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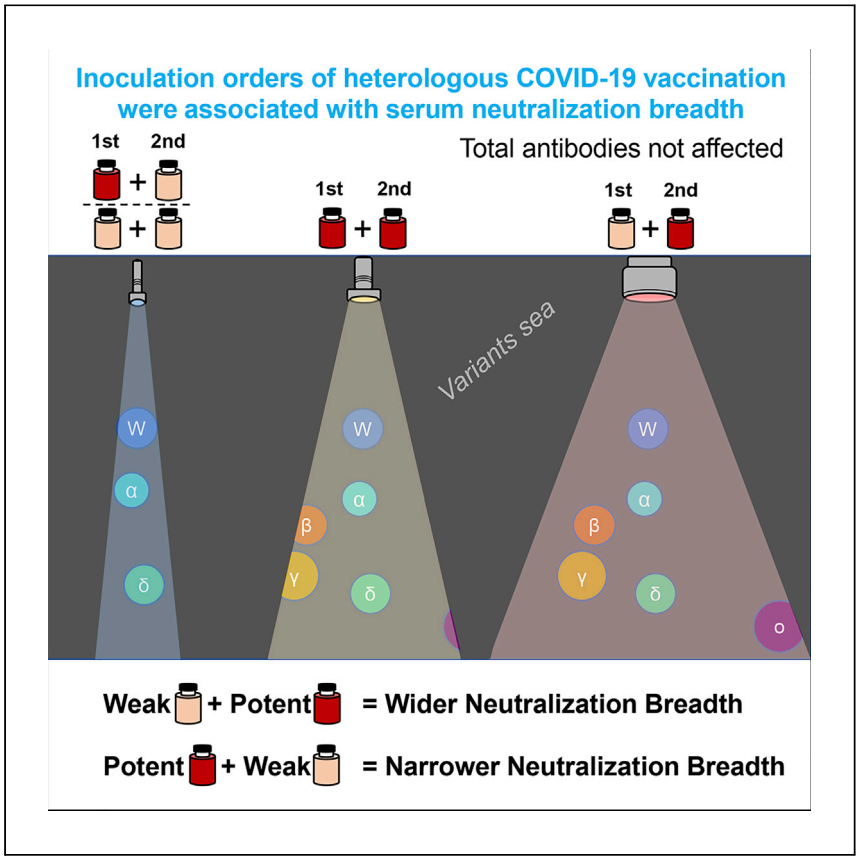
Clinical and Translational Report

Association of neutralizing breadth against SARS-CoV-2 with inoculation orders of heterologous prime-boost vaccines



Zhu et al. studied healthcare workers who had received homologous or heterologous prime-boost COVID-19 vaccination to find that the inoculation order of heterologous vaccines was associated with serum neutralization breadth against SARS-CoV-2 variants. The findings suggested using heterologous booster vaccines with high potency as a cost-efficient strategy against future variants.

Translation to Patients

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Highlights

Healthcare workers with heterologous COVID-19 vaccination were studied

Inoculation orders of heterologous vaccines affected serum neutralization breadth

Potency of heterologous booster vaccine was associated with neutralization breadth

Total neutralizing antibody levels were not associated with inoculation orders

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Clinical and Translational Report

Association of neutralizing breadth against SARS-CoV-2 with inoculation orders of heterologous prime-boost vaccines

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SUMMARY

Background: Emerging evidence suggests heterologous prime-boost COVID-19 vaccination as a superior strategy than homologous schedules. Animal experiments and clinical observations have shown enhanced antibody response against influenza variants after heterologous vaccination; however, whether the inoculation order of COVID-19 vaccines in a prime-boost schedule affects antibody response against SARS-CoV-2 variants is not clear.

Methods: We conducted immunological analyses in a cohort of health care workers (n = 486) recently vaccinated by three types of inactivated COVID-19 vaccines under homologous or heterologous prime-boost schedules. Antibody response against ancestral SARS-CoV-2 (Wuhan-Hu-1) was assessed by total antibody measurements, surrogate virus neutralization tests, and pseudovirus neutralization assays (PNA). Furthermore, serum neutralization activity against SARS-CoV-2 variants of concern was also measured by PNA.

Findings: We observed strongest serum neutralization activity against the widely circulating SARS-CoV-2 variant B.1.617.2 among recipients of heterologous BBIBP-CorV/CoronaVac and WIBP-CorV/CoronaVac. In contrast, recipients of CoronaVac/BBIBP-CorV and CoronaVac/WIBP-CorV showed significantly lower B.1.617.2 neutralization titers than recipients of reverse schedules. Laboratory tests revealed that neutralizing activity against common variants but not the ancestral SARS-CoV-2 was associated with the inoculation order of heterologous prime-boost vaccines. Multivariable regression analyses confirmed this association after adjusting for known confounders.

Conclusions: Our data provide clinical evidence of inoculation order-dependent expansion of neutralizing breadth against SARS-CoV-2 in recipients of heterologous prime-boost vaccination and call for further studies into its underlying mechanism.

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Context and significance

Many people around the world have received different types of COVID-19 vaccines in their two-dose vaccination schedules for various reasons. However, it is not clear whether the inoculation order of such heterologous vaccines was associated with subsequent immune responses. Here, an international team of physicians and scientists from China and the United States studied a cohort of healthcare workers who were among the earliest recipients of heterologous COVID-19 vaccination. The authors found that the inoculation order of heterologous vaccines was associated with the capability of neutralizing SARS-CoV-2 variants but not the original strain that the vaccines were based on. The results suggested that using heterologous booster vaccines with high potency could be a cost-efficient way to elicit protective immunity against future variants.

