Quality in Primary Care
ISSN: 1479-1064

Open access Research Article

Novel BA.4/5 Bivalent SARS-CoV-2 mRNA Vaccine Provides Broad Protection against SARS-CoV-2 Emerging Variants

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ABSTRACT

The worldwide pandemic is still being driven by the emergence of SARS-CoV-2 variants that appear to evade antibody neutralization, therefore, there is a constant demand to broaden vaccine-induced immunity. In this study, we assessed the immunogenicity of two bivalent mRNA vaccines that contain CS-2034, our first-generation monovalent vaccine, and an mRNA vaccine encoding the spike protein of one of the recent Omicron variants, either BA.1 or BA.4/5. When administered as a primary immunization series in BALB/c mice, the BA.4/5 bivalent vaccine induced broader neutralizing antibody responses against a variety of SARS-CoV-2 variants, including the original strain, as well as the BA.1, BA.2.75, BA.4/5, and XBB.1 variants, compared to other vaccine candidates. As a heterologous booster after the primary vaccination series with two doses of inactivated vaccine (BBIBP-CorV, Sinopharm), the BA.4/5 bivalent vaccines elicited a greater amount of neutralizing antibodies than the BBIBP-CorV homologous booster and constituent monovalent vaccines, even at lower doses. Thus, the BA.4/5 bivalent mRNA vaccine has excellent promise for clinical use against the present variants of concern (VOCs) of SARS-CoV-2.

Keywords: Bivalent mRNA vaccine; Primary immunization; Heterologous booster; COVID-19; Omicron variants

Received:	29-March-2023	**Manuscript No:**	IPQPC-23-15761
Editor assigned:	31-March-2023	**PreQC No:**	IPQPC-22-15761 (PQ)
Reviewed:	14-April-2023	**QC No:**	IPQPC-22-15761
Revised:	19-April-2023	**Manuscript No:**	IPQPC-22-15761 (R)
Published:	26-April-2023	**DOI:**	10.36648/1479-1064.23.31.007

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Citation Wang H, Li J, Chen Z, Wu D, Sui X, et al. (2023) Novel BA.4/5 Bivalent SARS-CoV-2 mRNA Vaccine Provides Broad Protection against SARS-CoV-2 Emerging Variants. Qual Prim Care. 31.007.

INTRODUCTION

The SARS-CoV-2 pandemic has resulted in more than 756 million infections and 6.8 million fatalities, making it a significant global public health emergency. In response to the worldwide public health crisis, several vaccine forms have been approved for emergency use during the SARS-CoV-2 pandemic including inactivated virion, mRNA-LNP, or viral-vectored vaccine, most of these vaccines use the spike protein derived from original strains that circulated in early 2020 as the target antigen. However, the emergence of SARS-CoV-2 variants with immune escapability dampened the effectiveness of global vaccination campaigns and virus transmission control [1-4].

To overcome the decrease in vaccine effectiveness against emerging variants, we have developed a vaccine, CS-2034, which is currently in clinical trials. This vaccine encodes the full-length SARS-CoV-2 spike protein based on the original strain and contains four convergent mutations found in different variants: K417N, E484K, N501Y, and D614G. To further broaden the immunity, especially against Omicron strains, we evaluated the neutralizing antibody responses against the prevailing circulating Omicron variants in mice with two bivalent mRNA vaccines which consist of a 1:1 mixture of CS-2034, our first-generation vaccine, and an mRNA vaccine based on either BA.1 or BA.4/5 variant. When administered as a primary immunization series, the BA.4/5 bivalent vaccine induced broader neutralizing antibody responses than the constituent monovalent vaccines against pseudoviruses displaying original virus, BA.1, BA.2.75, BA.4/5, or XBB.1 spike proteins. As a heterologous booster after two doses of inactivated vaccine (BBIBP-CorV, Sinopharm), the BA.5 bivalent mRNA vaccine can elicit stronger neutralizing antibodies based on live virus neutralization assays using the original strain and Omicron variant BA.5 compared to the BBIBP-CorV homologous booster and constituent monovalent vaccines. All of these preclinical results demonstrated that the BA.4/5 bivalent mRNA vaccine has significant clinical potential to induce protective immunity against past and current SARS-CoV-2 strains.

