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Therapeutic efficacy of antibodies and antivirals against a SARS-CoV-2 Omicron variant

Ryuta Uraki
National Center for Global Health and Medicine Research Institute https://orcid.org/0000-0003-0890-1922
Maki Kiso
Division of Virology, Department of Microbiology and Immunology, Institute of Medical Science, University of Tokyo
Masaki Imai
University of Tokyo https://orcid.org/0000-0001-6988-1975
Seiya Yamayoshi
University Of Tokyo https://orcid.org/0000-0001-7768-5157
Mutsumi Ito
University of Tokyo
Michiko Ujie
University of Tokyo
Yuri Furusawa
Institute of Medical Science, University of Tokyo
Kiyoko Iwatsuki-Horimoto
University of Tokyo https://orcid.org/0000-0002-8266-020X
Yuko Sakai-Tagawa
Institute of Medical Science, University of Tokyo
Yoshihiro Kawaoka (yoshihiro.kawaoka@wisc.edu)
University of Wisconsin-Madison https://orcid.org/0000-0001-5061-8296

Article

Keywords:

Posted Date: January 19th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1240227/v1

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Version of Record: A version of this preprint was published at Nature Microbiology on June 15th, 2022. See the published version at https://doi.org/10.1038/s41564-022-01170-4.

Abstract

The spike protein of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is the major antigen stimulating the host's protective immune response. A new SARS-CoV-2 variant, designated Omicron, first identified in South Africa and possess many spike protein mutations. Here, we assessed the efficacy of therapeutic monoclonal antibodies (mAbs) against an Omicron variant in Syrian hamsters. Of the mAbs tested (i.e., REGN10987/REGN10933, COV2-2196/COV2-2130, and S309), only COV2-2196/COV2-2130 efficiently inhibited the replication of the Omicron variant in the lungs of hamsters. We also found that treatment of Omicron-infected hamsters with molnupiravir (an inhibitor of the RNA-dependent RNA polymerase of SARS-CoV-2) or S-217622 (an inhibitor of the main protease of SARS-CoV-2) led to a dramatic reduction of virus replication in the lungs. These findings suggest that treatment with the mAb combination COV2-2196/COV2-2130 or the antiviral compounds molnupiravir and S-217622 may be effective against the Omicron variants.

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which is responsible for coronavirus disease 2019 (COVID-19), continues to spread around the world and has caused 5.4 million deaths to date. The Omicron variant (B.1.1.529) of SARS-CoV-2 was detected in November 2021 in South Africa and has spread rapidly around the world. As of January 2022, this variant has been reported in at least 110 countries (https://www.who.int/publications/m/item/enhancing-readiness-for-omicron-(b.1.1.529)-technical-brief-and-priority-actions-for-member-states). A notable feature of the Omicron variant is that it contains more than 30 amino acid substitutions in its spike (S) protein, which induces the immune responses of the host, specifically the production of neutralizing antibodies[1]. Importantly, 15 of these substitutions are in the receptor-binding-domain (RBD) of the S protein, which is the major target for monoclonal antibody (mAb)-based therapy, raising concerns of decreased effectiveness of current therapeutic mAbs for COVID-19 against this variant.

Recent studies using *in vitro* neutralization assays have demonstrated that the Omicron variants show reduced sensitivity to some monoclonal and polyclonal antibodies relative to past isolates or other SARS-CoV-2 variants[2–6]. However, the *in vivo* efficacy of mAbs against the Omicron variant is unknown. Here, we assessed the efficacy of FDA-approved therapeutic mAbs against an Omicron variant in Syrian hamsters, a well-established animal model for SARS-CoV-2 research[7–9]. In addition, using this model, we evaluated the therapeutic efficacy of small-molecule antiviral agents for COVID-19 against this variant.

Results

Therapeutic effects of monoclonal antibodies on the replication of the SARS-CoV-2 Omicron variant.

To date, the FDA has issued Emergency Use Authorizations (EUAs) to permit the emergency use of four different mAbs as therapeutic agents for the treatment and/or prevention of COVID-19 in adult and

