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COMMENT

Neutralizing antibodies and their cocktails against SARS-CoV-2 Omicron and other circulating variants

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Severe acute respiratory coronavirus 2 (SARS-CoV-2), the causative agent of coronavirus disease 2019 (COVID-19), has led to a pandemic with severe economic losses. The viral surface spike (S) protein comprises two subunits: S1 and S2. S1 contains an N-terminal domain (NTD) and a C-terminal domain (CTD) (i.e., the receptor binding domain (RBD)) [1, 2]. The RBD itself comprises core and receptor binding motif (RBM) regions **(**Fig. 1a**)**. During virus infection in humans, the RBD of S1 binds to the cellular receptor angiotensin-converting enzyme 2 (ACE2) (Fig. 1b), and S2 mediates viral entry and membrane fusion [1, 3]. Thus, the S protein is a critical vaccine and therapeutic target. The prefusion S protein exists as a trimer consisting of three RBDs; of these, the RBD in the "up" confirmation binds to ACE2 (Fig. 1c). SARS-CoV-2 has undergone frequent mutations since its emergence in 2019, and a number of mutated S protein residues have been identified, including in the RBD. Alpha (B.1.1.7), Beta (B.1.351), and Gamma (P.1) are previously circulating variants of concern (VOCs); in contrast, Delta (B.1.617.2), Omicron (B.1.1.529) BA.1, and other Omicron subvariants (BA.2, BA.3, BA.4, and BA.5), as well as BA.1/BA.2 circulating recombinant forms such as XE, are currently circulating VOC strains [4]. Compared with the original SARS-CoV-2, the Omicron variant carries more mutations than any other variant identified thus far, among which approximately 39 and 15 substitutions are within the S protein and RBD, respectively, of the BA.1 subvariant. Crystal and cryo-electron microscopy (cryo-EM) structures of Omicron S/RBD-ACE2 complexes demonstrate that the Omicron S trimer harbors substitutions at a number of RBD residues on the outer surface, with upright RBD(s) being responsible for receptor binding (Fig. 1d, e) [5–9]. However, these mutations in the RBD do not significantly reduce the binding affinity of the RBD for the ACE2 receptor. Many neutralizing monoclonal antibodies (mAbs) were developed based on the original SARS-CoV-2 strain with the aim of preventing and treating SARS-CoV-2 infection. Therefore, it is important to understand whether these mAbs neutralize SARS-CoV-2 VOCs and whether antibody cocktail treatments retain neutralizing activity against currently circulating variants. In a recent issue of *Nature Medicine*, Bruel et al. compared the neutralizing activity of therapeutic mAbs against the Omicron subvariants BA.1 and BA.2 and analyzed the serum-neutralizing activity of immunocompromised people after treatment with anti-COVID-19 mAb cocktails [10].

The majority of the neutralizing mAbs developed target the RBD, whereas only a few target the NTD or other regions of the SARS-CoV-2 S protein [2]. RBD-targeting mAbs neutralize SARS-CoV-2 in two ways: (1) by binding to the ACE2-binding region (RBM) of the RBD to compete with ACE2-RBD, thereby inhibiting viral attachment (ACE2-competitive mAbs); or (2) by binding to the non-ACE2 binding region (core) of the RBD to induce conformational changes in the S protein, thereby blocking viral entry and subsequent cell-cell fusion (non-ACE2-competitive mAbs) (Fig. 1f) [1, 2, 11]. Most RBD-targeting mAbs, including 55A8, 58G6, S2K146, S2X259, S2H97, THSC20.HVTR04, and THSC20.HVTR26, are in preclinical trials to examine whether they can prevent SARS-CoV-2 infection in cell culture or animal models [12–14]. Other RBD-targeting mAbs, including bamlanivimab (LY-CoV555), etesivimab (LY-CoV016), casirivimab (REGN-10933), imdevimab (REGN-10987), adintrevimab (AGD20), regdanvimab (CT-P59), sotrovimab (VIR-7831), VIR-7832, tixagevimab (AZD8895 or COV2-2196), cilgavimab (AZD1061 or COV2-2130), and bebtelovimab (LY-CoV1404), have progressed to full clinical trials or have received emergency use authorization (EUA) to treat COVID-19 [10, 13, 15].