MATERIALS AND METHODS

Materials and Cell Lines

Huh7 cells and African Green monkey kidney-derived Vero E6 cells (ATCC) were cultured at 37˚C with 5% CO_2 in Dulbecco's modified Eagle's medium (DMEM, GIBCO) containing 10% fetal bovine serum (FBS, GIBCO).

Animals

Groups of 6 week to 8-week-old female BALB/c mice were housed together and fed standard chow diets. For prime and boost doses, mice received 50 µL of BBIBP-CorV or formulations containing 1 µg, 3 µg, or 5 µg of mRNA *via* intramuscular injection into the same hind leg. The institutional animal care and use committee established the criteria for determining the sample size for animal experiments.

Mouse immune serum samples were heat-inactivated at 56˚C for 30 minutes before use in the neutralization assay. A pseudovirus-based neutralization assay or a live virus-based FRNT assay was used to determine the titers of neutralizing antibodies against various SARS-CoV-2 strains.

Vaccine Preparation

IVT (*in vitro* transcription) was used to generate the codon-optimized mRNAs for the prefusion-stabilized CS-2034, BA.1, and BA.4/5 from linear plasmid templates. The uridine was completely replaced by N1m-pseudouridine. The open reading frame of the S protein was included in the DNA templates, along with an optimized 5'-capped 5'-UTR and an optimized 3'-UTR, as well as a poly (A) tail. Before formulation, the mRNA was purified following RNA transcription to remove transcription enzymes, the linear DNA template, and mRNA-related impurities. Using a mixing system, a buffer solution of mRNA and an ethanol solution of lipid (ionisable lipid, DSPC, cholesterol, and DMG-PEG2000) were combined to encapsulate the mRNA in LNPs. The LNPs were then subjected to buffer exchange and sterile filtration to obtain the finished products.

Pseudo-virus Based Neutralization Assay

Mouse serum samples were heat-inactivated at 56˚C for 30 minutes to conduct the neutralization assay. Serial 3-fold dilutions were made using DMEM containing 10% FBS. After mixing the VSV-based SARS-Cov-2 pseudovirus (650 TCID50 per well) with the diluted sera in duplicate in a 1:1 (v/v) ratio, the hexaplicate was incubated at 37˚C for an hour with the virus control and cell control wells. Each well was then filled with freshly trypsinized Huh7 cells (2×10^4/well). The Luciferase Assay System (Promega) was used to measure the luminescence after 20-28 hours of incubation at 37˚C with 5% CO_2. The dilution at which the relative light units were reduced by 50% in comparison to the virus control wells (virus+cells) after subtraction of the background in the control groups was referred to as pVNT50. The Reed-Muench method was used to determine pVNT50.

FRNT Assay

After being seeded into 96-well plates (2×10^4 cells per well), Vero cells were incubated until they reached 100% confluence. Serum was inactivated for 30 minutes at 56˚C and then centrifuged for 1 minute at 2000 rpm. Serial 6-fold dilutions of the serum were prepared starting at 1:5 using DMEM containing 2% FBS. After that, the diluted sera were combined with the titrated virus in a 1:1 (v/v) ratio to produce a mixture containing between 150 FFU/50 µL and 220 FFU/50 µL of viruses. The mixture was then incubated for an hour at 37˚C. In duplicate (50 µL/well), the virus/serum mixture was added to 96-well plates containing Vero cell monolayers. The plates were then left to incubate for an hour at 37˚C. After the mixtures were removed, 125 µL/well of 1.6% CMC was applied to the cells. The cells were then fixed for one hour with 4% formaldehyde in PBS (200 µL/well) and washed three times with PBS after further incubation at 37˚C for 1 day. To permeabilize the cell for 20-30 minutes, a permeabilization buffer (1% BSA in PBS, 0.2% Triton) was added. An IgG-HRP antibody and an anti-SARS-CoV N primary antibody (Sino Biological, 40143-T62) were used to incubate the cells. TrueBlue (KPL, 50-78-02) was used to stain the cells for 5 to 10 minutes (50 µL/well). After the plates were rinsed with deionized water, the number of foci was recorded by CTL S6 ultra. The GraphPad Prism software was used to calculate the neutralization titre at 50% (FRNT50).