pediatric patients: a combination of etesevimab (LY-CoV016, CB6 or JS016) and bamlanivimab (LY-CoV555); REGEN-COV, a combination of imdevimab (REGN10987) and casirivimab (REGN10933); Xevudy, which is sotrovimab (VIR-7831); and Evusheld (AZD 7442), a combination of tixagevimab (COV2-2196 or AZD8955) and cilgavimab (COV2-2130 or AZD1061). However, in cell culture, we and others have previously shown that both LY-CoV016 and LY-CoV555, individually and combination, lack neutralizing activity against the Omicron variants [2–4, 6,10]. We therefore tested REGN10987/REGN10933, S309 (which is the precursor of sotrovimab), and COV2-2196/COV2-2130 for their therapeutic efficacy against Omicron. Sotrovimab for clinical use has been engineered with two amino acid mutations (M428L/N434S) in the Fc region, which extend the half-life of antibodies *in vivo*[11]. Both the COV2-2196 and COV2-2130 mAbs for clinical use contain the M252Y/S254T/T256E and L234F/L235E/P331S mutations in their Fc regions, which increase the half-life of antibodies and reduce the effector functions of antibodies, respectively[12–14]. All of the mAbs used in this study were synthesized according to publicly available sequences without any modifications (see methods). We treated five hamsters with a single dose of the REGN10987/REGN10933 or COV2-2196/COV2-2130 combination (2.5 mg/kg each), or S309 as monotherapy (5 mg/kg) by intraperitoneal injection on Day 1 after intranasal inoculation with 10^3 plaque-forming units (PFU) of CoV-2/UT-HP095-1N/Human/2020/Tokyo (D614G; HP095) or hCoV-19/Japan/NC928-2N/2021 (Omicron; NC928) (Fig. 1a). A human mAb specific to the hemagglutinin of influenza B virus was injected intraperitoneally to five hamsters on Day 1 post-infection as a control. The animals were sacrificed, and nasal turbinate and lung samples were collected for virus titrations on Day 4 post-infection. Sera were also collected at this timepoint and tested in an enzyme-linked immunosorbent assays (ELISA) for RBD-specific IgG antibodies to confirm successful antibody transfer. Hamsters that had low levels of ELISA titer (≤640; animals #1 and #6) were excluded from further analysis (Fig. 1b).

For the D614G (HP095)-infected groups, treatment with REGN10987/REGN10933 or COV2-2196/COV2-2130 resulted in a significant reduction in virus titers in both the nasal turbinates and lungs compared to control mAb-treated animals (Fig. 1b). These results were consistent with those of previous studies in which the combinations of REGN10987/REGN10933 or COV2-2196/COV2-2130 were shown to have therapeutic activity against infection with early (USA-WA1/2020) or variant (USA-WA1/2020 N501Y/D614G) SARS-CoV-2 strains in rhesus macaques, hamsters, and mice[15–17]. Virus titers in the lungs of the animals that were treated with S309 were significantly lower than those in the organs of the animals treated with the control mAb. In contrast, no differences in viral titers in the nasal turbinates were observed between the animals that were treated with this mAb and the animals treated with the control mAb. Thus, S309 showed less therapeutic effect against infection with D614G (HP095) compared with the other mAbs. For the Omicron (NC928)-infected groups, neither S309 nor REGN10987/REGN10933 had an effect on the virus titers in the nasal turbinates or the lungs of the animals (Fig. 1c). However, COV2-2196/COV2-2130 significantly reduced the virus titers in the lungs of the animals, whereas the virus titers in their nasal turbinates were not affected by this treatment. Taken together, these results indicate that the COV2-2196/COV2-2130 combination can restrict viral replication in the lungs of animals infected with Omicron even if the mAbs are administrated after the infection has occurred.

Therapeutic effects of antiviral compounds on the replication of the SARS-CoV-2 Omicron variant.

Molnupiravir (an inhibitor of the RNA-dependent RNA polymerase of SARS-CoV-2) was approved by the FDA for the treatment of adult patients with COVID-19 on December 23, 2021. S-217622, an inhibitor of the main protease (also called 3CLpro) of SARS-CoV-2, is currently in clinical trials. We previously showed that the susceptibility of Omicron (NC928) to molnupiravir was comparable to that of an early SARS-CoV-2 strain (NC002) and other variants of concern[10]. In this study, we confirmed that the Omicron variant and the early (SARS-CoV-2/UT- NC002-1T/Human/2020/Tokyo) strains have similar sensitivities to S-217622 *in vitro* (**Supplementary Table 1**).

After establishing the *in vitro* sensitivity of the Omicron variants to antiviral compounds, we assessed their therapeutic efficiencies in hamsters infected with the Omicron variant (NC928). The dosage of compounds for hamsters was determined based on previous studies to evaluate the effect of molnupiravir and S-217622 against SARS-CoV-2 in the mouse model[18,19]. Hamsters intranasally infected with 10^3 PFU of virus were treated by oral gavage twice daily (at 12-h intervals) for 3 days with 1,000 mg/kg/day or with 120 mg/kg/day of molnupiravir and S-217622, respectively, beginning 24 h post-infection (Fig. 2a). On Day 4 post-infection, the animals were sacrificed, and nasal turbinates and lungs were collected for virus titration. Treatment with molnupiravir had no effect on the virus titers in the nasal turbinates of the animals infected with the Omicron variant (NC928) (Fig. 2b). In marked contrast, both compounds dramatically reduced lung virus titers; no virus was recovered from the lungs of all four animals treated with molnupiravir or from the lungs of three of the four animals treated with S-217622. Treatment with S-217622 also resulted in a significant 9.9-fold reduction of virus titers in the nasal turbinates.