INTRODUCTION

The repeated emergence of severe acute respiratory distress syndrome coronavirus 2 (SARS-CoV-2) variants with varying levels of antigen drift limits the effectiveness of

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global vaccination programs and prolongs the COVID-19 pandemic.[1–3] Most existing COVID-19 vaccines are based on the spike protein of the ancestral SARS-CoV-2 strain, which have showed reduced protection against later variants such as B.1.351 (beta) and P.1 (gamma) with E484/K417 mutations of the spike.[4] Recently, heterologous prime-boost vaccination with adenovirus-based ChAdOx1 nCoV-19 and one of the mRNA-based vaccines has become a popular proposal to overcome the immune evasion of SARS-CoV-2 by eliciting stronger antibody response.[5–7] However, many countries without access to these vaccines rely on inactivated COVID-19 vaccines with weaker immunogenicity, which are at a natural disadvantage in protecting against immune evasive variants.[8,9] Inactivated COVID-19 vaccines are usually inoculated in a homologous prime-boost schedule to improve their immunogenicity.[10,11] Interestingly, heterologous prime-boost schedules of inactivated influenza vaccines have shown to elicit antibody response with increased neutralizing breadth against variants than homologous prime-boost schedules in both animal studies and clinical observations.[12,13] We therefore sought to assess serum neutralization activity against SARS-CoV-2 and variants of concern in those who received homologous or heterologous prime-boost vaccination and investigate whether the inoculation order was associated with neutralizing capacity or breadth.

RESULTS

We enrolled healthcare workers of Xiangyang Central Hospital, a large regional medical center in central China with more than 3,900 full-time employees, who were vaccinated prior to or during the institution-wide campaign in March to May 2021 and had thus received the second dose 1 to 3 months before blood collection, an ideal period to evaluate immune responses. Since heterologous prime-boost schedules were only allowed using inactivated vaccines, 486 recipients of three types of inactivated COVID-19 vaccines, BBIBP-CorV (BB, based on SARS-CoV-2 strain HB01) and WIBP-CorV (WB, based on WIV04) manufactured by SinoPharm,[14] and CoronaVac (CV, based on CN2) manufactured by Sinovac[15] in homologous or heterologous prime-boost schedules, were included in this study (Figure 1). All three vaccines were designed to be inoculated in homologous prime-boost schedules. However, due to unforeseeable availability of specific vaccines, recipients were allowed to voluntarily receive different inactivated vaccines as the booster dose to avoid long wait times of the booster dose. In this cohort, median (interquartile range [IQR]) dosing interval and interval between second dose and sample collection were 35 (29–46) and 53 (38–78) days, respectively. Regarding vaccination schedules, 283 (58%) received homologous of BB (24%), WB (13%), or CV (22%), whereas 203 (42%) received heterologous schedules in one of the six possible combinations of BB/WB/CV. Sex assigned at birth, allergy history prevalence, and adverse event rates were comparable among three homologous schedules and between each pair of heterologous schedules in reverse order (Table 1). Recipients of homologous BB had slightly older ages (median [IQR], BB/WB/CV, 32 [25–40]/25 [22–30]/27.5 [24–38], $p = 0.001$) and longer dosing intervals (median [IQR], BB/WB/CV, 39 [29–61]/37 [31–44]/33 [29–43], $p = 0.012$) and between second dose and blood collection (median [IQR], BB/WB/CV, 55.5 [38–100]/54 [43–77]/51 [31–64], $p = 0.017$) than recipients of other homologous schedules (Table 1), since BB was the first vaccine available in this region and was in short supply later. Clinical qualitative tests showed that 95.7%, 94.3%, and 96.7% of participants were positive for total, immunoglobulin (Ig) G, and neutralizing antibodies targeting SARS-CoV-2 spike protein, respectively, and homologous WB was the only group with less than 90% seroconversion (Table 1).

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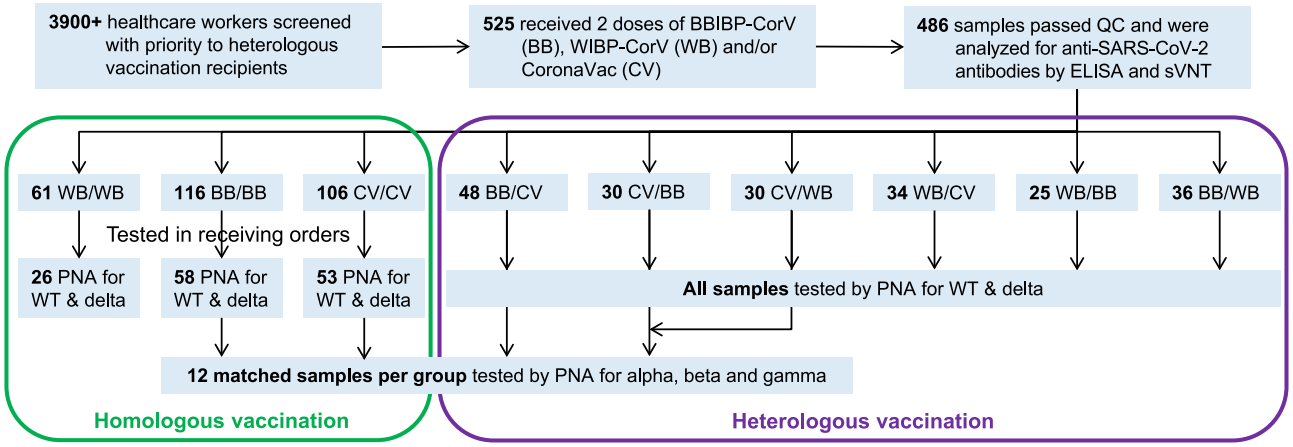