RESULTS

Neutralizing Antibody Responses in Naive BALB/C Mice

To evaluate the inhibitory activity of the bivalent vaccines, we immunized BALB/c mice twice at a 2-week interval with 3 μg or 5 μg (total dose) of two bivalent vaccines and their corresponding monovalent vaccines shown in **Figure 1A**. The inhibitory activities of serum antibodies from BALB/c mice that received two doses of the different mRNA vaccines were measured two weeks after the second dose (day 28), using a vesicular stomatitis virus (VSV)-based neutralization assay with pseudoviruses displaying spike proteins of the original virus, BA.1, BA.2.75, BA.4/5, or XBB.1 shown in **Figure 1B**. Serum obtained at day 28 from mice vaccinated with the BA.4/5 monovalent or bivalent vaccine showed robust neutralizing antibody responses against BA.4/5 (3 μg: GMT: 17371 and 6546, 5 μg: GMT: 15746 and 12237, respectively), while the other vaccine candidates induced a relatively low level of neutralizing antibodies against BA.4/5. The neutralizing antibody responses against the original strain elicited by both bivalent vaccines were much higher than that elicited by the BA.1 or BA.4/5 monovalent mRNA vaccine, but at a similar level to that induced by CS-2034. As expected, serum from the monovalent RNA vaccine encoding BA.1 spike protein and the bivalent BA.1 vaccine efficiently inhibited the infection of the BA.1 pseudovirus (3 μg: GMT: 7633 and 7300, 5 μg: GMT: 7316 and 6289, respectively). Meanwhile, the neutralizing antibody titres against the BA.1 variant induced by other candidates were quite acceptable (3 μg: GMT ranging from 681 to 1520, 5 μg: GMT ranging from 964 to 1665). The responses against BA.2.75 elicited by all of the two bivalent RNA vaccines were equivalent, if not slightly higher than the monovalent RNA vaccines. As for the newly emerged Omicron variants XBB.1, all the monovalent and bivalent vaccines induced a lower neutralizing antibody response compared with original strains and other Omicron variants (BA.1, BA.2.75 and BA.4/5), indicating that XBB.1 has stronger immune escape ability. Nevertheless, the BA.4/5 monovalent and bivalent vaccines still showed stronger neutralization against XBB.1 (3 μg: GMT: 512 and 648, 5 μg: GMT: 1659 and 563, respectively). Accounting for dose effects, 3 μg of mRNA vaccine in mice was sufficient to elicit strong antibody responses, and a higher dose only induced slightly greater neutralizing antibody responses. Overall, our data demonstrated that the BA.4/5 bivalent RNA vaccine offered the most neutralization breadth against the original, and the newly Omicron epidemic SARS-CoV-2 variants BA.1, BA.2.75, BA.4/5 and XBB.1.

