning of NAb was prone to be slower in the heterologous than the homologous group, except for the Beta strain. Albeit no statistical difference in these findings, it might be worthy to discuss about the benefits of priming with the whole virion inactivated vaccine. Wanlapakorn, et al. performed a study comparing heterologous and homologous vaccination as our regimen in the younger age group. They found that the heterologous CoVac/ChAd group could induce significantly higher NAb against the WT and no differences against the Delta, Alpha, and Beta variants.[23]

For the Omicron variant infection, although it caused less severe disease than the Delta variant with the admission rate of only 2.4%, the rates of severe disease from both variants in hospitalized patients were indifferent. Age ≥ 60 years was the significant risk factor of severe Omicron disease.[24] Among hospitalized patients, 18.5%, 1.6%, and 2.7% were ICU-admitted, mechanical ventilated, and dead, respectively.[25] The Omicron variant has several mutations at receptor-binding domain (RBD) which make it lower affinity to the vaccine-induced neutralizing antibody.[26] Vaccine effectiveness for the Omicron was reported to be lower than WT and other VOCs. Study on BNT162b2 vaccination showed that there were 22-fold drop in geometric mean titers of NAb against Omicron versus WT.[27] For the homologous inactivated vaccine regimen, the NAb response against Omicron were 11.2-12.5 and 3.4-5.8 folds lower than the WT and Delta variant, respectively.[28] NAb level for the Omicron was below the lower limit of quantification in 80% of subjects.[28] We then determined the level NAb against the Omicron strain and found that the median NAb levels against the Omicron after 4-week vaccination were lower than 30% threshold of NAb detection in both heterologous and homologous vaccination groups. None, except one, in each group had level of NAb above the cut-off point. It meant that both heterologous CoVac/ChAd and homologous ChAd/ChAd vaccinations had low efficacy in inducing NAb against the Omicron. Our result was compatible with the previous study done by Dejnirattisai, et al. who showed that levels of NAb against the Omicron following homologous ChAd/ChAd were below cut-off level in all, except one, subjects.[29] Our finding supported the lower effectiveness of the vaccine for the Omicron variant.

In term of cellular immune response, we investigated CD4 and CD8 T cells of participants obtained heterologous and homologous vaccines in response to the WT S peptides. Upon peptide stimulation, the frequencies of IFN-γ and TNF-α producing CD4 T cells were significantly higher in the heterologous CoVac/ChAd group compared with homologous ChAd/ChAd group. However, no difference in CD4 T cell producing IL-17A, IL-4, IL-10 and expressing of FasL. We did not observe the differences in CD8 T cell responses to the WT S peptides between the two regimens. These results indicated that, in the elderly, Th1-biased cellular immune response is preferred in heterologous CoVac/ChAd vaccination regimen, which is in line with the study published recently in non-aging group.[30] Th1 immune response is an important immunity that could lead to virus clearance. The induced T cell responses by vaccination might play role in reducing the severity of the disease after SARS-CoV-2 infection. T cell response to SARS-CoV-2 antigens which significant improvement by heterologous CoVac/ChAd might be the effect of the primed whole-virion in the CoronaVac vaccine.[23] The activation of innate immunity by immunodominance hierarchy of inactivated vaccine might play role in improving the immunogenic effect of heterologous CoVac/ChAd vaccination.[31] Importantly, T cell response induced by heterologous and homologous vaccination regimens were able to induce T cell responses against all tested VOCs including Alpha, Beta, and Delta variants. This confirms previous data that antigenic drift is generally less affected on T-cell response.[32] Priming with the whole-virion inactivated vaccine, which did not induce high antibody responses, could be a good priming vaccine for cellular immunity and beneficial for intensifying the immune system following boosting with other platforms of vaccine. T cell immune responses to SARS-CoV-2 antigens by infection or vaccination have been reported to be sustained in the body for years.[33,34] The T cell memory encompasses broad recognition of viral protein, estimated at 30 epitopes, and exhibit sustained immunity. A cross-reactive T cell response to SARS-CoV-2 and endemic coronaviruses was also demonstrated. Thus, unlike antibody responses, T cell responses are durable immunity lasting for years. In our study, even though, the T cell responses were determined at 4 weeks after vaccination, we believed that the same results will be obtained when determined at 3 months after vaccination.

