Heterologous BBIBP-CorV/ZF2001 vaccination augments neutralization against SARS-CoV-2 variants: A preliminary observation

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Vaccination to severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is pivotal for reducing deaths and severe illness from coronavirus disease 2019 (COVID-19).1 BBIBP-CorV as an inactivated virus vaccine and ZF2001 as a recombinant tandem-repeat dimeric receptor-binding domain (RBD) protein vaccine are two safe, immunogenic COVID-19 vaccines, used in China and several countries.2,3 Since the beginning of the COVID-19 pandemic, SARS-CoV-2 variants are continually emerging and circulating around the world.4 Variants of Concern (VOCs) designated by World Health Organization (WHO) currently include B.1.1.7 (Alpha), B.1.351 (Beta), P.1 (Gamma) and B.1.617.2 (Delta), B.1.1.529 (Omicron) lineages,5 with more rapid transmission and/or more challenges for the effectiveness of public health intervention and vaccines available. The emergence of SARS-CoV-2 variants is recognized as one of the reasons that led to breakthrough infections in the population of vaccination.2 Thus, one of the major public concerns is the administration of booster doses against COVID-19 pandemic, including the heterotypic vaccines.7,8

In this study, seven volunteers (two males and five females) were recruited and each received two doses of the inactivated SARS-CoV-2 vaccine (BBIBP-CorV) with a 28-days interval. Then, they received a dose of the recombinant ZF2001 at 4-5 months after receiving the second dose of BBIBP-CorV. Sera samples were collected from the volunteers at 1 month after receiving the second dose of BBIBP-CorV, and also at around 2-3 months and at 5-6 months after receiving a dose of ZF2001 (Figure 1A, Supplemental Table 1). The study was approved by the Ethics Committee, and all candidates signed the written informed consent.

Given the positive correlation between neutralizing titer and protection efficacy,5 we assessed 50% pseudovirus neutralization titer (pVNT50) of all sera from the volunteers before and after receiving heterologous BBIBP-CorV/ZF2001 vaccination (Figs. S1 and S2). The median of pVNT50 of sera from volunteers that received a dose of ZF2001 after 2-3 months showed 51.9 (p = 0.0625, interquartile range (IQR) 35.8–89.7, prototype (WH-01)), 66.5 (*, p = 0.0313, IQR 46.8–626.0, B.1.1.7), 221.3 (*, p = 0.0313, IQR 44.9–564.6, B.1.351), 64.8 (*, p = 0.0313, IQR 38.5–120.5, P.1), 55.4 (p = 0.0625, IQR 25.0–220.2, B.1.617.2) and 79.9 (p = 0.0625, IQR 46.8–566.3, B.1.617.1) fold, respectively, higher compared with the ones that received the second dose of BBIBP-CorV (Figures 1B and D, S3). Although pVNT50 against B.1.1.529 (Omicron) from the volunteers that received the second dose of BBIBP-CorV were below the lower limit, the GMT of pVNT50 was elevated to 234.1 after receiving a dose of ZF2001 was about 116.7 fold change of median (*, p = 0.0313, IQR 42.6–254.1) (Figure 1C, D). This result indicated that the humoral response to SARS-CoV-2 variants has been boosted when most volunteers received a dose of ZF2001 after receiving the two doses of BBIBP-CorV. And our results are concordant with the previous study about that heterologous vaccination of protein subunit vaccine significantly recalled and increased the humoral and cellular immune responses against SARS-CoV-2 and its variants.8,9,10

Moreover, we further evaluated the longevity of neutralizing activity against SARS-CoV-2 variants stimulated by the heterologous BBIBP-CorV/ZF2001 vaccination. Notably, at 5-6 months after the boost dose of ZF2001, the neutralization potency was still higher...
than after receiving the second dose of BBIBP-CorV, for
the median of pVNT\text{50} against all strains, i.e. prototype (20.6 fold, \( p = 0.125, \text{IQR} 14.7-38.6\)), B.1.1.7 (35.2 fold, \( p = 0.0625, \text{IQR} 28.1-403.2\)), B.1.351 (36.0 fold, \( p = 0.0625, \text{IQR} 18.1-249.6\)), P.1 (27.1 fold, \( p = 0.0625, \text{IQR} 9.3-58.2\)), B.1.617.2 (20.6 fold, \( p = 0.125, \text{IQR} 18.9-129.5\)), B.1.617.1 (45.9 fold, \( p = 0.125, \text{IQR} 14.1-304.4\)) and B.1.1.529 (20.4 fold, \( p = 0.0625, \text{IQR} 15.3-64.4\)) respectively (Figures 1B \(-D, S3\)). This result showed that the neutralizing antibody titer against variants was elicited and maintained a long-lasting recall humoral response. Recent research showed that the boosting 3-dose inactivated vaccinees could recall long-lasting humoral responses until 6 months.\(^{11}\)