Figure 1. Flow chart of participant enrollment and grouping

Chemiluminescent microparticle immunoassays (CMIAs) were used to quantitatively measure serum levels of total antibodies targeting the receptor binding domain (RBD) of SARS-CoV-2 spike protein. The linear range of CMIA was assessed by serial dilutions of CR3022 monoclonal antibody targeting the RBD (signal/cutoff [S/CO] 0.13–880, R^2 = 0.9984) and validated by serial dilutions of serum samples with the highest readout in this study (signal/cutoff [S/CO] 0.05–612, R^2 = 0.9980) (Figures S1A and S1B). Among recipients of homologous schedules, CV elicited more total anti-RBD antibodies than BBIBP and WIBP (Figure 2A). Heterologous BB/CV could further increase total anti-RBD antibody levels compared with homologous CV, while the inoculation orders of heterologous vaccines were not associated with antibody levels (Figure 2A).

Serum neutralization activity against the ancestral SARS-CoV-2 strain Wuhan-Hu-1 was measured by both surrogate virus neutralization tests (sVNT) for all samples and pseudovirus neutralization assays (PNA) in receiving order for homologous schedule samples and for all samples of heterologous schedules. Serum inhibition percentages of surrogate virus binding were in line with qualitative results and anti-RBD antibody levels, which showed superior neutralizing activity among both homologous and heterologous CV recipients regardless of inoculation orders (Figure 2B). Neutralization titers of Wuhan-Hu-1 PNA were largely in agreement with the sVNT results with the exception of a better performing homologous WB group (Figure 2C), which may arise from the difference of SARS-CoV-2 strains used in the manufacturing of BB and WB.[14] These data suggested that the immune response against the ancestral SARS-CoV-2 was not associated with the inoculation order of heterologous vaccines.

We next measured serum neutralization activity against circulating SARS-CoV-2 variants using PNA. Currently, the globally dominant variant B.1.617.2 (delta) has shown moderate resistance to neutralization by convalescent and vaccinated serum.[2,16] In this cohort, neutralization titers of homologous schedule groups against B.1.617.2 were proportionally reduced compared with their titers against Wuhan-Hu-1 (Figure 2D). In contrast, neutralization titers of heterologous schedule groups against B.1.617.2 were strongly depending on the inoculation order of vaccines with up to 10-fold difference in neutralization titers between reverse schedules (Figure 2D). Multivariable regression models confirmed that the inoculation order of heterologous vaccines was associated with neutralization titers against B.1.617.2

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Table 1. Demographic and serological characteristics of participants

		Homologous vaccination				Heterologous vaccination								
	Overall (n = 486)	BB (n = 116)	WB (n = 61)	CV (n = 106)	p[a]	WB/BB (n = 25)	BB/WB (n = 36)	p[a]	CV/BB (n = 30)	BB/CV (n = 48)	p[a]	CV/WB (n = 30)	WB/CV (n = 34)	p[a]
Age, years (IQR)	30 (24–43)	32 (25–40)	25 (22–30)	27.5 (24–38)	0.001	27 (23–40)	32 (26–45.5)	0.078	35.5 (25–47)	38 (28–45)	0.902	45 (25–50)	40 (27–53)	0.741
Female at birth, n (%)	319 (65.6)	81 (69.8)	44 (72.1)	75 (70.8)	0.950	14 (56.0)	25 (69.4)	0.416	15 (50.0)	28 (58.3)	0.493	17 (56.7)	20 (58.8)	1.000
Allergy history, n (%)	9 (1.9)	0 (0)	3 (4.9)	4 (3.8)	0.074	0 (0)	0 (0)	/	0 (0)	0 (0)	/	1 (3.3)	1 (2.9)	1.000
AE Grade 1, n (%)	24 (4.9)	5 (4.3)	3 (4.9)	5 (4.7)	0.787	1 (4.0)	3 (8.8)	0.395	0 (0)	1 (2.1)	1.000	4 (13.3)	2 (5.9)	0.407
AE Grade 2, n (%)	2 (0.4)	0 (0)	0 (0)	1 (0.9)		1 (4.0)	0 (0)		0 (0)	0 (0)		0 (0)	0 (0)	
Dosing interval, days, median (IQR)	35 (29–46)	39 (29–61)	37 (31–44)	33 (29–43)	0.012	40 (30–51)	36.5 (29.5–45)	0.437	35.5 (31–42)	35.5 (29–46.5)	0.992	32 (23–44)	34 (29–42)	0.264
Second dose to sample collection, days, median (IQR)	53 (38–78)	55.5 (38–100)	54 (43–77)	51 (31–64)	0.017	68 (40–89)	44 (40–50)	0.060	65 (39–80)	51 (40–81)	0.267	57 (24–70)	48 (42–64)	0.716
Positive anti-spike antibodies, n (%)	465 (95.7)	110 (94.8)	50 (82.0)	105 (99.1)	<0.001	24 (96.0)	34 (94.4)	1.000	30 (100)	48 (100)	/	30 (100)	34 (100)	/
Positive anti-spike IgG, n (%)	458 (94.2)	102 (87.9)	51 (83.6)	103 (97.2)	0.014	24 (96.0)	36 (100)	0.410	30 (100)	48 (100)	/	29 (96.7)	34 (100)	0.469
Positive sVNT, n (%)	470 (96.7)	111 (95.7)	53 (86.9)	105 (99.1)	0.002	24 (96.0)	35 (97.2)	1.000	30 (100)	48 (100)	/	30 (100)	34 (100)	/

Abbreviations: AE, adverse events; BB, BBIBP-CorV; CV, CoronaVac; IQR, interquartile range; sVNT, surrogate virus neutralization test; WB, WIBP-CorV.