To evaluate whether treatment with these compounds could result in the emergence of resistant variants, hamsters infected with 10^3 PFU of virus were treated, beginning 24 h post-infection, for 5 days with either molnupiravir or S-217622 (Fig. 2c). No virus was recovered from the lungs of all four animals that were treated with either molnupiravir or S-217622 on Day 7 post-infection (Fig. 2d), although low titers of virus were detected in the nasal turbinates of three of the four molnupiravir-treated animals (2.3, 1.7, and 2.4 $\log_{10}$ PFU/g) and in the nasal turbinates of one of the four S-217622-treated animals (3.0 $\log_{10}$ PFU/g). These results suggest that the possibility of the emergence of resistant variants in hamsters treated with molnupiravir or S-217622 may be limited under the conditions tested.

Collectively, our observations suggest that the two antiviral compounds tested here efficiently restrict viral replication upon infection with the Omicron variant in the lower respiratory tract, but not in the upper respiratory tract, although we did see a 9.9-fold reduction of virus titers in the nasal turbinates of animals treated with S-217622.

Discussion

The emergence of SARS-CoV-2 Omicron variants carrying a large number of mutations in the RBD of the S protein has raised concern that these variants may limit the therapeutic usefulness of mAbs. In this study, we observed that the combination of two mAbs, COV2-2196/COV2-2130, efficiently suppressed the replication of the Omicron variant in the lungs when these mAbs were administrated one day post-infection. This was consistent with recent studies using *in vitro* neutralization assays[2–4, 6,10]. These *in vitro* data also have shown that sotrovimab and its parental form (S309) neutralize the Omicron variants. In addition, prophylactic administration of hamster IgG2a S309 has been shown to prevent or significantly diminish the replication of SARS-CoV-2 in hamsters[20]. However, in this study, we observed that the therapeutic administration of this mAb had no effect on the virus titers in the respiratory tracts of hamsters infected with Omicron (NC928). The reason for this lack of therapeutic efficacy against the Omicron (NC928) variant may be that the antibody-binding activities detected in animals at the time of virus titration (three days after antibody administration) were lower with S309-treated animals compared to those observed with COV2-2196/COV2-2130-treated animals (Fig. 1). Another study has shown that Fc effector functions enhance the therapeutic activity of neutralizing mAbs against SARS-CoV-2 infections in hamsters[21]. Because the S309 tested in this study originated from a human, its Fc receptor may not be optimal to recruit effector functions in hamsters. Further investigations are required to determine whether the replication of the Omicron variants is efficiently inhibited by S309 possessing Fc domains modified to match the target host Fc receptors.

Our study has several potential limitations: (1) Although hamsters are one of the most susceptible animals to SARS-CoV-2 among those tested, including mice and non-human primates, the Omicron variants are attenuated in hamsters especially in the lungs. It is not known whether the Omicron variants in humans are as attenuated as in hamsters. The difference (if any) in the replication of the Omicron variants in humans and hamsters may affect the effectiveness of the mAbs and antiviral compounds; (2) We did not see efficacy with some of the mAbs or mAb cocktails. Because it is difficult to administer a large volume of antibodies intravenously to hamsters, we administered them intraperitonially. But, in humans, they are administered intravenously. The route of administration may affect the efficacy of antibodies; (3) The mAbs generated in this study are not identical to the mAbs in clinical use because amino acid substitutions have been introduced into clinical mAbs in an effort to enhance their half-lives and reduce their effector functions and this difference may have led to the lower efficacy in the hamster model; and (4) The mAbs tested in this study have the human IgG1 Fc region. As the Fcγ receptors of humans and hamsters are different, inefficient Fc-mediated effector functions elicited by interactions with Fcγ receptors may have affected the efficacy in hamsters; (5) We did not test the Omicron variant encoding the R346K mutation in the RBD; however, as of January 2022, approximately 20% of GISAID-registered the Omicron variants possess the S-R346K mutation, which is known to reduce susceptibility to S309 *in vitro* using a pseudotype virus[6].

