A randomized controlled trial of heterologous ChAdOx1 nCoV-19 and recombinant subunit vaccine MVC-COV1901 against COVID-19

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Heterologous prime-boost COVID-19 vaccine strategy may facilitate mass COVID-19 immunization. We reported early immunogenicity and safety outcomes of heterologous immunization with a viral vector vaccine (ChAdOx1) and a spike-2P subunit vaccine (MVC-COV1901) in a participant-blinded, randomized, non-inferiority trial (NCT05054621). A total of 100 healthy adults aged 20–70 years having the first dose of ChAdOx1 were 1:1 randomly assigned to receive a booster dose either with ChAdOx1 (n = 50) or MVC-COV1901 (n = 50) at an interval of 4–6 or 8–10 weeks. At day 28 post-boosting, the neutralizing antibody geometric mean titer against wild-type SARS-CoV-2 in MVC-COV1901 recipients (236 IU/mL) was superior to that in ChAdOx1 recipients (115 IU/mL), with a GMT ratio of 2.1 (95% CI, 1.4 to 2.9). Superiority in the neutralizing antibody titer against Delta variant was also found for heterologous MVC-COV1901 immunization with a GMT ratio of 2.6 (95% CI, 1.8 to 3.8). Both spike-specific antibody-secreting B and T cell responses were substantially enhanced by the heterologous schedule. Heterologous boosting was particularly prominent at a short prime-boost interval. No serious adverse events occurred across all groups. The findings support the use of heterologous prime-boost with ChAdOx1 and protein-based subunit vaccines.

The Oxford-AstraZeneca COVID-19 vaccine, also known as AZD1222 or ChAdOx1 nCoV-19 (ChAdOx1), was one of the earliest authorized and the most widely used vaccines (in 182 countries until January 2022) during the global fighting against COVID-191–3. It has been demonstrated in the clinical trials that ChAdOx1 given at a two-dose schedule was of 70.4% efficacy against laboratory-confirmed symptomatic COVID-194,5. Although with lower efficacy compared to the mRNA and protein-based vaccines, the real-world effectiveness data further revealed that ChAdOx1 with the original regimen was highly effective against severe COVID-19 diseases and fatal outcomes caused by the dominant variants of concern of SARS-CoV-2 virus6,7. Unfortunately, within three months of deployment, a public concern of the safety of ChAdOx1 was abruptly raised due to its linkage to a rare but potentially lethal blood clot disorder termed thrombosis and thrombocytopenia syndrome (TTS) or vaccine-induced thrombotic thrombocytopenia8–10. Replacement of the second dose with non-adenovirus vector vaccine was considered as an alternative immunization strategy against COVID-19 in ChAdOx1 recipients at risk of TTS. The consideration was later supported by clinical trials and observational studies on mix-and-match strategy displaying...
comparable safety profiles, enhanced and broadened immunogenicity, and improved clinical effectiveness against COVID-19 among ChAdOx1 recipients boosted with mRNA vaccines including BNT162b2 (Comirnaty, BioNTech/Pfizer) and mRNA-1273 (Spikevax, Moderna)\textsuperscript{10–15}. The heterologous schedule with vaccines from different platforms was widely adopted and officially recommended in many countries as the acceptable immunization strategy against COVID-19. The usefulness of the protein-based COVID-19 vaccine as the second shot to the adenovirus-vector vaccine recipients has been previously evaluated in a few clinical trials to our knowledge\textsuperscript{16}. COVID-19 vaccines from the two manufacturing platforms share similar characteristics including good thermostability, easy storage, transportation and are suitable for deployment, especially in resource-limited regions. We set up a pilot study to evaluate the reactogenicity and immunogenicity of the heterologous prime-boost vaccination strategy with the ChAdOx1 as the first dose and the MVC-COV1901 as the booster dose. The results including the antibody responses to the ancestral Wuhan strain and the predominant strain (Delta variant) in 2021 after the booster dose were presented in this report.