[a]p values were calculated by Kruskal-Wallis tests (between BB, WB, and CV) or Mann-Whitney *U* tests (between heterologous vaccination pairs) for continuous variables and chi-square tests (between BB, WB, and CV) or Fisher's exact tests (between heterologous vaccination pairs) for categorical variables. "/" indicates that tests were not performed due to categorical variables with only one value.

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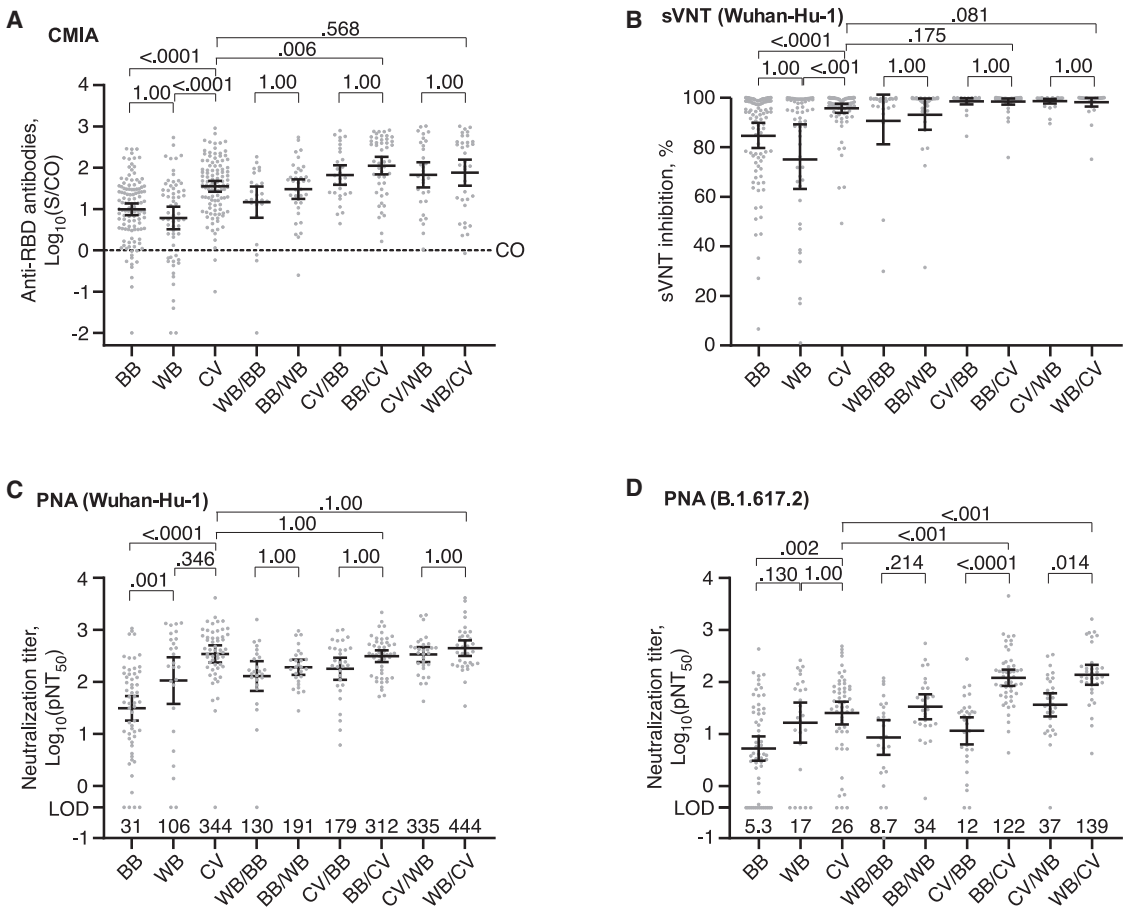

Figure 2. Antibody responses and neutralization activities elicited by homologous and heterologous COVID-19 vaccines

(A) Scatterplots of serum anti-RBD antibody levels measured by CMIA. Dotted line indicates the assay cutoff (CO) at 1. n (left to right) = 116, 61, 106, 25, 36, 30, 48, 30, 34. See also Figure S1.

(B) Scatterplots of sVNT inhibition percentages of serum samples. n (left to right) = 116, 61, 106, 25, 36, 30, 48, 30, 34.

(C and D) Scatterplots of serum neutralization titers against Wuhan-Hu-1 (C) or B.1.617.2 (D) measured by PNA. Geometric mean titer of each group is listed above respective x axis label. n (left to right) = 58, 26, 53, 25, 36, 30, 48, 30, 34. All plots display geometric means with 95% confidence interval (CI). Statistical significance was assessed by Kruskal-Wallis tests followed by Dunn's multiple comparisons tests and the adjusted p value of each post hoc comparison was indicated in the figure. Omnibus p values for all four panels were <0.0001. BB, BBIBP-CorV; WB, WIBP-CorV; CV, CoronaVac.

but not Wuhan-Hu-1 after adjusting for known confounders including age, sex assigned at birth,[17] dosing interval,[18] and interval between second vaccination and sample collection (Table S1).[19] These findings suggested that the inoculation order of heterologous vaccines was associated with vaccine-elicited neutralizing antibodies against variant B.1.617.2 but not the ancestral strain.

