CORRESPONDENCE

Efficacy of Antibodies and Antiviral Drugs against Omicron BA.2.12.1, BA.4, and BA.5 Subvariants

TO THE EDITOR: As of June 2022, the B.1.1.529 (omicron) variant of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) has been divided into five distinct sublineages: BA.1, BA.2, BA.3, BA.4, and BA.5.[1] Most circulating omicron variants belong to sublineage BA.2; however, the prevalence of BA.2.12.1 (a subvariant of BA.2), BA.4, and BA.5 is increasing rapidly in several regions of the world.[2] Previous studies have shown that the BA.2 subvariants have sensitivities to some monoclonal and polyclonal antibodies that are lower than those of the ancestral strains and other SARS-CoV-2 variants.[3-5]

As compared with BA.2, the BA.2.12.1 subvariant has substitutions L452Q and S704L in its spike protein, and both BA.4 and BA.5 have additional changes. Substitutions L452Q, L452R, and F486V are in the receptor-binding domain of the spike protein, the major target for monoclonal antibody therapies, which is worrisome with respect to the effectiveness of current monoclonal antibodies that have been approved by the Food and Drug Administration (FDA) against these variants. The efficacy of monoclonal antibodies against the BA.2.12.1, BA.4, and BA.5 subvariants that have been isolated from patients is unknown.

In this study, we examined the neutralizing ability of FDA-approved monoclonal antibodies, individually and in combination, against omicron BA.2.12.1 (hCoV-19/USA/NY-MSHSP-PV56475/2022), BA.4 (hCoV-19/USA/MD/HP30386/2022), and BA.5 (hCoV-19/Japan/TY41-702/2022) isolates. We confirmed that the BA.5 isolate had five additional amino acid changes (69–70del, L452R, F486V, and Q493) in its spike protein as compared with a BA.2 isolate (hCoV-19/Japan/UT-NCD1288-2 N/2022) (Fig. S1 in the Supplementary Appendix, available with the full text of this letter at NEJM.org). The BA.2.12.1 isolate consisted of a mixed viral population encoding either R or W at position 682, in addition to having L452Q and S704L substitutions. The BA.4 isolate contained a V3G mutation in the signal peptide region of the spike protein, in addition to the other five changes (i.e., 69–70del, L452R, F486V, and Q493).

Live-virus focus reduction neutralization testing (FRNT) showed that monoclonal antibody REGN10933 (marketed as casirivimab) lost neutralizing activity against BA.2.12.1, BA.4, and BA.5 (Table 1 and Fig. S2). However, REGN10987 (marketed as imdevimab) retained neutralizing activity against these isolates. The combination of casirivimab and imdevimab also inhibited BA.2.12.1, BA.4, and BA.5; however, the value of this combination was higher (indicating reduced neutralizing activity) on 50% focus reduction neutralization testing ($FRNT_{50}$) by a factor of 131.6 against BA.2.12.1, by a factor of 133.5 against BA.4, and by a factor of 317.8 against BA.5 than against the ancestral strain (SARS-CoV-2/ UT-NC002-1T/Human/2020/Tokyo) used in our study. COV2-2196 (marketed as tixagevimab) had neutralizing activity against BA.2.12.1 (although its $FRNT_{50}$ value for this virus was higher by a factor of 54.7 than against the ancestral strain) but not against BA.4 or BA.5. However, COV2-2130 (marketed as cilgavimab) neutralized BA.2.12.1, BA.4, and BA.5. The combination of tixagevimab and cilgavimab inhibited BA.2.12.1, BA.4, and BA.5, with a low $FRNT_{50}$ value (38.1 ng per milliliter, 37.8 ng per milliliter, and 192.5 ng per milliliter, respectively). However, as compared with the $FRNT_{50}$ value against the ancestral strain, the $FRNT_{50}$ value of this combination was higher by a factor of 6.1 against BA.2.12.1, by a factor of 6.0 against BA.4, and by a factor of 30.7 against BA.5. The precursor of sotrovimab (S309) lost inhibitory capability against BA.2.12.1, BA.4, and BA.5. Of the FDA-approved monoclonal antibodies that we tested, only LYCoV1404 (marketed as bebtelovimab) efficiently neutralized BA.2.12.1, BA.4, and BA.5; the $FRNT_{50}$ values for these isolates were similar to those for the ancestral strain.

Table 1. Efficacy of Monoclonal Antibodies and Antiviral Drugs against Omicron Subvariants in Vitro.*

Subvariant	Mean Neutralization Activity of Monoclonal Antibody† (ng per milliliter)								Susceptibility to Antiviral Drug‡ (μmol)		
	Imdevimab	Casirivimab	Tixagevimab	Cilgavimab	Sotrovimab Precursor	Bebtelovimab	Imdevimab+ Casirivimab	Tixagevimab+ Cilgavimab	Remdesivir	Molnupiravir	Nirmatrelvir
Reference§	7.4	6.1	6.1	7.0	95.1	2.5	3.4	6.3	1.7	2.8	2.7
BA.1	>50,000	>50,000	1552.7	2916.9	40727.1	5.8	>10,000	351.1	1.9	7.5	4.8
BA.1.1	>50,000	>50,000	603.5	>50,000	3769.2	3.9	>10,000	1296.8	2.0	6.0	3.9
BA.2	329.0	>50,000	2756.6	16.9	>50,000	3.3	835.1	34.6	5.9	8.7	6.9
BA.2.12.1	238.1	>50,000	335.2	21.0	>50,000	4.0	452.7	38.1	0.5	3.2	1.8
BA.4	132.6	>50,000	>50,000	53.6	>50,000	2.9	459.1	37.8	1.2	3.3	2.9
BA.5	583.4	>50,000	>50,000	56.8	>50,000	3.3	1093.1	192.5	2.0	4.1	4.4

