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Short Communication

Serological response and safety of heterologous ChAdOx1-nCoV-19/mRNA-1273 prime-boost vaccination with a twelve-week interval

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The appropriate interval between heterologous prime adenoviral vectored vaccination and boost mRNA vaccination remains unclear. We recruited 100 adult participants to receive a prime adenoviral vectored vaccine (ChAdOx1, AstraZeneca) and a boost mRNA vaccine (mRNA-1273, Moderna) 12 weeks apart and checked their serum SARS-CoV-2 anti-spike IgG titers and neutralizing antibody titers against B.1.1.7 (alpha) and B.1.617.2 (delta) variants on the 28th day after the boost dose. Results were compared with our previous study cohorts who received the same prime-boost vaccinations at 4- and 8-week intervals. Compared to other heterologous vaccination groups, the 12-week interval group had higher neutralizing antibody titers against SARS-CoV-2 variants than the 4-week interval group and was similar to the 8-week interval group at day 28. Adverse reactions after the boost dose were mild and transient. Our results support deploying viral vectored and mRNA vaccines in a flexible schedule with intervals from 8 to 12 weeks.

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Introduction

The outbreak of coronavirus disease 2019 (COVID-19) continues to spread with major impacts on healthcare systems worldwide.¹ Mass vaccination against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) remains the most effective measure to provide herd immunity and control its spread.

Among the currently authorized COVID-19 vaccines for use, the ChAdOx1 nCoV-19 adenovirus-based vector vaccine (ChAdOx1) and two mRNA vaccines (BNT162b2 and mRNA-1273) have been widely used in Taiwan.² Although homologous vaccination with the same formulation is standard practice in the vaccination program,^{3–5} the heterologous two-dose regimen of an adenovirus vectored vaccine followed by an mRNA vaccine has been reported to be more immunogenic than a two-dose homologous ChAdOx1 vaccine regimen.^{6–9} However, the most appropriate interval of heterologous prime-boost vaccination to induce the best protective effect remains uncertain.

We have previously addressed the immune responses and safety of heterologous ChAdOx1-nCoV-19/mRNA-1273 vaccination with prime-boost intervals of 4 and 8 weeks, compared with homologous ChAdOx1 vaccination and homologous mRNA-1273 vaccination separately.¹⁰ The SARS-CoV-2 anti-spike IgG titers and neutralization antibody titers against SARS-CoV-2 variants, as well as T-cell responses to heterologous vaccinations with either 4- or 8-week intervals, were significantly higher than that of homologous ChAdOx1 vaccination and comparable to homologous mRNA-1273 vaccination. Heterologous prime-boost ChAdOx1/mRNA-1273 vaccination with an 8-week interval had significantly higher mean neutralizing antibody titers than the 4-week interval of heterologous ChAdOx1/mRNA-1273 vaccination at day 28 after the booster dose.¹⁰

We investigated the immunogenicity and safety of an additional 100 participants vaccinated at a 12 week prime-boost interval. The first dose was the ChAdOx1 vaccine and the boost dose was the mRNA-1273 vaccine; results were compared with our previous study.

Materials and methods

Healthy volunteers (including healthcare workers and staff) from National Taiwan University Hospital, Taipei City were recruited. Two kinds of COVID-19 vaccines were used, the prime being the adenovirus vector vaccine (ChAdOx1, AstraZeneca, UK) and the booster was the messenger RNA vaccine (mRNA-1273, Moderna, USA). Participants were subject to a prime/boost vaccination schedule of 12 weeks. Enrollment criteria and test schedules were the same as those of our previous study.¹⁰ The serum SARS-CoV-2 anti-spike IgG titers and neutralizing antibody titers were determined as described in our previous report.¹⁰ The primary outcome was the analysis of serum SARS-CoV-2 anti-spike IgG titers and neutralizing antibody titers against B.1.1.7 (alpha) and the B.1.617.2 (delta) SARS-CoV-2 variants at day 28 after the booster. Adverse reactions were recorded until 84 days after boost. The statistical methods were the same as described in our previous report.¹⁰ This study was approved by the Institutional Review Boards

(Ethics Committee) of National Taiwan University Hospital (IRB No. 202106039 MINA).

