Virologica Sinica 38 (2023) 313–316

Contents lists available at ScienceDirect

Virologica Sinica

journal homepage: www.keaipublishing.com/en/journals/virologica-sinica
www.virosin.org

Letter

Identification of broad neutralizing antibodies against Omicron subvariants from COVID-19 convalescents and vaccine recipients

Jun Chen [a,c,1], Jing Yang [b,1], Fangfang Chang [d,1], Yabin Hu [c,1], Qian Wu [c,d,1], Shishan Teng [c], Yongchen Liu [d], Jian Zhang [c], Rongzhang He [c], Bo Liu [c], Xingyu Zheng [c], Ze Liu [e], Yanxi Peng [f], Zhenhua Xie [f], Yuanfang Zhang [g], Rui Lu [c], Dong Pan [c], You Wang [h], Liting Peng [c], Wenpei Liu [b,c,*], Yi-Ping Li [d,i,*], Xiaowang Qu [a,c,*]

[a] School of Public Health, Southern Medical University, Guangzhou, 510515, China
[b] School of Laboratory Medicine and Biotechnology, Southern Medical University, Guangzhou, 510515, China
[c] Translational Medicine Institute, The First People's Hospital of Chenzhou, Hengyang Medical School, University of South China, Chenzhou, 423000, China
[d] Institute of Human Virology, Department of Pathogen Biology and Biosecurity, and Key Laboratory of Tropical Disease Control of Ministry of Education, Zhongshan School of Medicine, Sun Yat-sen University, Guangzhou, 510080, China
[e] School of Nursing, Xiangnan University, Chenzhou, 423000, China
[f] College of Basic Medicine, Xiangnan University, Chenzhou, 423000, China
[g] College of Pharmacy, Xiangnan University, Chenzhou, 423000, China
[h] School of Public Health, Hengyang Medical School, University of South China, Hengyang, 421001, China
[i] Department of Infectious Diseases, The Third Affiliated Hospital of Sun Yat-sen University, Guangzhou, 510630, China

Dear Editor,

Omicron (B.1.1.529) was designated a variant of concern (VOC) on November 26, 2021 (Callaway, 2021), and its subvariants BA.1, BA.2, and BA.3 emerged and circulated almost simultaneously (Desingu et al., 2022). BA.2 was more efficient in transmission and quickly overtook BA.1 to become the variant most frequently detected worldwide (Yamasoba et al., 2022a). Compared to the prototype SARS-CoV-2 spike protein (S), the BA.1 and BA.2 spike proteins harbor more than 30 mutations, of which 21 are identical between the two subvariants, while the BA.3 spike differs from BA.1 and BA.2 by 3 mutations in the receptor binding domain (RBD) (Fig. 1A). More recently, BA.4 and BA.5 (hereafter BA.4/5) emerged, sharing the same spike sequence and containing four additional mutations, Del69–70, L452R, F486V, and R493Q, compared with BA.2. BA.4/5 were detected first in South Africa and evolved independently of BA.2; they have spread widely and replaced BA.2 as the predominant VOC (Gruell et al., 2022b; Tegally et al., 2022). In addition, BA.2.75, derived from the BA.2 subvariant, harbors nine additional mutations in the spike protein compared with BA.2 (Fig. 1A). BA.4/5 and BA.2.75 have led to the continuous emergence of novel Omicron subvariants, including BF.7 and BQ.1. These new subvariants may be driving waves of pandemics.

BA.1, BA.2, and BA.3 have proven to be considerably evasive of neutralization by sera from COVID-19 convalescents or vaccine recipients and by monoclonal antibodies (mAbs) approved as therapeutics (Ai et al., 2022; Kurhade et al., 2022). Moreover, the BA.4/5 subvariants evade neutralization more efficiently than BA.2 (Cao et al., 2022; Hachmann et al., 2022). BA.2.75 has shown a lower capacity for escape from neutralization than BA.5 (Tan et al., 2022). Recent studies have highlighted that most therapeutic antibodies lose neutralizing activity completely or partially against Omicron subvariants (Gruell et al., 2022b; Yamasoba et al., 2022b). To date, only bebtelovimab has demonstrated potent neutralization activity against the Omicron BA.1, BA.2, BA.4/5, and BA.2.75 subvariants (Gruell et al., 2022b; Gruell et al., 2022a; Hentzien et al., 2022; Yamasoba et al., 2022a). Thus, identifying additional antibody candidates for neutralizing SARS-CoV-2 variants, especially emerging Omicron subvariants, is urgently needed. In this study, we tested the neutralizing profiles of a panel of mAbs previously isolated from COVID-19 convalescents and vaccine recipients and demonstrated five mAbs showing potent broad neutralizing activity against five major SARS-CoV-2 variants and Omicron subvariants.