There were some limitations of our study. Firstly, this study did not have homologous CoVac/CoVac vaccination as another control group. The reason was that, during the study period, there was no national policy to vaccinate homologous CoVac/CoVac regimen in the elderly. In addition, data from a comparative study between homologous CoronaVac and homologous ChAdOx-1 vaccination in Thailand showed that NAb following homologous ChAdOx-1 regimen at 4 weeks and 12 weeks were significantly higher.[35] Secondly, this study aim to compare immunogenicity of 2 vaccination regimens used for the elderly in Thailand, the subjects younger than 60-year-old was not included and compared in this study. Thirdly, this is not a randomized controlled trial. Although the baseline demographic data were the same, selection bias could be possible because the participants could select their vaccination regimens by themselves. Moreover, the number of subjects in this study was low. The main reasons for this were that the number of elderly subjects that meet the inclusion criteria was limited.

In conclusion, our study demonstrated that, in the elderly, heterologous CoVac/ChAd vaccination regimen could induce NAb response against the WT and non-Omicron variants not different from the homologous ChAd/ChAd regimen. It, nevertheless, had a trend for induction of more subjects to have level of NAb above the threshold. Both of vaccine regimens induced T cell responses, but Th1 cell reactivity was significantly increased in heterologous vaccination. Our findings confirmed the conceptual benefits of heterologous vaccination regimen. This regimen which contained priming inactivated vaccine followed by boosting with adenoviral vaccine also took quicker time between priming and boosting to produce indifferent NAb response at both 4th week and 12th week post-vaccination. This sequential heterologous vaccination would be benefit in the situations with vaccine shortage and during pandemic of a new variant of virus which required rapidly developed immunization. Unfortunately, both regimens did not give adequate NAb for protection of the Omicron.

Conflict of interests

The authors declare that there is no conflict of interest.

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Authors contributions

- SP, WL, NT, KC and PS performed the experiments and data analysis and drafted the original manuscript.
- CL and WK, the principal investigator, provided a conceptual framework for the project, provided guidance for the methodology and interpretation of the data, and edited and finalized the manuscript.
- WC contributed to blood sample and clinical data collection and statistical analysis.
- JI carried out subject recruitment and funding management.
- PD, CW, AD, TT, AL, PT, NN, KT, and CP were responsible for subject recruitment and data collection.
- CP provided critical feedback and helped shape the research.
- All authors have read and approved the final manuscript.

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Supplemental material

Table S1. Demographic and clinical characteristics of participants.

Characteristics	CoVac/ChAd (N = 19)	ChAd/ChAd (N = 17)	*p-value*
Age (years)	69.9 ± 4.4 (min-max, 64 - 78)	70.4 ± 4.5 (min-max, 65 - 85)	0.756
Male (sex)	15 (78.9)	14 (82.4)	1.000
Height (cm)	160.3 ± 6.7	164.6 ± 8.4	0.091
Body weight (kg)	62.3 ± 8.5	67.4 ± 17.7	0.277
Body mass index (kg/m^2)	24.3 ± 3.1	24.7 ± 4.8	0.777
Co-morbidities			0.734
Cardiovascular	10 (52.6)	9 (52.9)	
Metabolic	1 (5.3)	2 (11.8)	
Cardiovascular + Metabolic	3 (15.8)	1 (5.9)	
Nones	5 (26.3)	5 (29.4)	
Smoking status			0.097
Non-smoker	9 (47.4)	13 (76.5)	
Ex-smoker	10 (52.6)	4 (23.5)	

CoVac, CoronaVac; ChAd, ChAdOx-1
Data are presented as mean ± SD or number (%)

Table S2. The percent decrement in % inhibition of neutralizing antibody against the wild type, Alpha, Beta, and Delta variants of SARS-CoV-2 between week 4 and week 12 after receiving 2nd dose of heterologous and homologous vaccinations.

Percent change	CoVac/ChAd (n = 18)	ChAd/ChAd (n = 17)	*p*-value
Wild type	-9.7 (-21.3, -5.3)	-22.5 (-24.9, -2.5)	0.692
Alpha (B.1.1.7)	-26.7 (-35.6, -13.7)	-33.6 (-41.3, -10.2)	0.373
Beta (B.1.351)	-35.2 (-52.4, -8.8)	-22.6 (-28.4, -7.3)	0.166
Delta (B.1.617.2)	-28.8 (-41.4, -15.3)	-26.7 (-32.8, -5.3)	0.322

CoVac, CoronaVac; ChAd, ChAdOx-1;
Data are presented as median (interquartile range, IQR)