Additionally, we also evaluated the changes in the proportion of the neutralizing titer against the SARS-CoV-2 variants as compared with the prototype (WH-01) after receiving a dose of ZF2001. At 2-3 months and 5-6 months after receiving a dose of ZF2001, the neutralizing titer against B.1.1.7 (Alpha) and B.1.617.2 (Delta) variants retain roughly equivalent as compared with the prototype, and the changes of median were 1.08 folds (\( p > 0.05, \text{IQR} 0.97-1.53\)) and 1.13 folds (\( p > 0.05, \text{IQR} 1.13-1.35\)) for Alpha to prototype, and 1.00 folds (\( p > 0.05, \text{IQR} 0.79-1.12\)) and 1.32 folds (\( p > 0.05, \text{IQR} 1.23-1.62\)) for Delta to prototype, respectively. However, the B.1.351 (Beta) and B.1.617.1 (Kappa) variants showed more pronounced reduction in sensitivity, and the changes of median were 0.64 folds (\( p > 0.05, \text{IQR} 0.26-0.95\)) and 0.39 folds (\( p > 0.05, \text{IQR} 0.21-0.70\)) for Beta to prototype, and 0.67 folds (\( p > 0.05, \text{IQR} 0.38-0.83\)) and 0.72 folds (\( p < 0.05, \text{IQR} 0.49-0.60\)) for Kappa to prototype, respectively (Figure 1B and E). Significantly, the median of pVNT\text{50} against B.1.1.529 (Omicron) strain have 0.2 folds (\( p < 0.05, \text{IQR} 0.1-0.2\)) and 0.1 folds (\( p < 0.05, \text{IQR} 0.01-0.2\)) reduction compared to the prototype. Thus, though neutralizing activity could be boosted against the newly emerging variants, the neutralizing titers against B.1.351 (Beta), B.1.617.1 (Kappa), and B.1.1.529 (Omicron) were significantly lower than the prototype (Figure 1C and E). These results were consistent with our previous findings about the multi-boost strategy with receiving 3-dose of ZF2001 or inactivated vaccine would be beneficial for NAb against SARS-CoV-2 variants.\(^{12,13}\)

This preliminary study indicated that the heterologous BBIBP-CorV/ZF2001 vaccination could boost and maintain a long-lasting recall of humoral immune

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**Figure 1.** Neutralization antibody titers to SARS-CoV-2 and its variants in heterologous BBIBP-CorV/ZF2001 vaccinated donors. (A) Schematic representation of vaccination and blood collection time of volunteers. Syringe: date for vaccination; Tube: blood collection. (B) 50% pseudovirus neutralization titer (pVNT\text{50}) against VSV-expressing spikes from prototype (WH-01) or five SARS-CoV-2 variants in sera from 7 volunteers receiving heterologous BBIBP-CorV/ZF2001 vaccination. The variants including four VOCs (B.1.1.7, B.1.351, P.1 and B.1.617.2 lineages), and one variant of interest (B.1.617.1 lineages) listed by WHO. (C) After the emerging of the Omicron, we added the data about the cross-responses to Omicron, and repeated the prototype (WH-01) as a control. pVNT\text{50} against prototype (WH-01) or B.1.1.529 (Omicron) variants in sera from 7 volunteers receiving heterologous BBIBP-CorV/ZF2001 vaccination. The geometric mean titer (GMT) was marked on top of each column and lined with 95% confidential interval (CI) shown as horizontal bars. The dashed line indicates the lower limit of detection. The GMT lower than 5 were considered negative and calculated as 2.5 in the statistical analysis. The statistical significance was analyzed by two-tailed Wilcoxon matched-pairs signed-rank test (*, \( p < 0.05\)). (D) After the heterologous BBIBP-CorV/ZF2001 vaccination, the fold increase of median of neutralization as compared with the ones after receiving the second dose of BBIBP-CorV. The value of median has been marked under the each of the boxplot. (E) Fold change of median in neutralization titer for each variant relative to prototype at different time points. The value of median has been marked above the each of the boxplot.
responses against SARS-CoV-2 and its VOCs. In our previous study, we showed the BBIBP-CorV vaccine given three times also have evoked cross-antibody responses to different VOCs, which is concordant to the current results of heterologous BBIBP-CorV/ZF2001 vaccination. In this study, we did not involve the control BBIBP-CorV given three times with the same interval, which may be a limitation for the current findings, and the safety and effectiveness of the strategy of heterologous BBIBP-CorV/ZF2001 vaccination should be further studied through clinical trials in the future. More types of vaccines, e.g live intranasal vaccines, peptide vaccines, etc. may be needed to induce more cross-protective immunity to combat SARS-CoV-2, VOCs and other potential emerging coronaviruses.

Author contributions
GFG, WJL, YZ and XZ conceived and designed the study. YZ, XZ and WJL designed, and coordinated the experiments. YZ, RZ, BY, DZ, YG, LL and JT performed experiments. YZ, XY and LL recruited volunteers and coordinated the blood samples. YZ, XZ, YG and WJL analyzed the data. GFG, YZ and WJL drafted and revised the manuscript. All authors reviewed and approved the final manuscript. WJL have accessed and verified the data, and takes responsibility for the decision to submit the manuscript.

Data sharing statement
Data will become publicly available upon request from the corresponding author.

Declaration of interests
G.F.G. are listed in the patent as the inventors of the RBD-dimer as a betacoronavirus vaccine. The patent has been licensed to Anhui Zhifei Longcom for protein subunit COVID-19 vaccine development. All other authors declare no competing interests.

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Supplementary materials
Supplementary material associated with this article can be found in the online version at doi:10.1016/j.lanwpc.2022.100440.

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