Collectively, our data show that the mAb combination COV2-2196/COV2-2130 and the two small-molecule antiviral agents molnupiravir and S-217622 may have therapeutic value against the Omicron variant of SARS-CoV-2.

Materials And Methods

Cells.

VeroE6/TMPRSS2 (JCRB 1819) cells[22,23] were propagated in the presence of 1 mg/ml geneticin (G418; Invivogen) and 5 μg/ml plasmocin prophylactic (Invivogen) in Dulbecco's modified Eagle's medium (DMEM) containing 10% Fetal Calf Serum (FCS) and antibiotics, and maintained at 37 °C with 5% CO_2. Chinese hamster ovary (CHO) cells were maintained in DMEM containing 10% FCS and antibiotics at 37 °C with 5% CO_2. Expi293 cells (Thermo Fisher Scientific) were maintained in Expi293 expression medium (Thermo Fisher Scientific) at 37 °C under 8% CO_2. The cells were regularly tested for mycoplasma contamination by using PCR, and confirmed to be mycoplasma-free.

Viruses.

hCoV-19/Japan/NC928-2N/2021 (Omicron; NC928)[10], SARS-CoV-2/UT- NC002-1T/Human/2020/Tokyo (NCGM02)[7], and SARS-CoV-2/UT-HP095-1N/Human/2020/Tokyo (D614G; HP095)[23] were propagated in VeroE6/TMPRSS2 cells in VP-SFM (Thermo Fisher Scientific). All experiments with SARS-CoV-2 were performed in enhanced biosafety level 3 (BSL3) containment laboratories at the University of Tokyo, which are approved for such use by the Ministry of Agriculture, Forestry and Fisheries, Japan.

Antibodies.

Amino acid sequences for the variable region of the heavy and light chains of the following human monoclonal antibodies against the S protein were used for gene synthesis: clones tixagevimab (COV2-2196/AZD8895; GenBank accession numbers QLI33947 and QLI33948), casirivimab (REGN10933; PDB accession numbers 6XDG_B and 6XDG_D), cilgavimab (COV2-2130/AZD1061; GenBank accession numbers QKY76296 and QKY75909), imdevimab (REGN10987; PDB accession numbers 6XDG_A and 6XDG_A), and S309 (PDB accession numbers 6WS6_A and 6WS6_F). An artificial signal sequence and the constant gamma heavy (lgG1, UniProtKB/Swiss-Prot accession number P01857) and kappa (UniProtKB/Swiss-Prot accession number P01834) or lambda (UniProtKB/Swiss-Prot accession number P0DOY2) light chain coding sequences were added before and after each variable region. Codon usage was optimized for expression in CHO cells. The synthesized genes were cloned into a plasmid for protein expression and transfected into CHO cells. Cell culture media were harvested after incubation for 10–14 days at 37 °C. A human monoclonal antibody (1430E3/9) against the hemagglutinin of influenza B virus[24] was previously cloned into the expression vector Mammalian Power Express System (TOYOBO) and was transiently expressed by Expi293 cells. Monoclonal antibodies were purified by using MabSelect SuRe LX (Cytiva) or a protein A column. Purity was confirmed by SDS-PAGE and/or HPLC before use. The

reactivities of these antibodies against SARS-CoV-2, including the Alpha, Beta, Delta, Gamma, and Omicron variants, have been tested previously[10].

Antiviral compounds.

Molnupiravir (EIDD-2801) was purchased from MedChemExpress. S-217622 was kindly provided by Shionogi Co., Ltd.. All compounds were dissolved in 0.5% methylcellulose prior to use in *in vivo* experiments.

Evaluation of therapeutic efficacy of mAbs and antiviral compounds in Syrian hamsters.

Five- to six-week-old male Syrian hamsters (Japan SLC Inc., Shizuoka, Japan) were used in this study. For the evaluation of mAb efficacy in hamsters, under *isoflurane* anesthesia, five hamsters per group were inoculated intranasally with 10^3 PFU (in 30 µl) of HP095 or NC928. On Day 1 post-infection, the hamsters were injected intraperitoneally with 1 ml of a mAb preparation (5 mg/kg). The animals were euthanized on Day 4 post-infection, and the virus titers in the nasal turbinates and lungs were determined by use of plaque assays on VeroE6/TMPRSS2 cells.