MVC-COV1901 was a CpG 1018- and aluminum hydroxide-adjuvanted recombinant subunit vaccine containing pre-fusion-stabilized spike protein S-2P\textsuperscript{16} developed by Medigen. It has been demonstrated the advantage of S-2P conformation in the designation of a vaccine against coronaviruses in both the immunogenicity and protective efficacy in animal models\textsuperscript{17,18}. MVC-COV1901 was officially authorized for emergency use in July 2021 in Taiwan after a large-scale phase 2 trial with more than four-thousand participants demonstrating a good safety profile and promising immunogenicity\textsuperscript{16}. It was the first S-2P protein-based vaccine being deployed against COVID-19 in the world. The clinical efficacy of MVC-COV1901 is under-evaluated in a global, randomized, placebo-controlled, phase 3 trial by World Health Organization (the Solidarity Trial Vaccines) since late 2021\textsuperscript{19}.

Results
The participants
A total of 100 one-dose ChAdOx1 participants at the ages of 22 to 62 years (median and mean ages, 40 and 40.9 years, respectively) were 1:1 randomly assigned to receive ChAdOx1 (n = 50) or MVC-COV1901 (n = 50) as the booster dose. The demographics, baseline vital signs, baseline laboratory values, and intervals between prime and boost vaccines were well balanced between both groups (Supplementary Table 1). Most of the participants were healthy Han Taiwanese without major systemic disorders. Type 2 diabetes and thyroid function disorders under medical control were respectively reported by three subjects (Supplementary Table 1). The other minor underlying conditions are listed in Supplementary Table 2. No withdrawals have occurred before the analysis at day 28 of booster vaccination (Fig. 1).

Safety and tolerability
The common solicited AEs occurring within one week after the boost dose for all recipients were pain at the injection site (63.0%), fatigue (43.0%), headache (28.0%), and myalgia (27.0%). The incidences of the common solicited AEs did not differ significantly between the two groups (Fig. 2 and Supplementary Table 3). However, the AEs of high-grade severity tended to be more commonly identified in ChAdOx1 recipients. Of them, the greater incidence of high-grade fatigue (≥ grade 2 severity) in the ChAdOx1 recipients than in MVC-COV1901 reached statistical significance (18.0% versus 6.0%, \(P = 0.0160\), Supplementary Table 3 and Fig. 2). There was no case with serious AE in both groups before day 28 of booster vaccination in this analysis.

Neutralizing antibody response
Neutralizing antibody (nAb) titer was assayed using a surrogate ELISA-based assay\textsuperscript{17}. Before administration of the booster dose, the baseline nAb titers were at low levels, with similar GMT of 32.2 IU/mL and 30.2 IU/mL respectively for day 28 ± 3 vaccine recipients of two groups (\(P = 0.7900\), Fig. 3a and Supplementary Table 4). After the booster dose, the nAb GMT significantly elevated to 202.1 IU/mL at day 10 ± 3 (95% confidence interval [CI], 162.1–252.1 IU/mL) and 235.5 IU/mL at day 28 ± 3 (95% CI, 186.7–297.1 IU/mL) in recipients boosted with MVC-COV1901, which were 2.6-fold (95% CI, 1.7–4.0 folds) and 2.1-fold (95% CI, 1.4–2.9 folds) higher than in those boosted with ChAdOx1 at the two respective time points (two-tailed Mann–Whitney, \(P < 0.0010\) for day 10 ± 3; \(P < 0.0010\) for day 28 ± 3) (Fig. 3a and Supplementary Table 4).