Previously, we constructed a panel of mAbs from B cells of COVID-19 convalescents and vaccine recipients and demonstrated that 77 mAbs could neutralize pseudotyped prototype SARS-CoV-2 spike virus (Wuhan-Hu-1, GenBank no. YP_009724390.1; defined by $\geq$ 50% inhibition observed at 1 μg/mL mAb) (Fig. 1B). Whether these mAbs show broad neutralizing activity against SARS-CoV-2 variants, especially

* Corresponding authors.
E-mail addresses: wenpeiliu_2008@foxmail.com (W. Liu), lyiping@mail.sysu.edu.cn (Y.-P. Li), quxiaowang@163.com (X. Qu).
[1] Jun Chen, Jing Yang, Fangfang Chang, Yabin Hu and Qian Wu contributed equally to this study.

https://doi.org/10.1016/j.virs.2023.01.005
Received 4 October 2022; Accepted 13 January 2023
Available online 19 January 2023

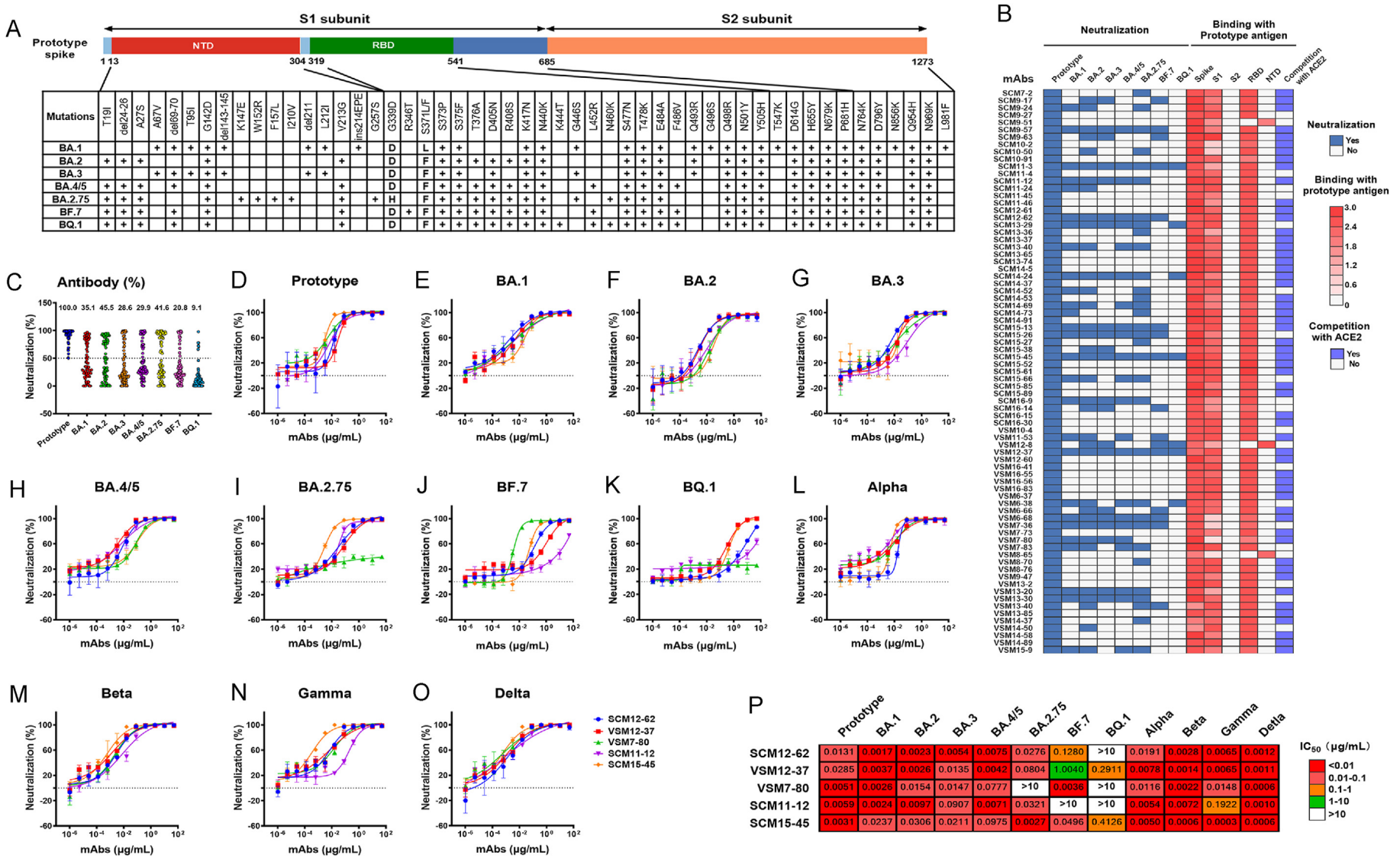

Fig. 1. Neutralizing activity of mAbs against prototype SARS-CoV-2, Omicron subvariants, and other VOCs. **A** Omicron subvariants spike mutations compared to prototype SARS-CoV-2. **B** Heatmap of pseudovirus neutralization, binding, and ACE2 competition of mAbs. Pseudotype-based virus assays were used to test for neutralization. Binding and ACE2 competition of mAbs to prototype SARS-CoV-2 measured by enzyme linked immunosorbent assay (ELISA). Neutralization was defined as $\geq$ 50% inhibition at 1 μg/mL mAb; binding was defined as an $OD_{450nm} \geq 0.3$ at 1 μg/mL mAb; and ACE2 competition was defined as an antibody binding to the RBD and inhibiting ACE2 binding by $\geq$ 50%. **C** Neutralization of mAbs against prototype SARS-CoV-2 and Omicron subvariants. Seventy-seven mAbs that neutralized prototype SARS-CoV-2 were tested for their capacity to neutralize the Omicron subvariants ($\geq$ 50% inhibition at 1 μg/mL mAb was considered neutralization). The upper numbers in