Although anti-RBD mAbs may neutralize the original strain of SARS-CoV-2, many show reduced neutralizing activity against SARS-CoV-2 VOCs, particularly the Omicron variant. Bruel et al. conducted S-Fuse neutralization assays to compare the neutralizing activity of the nine RBD-targeting mAbs used clinically, as described above, against Delta and Omicron VOCs [10]. Different from traditional plaque-based neutralization assays, the S-Fuse neutralization assay is a fluorescence-based method for calculating the formation of syncytia after virus infection, and the results can be used to calculate the neutralization percentage. The presence of green fluorescent protein allows for rapid measurement of viral infectivity and mAb neutralizing activity. According to the results, except for bamlanivimab (which did not neutralize the Delta variant at >9000 ng/ml), the other eight mAbs neutralized this variant to some degree at doses ranging from 0.58 to 280 ng/ml. Nevertheless, only two mAbs (imdevimab and cilgavimab) neutralized the Omicron BA.2 subvariant, and only three (adintrevimab, sotrovimab, and cilgavimab) neutralized the Omicron BA.1 subvariant when used at relatively high concentrations. Similar to the above report, the RBD (RBM)-targeting mAbs bamlanivimab, etesivimab, casirivimab, imdevimab, COV2-2196, and regdanvimab showed reduced or a complete lack of neutralizing activity against Omicron in cell culture, as reported by other groups, potentially due to mutation of the epitopes recognized [9, 15].

Conversely, other RBD-targeting mAbs, such as bebtelovimab, which binds to the RBD outside of the ACE2-binding region,

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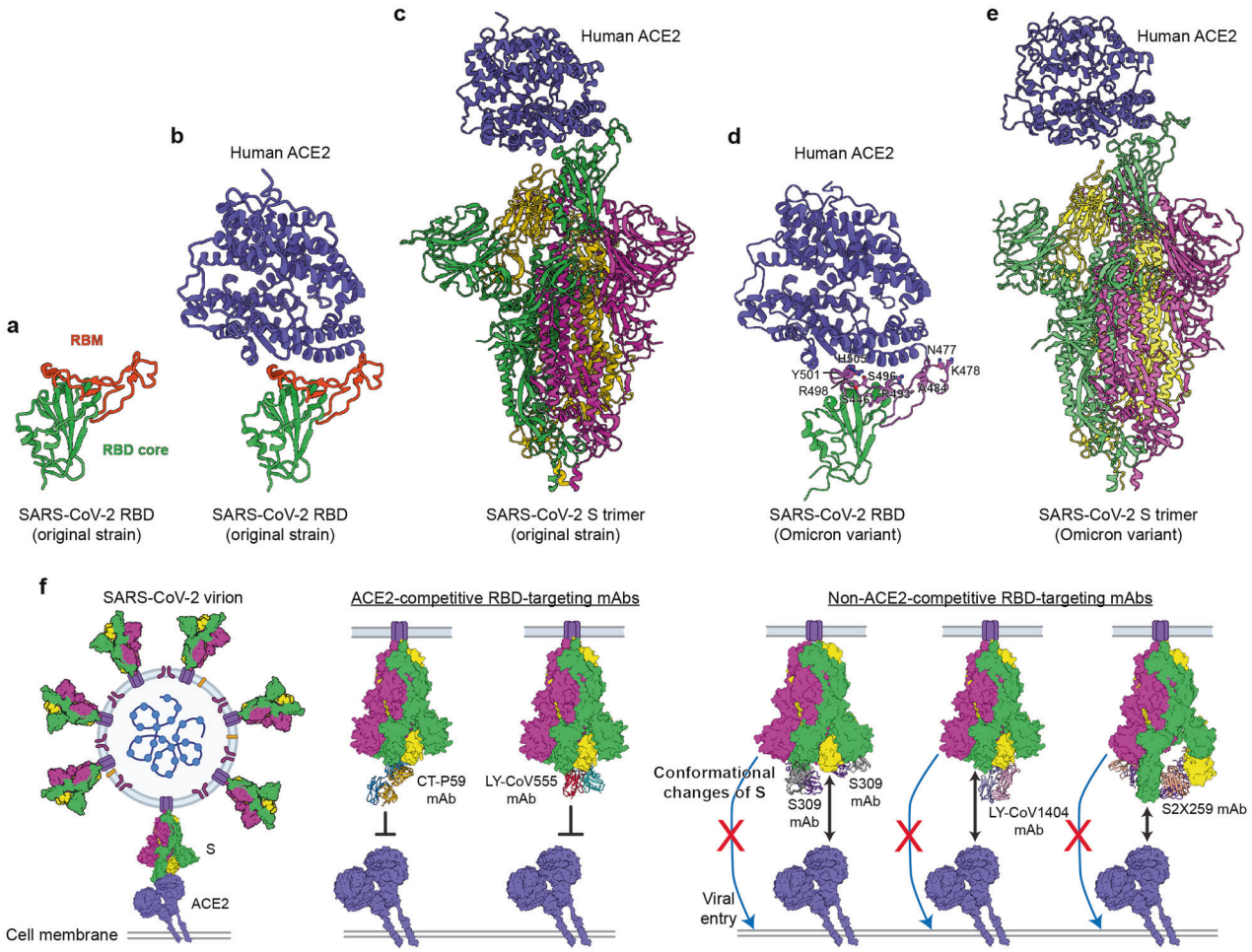