![Fig. 1 | Consort diagram of study design.](https://doi.org/10.1038/s41467-022-33146-7)

![Fig. 2 | Solicited local and systemic adverse events in the 7 days after the booster dose of the heterologous ChAdOx1/MVC-COV1901 (n = 50) and homologous ChAdOx1/ChAdOx1 (n = 50) group.](https://doi.org/10.1038/s41467-022-33146-7)
Neutralizing titer of day 28 ± 3 serum against live SARS-CoV-2 virus of wild-type Wuhan and Delta variant were measured with plaque reduction neutralization assay. Consistent with the finding by ELISA method, the live virus nAb titers against wild-type Wuhan and Delta variant were both higher in recipients boosted with MVC-COV1901 than those boosted with ChAdOx1, with GMT ratios of 2.5-fold (95% CI, 1.8–3.5 folds) and 2.6-fold (95% CI, 1.8–3.8 folds), respectively (two-tailed Mann–Whitney, \( P < 0.001 \) for wild-type Wuhan; \( P < 0.001 \) for Delta variant) (Fig. 3b and Supplementary Table 4). When comparing the results of nAb titers generated by live virus neutralization and the ELISA, we found a high degree of correlation between the assays, with \( R^2 \) values of 0.718 against the wild-type Wuhan and 0.663 against Delta variant (Supplementary Table 5).

**Spike S1- and RBD-binding antibody responses**

The binding antibody (bAb) titers against S1 protein and RBD in both groups are shown in Fig. 3c and Supplementary Table 4. Consistent with the trend observed for nAb, the RBD-binding antibody titer was higher in recipients boosted with MVC-COV1901 than those boosted with ChAdOx1, with GMT ratios of 1.5-fold and 1.8-fold at day 10 ± 3 and day 28 ± 3, respectively (two-tailed Mann–Whitney, \( P < 0.001 \) for day 10 ± 3; \( P < 0.001 \) for day 28 ± 3). Similar result is observed for the SI-binding antibody response (Fig. 3c and Supplementary Table 4).

**Association of dose intervals and nAb titers**

There was no significant difference in the baseline nAb titer between heterologous and homologous groups for each prime-boost interval stratum (Supplementary Table 6). For the heterologous MVC-COV1901 group, there was no significant difference in the nAb titer at baseline between two prime-boost interval strata (Supplementary Table 6). After boosting, the recipients with short prime-boost interval (4–6 weeks) had higher nAb titers compared to those with long interval (8–10 weeks) at day 10 ± 3 (GMT, 258.4 IU/mL versus 138.2 IU/mL, \( P = 0.0250 \)) and day 28 ± 3 (GMT, 325.3 IU/mL versus 170.5 IU/mL, \( P = 0.0050 \)) (Fig. 4 and Supplementary Table 6) and those with short interval had higher nAb titer change from baseline at day 28 ± 3 (Supplementary Fig. 1). A similar trend of higher nAb titers favoring the short interval was also identified in the homologous ChAdOx1 group at day 10 ± 3 though the difference of GMTs did not reach statistical significance at day 28 ± 3 (134.4 IU/mL versus 98.0 IU/mL, \( P = 0.2630 \), Fig. 4 and Supplementary Table 6). No significant difference in nAb titer change from baseline was observed between subgroups with short and long intervals at day 10 ± 3 and day 28 ± 3 (Supplementary Fig. 1).
Spike-specific antibody-secreting B cell response

We assessed the levels of SARS-CoV-2 spike-specific antibody-secreting B cells in the peripheral blood over the course of booster vaccination. The predominance of an IgG response following the booster dose was observed in both groups of MVC-COV1901 and ChAdOx1 recipients by assessment of spike binding of IgG-, IgM- and IgA-secreting B cells by ex vivo ELISpot (Fig. 5a, b).

Significant increase in the frequency of spike-specific IgG-secreting cells was observed on day 10 ± 3 after the booster dose in both groups (one-way ANOVA, P < 0.0001 for either group). Such IgG response was accompanied with a lower IgA-secreting cell response while IgM-secreting cell response was barely detectable in both groups (one-way ANOVA, P > 0.0001 for either group). The frequency of day 10 ± 3 spike-specific IgG-secreting cells was significantly higher in MVC-COV1901 recipients than that in ChAdOx1 recipients (two-tailed Mann–Whitney, P = 0.0007). Similar result is observed for IgA-secreting cell response (two-tailed Mann–Whitney, P = 0.0010), but no significant difference was observed for IgM response (Fig. 3b).