the figure represent the percentage of antibodies with neutralization. **D–O**. The neutralization curves of five mAbs against prototype SARS-CoV-2 (**D**), Omicron subvariants (**E–K**), and other VOCs (**L–O**). **P** Heatmap of the IC_{50} of five mAbs against prototype SARS-CoV-2, Omicron subvariants, and other VOCs. Data are calculated from panels **D–O**, and data were shown as mean with standard deviation. Data were analyzed and plotted by GraphPad Prism (version 8.0).

Omicron subvariants, has not been demonstrated. Here, we tested the neutralizing profile against SARS-CoV-2 VOCs by using pseudotyped virus. Our results showed that only a proportion of these mAbs were able to neutralize pseudotyped viruses of Omicron subvariants, with 27 for BA.1 (35.1%), 35 for BA.2 (45.5%), 22 for BA.3 (28.6%), 23 for BA.4/5 (29.9%), 32 for BA.2.75 (41.6%), 16 for BF.7 (20.8%), and 7 for BQ.1 (9.1%) (Fig. 1B and C). These data indicated that the emerging Omicron subvariants represented by BF. 7 and BQ.1 had a greater ability to escape neutralization, especially BQ.1, which exhibited the largest neutralization resistance (Fig. 1C). All the mAbs bound to prototype S1, and 96.1% of the mAbs (74/77) bound to the RBD, of which 72.7% (56/77) of the mAbs showed competition for ACE2 binding (Fig. 1B). Of the Omicron-neutralizing mAbs, approximately two-thirds competed with ACE2, with 63.0% for BA.1 (17/27), 62.9% for BA.2 (22/35), 68.2% for BA.3 (15/22), 65.2% for BA.4/5 (15/23), 75.0% for BA.2.75 (24/32), 81.3% for BF.7 (13/16), and 57.1% for BQ.1 (4/7) (Fig. 1B). Collectively, these results suggest that most of these broadly neutralizing mAbs neutralized the prototype SARS-CoV-2 and Omicron subvariants by competitively binding to the RBD, thus interfering with the RBD-ACE2 interaction.