Fig. 1 Receptor recognition and cell entry mediated by the SARS-CoV-2 spike (S) protein and its inhibition by neutralizing antibodies. **a** Crystal structure of the receptor-binding domain (RBD) of the original strain of SARS-CoV-2 (extracted from PDB 6M0J). The core region is colored green, and the receptor-binding motif (RBM) is colored orange-red. **b** Crystal structure of the original strain of SARS-CoV-2 RBD in complex with the human angiotensin-converting enzyme 2 (ACE2) receptor (PDB 6M0J). ACE2 is colored blue. **c** Cryo-EM structure of the original strain of SARS-CoV-2 S trimer in complex with human ACE2 (PDB 7DF4). The three S subunits are colored green, yellow, and magenta, respectively. **d** Cryo-EM structure of the SARS-CoV-2 Omicron variant RBD in complex with human ACE2 (PDB 7WPB). The RBM is colored purple. RBM residues that have undergone mutations from the original strain to the Omicron variant are labeled and shown as sticks. **e** Cryo-EM structure of the SARS-CoV-2 Omicron variant S trimer in complex with human ACE2 (PDB 7WPA). **f** Mechanisms of neutralization by ACE2-competitive and non-ACE2-competitive RBD-targeting monoclonal antibodies (mAbs). Left, schematic map of the SARS-CoV-2 virion and its binding with the cellular ACE2 receptor through the RBD of the S protein. Middle, CT-P59 (regdanvimab) and LY-CoV555 (bamlanivimab) are representatives of ACE2-competitive mAbs. The composite structural model of the SARS-CoV-2 S trimer/CT-P59 mAb complex was generated by docking the CT-P59 mAb to the S trimer based on the alignment of RBD regions between the crystal structure of the RBD/CT-P59 mAb complex (PDB 7CM4) and the cryo-EM structure of the SARS-CoV-2 S trimer (PDB 6VYB). The illustration of the SARS-CoV-2 S trimer/LY-CoV555 mAb was prepared using PDB 7L3N. Right, S309, LY-CoV1404 (bebtelovimab), and S2X259 are representatives of non-ACE2-competitive mAbs. The composite structural model of the SARS-CoV-2 S trimer/LY-CoV1404 mAb complex was generated by docking the LY-CoV1404 mAb to the S trimer based on the alignment of RBD regions between the crystal structure of the RBD/LY-CoV1404 mAb complex (PDB 7MMO) and the cryo-EM structure of the SARS-CoV-2 S trimer (PDB 6VYB). The illustrations of the SARS-CoV-2 S trimer/S309 mAb and SARS-CoV-2 S trimer/S2X259 mAb were prepared using PDB 6WPS and PDB 7RA8, respectively

exhibit neutralizing activity against the BA.1 and/or BA.2 subvariants [9]. Of note, bebtelovimab shows high potency for neutralizing other VOCs, including Alpha, Beta, Gamma, Delta, B.1.427/B.1.429, and B.1.526, and is capable of binding effectively to S proteins harboring the RBD mutations K417N, L452R, E484K, and N501Y. Furthermore, sotrovimab, S2X259, and S2H97, which recognize conserved neutralizing epitopes outside of the RBM, display neutralizing activity against the Omicron variant [13]. These studies suggest that neutralizing mAbs that recognize highly conserved epitopes, particularly those outside of the RBD RBM region, may retain potent binding and neutralizing activity against VOCs, including the Omicron variant, and thus are unlikely to be affected by the most common RBD mutations.