For the evaluation of antiviral compound efficacy in hamsters, under *isoflurane* anesthesia, four hamsters per group were inoculated intranasally with 10^3 PFU (in 30 µl) of Omicron (NC928). At 24 h after inoculation, hamsters were treated with the following antiviral compounds: (1) molnupiravir, 500 mg/kg (in 1 ml) administered orally twice daily; (2) S-217622, 60/kg (in 1 ml) administered orally twice daily; or (3) methylcellulose (1 ml) as a control for oral treatment. The animals were euthanized on Day 4 post-infection, and the virus titers in the nasal turbinates and lungs were determined by use of plaque assays on VeroE6/TMPRSS2 cells.

All experiments with hamsters were performed in accordance with the Science Council of Japan's Guidelines for Proper Conduct of Animal Experiments and the guidelines set by the Institutional Animal Care. The protocols were approved by the Animal Experiment Committee of the Institute of Medical Science, the University of Tokyo (approval number PA19-75).

Enzyme-Linked Immunosorbent Assay (ELISA).

ELISAs were performed as previously reported[25]. Briefly, ninety-six-well Maxisorp microplates (Nunc) were incubated with the recombinant receptor-binding domain (RBD) of the S protein (50 µl/well at 2 µg/ml), or with PBS at 4 °C overnight and were then incubated with 5% skim milk in PBS containing 0.05% Tween-20 (PBS-T) for 1 h at room temperature. The microplates were reacted for 1 h at room temperature with

hamster serum samples that were initially diluted 40-fold in PBS-T containing 5% skim milk and subsequently serially 2-fold diluted, followed by peroxidase-conjugated goat anti-human IgG, Fcγ Fragment specific antibody (Jackson Immuno-Research) for 1 h at room temperature. Then, 1-Step Ultra TMB-Blotting Solution (Thermo fisher scientific) was added to each well and incubated for 3 min at room temperature. The reaction was stopped by the addition of 2 M H_2SO_4 and the optical density at 450 nm (OD_{450}) was immediately measured. The average OD_{450} values of two PBS-wells were subtracted from the average OD_{450} values of the two RBD-wells for background correction. A subtracted OD_{450} value of 0.1 or more was regarded as positive; the minimum dilution to give a positive result was used as the ELISA titer.

Determination of half-maximal effective concentration (EC_{50}) values.

VeroE6/TMPRSS2 cells were seeded in 96-well plates one day prior to infection, and were incubated at a multiplicity of infection (MOI) of 0.01 with SARS-CoV-2 at 37 °C for 1 h. The inocula were then replaced with MEM containing 5% FCS and serially diluted S-217622, and the cells were incubated at 37 °C with 5% CO_2 for 2–3 days to observe cytopathic effects (CPE). The 50% effective concentration (EC_{50}) was determined by using the Spearman–Karber formula[26] based on the appearance of visually detectable CPE in quadruplicate experiments.

Statistical analysis.

GraphPad Prism software was used to analyze all the data. We compared virus titers in hamster organs with the control by using a one-way analysis of variance (ANOVA) followed by Dunnett's multiple comparisons test or the Kruskal-Wallis test followed by Dunn's test with multiple comparisons. Differences between groups were considered significant for P values < 0.05.

Data availability.

All data supporting the findings of this study are available within the paper and from the corresponding author upon request. There are no restrictions to obtaining access to the primary data.

Code availability.

No code was used in the course of the data acquisition or analysis.

Reagent availability.

All reagents described in this paper are available through Material Transfer Agreements.

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Declarations

Acknowledgements

We thank Shionogi Co., Ltd. for providing S-217622. We thank Susan Watson for scientific editing. We also thank Kyoko Yokota for technical assistance. This work was supported by a Research Program on Emerging and Re-emerging Infectious Diseases (JP20fk0108412, JP21fk0108615, and JP 20fk0108472), a Project Promoting Support for Drug Discovery (JP20nk0101632), the Japan Program for Infectious Diseases Research and Infrastructure (JP21wm0125002) from the Japan Agency for Medical Research and Development (AMED), the National Institutes of Allergy and Infectious Diseases Center for Research

on Influenza Pathogenesis (HHSN272201400008C), and the Center for Research on Influenza Pathogenesis and Transmission (CRIPT) (75N93021C00014).

Author information

These authors contributed equally: Ryuta Uraki, Maki Kiso, and Masaki Imai

Affiliations

Division of Virology, Institute of Medical Science, University of Tokyo, Tokyo, Japan

Ryuta Uraki, Maki Kiso, Masaki Imai, Seiya Yamayoshi, Mutsumi Ito, Michiko Ujie, Yuri Furusawa, Kiyoko Iwatsuki-Horimoto, Yuko Sakai-Tagawa, &Yoshihiro Kawaoka

The Research Center for Global Viral Diseases, National Center for Global Health and Medicine Research Institute, Tokyo, Japan.