Next, we further characterized five mAbs that neutralized prototype SARS-CoV-2 by $\geq$ 95% inhibition (observed at 1 μg/mL mAb) and maintained potent neutralizing activity against Omicron subvariants BA.1 and BA.2 ($\geq$ 80% inhibition at 1 μg/mL), which represent two distinct Omicron sublineages. The half-maximal inhibitory concentrations (IC_{50}) of these antibodies for prototype SARS-CoV-2 and Omicron subvariants were initially evaluated (Supplemental Table S1). These five antibodies showed potent neutralizing capacity in the majority of subvariants with a distinct neutralizing profile in the different subvariants (Fig. 1D–K). As expected, the BF.7 and BQ.1 subvariants exhibited greater neutralization resistance to these antibodies than the other subvariants. Compared to other antibodies, SCM15-45 was shown to be the most potent mAb for neutralizing prototype SARS-CoV-2, showing broad neutralizing profiles against Omicron subvariants (Fig. 1P). We further assessed the cross-binding reactivity and affinity of these five antibodies to different spike protein variants. All antibodies showed good reactivity and affinity for the spike proteins tested, with the exception of BA.1, which showed relatively weak activity (Supplementary Table S1). Among them, SCM11-12 bound to all tested spike proteins with a high activity, followed by SCM15-45. Sequence analysis revealed that these five mAbs originated from *IGHV3*, a *V* gene frequently used in the human antibody repertoire. The heavy chain CDR3 had a median length of 11 amino acids (aa) (ranging from 10 to 19 aa) and diverged from the germline sequence by 93.8%–98.0%. In addition, the light chain of these five mAbs was derived from *IGKV1*, *IGLV1* and *IGLV3*. The light chain CDR3 had a median length of 9 aa (ranging from 9 to 11 aa) and diverged from the germline sequence by 94.4%–98.3% (Supplementary Table S2). To test whether these five mAbs neutralized other VOCs, we performed neutralization assays for Alpha (B.1.1.7), Beta (B.1.351), Gamma (P.1), and Delta (B.1.617.2). All five mAbs efficiently neutralized these VOCs (Fig. 1L–O). SCM15-45 was the most potent mAb neutralizing these VOCs compared with the other mAbs (Fig. 1L–O). Together, we identified five mAbs that potently neutralized prototype SARS-CoV-2, five VOCs, and Omicron subvariants, with SCM15-45 showing a broad neutralizing profile.

The development and characterization of mAbs with broad neutralizing activity are vitally important for the prevention and therapy of infections with SARS-CoV-2 and its variants. Here, we identified a proportion of mAbs that had neutralizing activity against prototype SARS-CoV-2 and could also neutralize five major VOCs and Omicron subvariants recently shown to be prevalent worldwide. In addition, most of the mAbs we studied here lost neutralizing activity for Omicron subvariants to varying extents and exhibited distinct antibody escape patterns, such as in BF.7 and BQ.1, which are substantially more resistant, a finding consistent with serological results recently reported by other groups (Qu et al., 2022). The N460K and K444T mutations (present in BQ.1) and the R346T mutation (present in BF.7) are essential for the enhanced resistance of the BQ.1 and BF.7 subvariants. The majority of the mAbs examined in this study showed good reactivity and affinity for the spike proteins, simultaneously showed binding to RBD and competition for ACE2 binding, which may explain that the potency in neutralizing the prototype and VOCs occurs primarily through interfering with RBD-ACE2 binding. Nevertheless, the mechanisms of neutralization require further investigation. A caveat of this study is that pseudotyped viruses were used to test for neutralization. Although previous studies have demonstrated a good correlation between pseudotype-based virus assays and assays using real viruses (Graham et al., 2021), the neutralizing profile of these mAbs needs to be further confirmed by authentic virus. The mAbs with broad neutralizing activity against SARS-CoV-2 and related variants developed here provide candidates for antibody therapy of COVID-19 and future vaccine design toward prophylactics of pancoronaviruses.