In general, mAb cocktails have been used extensively to prevent and treat infection by SARS-CoV-2 as well as its variants. For example, several mAb cocktails, such as casirivimab/imdevimab (REGEN-COV in the U.S. or Ronapreve in the European Union and other countries) and cilgavimab/tixagevimab (Evusheld) have received EUA as pre- or postexposure prophylactics to prevent and treat SARS-CoV-2 infection [10]. In some cases, combining mAbs may improve the neutralizing activity of the individual component mAbs, thereby reducing COVID-19 symptoms. Nonetheless, other cocktails of mAbs show decreased neutralizing activity against SARS-CoV-2 variants, particularly Omicron, compared with that against the original virus strain [16].

Bruel et al. evaluated SARS-CoV-2 S-specific IgG antibodies and the neutralizing activity of antibodies from the serum of

immunocompromised individuals receiving Ronapreve and/or Evusheld mAb cocktails [10]. The authors reported that treatment with a single (Ronapreve or Evusheld) mAb cocktail, but not two successive mAb cocktails (Ronapreve + Evusheld), increased serum IgG antibody titers. Although Ronapreve treatment effectively neutralized the Delta variant and poorly neutralized the Omicron BA.2 subvariant, it did not neutralize the Omicron BA.1 subvariant. In addition, Evusheld or Ronapreve + Evusheld effectively neutralized the Delta variant and BA.2 subvariant but not the BA.1 subvariant, suggesting that Evusheld is more active against the BA.2 than the BA.1 subvariant.

Other studies have evaluated the combined effects of mAb cocktails against SARS-CoV-2 Omicron and other variants in vitro and/or in vivo. A cocktail comprising casirivimab and imdevimab (REGEN-COV) exhibited reduced neutralizing ability against Omicron containing the RBD mutations K417N, N440K, L484A, and Q498R, despite an increased ability to bind the RBD compared with the respective component mAbs and despite efficient neutralization of the Delta variant [17]. A cocktail of bamlanivimab and etesivimab, or a combination of COV2-2196 and COV2-2130, completely lost the ability (or it was attenuated) to neutralize the Omicron variant in cell culture, and Ronapreve failed to prevent Omicron infection in human ACE2-transgenic (hACE2-Tg) mice [13, 15, 18]. In contrast, combinations of COV2-2196 + COV2-2130, B1-182.1 + A19-46.1, and B1-182.1 + S309 mAbs showed better neutralizing activity against Omicron than the individual antibodies [9]. Additionally, 55A8 and 58G6 mAb cocktails demonstrated synergetic neutralizing activity in cell culture and protected hamsters from challenge with Omicron (BA.1) [14]. Notably, cocktails of neutralizing mAbs that target nonoverlapping epitopes on the SARS-CoV-2 S RBD may bind to the domain simultaneously, with synergistic effects for preventing and treating infection by SARS-CoV-2 variants [9, 12].

In conclusion, the results presented by Bruel et al. demonstrate that SARS-CoV-2 RBD-targeting therapeutic mAbs or mAb cocktails have different abilities to neutralize Omicron, particularly the BA.1 and BA.2 subvariants [10]. There are some potential limitations, however. For example, the small number of immunocompromised patients receiving mAb treatment might not reflect the efficacy of mAbs against SARS-CoV-2 variants; therefore, studies enrolling more immunocompromised and nonimmunocompromised individuals would be of value. In addition, it is necessary to confirm the data from fluorescence-based neutralization assays using other neutralization assays. Bruel et al. also reported that despite a clear correlation between serum IgG titers and neutralizing antibody titers against Delta, such a correlation was not obvious with respect to neutralizing antibodies against Omicron [10]; this may be due (in part) to the fact that the RBD of this variant S protein contains many mutations. It should be noted that, in addition to mAbs targeting the RBD, mAbs targeting other regions of the SARS-CoV-2 S protein (particularly the conserved S2 subunit) have the potential to neutralize multiple variants, including Omicron, and these mAbs may be developed as alternative therapeutics. Overall, anti-SARS-CoV-2 mAbs can be developed to target conserved neutralizing epitopes of the S protein. In particular, cocktails comprising mAbs targeting conserved and nonoverlapping epitopes on the RBD, S2, and/or NTD regions are expected to have synergistic effects and show broad and improved neutralizing activity for preventing and treating infection by SARS-CoV-2 (including Omicron and its subvariants, future variants) and other coronaviruses with pandemic potential.