Ryuta Uraki, Masaki Imai, Seiya Yamayoshi, &Yoshihiro Kawaoka

Laboratory of Ultrastructural Virology, Institute for Frontier Life and Medical Sciences, Kyoto University, Kyoto, Japan

Yuri Furusawa

Influenza Research Institute, Department of Pathobiological Sciences, School of Veterinary Medicine, University of Wisconsin-Madison, WI, USA

Yoshihiro Kawaoka

Contributions

R.U., M.K., and M.Imai performed the *in vivo* experiments. S.Y., M.Ito, M.U., Y.F., K.I.-H., and Y.S.-T. performed the *in vitro* experiments. R.U., M.K., M.Imai, S.Y., and Y.K. planned the experiments and/or analyzed the data. R.U., M.K., M.Imai, S.Y, and Y.K. wrote the manuscript.

Corresponding author

Correspondence to Yoshihiro Kawaoka.

Ethics declarations

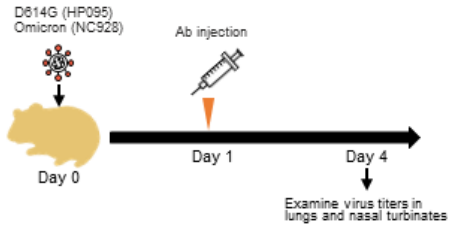
Competing interests

Y.K. has received unrelated funding support from Daiichi Sankyo Pharmaceutical, Toyama Chemical, Tauns Laboratories, Inc., Shionogi & Co. LTD, Otsuka Pharmaceutical, KM Biologics, Kyoritsu Seiyaku, Shinya Corporation, and Fuji Rebio. The remaining authors declare that they have no competing interests.

Figures

Figure 1

a



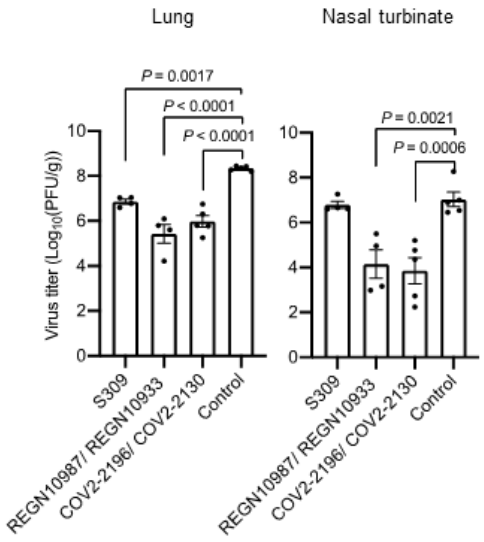
Virus	Infection route	Infection titer
D614G (HP095)	Intranasal	10^3 PFU/30 μL
Omicron (NC928)	Intranasal	10^3 PFU/30 μL

Antibody	Inoculation route	Concentration (mg/kg)
S309	Intraperitoneal	5
REGN10987 REGN10933	Intraperitoneal	2.5 2.5
COV2-2196 COV2-2130	Intraperitoneal	2.5 2.5
Control (1430E3/9)	Intraperitoneal	5

b

D614G(HP095)

Antibody Treatment	Animal ID #	Antibody Endpoint Titer	Virus titers (log_{10}(PFU/g)) Lung	Nasal turbinate
S309	#1	160	8.22	6.75
	#2	20480	7.03	6.66
	#3	40960	6.78	7.27
	#4	20480	7.05	6.61
	#5	40960	6.60	6.62
REGN10987 REGN10933	#6	160	8.14	7.76
	#7	81920	5.61	5.51
	#8	81920	4.22	3.17
	#9	81920	5.80	4.96
	#10	81920	6.09	2.99
COV2-2196 COV2-2130	#11	40960	6.31	4.36
	#12	40960	5.78	4.77
	#13	81920	6.76	2.73
	#14	81920	5.26	5.21
	#15	81920	5.80	2.24
Control mAb	#16	<40	8.37	6.90
	#17	<40	8.22	8.27
	#18	<40	8.44	7.00
	#19	<40	8.27	6.46
	#20	<40	8.43	6.54



c

Omicron (NC928)