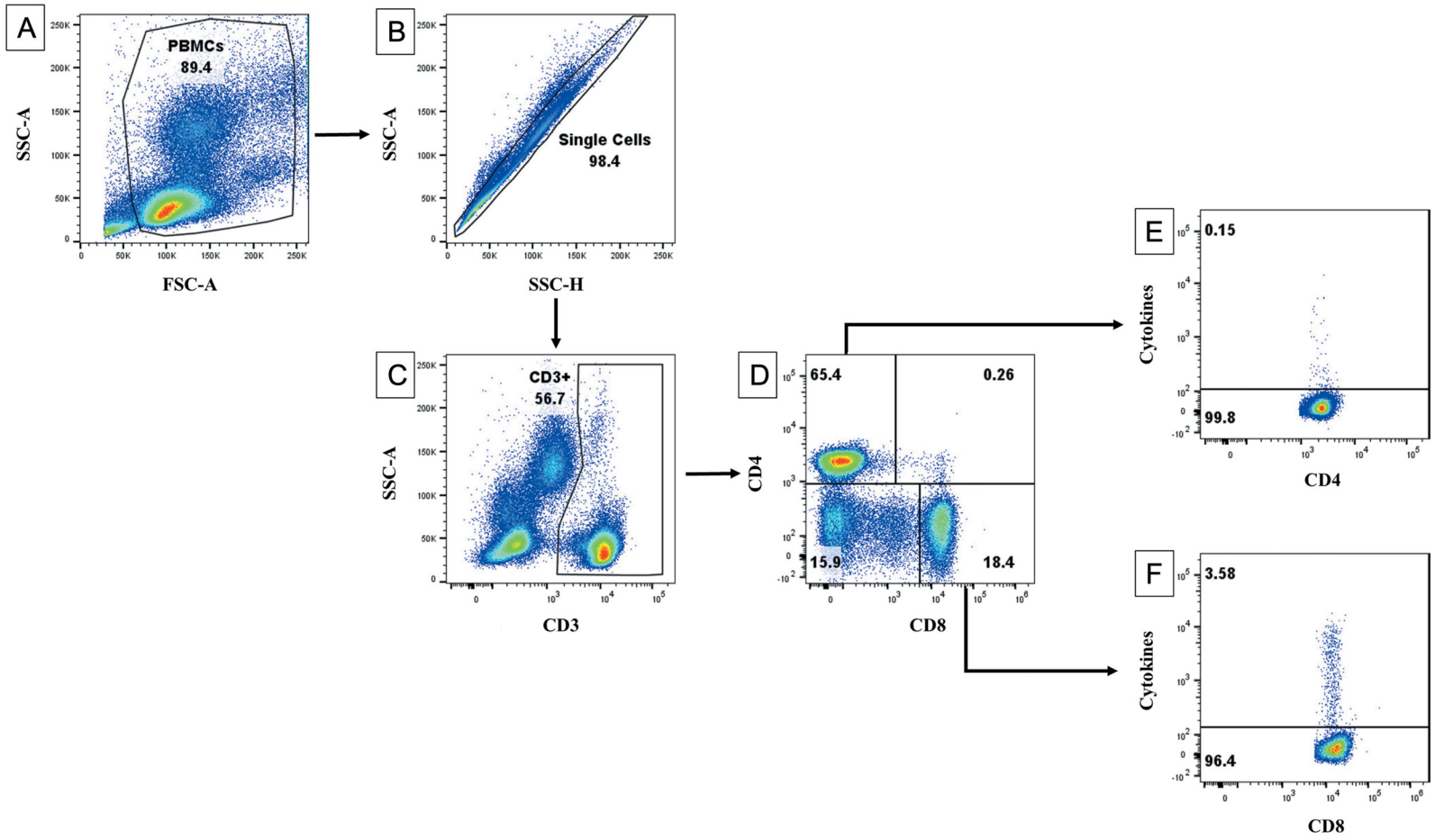

Figure S1. Flow cytometry and gating strategy. PBMCs were stimulated with the SARS-CoV-2 peptide pool of spike proteins, and cytokines and FASL expression levels were determined by flow cytometry. The gating strategies are shown.
(A) The size (FSC-A) and granularity (SSC-A) of PBMCs were plotted and gated as indicated.
(B) The gated cells were represented in an SSC-H vs. SSC-A dot plot to eliminate doublets.
(C) CD3 T cells were gated by plotting CD3 staining vs. SSC-A.
(D) CD4 T cells and CD8 T cells were gated from CD3 T cells by plotting CD8 vs. CD4 staining. The CD4 T cells
(E) and CD8 T cells
(F) were plotted against CD4 or CD8 staining and molecule of interest. The frequency in each population were determined by FlowJo software.