REFERENCES

1. Jiang S, Zhang X, Du L. Therapeutic antibodies and fusion inhibitors targeting the spike protein of SARS-CoV-2. Expert Opin Ther Targets. 2021;25:415–21.
2. Du L, Yang Y, Zhang X. Neutralizing antibodies for the prevention and treatment of COVID-19. Cell Mol Immunol. 2021;18:2293–306.
3. Tai W, He L, Zhang X, Pu J, Voronin D, Jiang S, et al. Characterization of the receptor-binding domain (RBD) of 2019 novel coronavirus: implication for development of RBD protein as a viral attachment inhibitor and vaccine. Cell Mol Immunol. 2020;17:613–20.
4. World Health Organization. Tracking SARS-CoV-2 variants. Updated on 30 Aug. 2022. https://www.who.int/activities/tracking-SARS-CoV-2-variants.
5. McCallum M, Czudnochowski N, Rosen LE, Zepeda SK, Bowen JE, Walls AC, et al. Structural basis of SARS-CoV-2 Omicron immune evasion and receptor engagement. Science 2022;375:864–8.
6. Ye G, Liu B, Li F. Cryo-EM structure of a SARS-CoV-2 omicron spike protein ectodomain. Nat Commun. 2022;13:1214.
7. Han P, Li L, Liu S, Wang Q, Zhang D, Xu Z, et al. Receptor binding and complex structures of human ACE2 to spike RBD from omicron and delta SARS-CoV-2. Cell 2022;185:630–40. e10
8. Mannar D, Saville JW, Zhu X, Srivastava SS, Berezuk AM, Tuttle KS, et al. SARS-CoV-2 Omicron variant: antibody evasion and cryo-EM structure of spike protein-ACE2 complex. Science 2022;375:760–4.
9. Zhou T, Wang L, Misasi J, Pegu A, Zhang Y, Harris DR, et al. Structural basis for potent antibody neutralization of SARS-CoV-2 variants including B.1.1.529. Science 2022;376:eabn8897.
10. Bruel T, Hadjadj J, Maes P, Planas D, Seve A, Staropoli I, et al. Serum neutralization of SARS-CoV-2 Omicron sublineages BA.1 and BA.2 in patients receiving monoclonal antibodies. Nat Med. 2022. https://doi.org/10.1038/s41591-022-01792-5.
11. Starr TN, Czudnochowski N, Liu Z, Zatta F, Park YJ, Addetia A, et al. SARS-CoV-2 RBD antibodies that maximize breadth and resistance to escape. Nature 2021;597:97–102.
12. Hingankar N, Deshpande S, Das P, Rizvi ZA, Wibmer CK, Mashilo P, et al. A combination of potently neutralizing monoclonal antibodies isolated from an Indian convalescent donor protects against the SARS-CoV-2 Delta variant. PLoS Pathog. 2022;18:e1010465.
13. Cameroni E, Bowen JE, Rosen LE, Saliba C, Zepeda SK, Culap K, et al. Broadly neutralizing antibodies overcome SARS-CoV-2 Omicron antigenic shift. Nature 2022;602:664–70.
14. Zhang X, Luo F, Zhang H, Guo H, Zhou J, Li T, et al. A cocktail containing two synergetic antibodies broadly neutralizes SARS-CoV-2 and its variants including Omicron BA.1 and BA.2. bioRxiv [Preprint]. 2022. Available from https://doi.org/10.1101/2022.04.26.489529.
15. VanBlargan LA, Errico JM, Halfmann PJ, Zost SJ, Crowe JE, Purcell LA, et al. An infectious SARS-CoV-2 B.1.1.529 Omicron virus escapes neutralization by therapeutic monoclonal antibodies. Nat Med. 2022;28:490–5.
16. O'Brien MP, Forleo-Neto E, Sarkar N, Isa F, Hou P, Chan KC, et al. Effect of subcutaneous casirivimab and imdevimab antibody combination vs placebo on development of symptomatic COVID-19 in early asymptomatic SARS-CoV-2 infection: a randomized clinical trial. JAMA 2022;327:432–41.
17. Nguyen H, Lan PD, Nissley DA, O'Brien EP, Li MS. Cocktail of REGN antibodies binds more strongly to SARS-CoV-2 than its components, but the Omicron variant reduces its neutralizing ability. J Phys Chem B. 2022;126:2812–23.
18. Tatham L, Sharp J, Kijak E, Herriott J, Neary M, Box H, et al. Lack of Ronapreve (REGN-CoV; casirivimab and imdevimab) virological efficacy against the SARS-CoV-2 Omicron variant (B.1.1.529) in K18-hACE2 mice. bioRxiv [Preprint]. 2022. Available from https://doi.org/10.1101/2022.01.23.477397.

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AUTHOR CONTRIBUTIONS

LD wrote and revised the paper. YY prepared the figure.

COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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