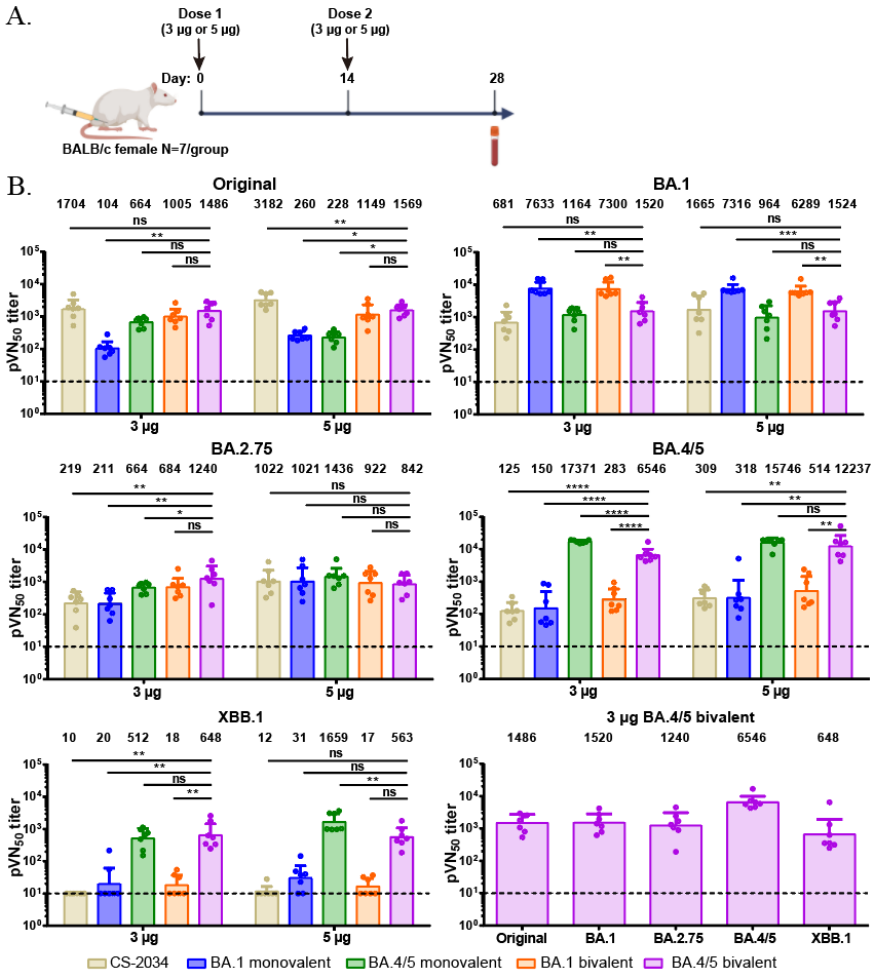

Figure 1: Neutralizing antibody responses in naïve BALB/c mice with monovalent and bivalent mRNA vaccines. (A) Scheme of immunization and blood draws. (B) Neutralizing activity of serum at day 28 against VSV pseudo-viruses displaying the spike proteins of original, BA.1, BA.2.75, BA.4/5, or XBB.1 strains (n=7, boxes illustrate geometric mean values, dotted lines show the limit of detection [LOD]). GMT values are indicated above the columns. P values result from a comparison between the two treatment groups using t-tests for log-transformed antibody titers. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001, ns, not significant. The lower detection limit (LL) was set as 10.

Neutralizing Antibody Responses after Booster with Bivalent mRNA Vaccine

Inactivated COVID-19 vaccines were widely offered around the world, to evaluate the neutralizing antibody responses of the bivalent vaccines as a heterologous booster following inactivated COVID-19 vaccines, two doses of BBIBP-CorV were given to BALB/c mice at a 2-week interval and we immunized them with either saline (sham control), the heterologous monovalent or bivalent mRNA vaccines, or the homologous BBIBP-CorV vaccine at day 109 shown in **Figure 2A**. Blood samples were collected 14 days after booster immunization (day 123) and serum samples were isolated for determining neutralized antibody titers based on live-virus FRNT assay. At 109 days (about 3.6 months) after completion of the primary BBIBP-CorV vaccination series, neutralizing antibody levels against the original strain (GMT: 84) and BA.5 (GMT: 46) showed low neutralizing activity shown in **Figure 2B**. Although neutralizing antibody levels were increased after the third homologous booster with BBIBP-CorV, they remained relatively low compared with the heterologous booster with the mRNA monovalent or bivalent vaccines. The third dose of the heterologous booster with mRNA vaccine dramatically and significantly increased neutralized antibody levels against both the original strain and Omicron variant BA.5 compared to that of received two doses of BBIBP-CorV shown in **Figure 2B**. The neutralizing titers against the original strain after boosting with CS-2034, BA.4/5 monovalent, and BA.4/5 bivalent vaccines had a similar trend, consistent with a 491-fold to 3473-fold increase in neutralizing antibody titers. Among them, booster immunization with 3 μg BA.4/5 bivalent vaccine can elicit the highest neutralizing titers (3473-fold) against the original strain. Boosting with BA.4/5 monovalent or bivalent vaccines resulted in significantly increased neutralizing titers against Omicron variant BA.5 (487 to 2116-fold), whereas CS-2034 boosted titers by a lesser degree (28.7-fold) shown in **Figure 2B**. As for dose effects, 1 μg BA.4/5 bivalent vaccine induced lower neutralizing antibody titers compared to 3 μg or 5 μg per dose, while the responses elicited by 3 μg and 3 μg bivalent vaccine have no statistical differences shown in **Figure 2B**. These data indicate that the Omicron-matched bivalent boosters have greater neutralizing activity against BA.5 than the constituent CS-2034 booster.