The frequency of spike-specific IgG-secreting cells was significantly correlated with the surrogate neutralizing titer measured by ELISA method on day 10 ± 3 (P = 0.0002) and 28 ± 3 (P = 0.0024) after the booster dose (Fig. 5c).

Day 10 ± 3 spike-specific antibody-secreting cell response was compared between short (4–6 weeks) and long (8–10 weeks) vaccination intervals. A strongest IgG-secreting cell response was detected in the subgroup of MVC-COV1901 recipients with short vaccination interval (one-way ANOVA, P = 0.0004) (Fig. 5d). No significant changes were seen in the frequency of IgM- and IgA-secreting cells among subgroups (Fig. 5d).

Spike-specific T cell response

SARS-CoV-2-specific T cell responses to spike antigens were measured by ex vivo IFN-γ-ELISpot prior to, on day 10 ± 3, and day 28 ± 3 after their booster dose of ChAdOx1 or MVC-COV1901 vaccines (Fig. 6a). T cell responses were detected prior to and during the course of vaccination for all recipients (Fig. 6b, c). A boosting effect was observed in ChAdOx1/MVC-COV1901 recipients on days 10 ± 3 (one-way ANOVA, P < 0.0001) and 28 ± 3 (one-way ANOVA, P = 0.0486). On day 28 ± 3, the spike-specific T cell response had contracted from the peak response but remained nearly 1.5-fold higher than that prior to the booster dose in ChAdOx1/MVC-COV1901 recipients. By contrast, no significant boosting effect was observed in ChAdOx1/ChAdOx1 recipients (Fig. 6b).

ChAdOx1/MVC-COV1901 recipients had significantly higher spike-specific T cell responses than ChAdOx1/ChAdOx1 recipients on days 10 ± 3 (two-tailed Mann–Whitney, P = 0.0039) and 28 ± 3 (two-tailed Mann–Whitney, P = 0.0053) (Fig. 6b). These stronger T cell responses were mapped to both S1 and S2 antigens for both day 10 ± 3 and day 28 ± 3 time points (Fig. 6b, c and Supplementary Fig. 2). The S1 subunit responses was higher than S2 subunit response for both groups of recipients, but the difference was not significant (Fig. 6c and Supplementary Fig. 2).

We compared the fold change of T cell responses after the booster dose between short (4–6 weeks) and long (8–10 weeks) vaccination regimens. Vaccine recipients that had their 1st dose of ChAdOx1 4–6 weeks before had a significantly higher T cell response than those that had their 1st dose 8–10 weeks ago (one-way ANOVA, P = 0.0013) (Supplementary Fig. 3). On day 10 ± 3, no significant difference in fold change of T cell response was observed between subgroups with short and long vaccination intervals (Fig. 6d). On day 28 ± 3, a significantly higher fold change of T cell response was detected in the subgroup of ChAdOx1/MVC-COV1901 recipients with long vaccination interval than the subgroup of ChAdOx1/ChAdOx1 recipients with short vaccination interval (one-way ANOVA, P = 0.0234) (Fig. 6d).

Correlation between T cell response and fold increase in spike-specific response and antibody response post vaccination

The impact of pre-existing spike-specific T cells on induction of T cell responses post vaccination was next investigated. An inverse correlation was found between spike-specific T cell responses at baseline and the fold increase in spot-forming cells in IFN-γ-ELISpot post-vaccination (Supplementary Fig. 4a). The inverse correlations between total spike-specific T cell response in the baseline and fold-change post-vaccination were strongly statistically significant after the
booster dose in all recipients ($P = 0.0395$ for day $10 \pm 3$ fold change, $P = 0.0308$ for day $28 \pm 3$ fold change) (Supplementary Fig. 4a).

