# Efficacy of antivirals and mRNA vaccination against an XBF clinical isolate

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The Lancet Regional Health - Western Pacific 2023;34: 100777
Published Online 11 May 2023
https://doi.org/10.1016/j.lanwpc.2023.100777

Recombination events occur frequently in severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), resulting in genetic diversity. Because these events contribute to altered host immune evasion and antiviral susceptibility, it is crucial to evaluate the efficacy of COVID-19 vaccines and antivirals against recombinant variants. As of March 2023, XBB.1.5, a recombinant sublineage of XBB, is currently the dominant form globally (Fig. S1A, Supplementary Appendix). XBB emerged as a result of recombination between two BA.2 descendants, BJ.1 and BM.1.1.1 (a progeny of BA.2.75). We and other groups have shown that XBB.1.5 is resistant to several therapeutic monoclonal antibodies and effectively evades humoral immunity elicited by natural infection or COVID-19 vaccination.[1–3] By March 30, 2023, an additional recombinant variant, XBF, had been sampled 8966 times in 47 countries and territories in GISAID, reaching it highest prevalence in Australia and New Zealand (Fig. S2, Supplementary Appendix). XBF is still increasing in frequency, although the prevalence of XBB.1.5 appears to be increasing at a faster pace in most regions (Fig. S1B, Supplementary Appendix). XBF is a recombinant of BA.5.2.3 (a descendant of BA.5) and CJ.1 (a descendant of BA.2.75) and, like CJ.1, has an additional three substitutions (R346T, F486P, and F490S) in the receptor-binding domain (RBD) of the consensus form of its spike protein compared to baseline BA.2.75 (Fig. S3A, Supplementary Appendix). CJ.1 and its related sublineage CJ.1.1 did not expand as extensively as XBF, being sampled in GISAID only 1589 and 142 times respectively. CJ.1 was found circulating in many countries, but most commonly sampled in South Korea, where it is still increasing and has currently reached about 5% of the sampled population. CJ.1.1 was most frequently sampled in Malaysia and Singapore, but remained rare in both nations, peaking at about 1% of the sample in December of 2022. Despite the importance of these related variants, we have no information about the antiviral efficacy and immunity induced by COVID-19 vaccines against a clinical isolate of XBF.

Accordingly, here, we assessed the reactivity of COVID-19 therapeutic monoclonal antibodies (mAbs) against omicron XBF (hCoV-19/Japan/UT-OM110/2022) obtained from a patient. We used a live virus neutralisation assay with Vero E6-TMPRSS2-T2A-ACE2 cells to determine the 50% focus reduction neutralization test ($FRNT_{50}$) titres of the mAbs. We found that LYCoV1404 (marketed as bebtelovimab) efficiently inhibited XBF with a very low $FRNT_{50}$ value (44 ng/ml), similar to that for the ancestral strain, but REGN10987 (known as imdevimab), REGN10933 (known as casirivimab), COV2-2196 (known as tixagevimab), COV2-2130 (known as cilgavimab), and S309 (known as the precursor of sotrovimab) lost neutralising activity against the XBF isolate (Fig. S4, Supplementary Appendix). We recently showed that none of the antibodies tested in this study neutralised XBB.1.5 in Vero E6-

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TMPRSS2-T2A-ACE2 cells, demonstrating that the antibody escape capability of XBF is different from that of XBB.1.5.[1] A CJ.1.1 clinical isolate (hCoV-19/Japan/UT-OM039/2022), which has an additional substitution (V445A) in the RBD compared to XBF, showed remarkably reduced susceptibility to LYCoV1404, indicating that the V445A substitution contributes to LYCoV1404 resistance. We also determined $FRNT_{50}$ values for S309 in Vero E6-TMPRSS2 cells to evaluate the possibility that the activity of S309 was underestimated in cells overexpressing host proteins.[4,5] S309 did not neutralise either CJ.1.1 or XBF isolates even at the highest concentration (>50,000 ng/ml) in either Vero E6-TMPRSS2 cells or Vero E6-TMPRSS2-T2A-ACE2 cells (Fig. S4B, Supplementary Appendix).

Next, we examined the efficacy of antiviral drugs against XBF. The Food and Drug Administration (FDA) has authorized remdesivir (an RNA-dependent RNA polymerase (RdRp) inhibitor), molnupiravir (also an RdRp inhibitor), and nirmatrelvir (a main protease inhibitor) for COVID-19 treatment. In Japan, ensitrelvir (a main protease inhibitor) has been approved for emergency use since November 2022. We determined their *in vitro* 50% inhibitory concentration ($IC_{50}$) values against the XBF isolate. The susceptibilities of XBF to these four antivirals were similar to those of the ancestral strain as well as those of CJ.1.1 isolate (Fig. S4C, Supplementary Appendix), suggesting that the substitutions in CJ.1.1 and XBF in the RdRp (P314L and G662S) and main protease (P3395H), respectively, do not affect drug susceptibility (Fig. S3B, Supplementary Appendix). These results suggest that remdesivir, molnupiravir, nirmatrelvir, and ensitrelvir are effective against XBF *in vitro*.