To investigate whether such variant-specific neutralization boost was due to an overall increased neutralizing breadth or sporadic cases of "super-immunity,"[20] we calculated B.1.617.2 to Wuhan-Hu-1 ratios of neutralization titers for heterologous vaccinated samples and found few outliers but rather uniformed swing of ratios between groups of reverse schedules (Figure 3A). Moreover, the data indicated that the choice of booster vaccine alone in heterologous schedule was associated with neutralizing breadth (Figure 3C), and there was an apparent correlation of neutralizing breadth with the immunogenicity of booster vaccines (Figures 2A–2C). To further validate the neutralizing breadth against other variants, we chose 12 age- (±5 years), gender-, and second vaccination-to-sample-collection interval-matched

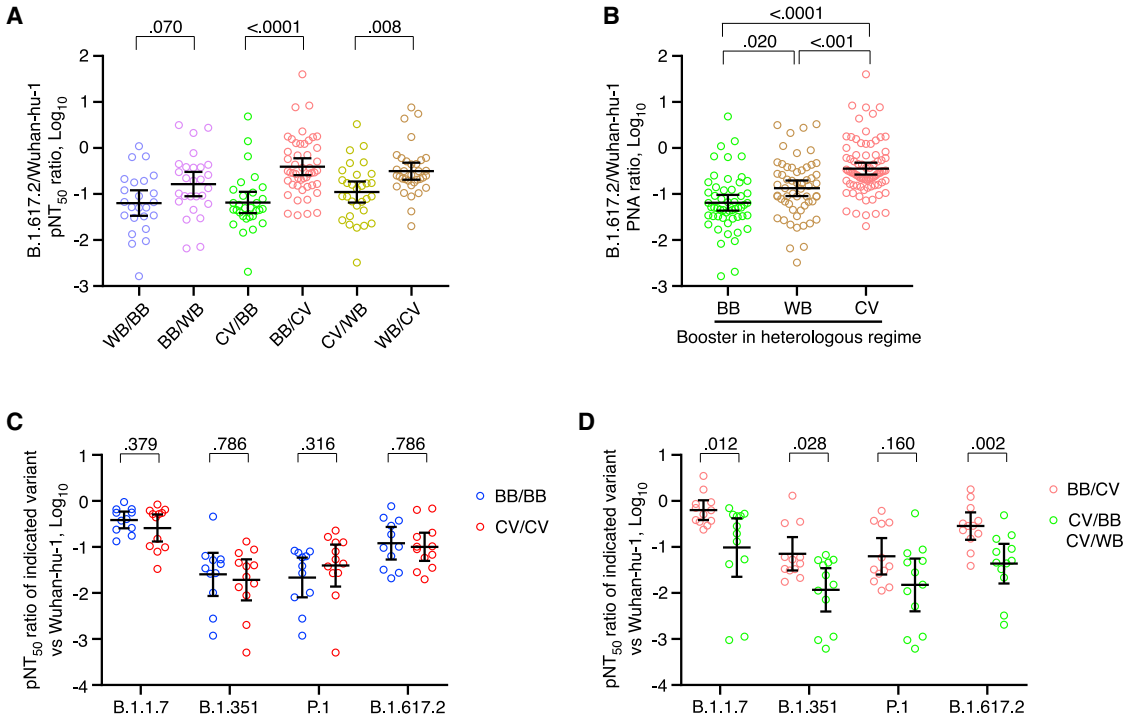

Figure 3. Inoculation order-dependent neutralizing breadth against SARS-CoV-2 variants after heterologous prime-boost vaccination

(A and B) Scatterplots of serum neutralization titer ratios of B.1.617.2 versus Wuhan-Hu-1 grouping by vaccination schedules (A) or the booster type of heterologous regimes (B). n (left to right) = 25, 36, 30, 48, 30, 34 (A) and 54, 57, 82 (B). Statistical significance was assessed by Kruskal-Wallis tests followed by Dunn's multiple comparisons tests and the adjusted p value of each post hoc comparison was indicated in the figure. Omnibus p values for both panels were <0.0001.

(C and D) Scatterplots of serum neutralization titer ratios of each variant of concern versus Wuhan-Hu-1 from matched samples that received homologous BB or CV (C) or heterologous BB/CV or CV/BB + CV/WB vaccination. n = 12. Statistical significance was assessed by independent Mann-Whitney *U* tests and the p value of each test is indicated in the figure. See also Figure S2. BB, BBIBP-CorV; WB, WIBP-CorV; CV, CoronaVac.

(±10 days) samples from each group of homologous or heterologous schedules of the stronger CV and less potent BB vaccines to undergo PNA of other variants of concern, B.1.1.7 (Alpha), B.1.351, and P.1 (Figures S2A–S2D). Of note, CV/BB and CV/WB groups were combined due to fewer than 12 matched samples in the CV/BB group alone and their similar neutralization profiles against Wuhan-Hu-1 and B.1.617.2 (Figures 2C and 2D). The variant-to-ancestral ratios showed that while homologous BB and CV showed comparable neutralizing breadth against all four variants (Figure 3C), BB/CV exhibited significantly enhanced neutralizing breadth than CV/BB, with only the ratios of P.1 not reaching statistical significance due to higher intra-group variation and limited sample size (Figure 3D). In addition, PNA of the recently emerged B.1.1.529.1 variant (omicron BA.1) showed enhanced serum neutralizing activity after heterologous BB/CV vaccination compared with CV/BB vaccination (Figure S3), which was largely in agreement with recent studies of heterologous CV and BNT162b2 (Pfizer-BioNTech) vaccines.[21–23] These results together suggested that the inoculation order of heterologous prime-boost vaccines was associated with neutralizing breadth against SARS-CoV-2.