Results

Between August 6th and 27th, 2021, 100 participants were enrolled for heterologous prime-boost vaccination with a 12 week interval (Group 5, [Supplementary Fig 1](#)). The median age was 44 years (interquartile ranges, 37 and 52, ranged from 24 to 63 years; mean \pm standard deviations, 43.9 ± 10.1 years) with 89% women. There were no significant differences in demographic characteristics, underlying medical illnesses or concurrent medication compared to the four groups of our previous study, except that there were significant differences in the male/female ratios among the five groups ($P < 0.001$, [Supplementary Table 1](#)).

The SARS-CoV-2 anti-spike IgG titers and neutralizing antibody titers against the alpha and delta variants of the 12-week interval group, before and after the boost vaccination, are shown in [Table 1](#). Similar to our previous findings, the SARS-CoV-2 anti-spike IgG titers and neutralizing antibody titers against both variants, increased significantly at day 14, 28 and 84 after boost vaccination compared to the baseline titer before boost vaccination (all $P < 0.0001$). The antibody titers reached a peak around 14 days after boost vaccination before declining. Therefore, the antibodies at 28 days after boost dose were significantly lower than those at 14 days after the boost dose ([Table 1](#)). Participants with 12-week prime-boost vaccination had significantly lower SARS-CoV-2 anti-spike IgG titers before boost than those with the 4- and 8-week prime-boost vaccination schedule. At day 28 after the boost, the SARS-CoV-2 anti-spike IgG titers and neutralizing antibody titers were similar between the 12 and 8-week interval groups, but higher than those in the 4-week interval group. In comparison with our previous study, the SARS-CoV-2 anti-spike IgG titers and neutralizing antibody titers against alpha and delta variants of the 12-week interval group were significantly higher than those of homologous ChAdOx1 vaccination group (Group 1), but lower than the homologous mRNA-1273 vaccination group (Group 4). The comparisons of antibody response and neutralizing

Table 1 Anti-SARS-CoV-2 antibody responses of Group 5 at Day 1, Day 14, Day 28, and Day 84 post booster dose.

	SARS-CoV-2 S-IgG (BAU/mL) Geometric mean (95%CI) ^a	Geometric mean neutralization titer (NT ₅₀) (IU/mL)	
		Alpha Variant	Delta Variant
Day 1	43.86 (36.71–52.41)	2.14	1.00
Day 14	2609.93 (2276.71–2991.92)	1042.30	288.36
Day 28	1661.39 (1451.46–1901.69)	670.12	193.59
Day 84	561.28 (491.57–640.87)	296.89	40.24

BAU, binding antibody units; CI, confidence interval; NT₅₀, 50% neutralization titer; IU, international unit.

^a Antibody values were transformed to log values, and the average values were expressed as geometric means with 95% confidence interval.

Table 2 Anti-SARS-CoV-2 antibody responses of 4 vaccine groups compared with Group 5 at 1st, 14th, 28th and 84th Days after boost vaccination. (A) SARS-CoV-2 S-IgG antibody titers. (B) Neutralization antibody titers against SARS-CoV-2 variants (Alpha and Delta).