Antibody Treatment	Animal ID #	Antibody Endpoint Titer	Virus titer (log_{10}(PFU/g)) Lung	Nasal turbinate
S309	#21	1280	5.84	6.49
	#22	20480	3.71	6.17
	#23	20480	6.34	6.12
	#24	20480	6.48	6.61
	#25	40960	6.45	6.79
REGN10987 REGN10933	#26	40960	6.97	8.06
	#27	81920	5.72	7.53
	#28	81920	4.32	6.65
	#29	2560	6.02	6.84
	#30	81920	6.22	7.07
COV2-2196 COV2-2130	#31	81920	3.96	5.71
	#32	81920	1.50	5.96
	#33	81920	3.86	6.90
	#34	40960	4.94	6.50
	#35	20480	5.05	7.55
Control mAb	#36	<40	7.00	7.25
	#37	<40	6.95	7.94
	#38	<40	6.23	6.53
	#39	<40	6.32	7.29
	#40	<40	6.36	6.91

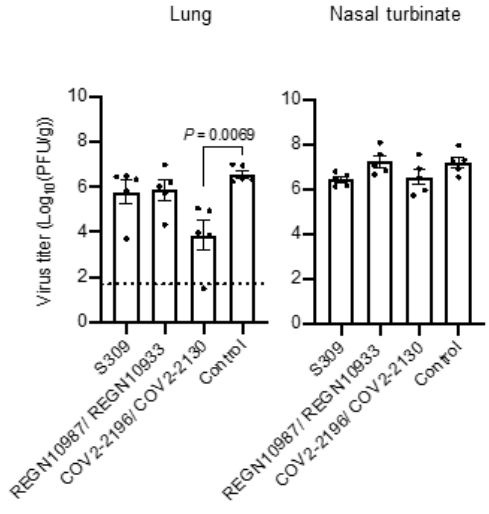


Figure 1

Therapeutic effects of monoclonal antibodies on the replication of the SARS-CoV-2 the Omicron variant.

a, Schematic diagram of the experimental workflow for assessing the therapeutic effects of monoclonal antibodies. **b, c,** Syrian hamsters were intranasally inoculated with 10^3 PFU of D614G (HP095) (**b**) or Omicron (NC928) (**c**). On Day 1 post-infection, the hamsters were intraperitoneally injected with a single

dose of the REGN10987/REGN10933 or COV2-2196/COV2-2130 combination (2.5 mg/kg each), or S309 as monotherapy (5 mg/kg). As a control, a human monoclonal antibody (1430E3/9) against the hemagglutinin of influenza B virus was injected. Hamsters were euthanized on Day 4 post-infection for virus titration. Sera were also collected at this timepoint, and titers of RBD-specific IgG antibodies in the sera were determined by using ELISAs coated with recombinant RBD derived from the S protein of Wuhan/Hu-1/2019 (GenBank accession #. MN908947). The endpoint titer is defined as the reciprocal of the highest dilution with an OD_{450} cutoff value ≧ 0.1. Virus titers in the nasal turbinates and lungs were determined by use of a plaque assay on VeroE6/TMPRSS2 cells. The detection limit for virus titers was 1.7 $\log_{10}$ (PFU/g). Vertical bars show the mean ± s.e.m. Points indicate data from individual hamsters (*n*=4 or 5/group). The lower limit of detection is indicated by the horizontal dashed line. To compare the lung titers of the different groups in the Omicron (NC928)-infected hamsters, we used a Kruskal-Wallis test followed by Dunn's multiple comparisons test. To compare the nasal turbinate and lung titers of the different groups in the D614G (HP095)-infected hamsters and the nasal turbinate titers of the different groups in the Omicron (NC928)-infected hamsters, we used a one-way ANOVA followed by Dunnett's multiple comparisons test. *P* values of < 0.05 were considered statistically significant.