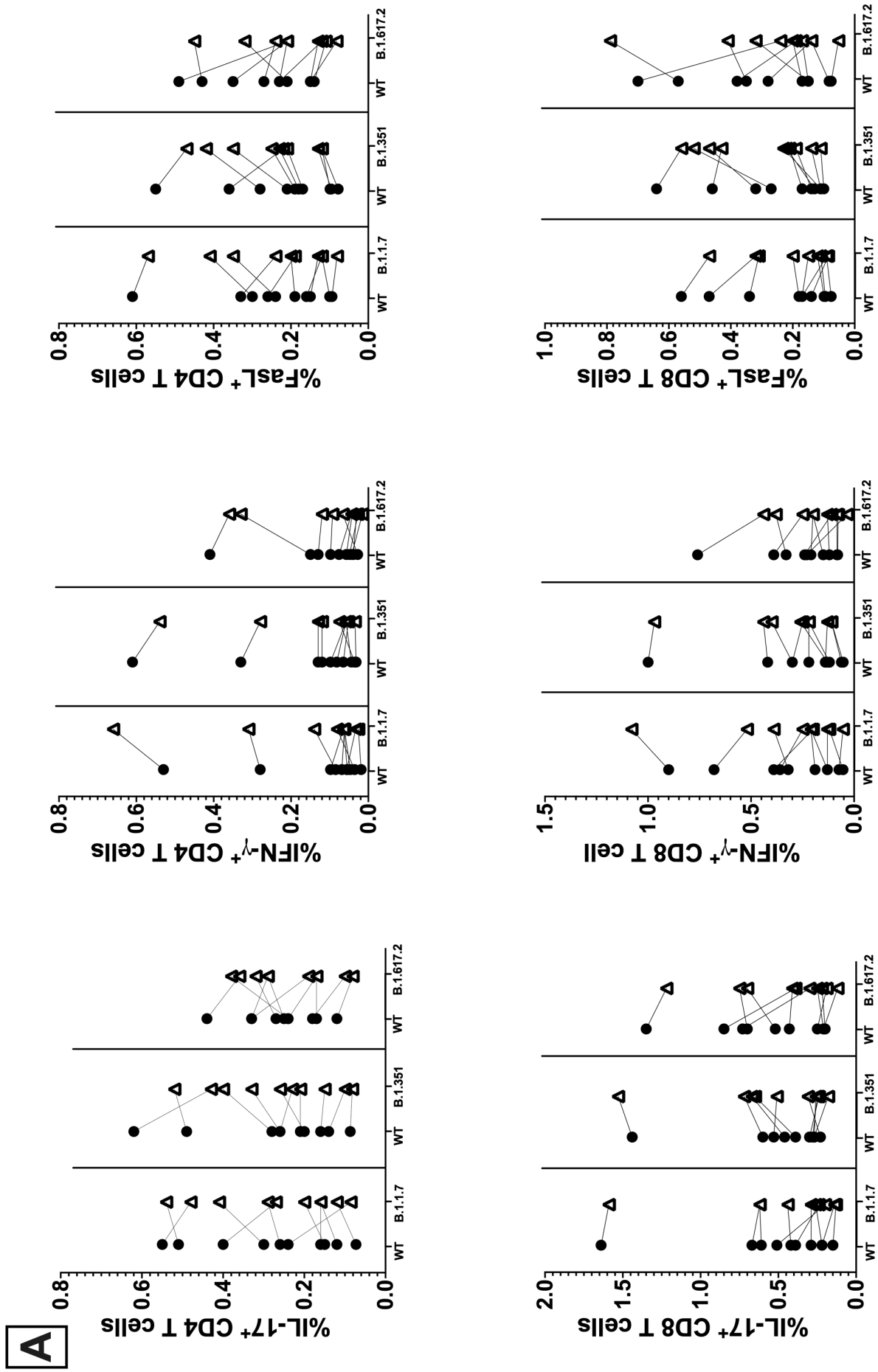

Figure S2. T cell responses to pooled spike peptides of SARS-CoV-2 variants and their wild type peptide homologues. PBMCs (n = 10) at 4 weeks after heterologous (CoVac/ChAd) or homologous (ChAd/ChAd) vaccination were stimulated with spike peptide pools of B.1.1.7 (Alpha), B.1.351 (Beta), or B.1.617.2 (Delta) mutants or their WT peptide homologue. By immunofluorescence staining and flow cytometry, CD4 T cells (A) or CD8 T cells (B) were gated to determine the frequency of cells expressing IL-17, IFN-γ and FasL. Individual data in each tested condition are presented. The Wilcoxon matched pairs signed-ranks test was used for comparison. No significant difference ($p > 0.05$) between mutants and their homologous WT peptides was observed.