(A)					
Groups	Days after boost vaccination	SARS-CoV-2 S-IgG (BAU/mL) Geometric mean (95%CI) ^a		P value ^b	
Group 5	Day 1	43.86 (36.71–52.41)		—	
	Day 14	2609.93 (2276.71–2991.92)		—	
	Day 28	1661.39 (1451.46–1901.69)		—	
	Day 84	561.28 (491.57–640.87)		—	
Group 1	Day 1	72.10 (60.06–86.56)		<0.0001	
	Day 14	194.07 (165.50–227.57)		<0.0001	
	Day 28	170.09 (146.79–197.08)		<0.0001	
	Day 84	88.96 (77.50–102.12)		<0.0001	
Group 2	Day 1	76.38 (64.82–89.99)		<0.0001	
	Day 14	2330.81 (2038.83–2664.60)		NS	
	Day 28	1534.82 (1350.72–1744.02)		NS	
	Day 84	517.36 (456.46–586.39)		NS	
Group 3	Day 1	93.47 (76.77–113.80)		<0.0001	
	Day 14	3283.76 (2905.02–3711.87)		0.0278	
	Day 28	1789.50 (1588.75–2015.62)		NS	
	Day 84	553.68 (494.20–620.31)		NS	
Group 4	Day 1	449.28 (383.46–526.40)		<0.0001	
	Day 14	3791.72 (3457.41–4158.35)		<0.0001	
	Day 28	2516.60 (2285.50–2771.06)		<0.0001	
	Day 84	903.10 (813.62–1002.43)		<0.0001	
(B)					
Groups	Days after boost vaccination	Neutralization antibody titers Geometric mean (NT ₅₀) (IU/mL) ^a		P value ^b	
		Alpha variant	P value ^b	Delta variant	P value ^b
Group 5	Day 1	2.14	—	1.00	—
	Day 14	1042.30	—	288.36	—
	Day 28	670.12	—	193.59	—
	Day 84	296.89	—	40.24	—
Group 1	Day 1	8.73	NS	1.07	NS
	Day 14	125.94	<0.0001	3.73	<0.0001
	Day 28	97.02	<0.0001	4.72	<0.0001
	Day 84	32.22	<0.0001	3.66	<0.0001
Group 2	Day 1	4.35	NS	1.00	NS
	Day 14	1237.61	NS	274.55	NS
	Day 28	928.72	0.0039	204.42	NS
	Day 84	282.36	NS	73.51	0.0249
Group 3	Day 1	11.03	NS	1.42	NS
	Day 14	993.21	NS	263.07	NS
	Day 28	510.66	0.0203	89.81	<0.0001
	Day 84	180.98	0.0001	35.01	NS
Group 4	Day 1	51.13	<0.0001	2.41	0.0108
	Day 14	1524.16	0.0015	342.12	NS
	Day 28	961.98	0.0018	195.36	NS
	Day 84	403.75	0.0191	105.72	<0.0001

BAU, binding antibody units; CI, confidence interval; NT₅₀, 50% neutralization titer; IU, international unit.

NS, not significant (P > 0.05).

^a The antibody titers of Group 1 to Group 4 have been published in our previous report (Reference 10 in this report).

^b The P value was the result of comparison of the antibody titers at each testing day of each group (Group 1 to Group 4) with the titer at the same day of Group 5. Mann–Whitney U test was performed to compare the antibody responses between groups.

antibody titers against the alpha and delta variants between Group 5 and other groups are shown in [Supplementary Figs. 2 and 3](#) and [Table 2](#). In general, the heterologous vaccination groups had higher antibody titers than the homologous ChAdOx1 vaccination group but lower than the homologous mRNA-1273 vaccination group. Among the heterologous vaccination groups, the 12-week interval group had similar SARS-CoV-2 anti-spike IgG titers to the 4- and 8-week interval groups ([Table 2A](#)), but had higher neutralizing antibody titers against SARS-CoV-2 variants than the 4-week interval group and similar to the 8-week interval group at day 28 ([Table 2B](#) and [Supplementary 3A and 3B](#)).

The comparison of adverse reactions with the other four groups of our previous study are shown in [Supplementary Table 2](#). Group 5 adverse reactions were all mild and transient. The longer intervals (8- and 12-weeks) of the heterologous vaccination groups (Groups 2 and 5) seemed to have lower incidence of adverse reactions, such as pain, erythema, swelling, chills, myalgia, fatigue and arthralgia/arthritis than the shorter interval (4 week) group (Group 3). No serious adverse reactions occurred during the observation period.