Figure 2

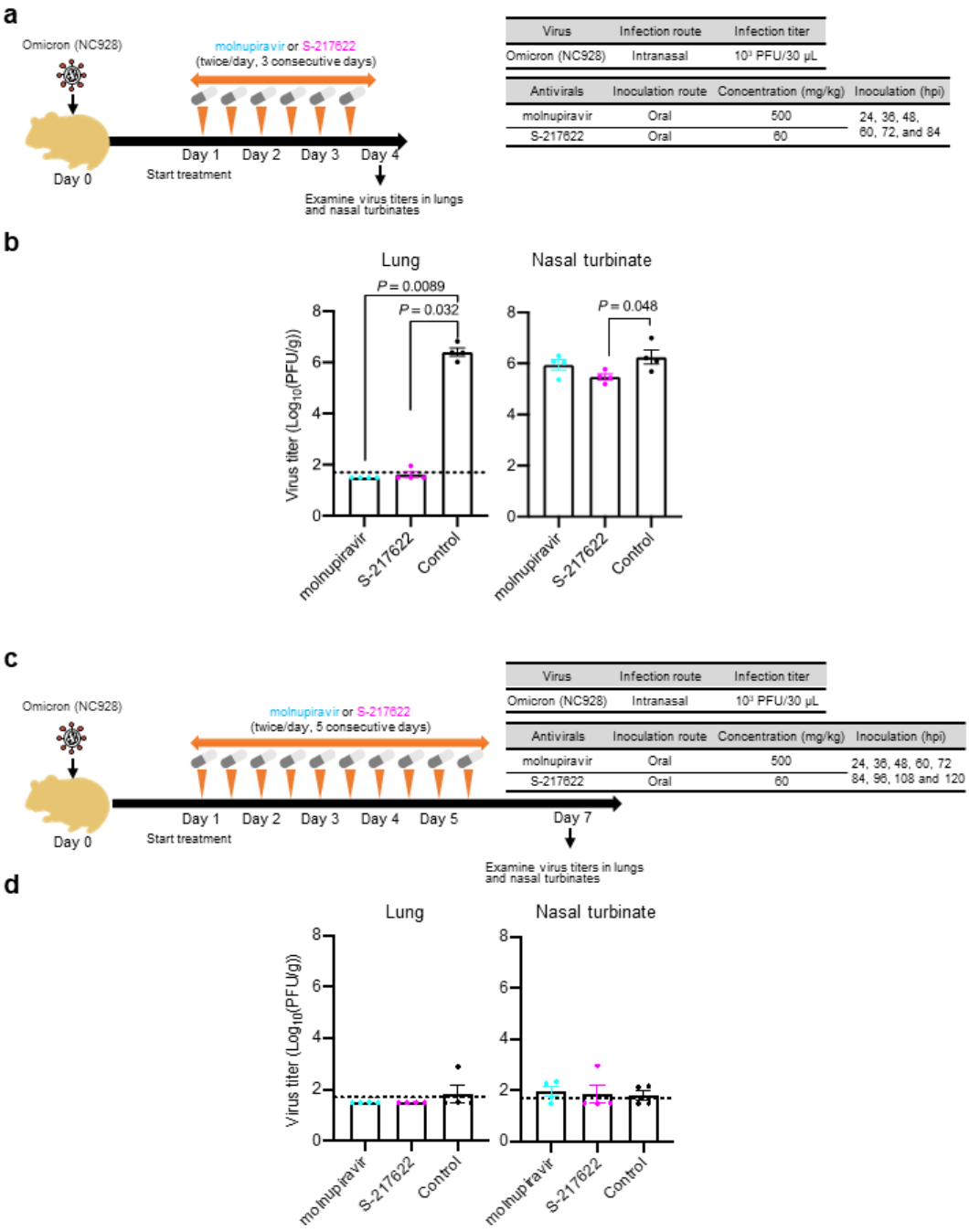


Figure 2

Therapeutic effects of antiviral compounds on the replication of the SARS-CoV-2 Omicron variant.

a, Schematic diagram of the experimental workflow for assessing the therapeutic effects of antiviral compounds. **b,** Syrian hamsters were intranasally inoculated with 10^3 PFU of Omicron (NC928). At 24 h post-infection, hamsters were treated with: 500 mg/kg molnupiravir orally twice daily for 3 days or 60

mg/kg S-217622 orally twice daily for 3 days. Methylcellulose served as a control for oral treatment. Hamsters were euthanized on Day 4 post-infection for virus titration. Virus titers in the nasal turbinates and lungs were determined by use of a plaque assay on VeroE6/TMPRSS2 cells. Vertical bars show the mean ± s.e.m. Points indicate data from individual hamsters (n=4/group). The lower limit of detection is indicated by the horizontal dashed line. To compare the lung and nasal turbinate titers of the different groups in the Omicron (NC928)-infected hamsters, we used a Kruskal-Wallis test followed by Dunn's multiple comparisons test and a one-way ANOVA followed by Dunnett's multiple comparisons test, respectively. *P* values of < 0.05 were considered statistically significant. **c,** Schematic diagram of the experimental workflow for investigating the emergence of resistant variants. **d,** Syrian hamsters were intranasally inoculated with 10^3 PFU of Omicron (NC928). At 24 h post-infection, hamsters were treated with: 500 mg/kg molnupiravir orally twice daily for 5 days or 60 mg/kg S-217622 orally twice daily for 5 days. Methylcellulose served as a control for oral treatment. Hamsters were euthanized on Day 7 post-infection for virus titration. Virus titers in the nasal turbinates and lungs were determined by use of a plaque assay on VeroE6/TMPRSS2 cells. Vertical bars show the mean ± s.e.m. Points indicate data from individual hamsters (n=4/group). The lower limit of detection is indicated by the horizontal dashed line.

Supplementary Files

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