Last, we evaluated the neutralising ability of plasma from three different cohorts against the XBF isolate[1]: individuals who received four doses of either monovalent mRNA vaccine [BNT162b2 (Pfizer–BioNTech), mRNA-1273 (Moderna), or both][2]; individuals who received the bivalent (ancestral and BA.4/5) mRNA vaccine as a fifth dose; and[3] individuals who experienced a BA.2 infection after receiving three doses of mRNA vaccine. The $FRNT_{50}$ geometric mean titres against XBF were lower than those against the ancestral strain, BA.5, or BA.2.75 in plasma from individuals who received a fourth dose of the monovalent mRNA vaccine or the bivalent mRNA vaccine as a fifth vaccine (Fig. S5A and Tables S1 and S2 in the Supplementary Appendix). Of note, the bivalent vaccine administered as a fifth dose increased the neutralising activities against XBF by a factor of 3.0 (Fig. S5B and Tables S1 and S2 in the Supplementary Appendix), which was greater than the change in neutralising titres against the ancestral strain (1.6-fold) and similar to that against BA.5 (3.0-fold). For plasma from vaccinees with BA.2 breakthrough infection after a third dose of the mRNA vaccine, while the $FRNT_{50}$ geometric mean titres against XBF were lower than those against the ancestral strain, BA.5, or BA.2.75 (Fig. S5A and Table S3 in the Supplementary Appendix), most of the samples (9 of 10 samples) showed neutralising activity, which was similar to the findings with the plasma from individuals who received the bivalent mRNA vaccine (20 of 22 samples with neutralising activity). The $FRNT_{50}$ geometric mean titres against CJ.1.1 were similar to those against XBF in all tested groups. These results suggest that the bivalent vaccine can enhance neutralising activities but XBF evades humoral immunity induced by mRNA vaccines or natural infection.

Overall, our data suggest that bebtelovimab, remdesivir, molnupiravir, nirmatrelvir, and ensitrelvir remain effective therapeutic options against the omicron sublineage XBF, although bebtelovimab is no longer authorized for use by the FDA. In addition, bivalent mRNA vaccine boosters may augment humoral immunity against XBF and XBB.1.5 infection.[1]

#### Contributors

R.U.: conceptualization, formal analysis, validation, visualization, and writing the first draft. M. Ito, M. Kiso: data curation, formal analysis, and methodology. S. Yamayoshi: conceptualization and methodology. K.I-H.: resources and validation. Y.S-T., M. Imai, M. Koga, S. Yamamoto, Y. Kashima, E.A., M.S., T.T, A.O., T.K., H.Y.,Y.S.: resources. B.K. and J.T.: formal analysis and methodology. Y.Kawaoka: conceptualization, supervision, writing (review and editing), and funding acquisition. R.U., M. Ito, and M. Kiso contributed equally.

#### Declaration of interests

Y.Kawaoka 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. T.K. is employed by Nihon Sumo Kyokai. The remaining authors declare that they have no competing interests.

#### Acknowledgements

We thank Susan Watson for scientific editing. We also thank the Medical Association of Kashiwa for collecting clinical samples. In addition, we thank Mashiho Yanagi, Kyoko Yokota, Kyoko Tada, Tomoka Nagashima, Naoko Mizutani, Rie Onoue, and Madoka Yoshikawa for technical assistance. Vero E6-TMPRSS2-T2A-ACE2 cells were provided by Dr. Barney Graham, NIAID Vaccine Research Center.

We thank the global community of scientists who share their current SARS-CoV-2 sequence data through GISAID. In this study we used sequences deposited in the database by Mar. 20, 2023. We used the full set of GISAID sequences sampled after Oct. 1, 2022 and all sequences that were been designated XBF. To view the contributors of each individual sequence with the sequence details see:

XBF designated SARS-CoV-2 sequences: 8127 individual genome sequences with collection dates ranging from 07-27-2022 to 03-16-2023, GISAID Identifier: EPI_SET_230320mo https://doi.org/10.55876/gis8.230320mo. Data were collected in 47 countries and territories.

All GISAID SARS-CoV-2 sequences with a sampling date after 10-01-2022: 1,337,360 individual genome sequences EPI_SET_230320po with collection dates ranging from 10-01-2022 to 03-16-2023.

Data were collected in 157 countries and territories.

This study was supported by grants from the Center for Research on Influenza Pathogenesis and Transmission (75N93021C00014) by the National Institute of Allergy and Infectious Diseases, and a research programme on emerging and re-emerging infectious diseases (JP21fk0108552 and JP21fk0108615), a project promoting

support for drug discovery (JP21nf0101632), the Japan Program for Infectious Diseases Research and Infrastructure (JP22wm0125002), and the Japan Initiative for World-leading Vaccine Research and Development Centers (JP223fa627001) from the Japan Agency for Medical Research and Development. B.K. and J.T. are supported by the NIH SAVE project (AAI12018-001-00000). The funders had no role in the study design, data collection, data analysis, interpretation, or writing of the paper.

**Appendix A. Supplementary data**

Supplementary data related to this article can be found at https://doi.org/10.1016/j.lanwpc.2023.100777.

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