DISCUSSION

Heterologous vaccination has immense potential not only thanks to its superior immunogenicity but also due to the ongoing logistic burden of vaccine distribution that frequently delays the vital booster dose of homologous schedules.[7,24] To our knowledge, our analyses are the first report of inoculation order-dependent

expansion of neutralizing breadth against SARS-CoV-2 after heterologous vaccination, which could be translated into a promising strategy of using potent booster vaccines in heterologous prime-boost schedules to counter the antigen drift of SARS-CoV-2 variants. As we learned from seasonal influenza vaccines, mass production of variant-specific vaccines may never catch up the rate of viral mutations,[25] and heterologous vaccination might represent a more efficient strategy using existing stockpiles to protect against future variants. Furthermore, our data provide preliminary evidence that using less immunogenic vaccines as primer might not compromise the neutralization activity against variants elicited by more potent booster vaccines because of enhanced neutralizing breadth. Therefore, using abundantly available vaccines as the primer in tandem with a more potent booster vaccine may achieve the same coverage as single-dose strategy of the potent vaccine but with protection comparable with the two-dose schedule.[26]

A number of factors including antigen sequences, vaccine platforms, and adjuvants may contribute to the inoculation sequence-dependent expansion of neutralizing breadth. While our data could not provide a definitive answer regarding these possibilities, recent progress in the field suggested that vaccine platforms or antigen sequences were unlikely to play a major role here. Reference sequences of SARS-CoV-2 HB02 (used in BB), WIV04 (WB), and CN2 (CV) strains are available in GenBank. A detailed analysis of those sequences found only minimal sequence variations between these strains, which was as expected due to their close timing of isolation and geographic localization.[10,11,14] These sequence variations were unlikely to alter the immunogenic profile compared with the larger number of mutations in known variants.[27] Furthermore, the results from ChAd(Oxford–AstraZeneca)/BNT(Pfizer-BioNTech) heterologous prime-boost studies showed similar expansion of variant-reactivity breadth of neutralizing antibodies as higher beta/alpha or beta/D614G ratios of neutralization titers after heterologous ChAd/BNT vaccination compared with homologous prime-boost vaccination of either vaccine on similar schedules,[7,28] suggesting that our findings may not be platform-specific. Similar with inactivated vaccines in our study, ChAd and BNT were also based on the ancestral spike sequence with minor optimizations that should not alter their antigenicity.[29,30] In contrast, delta breakthrough patients did not gain such expanded breadth of neutralizing antibodies against the beta variant, despite a significant increase of neutralization titers against D614G and delta variants than those who received three doses of homologous mRNA vaccines.[31] Considering delta infection as an immunization event, these findings suggest that a booster based on a novel variant spike could induce stronger neutralization against this specific variant only, which is different from heterologous prime-boost vaccination that could expand neutralizing breadth not limited to specific variants. Of note, the adjuvants of BB, WB, and CV are the same according to manufacturers' instructions. Together, our and others' data strongly suggest that antigen sequence or platform variation between each vaccination is not associated with the inoculation order-dependent expansion of neutralizing breadth.

Nonetheless, our data did not rule out the association of heterologous vaccine antigens, regardless of inoculation order, with the neutralizing breadth, since prior studies have shown that immunization using different variants of the same antigen could further expand antibody cross-reactivity against other variants in an animal model.[32] Therefore, development of variant-specific vaccines would still be of high value in containing future variants, while our findings would offer an anti-variant strategy based on existing vaccine stockpile, which is currently more feasible given the shortage of potent vaccines in many regions.

We are also curious about the underlying molecular mechanism of our findings. Increasing the neutralizing breadth of vaccine-induced antibodies is a fundamental goal in vaccine development, and heterologous vaccination has been tested in this aim before. Despite the long-known advantage of heterologous vaccination, the effect of inoculation order was often overlooked. One of the few relevant studies examined DNA/Ad heterologous vaccination against HCV.[33] Homologous Ad vaccination was more potent than homologous DNA vaccination. Similar to our findings, among all four schedules (DNA/DNA, Ad/Ad, DNA/Ad, and Ad/DNA), the DNA/Ad schedule induced not only the highest T cell response but also T cells binding strongly with more E2 epitopes. On the contrary, Ad/DNA schedule induced T cells binding most weakly with E2 epitopes, despite a decent T cell response. These findings resonate with ours that the breadth of immune response may be associated with the immunogenicity of the booster vaccine in a heterologous prime-boost schedule. Mechanistically, it was found that DNA/Ad induced strikingly high $CD4^+$ T cells against target antigens. Intriguingly, a recent report of heterologous ChAd/BNT vaccination found that spike-specific $CD8^+$, not $CD4^+$, T cells were highly enriched after ChAd/BNT vaccination compared with ChAd/ChAd or BNT/BNT vaccination.[34] We therefore speculate that certain adaptive immune pathways might be involved in this expansion of immune responses. In fact, recent studies have revealed that prolonged or repeated exposure to SARS-CoV-2 antigens could enhance neutralizing potency and breadth via affinity maturation.[35] We have also shown that unvaccinated survivors of severe COVID-19 underwent long-term post-recovery expansion of neutralizing breadth, a feature that was not found among mild to moderate COVID-19 survivors or vaccine recipients.[36] Since our data have shown that CV was more immunogenic than the other two vaccines, it is possible that this superior immunogenicity led to enhanced or prolonged affinity maturation only after the inoculation of a booster dose.

In summary, our data provide clinical evidence of inoculation order-dependent expansion of neutralizing breadth against SARS-CoV-2. Pending further validation of our conclusions, heterologous vaccination in a weak/potent order may represent an efficient strategy to achieve optimal protection against SARS-CoV-2 variants with existing vaccine stockpiles.