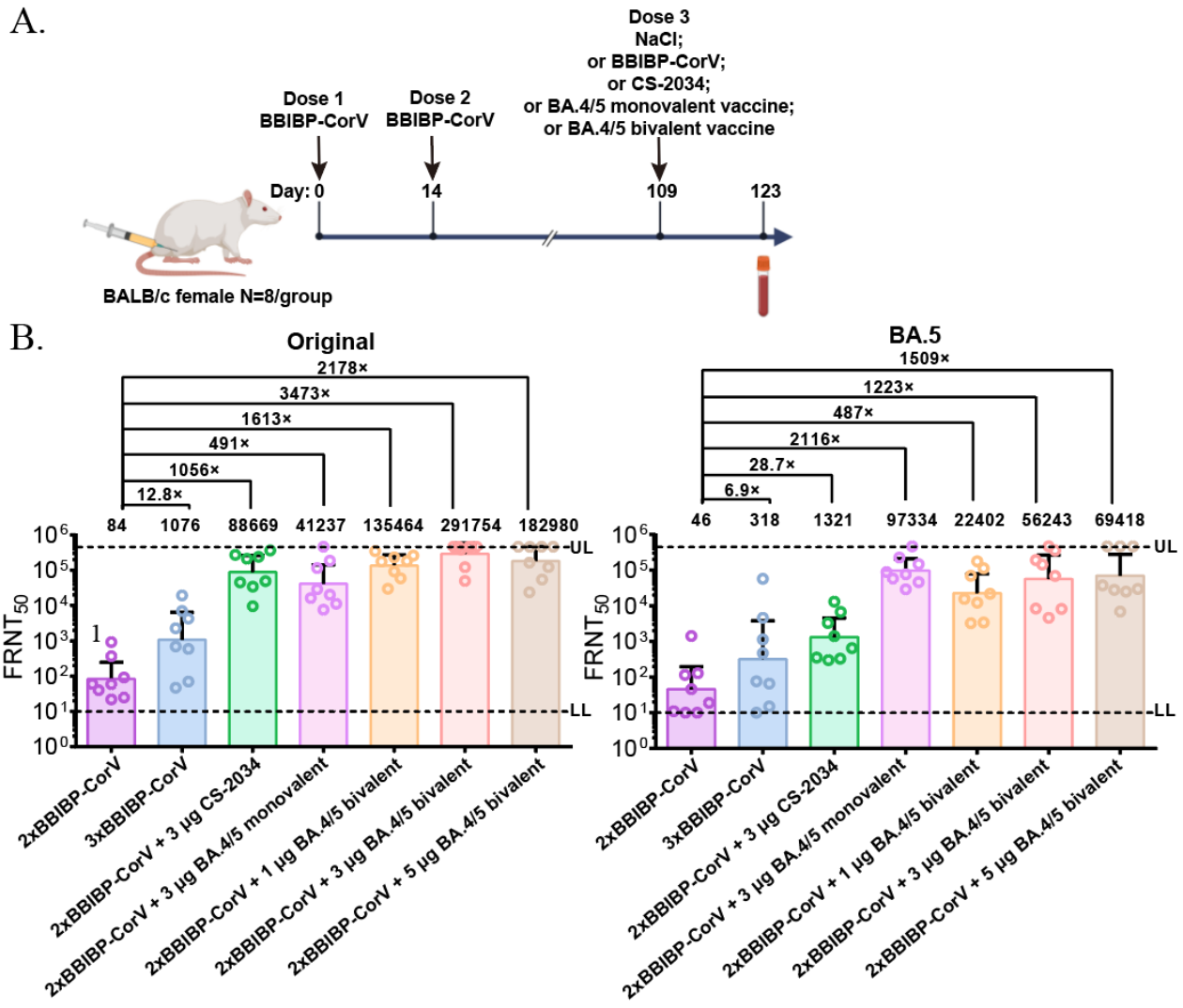

Figure 2: Neutralizing antibody responses in BALB/c mice after heterologous and heterologous booster. (A) Scheme of immunization and blood draws. (B) Neutralizing activity of serum at day 123 against original live virus and BA.5 live virus (n=8, boxes illustrate geometric mean values, dotted lines show the limit of detection [LOD]). GMT values are indicated above the columns. Fold change is the value compared to the two doses of BBIBP-CorV. The lower detection limit (LL) was set as 10, the upper detection limit (UL) was set as 466560.