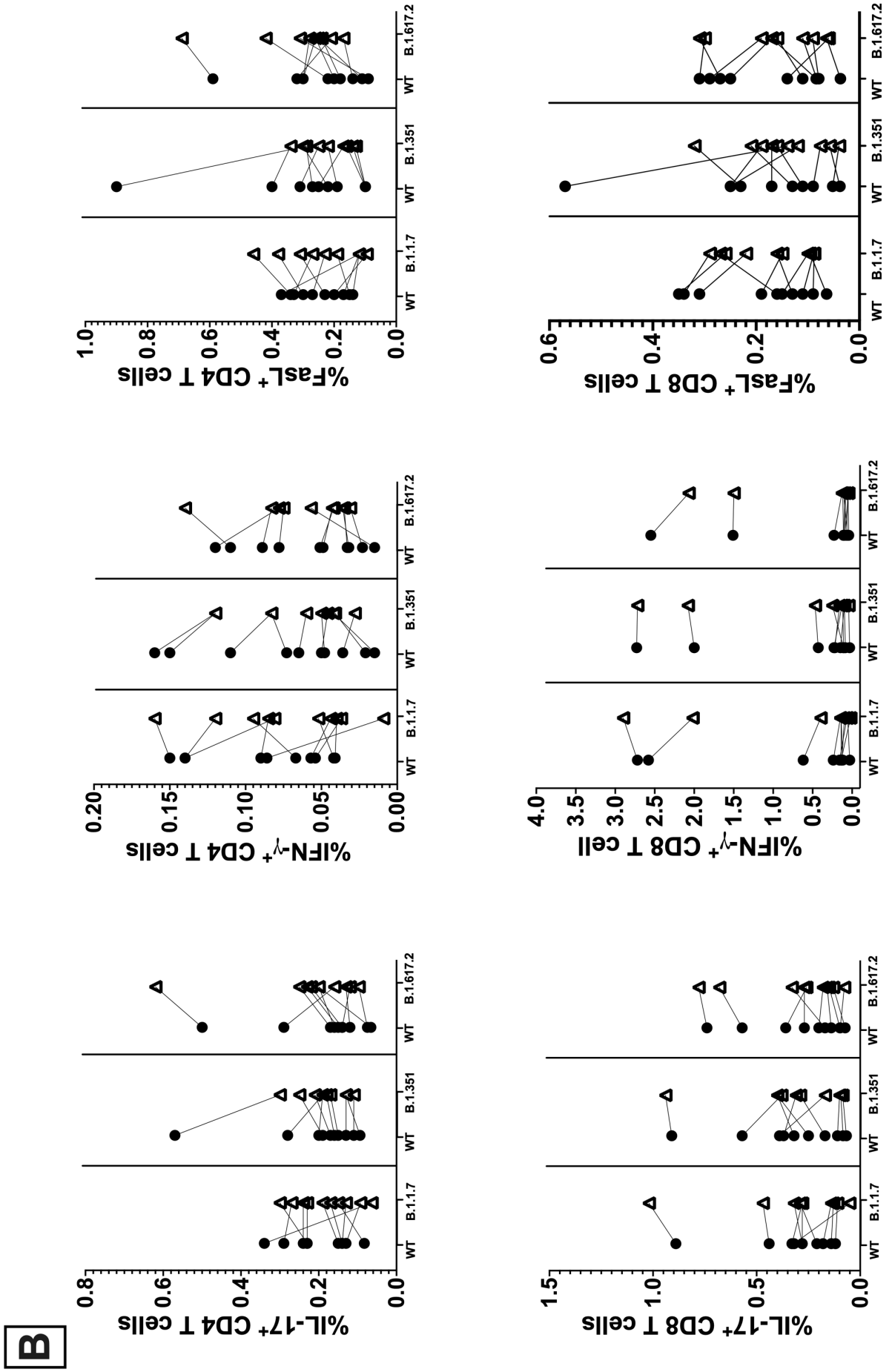

Figure S2. (Continued)