Limitations of the study

This study has several limitations. First, the non-randomized and observational nature made the conclusions prone to confounding effects and precluded any causal interpretation of the findings. Since heterologous vaccination was approved later than homologous schedules due to lengthy waiting for a matched vaccine, time intervals between the second vaccination and sample collection were shorter in contrast to longer dosing intervals among recipients of heterologous schedules in this cohort. Furthermore, since heterologous vaccination in the prime-boost schedule with 1-month interval between shots was not a standard practice for the public at the time of this study, the participants were limited to healthcare workers and in limited quantity. Of note, we have used multivariable regression models to adjust for these potential confounders in relevant analyses. Nonetheless, we would caution against interpreting our data as the indicator of immunogenicity or efficacy of homologous vaccination schedules. Also, it is worth noting that previous evidence of heterologous vaccination-induced enhancement of variant-reactivity was found among recipients of inactivated vaccines and all three vaccines used in this study are also inactivated vaccines, which may limit the generalization of the above conclusions to vaccines developed on other platforms.

Other limitations of this study include the lack of neutralization assays based on live viruses. The use of PNA instead of live virus neutralization assays limited the interpretation to the inhibition of first-round viral entry, while the inhibition of viral replication and secondary infection was not assessed in this study. Last, our sample size was not sufficient to explore potential adverse events associated with heterologous vaccination, which were reported to be more frequent than those associated with homologous vaccination.[37] We thus caution proactive implementation of heterologous vaccination before more evidence on its adverse effects emerge.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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  - Pseudovirus neutralization assays
- QUANTIFICATION AND STATISTICAL ANALYSIS

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.medj.2022.05.003.

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AUTHOR CONTRIBUTIONS

Y.Zhu and P.H. conceived and designed the study; Y.Zhu, C.Z., T.G., Y.W., R.L., Y.Li, L.W., Y.Zhan, J.C., J.G., J.W. and Linyu Zhu recruited participants and collected specimens; Y.Zhu, C.Z., Y.W., R.L., Y.Li, T.G., L.W., and Z.C. conducted clinical laboratory tests; Y.Lu, G.T., M.G., W.Z., J.Q., Longchao Zhu, and F.X. conducted PNA; Y.Zhu, Y.Lu, and P.H. had unrestricted access to all data and conducted statistical analyses; M.C., Y.X., Z.Zheng, Z.Zhou, and Z.C. interpreted clinical and statistical findings; Y.Lu and P.H. wrote the original draft; Y.Zhu, Y.Lu, Z.Zhou, Z.C., and P.H. reviewed and revised the manuscript. All authors agreed to submit the manuscript, read and approved the final draft, and take full responsibility for its content, including the accuracy of the data and the fidelity of the study to the registered protocol and its statistical analysis.

DECLARATION OF INTERESTS

The authors declare no competing interests.

INCLUSION AND DIVERSITY

We worked to ensure gender balance in the recruitment of human subjects. The author list of this paper includes contributors from the location where the research was conducted who participated in the data collection, design, analysis, and/or interpretation of the work.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Recombinant Anti-SARS-CoV-2 Spike Glycoprotein S1 antibody [CR3022] - Chimeric	Abcam	AB_2847846
Critical commercial assays		
2019-nCoV antibody detection kit [ELISA]	InnoDx	2019-nCoV-Ab
2019-nCoV IgG antibody detection kit [ELISA]	InnoDx	2019-nCoV-IgG
2019-nCoV neutralizing antibody detection kit	InnoDx	2019-nCoV-NAb
2019-nCoV antibody detection kit [CMIA]	InnoDx	2019-nCoV-CMIA
Experimental models: Cell lines		
HEK293/ACE2 cells, replication-defective	GenScript	M00770
Experimental models: Organisms/strains		
SARS-CoV-2 Wuhan-Hu-1 pseudovirus, Luc reporter	GenScript	SC2087A
SARS-CoV-2 B.1.1.7 pseudovirus, Luc reporter	GenScript	SC2087K
SARS-CoV-2 B.1.351 pseudovirus, Luc reporter	GenScript	SC2087L
SARS-CoV-2 P.1 pseudovirus, Luc reporter	Vazyme Biotech	DD1546
SARS-CoV-2 B.1.617.2 pseudovirus, Luc reporter	GenScript	SC2087V
SARS-CoV-2 B.1.1.529.1 pseudovirus, Luc reporter	Vazyme Biotech	DD1768
Software and algorithms		
SPSS 26	IBM	https://www.ibm.com/analytics/spss-statistics-software
Prism 9	GraphPad	https://www.graphpad.com
Other		
Caris 200 CMIA analyzer	UMIC Medical Instrument	Caris200

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Peng Hong (peng.hong@downstate.edu).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- All data reported in this paper will be shared by the lead contact upon request.
- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Human sample

This study was based on a prospective observational cohort of hospital staff located in Xiangyang city of Hubei, China, where eligible healthcare workers were required to receive prime-boost COVID-19 vaccines. The cohort included participants 18 years of age or older who had received at least two doses of COVID-19 vaccines at least 7 days before the date of blood collection. Participants with the following conditions were excluded: (a) prior COVID-19 diagnosis or positive serological tests, (b) under immune-modulatory medication, (c) active infection or any inflammatory

disease, (d) coagulation disorder or other conditions precluding safe blood collection, (e) physician assessment, laboratory examination, or any other conditions making the subject unsuitable for the study. For an effect size of 0.15 at 5% significance level and 90% power for the neutralization titers, the cohort requires 459 subjects that complete the study with equal distribution between two groups. A maximum of 1,000 subjects will be enrolled. The study protocol (#2021-034) was approved by the Medical Ethics Committee of Xiangyang Central Hospital.