Footnotes

This work was supported by the National Natural Science Foundation of China (82061138020, 32270996, 82102365), The Science and Technology Innovation Program of Hunan Province of China (2022RC3079), the Educational Commission of Hunan Province of China (21A0529), the Natural Science Foundation of Hunan Province of China (2021JJ40006, 2022JJ30095), the Clinical Medical Innovation Technology Guide Project of Hunan Province (2021SK50304, 2021SK50306 and 2021SK50312) and "SC1-PHE-CORONAVIRUS-2020: Advancing knowledge for the clinical and public health response to the 2019-nCoV epidemic" from the European Commission (CORONADX, no. 101003562) (to Y-P.L.). This study was approved by the Institutional Ethical Review Board of The Central Hospital of Shaoyang, Hunan Province, China (V.1.0, 20200301) and The First People's Hospital of Chenzhou, Hunan Province, China (V.3.0, 2021001). Each participant signed a written consent form.

Supplementary data to this article can be found online at https://doi.org/10.1016/j.virs.2023.01.005.

References

Ai, J., Wang, X., He, X., Zhao, X., Zhang, Y., Jiang, Y., Li, M., Cui, Y., Chen, Y., Qiao, R., Li, L., Yang, L., Li, Y., Hu, Z., Zhang, W., Wang, P., 2022. Antibody evasion of sars-cov-2 omicron ba.1, ba.1.1, ba.2, and ba.3 sub-lineages. Cell Host Microbe 30, 1077–1083.

Callaway, E., 2021. Heavily mutated omicron variant puts scientists on alert. Nature 600, 21.

Cao, Y., Yisimayi, A., Jian, F., Song, W., Xiao, T., Wang, L., Du, S., Wang, J., Li, Q., Chen, X., Yu, Y., Wang, P., Zhang, Z., Liu, P., An, R., Hao, X., Wang, Y., Wang, J., Feng, R., Sun, H., Zhao, L., Zhang, W., Zhao, D., Zheng, J., Yu, L., Li, C., Zhang, N., Wang, R., Niu, X., Yang, S., Song, X., Chai, Y., Hu, Y., Shi, Y., Zheng, L., Li, Z., Gu, Q., Shao, F., Huang, W., Jin, R., Shen, Z., Wang, Y., Wang, X., Xiao, J., Xie, X.S., 2022. Ba.2.12.1, ba.4 and ba.5 escape antibodies elicited by omicron infection. Nature 608, 593–602.

Desingu, P.A., Nagarajan, K., Dhama, K., 2022. Emergence of omicron third lineage ba.3 and its importance. J. Med. Virol. 94, 1808–1810.

Graham, C., Seow, J., Huettner, I., Khan, H., Kouphou, N., Acors, S., Winstone, H., Pickering, S., Galao, R.P., Dupont, L., Lista, M.J., Jimenez-Guardeno, J.M., Laing, A.G., Wu, Y., Joseph, M., Muir, L., van Gils, M.J., Ng, W.M., Duyvesteyn, H.M.E., Zhao, Y., Bowden, T.A., Shankar-Hari, M., Rosa, A., Cherepanov, P., McCoy, L.E., Hayday, A.C., Neil, S.J.D., Malim, M.H., Doores, K.J., 2021. Neutralization potency of monoclonal antibodies recognizing dominant and subdominant epitopes on sars-cov-2 spike is impacted by the b.1.1.7 variant. Immunity 54, 1276–1289 e1276.

Gruell, H., Vanshylla, K., Tober-Lau, P., Hillus, D., Sander, L.E., Kurth, F., Klein, F., 2022a. Neutralisation sensitivity of the sars-cov-2 omicron ba.2.75 sublineage. Lancet Infect. Dis. 22, 1422–1423.

Gruell, H., Vanshylla, K., Korenkov, M., Tober-Lau, P., Zehner, M., Munn, F., Janicki, H., Augustin, M., Schommers, P., Sander, L.E., Kurth, F., Kreer, C., Klein, F., 2022b. Sars-cov-2 omicron sublineages exhibit distinct antibody escape patterns. Cell Host Microbe 30, 1231–1241.

Hachmann, N.P., Miller, J., Collier, A.Y., Ventura, J.D., Yu, J., Rowe, M., Bondzie, E.A., Powers, O., Surve, N., Hall, K., Barouch, D.H., 2022. Neutralization escape by sars-cov-2 omicron subvariants ba.2.12.1, ba.4, and ba.5. N. Engl. J. Med. 387, 86–88.