Positive correlations were found between spike-specific T cell response on day $10 \pm 3$ and peak antibody-secreting B cell response ($P < 0.0001$) and serological antibody response ($P = 0.0044$) on day $28 \pm 3$ (Supplementary Fig. 4b). Similar correlation results were observed for day $28 \pm 3$ T cell response (Supplementary Fig. 4c).

**Discussion**

Results from the study demonstrated that the heterologous prime-boost schedule with ChAdOx1 followed by MVC-COV1901 in healthy adult subjects elicited significantly greater humoral and cellular immunogenicity when compared to the homologous schedule with two doses of ChAdOx1 vaccination. The observed increase in immunogenicity for the heterologous schedule was particularly prominent when the prime and boost vaccines were administered at a short interval between 4 to 6 weeks. This study has shown that the protein-based subunit vaccine MVC-COV1901 was able to evoke a strong booster response in recipients primed with the adenovirus-vector vaccine and may provide better protection against the ancestral and Delta variant of SARS-CoV-2 virus than a ChAdOx1 vaccine boost.

The reactogenicity profiles were generally consistent with the safety data published for the homologous schedule of both vaccines in their respective clinical trials. Although the incidences of most local and systemic AEs irrespective of severity did not differ with statistical significance between the two groups, the AEs of grade 3 severity including pain at the injection site and headache were exclusively identified in the participants on homologous schedule. The only headache event of grade 3 severity occurred in a ChAdOx1 recipient after the booster shot and persisted for three days which required analgesic treatment in the emergency department. The fatigue of grade 2 severity was also significantly more common after the ChAdOx1 vaccination. It has been reported that the booster shot was associated with lower incidences of AEs compared to the prime dose for the vaccine at a homologous schedule.

A growing body of evidence has demonstrated the superiority of heterologous prime-boost regimes with ChAdOx1 followed by mRNA vaccines over the homologous two-dose ChAdOx1 vaccination in both the humoral and cellular immune responses against SARS-CoV-2. On day 28 after a boost, the anti-spike IgG titer and live virus nAb titer were 9.2-fold and 6.4-fold higher in heterologous ChAdOx1 followed by MVC-COV1901.

![Image](https://doi.org/10.1038/s41467-022-33146-7)
The ELISA method for measuring nAb was developed by our group based on the binding affinity of antibodies to both the S1 protein and the receptor-binding domain. Comparing the results of surrogate neutralization assay, we observed a high degree of correlation between both assays. A similar magnitude of fold increase in neutralizing activity in recipients on heterologous schedule versus those on the homologous schedule was also consistently demonstrated by the two assays. Taken together, the finding suggested that the binding antibody-based ELISA method was a robust assay in the prediction of neutralizing activity to SARS-CoV-2 and was useful especially in large scales studies given its automation characteristic.

The finding that short dose intervals of 4 to 6 weeks tended to be associated with higher nAb titers was unexpected especially for the homologous schedule given its automation characteristic.
from <6 weeks to ≥12 weeks\(^{26}\). The reason for the conflict results between our study and previous observations remained unclear. During the period of this heterogenous schedule trial, there was an extremely low incidence of indigenous COVID-19 cases in Taiwan. It was different in the nations including the UK, Brazil and South Africa where the epidemic waves were occurring when the phase 2/3 trials of the ChAdOx1 vaccine were conducted. The possibility of immunogenicity data confounded by natural infections cannot be completely excluded. Further, the effect of interval on immunogenicity was an exploratory outcome in the pooled analysis of the phase 2/3 trials. In the current study, the dosing interval was an important parameter that was well controlled and the favourable neutralizing activity toward short interval was confirmed in the experiments on spike-specific antibody-secreting B cell response. However, it was noteworthy that the significance of increased immunogenicity for the short interval was lost at day 28 post the booster dose in recipients on homologous schedule but remained in those on heterologous schedule. The ongoing follow-up immunogenicity data on day 56 and 168 post the booster dose in this trial will add further insight into the impact of interval and schedule on the kinetics of the vaccine-evoked immunogenicity.