Participants information on sex, age, and race was self-reported. Information on socioeconomic status was not collected. Dates of vaccination and manufacturers of vaccines were provided by participants after signing the informed consent. The participants were asked for the history of allergy and chronic disease and medication taken since the first vaccine dose. Adverse effects potentially related to vaccination were also recorded. After confirming the eligibility of participation, 5 mL of venous blood was collected from the participant and proceeded to serum isolation immediately. Serum samples were aliquoted for each assay to minimize freeze-thaw cycles.

METHOD DETAILS

Serological assays

SARS-CoV-2 serology was determined by both qualitative and quantitative assays. Anti-spike total and IgG antibodies were separately assessed by qualitative ELISA kits (2019-nCoV antibody detection kit [ELISA], InnoDx; 2019-nCoV IgG antibody detection kit [ELISA], InnoDx) according to manufacturer's instructions. Cutoff value was calculated as 0.16 + the average of negative control readouts. Samples with readouts equal to or higher than the cutoff value were deemed positive.

Neutralizing antibodies were assessed by a surrogate virus neutralization assay kit (2019-nCoV neutralizing antibody detection kit, InnoDx) according to manufacturer's instructions. Results were expressed as inhibition percentages calculated by the following formula: inhibition percentage = (negative control value – sample value) × 100%/negative control value. Samples with inhibition percentages equal to or higher than 50% were deemed positive.

Quantitative CMIA for total anti-RBD antibodies (2019-nCoV antibody detection kit [CMIA], InnoDx) was performed on a Caris200 analyzer (UMIC Medical Instrument) following manufacturer's instructions. Cutoff value was calculated according to manufacturer's instructions. The assay sensitivity and specificity were 94.8 and 99.7% according to the manufacturer, and 90.8 and 98.9% in an independent study,[38] respectively. S/CO values less than 0.01 were recorded as 0.01 for all analyses.

Quality control checks of all serological assays were conducted according to manufacturer's instructions. Samples failing any checks were re-tested after necessary procedures to improve quality. Samples repeatedly failing checks or without sufficient volume for further re-test were excluded from the analysis.

Pseudovirus neutralization assays

Seum neutralization titers against Wuhan-Hu-1 (GenScript), B.1.1.7 with Δ69-70, Δ144, N501Y, A570D, D614G, P681H, T716I, S982A and D1118H mutations (GenScript), B.1.351 with L18F, D80A, D215G, Δ242–244, R246I, K417N, E484K, N501Y, D614G and A701V mutations (GenScript), P.1 with L18F, T20N, P26S, D138Y, R190S, K417T, E484K, N501Y, D614G, H655Y and T1027I mutations (Vazyme Biotech), B.1.617.2 with T19R, Δ156–158, L452R, T478K, D614G, P681R

and D950N mutations (GenScript), and B.1.1.529.1 pseudoviruses with A67V, Δ69–70, T95I, Δ142-144, Y145D, Δ211, L212I, ins214EPE, G339D, S371L, S373P, S375F, K417N, N440K, G446S, S477N, T478K, E484A, Q493R, G496S, Q498R, N501Y, Y505H, T547K, D614G, H655Y, N679K, P681H, N764K, D796Y, N856K, Q954H, N969K, L981F mutations (Vazyme Biotech) were measured by PNA. All PNA procedures were performed in a Biosafety Level 2 laboratory. In brief, HIV pseudoviruses carrying a luciferase reporter and encapsulated in ancestral or variant spike proteins were incubated with eight 4-fold serial dilutions of the serum sample by Opti-MEM (Gibco) for 1 h at room temperature. The mixture was then added to the culture of replication-deficient HEK293/ACE2 cells in 96-well plates with DMEM (Gibco)/10% FBS (Gibco)/1× antibiotics (Gibco) and incubated in a humidified cell culture chamber at 37 °C with 5% CO2 for 48 h. Medium was removed at the end of incubation, and 50 μL one-step luciferase detection reagent (GenScript) was added to each well. Luminescence in relative light units (RLUs) was measured by a luminometer (Synergy H1, BioTek Instruments) after 3 min of incubation at room temperature. Serum samples may be diluted to meet the initial volume requirement. Samples without maximum RLUs equal to 100 times of cell-only controls were tested again with dilution of the initial sample when necessary. Samples failed to yield meaningful results due to quality issues or limited volumes were excluded from analyses. RLUs of sample wells were normalized with positive control wells and pNT_{50} was calculated as EC_{50} by normalized four-parameter sigmoid curve fit in Prism 9.0 (GraphPad). pNT_{50} was arbitrarily set to 0.39, the limit of detection (LOD) of PNA, when EC_{50} was lower than the LOD or not computable due to low neutralization activity. The LOD was calculated as mean +1.96 × SD of the Wuhan-Hu-1 pNT_{50} of 12 serologically negative samples from healthy donors.

QUANTIFICATION AND STATISTICAL ANALYSIS

Participant characteristics and serological data were assessed with Chi-square tests for categorical variables or Kruskal-Wallis tests for continuous variables. Post hoc comparison methods were detailed in figure legends. Serological data were log-transformed before being analyzed by regression models. Confounders in multivariable regression models were selected based on documented associations and listed in Table S1. Analyses were performed using SPSS 26 (IBM) or Prism 9 (GraphPad). Missing data were excluded pairwise from analyses. Significance was evaluated at $\alpha = 0.05$ and all tests were 2-sided.