* The antibodies that were used in this analysis are listed by their commercial names for readability although they were produced in the authors' laboratories in their generic formulations. Omicron subvariants of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) are listed according to the World Health Organization labels for the Pango lineage.
† Individual monoclonal antibodies were tested at a starting concentration of 50,000 ng per milliliter on 50% focus reduction neutralization testing. The monoclonal antibody combinations were tested at a starting concentration of 10,000 ng per milliliter for each antibody.
‡ The susceptibility to antiviral drugs was measured as the 50% inhibitory concentration of the mean micromole value of triplicate reactions. GS-441524 is the main metabolite of remdesivir and EIDD-1931 is the active form of molnupiravir, both of which are RNA-dependent RNA polymerase inhibitors. Nirmatrelvir (PF-07321332) is a protease inhibitor.
§ The reference strain was SARS-CoV-2/UT-NC002–1T/Human/2020/Tokyo.

Remdesivir and molnupiravir (inhibitors of the RNA-dependent RNA polymerase of SARS-CoV-2) and nirmatrelvir (an inhibitor of the main protease) have been approved by the FDA for the treatment of coronavirus disease 2019 (Covid-19). We therefore tested these antiviral drugs by determining the in vitro 50% inhibitory concentration (IC_{50}) of each compound against BA.2.12.1, BA.4, and BA.5. As compared with the amino acid sequence of the reference strain Wuhan/Hu-1/2019, all three isolates encoded the P314L mutation in their RNA-dependent RNA polymerase and the P3395H mutation in their main protease (Fig. S3). The susceptibilities of BA.2.12.1, BA.4, and BA.5 to the three compounds (with higher values indicating reduced susceptibility) were similar to those of the ancestral strain (SARS-CoV-2/UT-NC002-1T/Human/2020/Tokyo). For the BA.2.12.1 subvariant, the IC_{50} was lower by a factor of 0.3 with remdesivir, was higher by a factor of 1.1 with molnupiravir, and was lower by a factor of 0.7 with nirmatrelvir; for the BA.4 subvariant, the IC_{50} was lower by a factor of 0.7 with remdesivir and was higher by factors of 1.2 and 1.1 with molnupiravir and nirmatrelvir, respectively; and for the BA.5 subvariant, the IC_{50} was higher by factors of 1.2, 1.5, and 1.6 with remdesivir, molnupiravir, and nirmatrelvir, respectively (Table 1 and Fig. S4).

The main limitation of our study is the lack of clinical data on the efficacy of these monoclonal antibodies and antiviral drugs for the treatment of patients infected with BA.2.12.1, BA.4, or BA.5 subvariants. Overall, our data suggest that the three small-molecule antiviral drugs remdesivir, molnupiravir, and nirmatrelvir may have therapeutic value against the sublineages BA.2.12.1, BA.4, and BA.5 of SARS-CoV-2 omicron variants. Our data also indicate that bebtelovimab is effective against BA.2.12.1, BA.4, and BA.5. However, in clinical use, these variants may be less susceptible to combination therapy with casirivimab and imdevimab and with tixagevimab and cilgavimab. In addition, sotrovimab may not provide effective treatment against BA.2.12.1, BA.4, or BA.5. Our findings show that the selection of monoclonal antibodies to treat patients who are infected with omicron variants should be carefully considered.

Emi Takashita, Ph.D.
National Institute of Infectious Diseases
Tokyo, Japan

Seiya Yamayoshi, D.V.M., Ph.D.

University of Tokyo
Tokyo, Japan

Viviana Simon, M.D., Ph.D.
Harm van Bakel, Ph.D.
Emilia M. Sordillo, M.D., Ph.D.

Icahn School of Medicine at Mount Sinai
New York, NY

Andrew Pekosz, Ph.D.

Johns Hopkins University
Baltimore, MD

Shuetsu Fukushi, D.V.M., Ph.D.
Tadaki Suzuki, M.D., Ph.D.
Ken Maeda, D.V.M., Ph.D.

National Institute of Infectious Diseases
Tokyo, Japan

Peter Halfmann, Ph.D.

University of Wisconsin–Madison
Madison, WI

Yuko Sakai-Tagawa, Ph.D.
Mutsumi Ito, D.V.M.

University of Tokyo
Tokyo, Japan

Shinji Watanabe, D.V.M., Ph.D.

National Institute of Infectious Diseases
Tokyo, Japan

Masaki Imai, D.V.M., Ph.D.

University of Tokyo
Tokyo, Japan

Hideki Hasegawa, M.D., Ph.D.

National Institute of Infectious Diseases
Tokyo, Japan

Yoshihiro Kawaoka, D.V.M., Ph.D.

University of Tokyo
Tokyo, Japan
yoshihiro.kawaoka@wisc.edu

Drs. Takashita and Yamayoshi contributed equally to this letter.

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