We showed that both vaccines, which have proven to be highly immunogenic\(^{4,16,23}\), induced spike-specific T cells against both S1 and S2 antigens after boosting. Spike-specific T cell responses to ChAdOx1 could be primarily mediated by Th1-dominated CD4 + T cell helper type 1 and also CD8 + T cells that might help control or prevent SARS-CoV-2\(^{27}\). A stronger T cell response to heterologous vaccination primed with ChAdOx1 has been reported\(^{12}\). This was associated with a broader antibody response that cross-reacts with other variants\(^{14}\), which is in line with our findings of antibody profiles after heterologous boosting.

A rapid elicitation of IgG dominated antibody-secreting B cell response was detected after boosting in both heterologous and homologous prime-boost groups, indicative of the generation of secondary immune response. Nevertheless, stronger spike-specific antibody-secreting B cell response with higher levels of neutralizing and spike-binding antibodies were observed in the heterologous group. Similar results were found in the study of ChAdOx1/mRNA-1273 prime-boost vaccination\(^{28}\). Extended studies are needed to understand the breadth and function of antibody repertoire derived from spike-specific B cell population among heterologously immunized individuals. It would be of great importance to formulate optimal vaccination strategy to achieve protective immunity against emerging variants in the near future.

Although a relatively small number of participants were enrolled, the trial results of vaccine-elicited immunity demonstrate strong neutralizing antibody and spike-specific cellular responses after a heterologous ChAdOx1 and MCV-COV1901 immunization. Importantly, this heterologous booster immunization is well tolerated. Recent studies have shown the safety and non-inferior immunogenicity of combination of ChAdOx1 and mRNA vaccines\(^{12,29}\), subunit vaccine\(^{30}\) or inactivated vaccines\(^{31}\). The current data support the use of heterologous prime-boost vaccination with ChAdOx1 and MCV-COV1901 vaccines.

**Methods**

**Study design**

This was an investigator-initiated, single-blinded, 1:1 randomized vaccine clinical study, designed to assess the reactogenicity and immunogenicity of heterologous prime-boost immunization with ChAdOx1 (AZD1222) followed by the subunit vaccine MCV-COV1901, compared with the homologous immunization with two doses of ChAdOx1 (Fig. 1). Participants were healthy adults without severe disorders at the age of 20–70 years who have had their first dose of the ChAdOx1 vaccine. There was no TTS or other serious adverse events following the first ChAdOx1 vaccination in all participants. For female participants, they must be either of non-childbearing potential (i.e., surgically sterilized or one year post-menopausal) or, if of childbearing potential, be abstinent or agree to use medically effective contraception on enrollment continuously until 90 days after boost immunization of study intervention. A negative pregnancy test was required before enrollment.

The primary objective of this trial was to determine if the immune response (neutralizing antibody titer at day 28 after the booster dose) of heterologous group was non-inferior to that observed in the homologous group (Supplementary Note 1). The non-inferiority study design was based on the immuno-bridging standards in granting Emergency Use Authorization for COVID-19 vaccine (including MCV-COV1901) by Taiwan FDA (https://www.fda.gov.tw)\(^{20}\). The immuno-bridging success criteria was the lower limit of the 2-sided 95% confidence interval (CI) for geometric mean titer (GMT) ratio >0.67\(^{20}\).

The study was conducted in a single institute in Chang Gung Memorial Hospital, Linko branch, in Taiwan. After receiving the treatment, the participants remained in the study for 168 days following booster vaccination.