Hentzien, M., Autran, B., Piroth, L., Yazdanpanah, Y., Calmy, A., 2022. A monoclonal antibody stands out against omicron subvariants: a call to action for a wider access to bebtelovimab. Lancet Infect. Dis. 22, 1278.

Kurhade, C., Zou, J., Xia, H., Cai, H., Yang, Q., Cutler, M., Cooper, D., Muik, A., Jansen, K.U., Xie, X., Swanson, K.A., Shi, P.Y., 2022. Neutralization of omicron ba.1, ba.2, and ba.3 sars-cov-2 by 3 doses of bnt162b2 vaccine. Nat. Commun. 13, 3602.

Qu, P., Evans, J.P., Faraone, J., Zheng, Y.M., Carlin, C., Anghelina, M., Stevens, P., Fernandez, S., Jones, D., Lozanski, G., Panchal, A., Saif, L.J., Oltz, E.M., Xu, K., Gumina, R.J., Liu, S.L., 2022. Distinct neutralizing antibody escape of sars-cov-2 omicron subvariants bq.1, bq.1.1, ba.4.6, bf.7 and ba.2.75.2. bioRxiv.

Tan, C.W., Lim, B.L., Young, B.E., Yeoh, A.Y., Yung, C.F., Yap, W.C., Althaus, T., Chia, W.N., Zhu, F., Lye, D.C., Wang, L.F., 2022. Comparative neutralisation profile of sars-cov-2 omicron subvariants ba.2.75 and ba.5. Lancet Microbe 3, e898.

Tegally, H., Moir, M., Everatt, J., Giovanetti, M., Scheepers, C., Wilkinson, E., Subramoney, K., Makatini, Z., Moyo, S., Amoako, D.G., Baxter, C., Althaus, C.L., Anyaneji, U.J., Kekana, D., Viana, R., Giandhari, J., Lessells, R.J., Maponga, T., Maruapula, D., Choga, W., Matshaba, M., Mbulawa, M.B., Msomi, N., consortium, N.-S., Naidoo, Y., Pillay, S., Sanko, T.J., San, J.E., Scott, L., Singh, L., Magini, N.A., Smith-Lawrence, P., Stevens, W., Dor, G., Tshiabuila, D., Wolter, N., Preiser, W., Treurnicht, F.K., Venter, M., Chiloane, G., McIntyre, C., O'Toole, A., Ruis, C., Peacock, T.P., Roemer, C., Kosakovsky Pond, S.L., Williamson, C., Pybus, O.G., Bhiman, J.N., Glass, A., Martin, D.P., Jackson, B., Rambaut, A., Laguda-Akingba, O., Gaseitsiwe, S., von Gottberg, A., de Oliveira, T., 2022. Emergence of sars-cov-2 omicron lineages ba.4 and ba.5 in South Africa. Nat. Med. 28, 1785–1790.

Yamasoba, D., Kosugi, Y., Kimura, I., Fujita, S., Uriu, K., Ito, J., Sato, K., et al., 2022a. Neutralisation sensitivity of SARS-CoV-2 omicron subvariants to therapeutic monoclonal antibodies. Lancet Infect. Dis. 22, 942–943.

Yamasoba, D., Kimura, I., Nasser, H., Morioka, Y., Nao, N., Ito, J., Uriu, K., Tsuda, M., Zahradnik, J., Shirakawa, K., Suzuki, R., Kishimoto, M., Kosugi, Y., Kobiyama, K., Hara, T., Toyoda, M., Tanaka, Y.L., Butlertanaka, E.P., Shimizu, R., Ito, H., Wang, L., Oda, Y., Orba, Y., Sasaki, M., Nagata, K., Yoshimatsu, K., Asakura, H., Nagashima, M., Sadamasu, K., Yoshimura, K., Kuramochi, J., Seki, M., Fujiki, R., Kaneda, A., Shimada, T., Nakada, T.A., Sakao, S., Suzuki, T., Ueno, T., Takaori-Kondo, A., Ishii, K.J., Schreiber, G., Japan, C., Sawa, H., Saito, A., Irie, T., Tanaka, S., Matsuno, K., Fukuhara, T., Ikeda, T., Sato, K., 2022b. Virological characteristics of the sars-cov-2 omicron ba.2 spike genotype to phenotype. Cell 185, 2103–2115.