Discussion

Our previous study results demonstrated that heterologous prime-boost vaccination of ChAdOx1/mRNA-1273 provides better immunological response than homologous ChAdOx1/ChAdOx1 prime-boost vaccination. Our current study results revealed that heterologous ChAdOx1/mRNA-1273 vaccination with an interval of 12 weeks is safe and provides humoral immune response similar to the 8-week heterologous vaccination schedule, and better humoral immune response than the 4-week heterologous vaccination schedule.

The World Health Organization recommends shorter interval between prime and boost vaccination for homologous mRNA-vaccination (3–4 weeks) than that for adenoviral vector vaccination (8–12 weeks).^{3–5} However, the optimum interval for heterologous prime-boost ChAdOx1/mRNA vaccination remains uncertain.^{6–9,11} Evidence suggests that longer dosing intervals (12 weeks or more) of homologous ChAdOx1 vaccination provide higher binding and neutralizing antibody titers than shorter intervals (less than 6 weeks).¹² A study of German healthcare workers, showed that a comparable interval (2–3 months after prime dose) of heterologous ChAdOx1/BNT-162b2 (BioNTech/Pfizer, Germany) vaccination induced better humoral immunity than the homologous ChAdOx1 vaccination.⁷ Similar findings were reported by a Swedish cohort with an interval of 9–12 weeks for heterologous ChAdOx1/mRNA-1273 prime-boost vaccination, compared with those of homologous ChAdOx1 vaccination.¹¹ A study of United Kingdom healthcare workers revealed that extending the dosing interval (6–14 weeks) for the homologous BNT162b2 vaccination had higher neutralizing antibody responses and sustained B and T cell responses to the spike protein, compared with 3–4 week intervals.¹³ Another German observational cohort showed that heterologous ChAdOx1/BNT-162b2 vaccination at a 10–12 week interval provide higher SARS-CoV-2 anti-RBD IgG titers and neutralization antibody

titers against B.1.1.7 and B.1.351 variants than homologous BNT-162b2 vaccination at a 3-week interval.¹⁴ Our study provides additional evidence that heterologous ChAdOx1/mRNA-1273 vaccination with an 8–12 week interval is a reasonable recommendation.

Significant decline of SARS-CoV-2 S-IgG titers were detected 12 weeks after prime ChAdOx1 vaccination, compared to the titers 4 and 8 weeks after prime ChAdOx1 vaccination. Nevertheless, the elevation of antibody titers in all these three groups were similar after boost with mRNA vaccine. A longitudinal study from France recruited a cohort of healthcare workers without comorbidities who received the homologous BNT-162b2 vaccination at a 4-week interval and heterologous ChAdOx1/BNT-162b2 vaccination at a 12-week interval.⁸ They found mRNA vaccination could enhance neutralizing potential correlated with increased frequencies of activated memory B cells that recognize the SARS-CoV-2 receptor binding domain. Although the ChAdOx1 vaccination induced a weaker antibody response, a stronger T cell response than the BNT162b2 vaccination after the priming dose was detected, which could explain the complementarity of both vaccines when used in combination.

In our previous report, the adverse reactions were less frequent when heterologous boosters were given at 8 weeks rather than at 4 weeks.¹⁰ In combination with our present study results, a longer interval between heterologous prime and booster vaccination (8–12 weeks) seemed to be associated with lower incidence of pain, swelling, fever, myalgia, and fatigue than the 4-week interval. Most adverse reactions were mild and transient. In this study we confirm the safety of heterologous prime-boost ChAdOx1/mRNA vaccination with a longer interval.

In conclusion, the heterologous prime-boost ChAdOx1/mRNA vaccination with a longer interval of 12 weeks provided similar immunogenicity responses to that of the 8-week interval and better than that of the 4-week interval. Our results support the flexible range of heterologous prime-boost vaccination intervals from 8 to 12 weeks.

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Declaration of competing interest

The authors have no conflicts of interest relevant to this article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jfma.2022.07.010>.

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