Written informed consent was obtained from all participants, and the trial is being done in accordance with the principles of the Declaration of Helsinki and Good Clinical Practice. This study was approved by the Taiwan Food and Drug Administration and the ethics committee at Chang Gung Medical Foundation (Taiwan). The study was registered in ClinicalTrials.gov with ID NCT05054621 and the protocol in detail is available in Supplementary information.

**Outcomes**

For the study primary objective, humoral immunogenicity including serologic neutralizing antibody titers against SARS-CoV-2 and serological quantification of binding antibody to SARS-CoV-2 antigen was assessed during the duration of the study at baseline and after booster vaccination at day 10 ± 3, day 28 ± 3, day 56 ± 3 and day 168 ± 7. The SARS-CoV-2 antigen specific B cell and T cell frequencies were assessed in day 0, day 10 ± 3 and day 28 ± 3 after booster vaccination. Safety was assessed during the duration of the study. The solicited adverse events (AEs) occurring locally or systemically were assessed for 7 days following each vaccination from day 0 through day 7. Unsolicited AEs were recorded for 28 days after the boost dose. Serious AEs (SAEs) were recorded from signing of the informed consent form through day 168. Adverse events of special interest (AESIs) were recorded from the booster vaccination through day 168.

**Randomization and blinding**

All eligible participants were 1:1 randomly assigned to receive a single dose of either the same vaccine as their prime dose ChAdOx1 (homologous group) or the Medigen COVID-19 vaccine MCV-COV1901 (heterologous group). Stratified randomization was used based on the intervals between prime and boost vaccination. Participants were stratified according to the prime-boost intervals of 4–6 weeks and 8–10 weeks, respectively, with equal-sized strata. Randomization was applied to each stratum and the random number list was generated by an independent study statistician using SAS software.

The treatment phase was conducted in a single-blinded fashion such that the participants were masked to the vaccine received but not to the prime-boost interval. Clinical staffs who involved in the vaccine delivery were aware of which vaccine the participant received, but the participant remained blinded by preparing the vaccine out of sight and applying a masking tape over the vaccine syringe. Laboratory staffs were also blinded to the vaccine the participant received, which may minimize the evaluation bias from the knowledge about the treatment assignment of the participant.

**Surrogate neutralizing titers by ELISA method**

All serum samples were analyzed by the SARS-CoV-2 antibody ELISA kit according to the manufacturers’ instructions (MediPro, Taiwan)\(^{12,25}\).
MediPro was a Taiwan FDA-approved kit for quantifying Spike S1-and receptor-binding domain (RBD)-binding antibodies which were surrogates of live virus neutralization titers with high correlation. With the cutoff of <34.47 IU/mL defining negative result, the sensitivity and specificity of the test was 92.2% (95% CI, 84.0%–96.4%) and 93% (95% CI, 81.4%–97.6%), respectively.

**Live virus neutralization assay**

The neutralization assay following the standard protocol of a plaque reduction neutralization test was performed on the serum samples collected at day 28 after booster dose of vaccination. Vero cells were regularly maintained in minimal essential medium (MEM) supplemented with 10% (v/v) fetal bovine serum. Wild type virus ( Wuhan strain) and Delta variant of SARS-CoV-2 were propagated in Vero cells in MEM. Serum samples were inactivated at 56 °C for 30 min before use. Serum were two-fold diluted serially and were mixed with equal volumes of SARS-CoV-2 suspension containing 100 folds of the median tissue culture infectious dose. The mixture was incubated for 2 h at 37 °C, and then an equal volume of suspended Vero E6 cells (approximately 30,000 cells/well) was added to each well. Following incubation for 1 week at 37 °C, cells were fixed with 5% glutaraldehyde and stained with 0.1% crystal violet. Serum neutralization titers were calculated and expressed as the reciprocals of the highest serum dilution that inhibits cytopathic effects.