DISCUSSION

In this study, we evaluated neutralizing antibody responses of the bivalent mRNA vaccines when administered as a primary vaccination series or as a heterologous booster in BALB/c mice. To analyse the potential breadth of the bivalent RNA vaccines against different Omicron variants, we compared the immunogenicity of the bivalent and constituent monovalent mRNA vaccines in BALB/c mice in the context of the primary immune series with two doses. The BA.4/5 bivalent RNA vaccine offered the most neutralization breadth against both the original, and the newly Omicron epidemic SARS-nCoV-2 variants BA.1, BA.2.75, BA.4/5, and XBB.1. Interestingly, contrary to other studies, where the first-generation vaccine based on the original strain elicited a much lower neutralizing antibody response to BA.4/5 compared to BA.1-based vaccine, the neutralizing antibody responses induced by CS-2034 and BA.1 monovalent mRNA vaccine to BA.4/5 were statistically at the same level, indicating that the CS-2034 antigen design is prone to elicit a humoral response with better cross-reactivity than other first-generation vaccines designed simply based on the original strain. In the context of the heterologous booster studies, the bivalent BA.4/5 vaccine booster can significantly increase the neutralizing titers against the original strain and BA.5 variants. These results are consistent with bivalent vaccine formulations or boosters with inactivated, spike protein-based, and mRNA-based SARS-CoV-2 bivalent vaccine candidates [5-10].

There are some limitations to this study. Firstly, we did not analyse T-cell responses after the primary immunization series or heterologous booster with bivalent mRNA vaccines, which could influence protective immunity. Besides, we evaluated the neutralizing titres with mRNA vaccines heterologous booster using the original strain and BA.5 live virus, while BA.5 is the predominant circulating strain in China, the new emerging variants like XBB.1, CH.1.1, and BN.1.1 may be informative in determining the breadth of protection.

Taken together, our data demonstrated that the BA.4/5 bivalent RNA vaccine (1:1 CS-2034 and BA.4/5 monovalent) offered the most neutralization breadth against the original, and the newly Omicron epidemic SARS-nCoV-2 variants BA.1, BA.2.75, BA.4/5 and XBB.1. Heterologous booster with bivalent mRNA vaccines can elicit extremely higher neutralizing antibodies compared to the BBIBP-CorV homologous booster and constituent monovalent vaccines even in lower doses. All of these data indicated the bivalent mRNA vaccines have significant clinical potential to overcome the SRAS-Cov-2 pandemic.

CONCLUSION

GraphPad Prism was utilized for the analysis of all of the data. Unless otherwise specified, no statistical methods were utilized to predetermine the sample size. Unless otherwise specified, neither the allocation nor the evaluation of the results was made known to the researchers. The geometric mean (± 95% CI) was used to represent quantification for each group. According to the legends of the figures, statistical significance was determined between various groups using analysis of variance (ANOVA) and Tukey's correction for multiple comparisons. When the P value was less than 0.05, it was deemed statistically significant, and ns, not significant.

AUTHOR CONTRIBUTION

J.L. and H.M.W. designed the vaccines and experiments. C.L.H. and Z.H.Y. prepared and provided the vaccines. J.D., Q.L., and L.Q.M. were responsible for quality control. X.W.S. was in charge of the VSV-pseudovirus neutralization assays and analyses. Z.C., C.J.C, J.D., C.C., S.X.H., L.C., J.C.Z. performed and analyzed live virus neutralization assays. D.W., J.Li, X.O.D., J.Liu, and T.Z. wrote, reviewed, and edited the manuscript. All authors critically reviewed and approved the final version. All authors have read and agreed to the published version of the manuscript.

FUNDING

This work was supported by a grant from the National Key R and D Program of China (No. 2021YFC2301700, 2021YFC2300101 YW, and 2022YFC2303700), Guangdong Basic and Applied Basic Research Foundation (2021B1515130005 and 2022B1515020059), National Natural Science Foundation of China (82201933, 82172240 YW and 82025001 JZ), China Postdoctoral Science Foundation (2022M710892), Guangzhou Science and Technology Planning Program Municipal School/Institute-Joint Funded Program (202102010143), State Key Laboratory of Respiratory Diseases Funded Program (SKLRD-Z-202330), Zhong Nanshan Medical Foundation of Guangdong Province (ZNSA-202001, ZNSXS-2020012, ZNSA-2020013, and ZNSXS-20220015) and Science and technology project of Guangzhou Customs District Technology Center (2021A1111100009).

DISCLOSURE STATEMENT

No potential conflict of interest was reported by the authors.

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