**PBMC preparation**

PBMCs were separated from heparinized blood by density gradient centrifugation using lymphoprep (Stemcell Technologies, Canada) for 20 min, 800 g at 20 °C. The PBMC band was collected, transferred to a sterile, clear 96-well dish, and resuspended in RPMI medium containing 10% fetal bovine serum for 1 h at 37 °C. Blocking medium was removed and plates were washed with PBS. 100 μL of BCIP/NBT-plus substrate (Mabtech, United States) was added to each well. Following incubation, the supernatant was discarded, the reaction was stopped using distilled water. Plates were air-dried and spots were measured and counted with an automatic ELISpot reader.

**Sample size**

The primary objective of this trial was to determine if the immune response of heterologous group was non-inferior to that observed in homologous group, and the primary endpoints was neutralizing antibody titer at day 28 after booster vaccination. By assuming the non-inferiority margin was 0.67-fold-difference or -0.401 absolute difference of log GMT between heterologous group and homologous group with the standard deviation 0.66, and the true difference of log GMT was 0, the study needed to recruit 44 evaluable participants per group (total 88 participants) to achieve 80% of power at one-sided 2.5% significance level. According to the missing rate 10% and the stratification of subjects with serious AEs, AEs leading to withdrawal, AEs by severity and AEs by relationship to study treatment. All other safety measures were recorded. Frequency counts and percentages were also be presented.

**Statistical analysis**

Descriptive statistics on continuous measurements included means, medians, standard deviations, and ranges, while categorical data was summarized using frequency counts and percentages. For the immunogenicity endpoints including SARS-CoV-2 neutralizing antibody levels and cell-mediated immune responses, the point estimates were reported with 95% confidence intervals. The secondary endpoints for comparisons of continuous scale between groups, independent t-test was used. For the comparison of nAb titer changes from baseline between groups of short and long prime-boost intervals, a value of 1 was used to substitute a change value with zero or a value less than zero, so that the information contained in these data was not lost in calculation of geometric mean.

For safety analysis, the number (%) of subjects with AEs was reported. Frequency counts and percentages were also be presented of subjects with serious AEs, AEs leading to withdrawal, AEs by severity and AEs by relationship to study treatment. All other safety measures were analyzed for the safety population.

Evidence of significant interaction was assessed at the 5% level. All analyses were performed using the Statistical Analysis System (SAS) statistical software package, version 9.4. (SAS Institute Inc, Cary, NC) and Graphpad Prism (Version 9.1.1, GraphPad Software, US).
**Interim report and enrolment status**

The study duration for each participant would be nearly or more than 6 months following the enrolment (visit day −70−→0, 7, 10 ± 3, 28 ± 3, 56 ± 3 and 168 ± 7, Supplementary Note 1). This analysis was prospectively specified in the protocol (Supplementary Note 1) and reported based on the data collected from all enrolled participants until day 28. As of 01 February 2022, a total of 101 subjects were enrolled in the study. One subject failed screening because he was unable to visit the study site in the scheduled time points. The remaining 100 were followed up, and 0 completed the study when this analysis was reported. The earliest vaccination campaign start date was 15 September 2021.

**Reporting summary**

Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Data availability**

The data associated with this study are available within the article, its supplementary information and Source Data file. This trial is registered on ClinicalTrials.gov under the identifier NCT05054621. Source data are provided with this paper.

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Author contributions
K.-Y.A.H. conceived the study. K.-Y.A.H. and C.-J.C designed the study and produced the protocol. L.-Y.Y. and W.-Y.C. assisted statistical design and analysis of the study. K.-Y.A.H. designed and performed B and T cell experiments. C.-G.H. designed and performed serological experiments. Y.-C.H., C.-H.-C., and S.-R.S. helped prepare materials, perform experiments and analyse data. K.-Y.A.H. and C.-J.C. wrote the first draft of manuscript. All authors read and approved the manuscript.

Competing interests
The authors declare no competing interests.

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