Receptor-mediated host cell recognition and internalization of SARS-CoV-2 are essential for its propagation; therefore, multiple spike-binding SARS-CoV-2 neutralizing antibodies (nAbs) have been approved by the FDA to combat the COVID-19 pandemic. The spike protein comprises S1 and S2 subunits responsible for the receptor recognition/binding and membrane fusion, respectively. The N-terminal domain (NTD) in S1 contributes to the overall structural and conformational stability of the spike, while the C-terminal receptor-binding domain (RBD) recognizes ACE2 receptor on host cells (Figure 1A). Serological investigation of SARS-CoV-2 patients indicates that over 90% of neutralizing antibodies in serum/plasma target the RBD of the spike. This could be due to the relatively lower glycan-shielding and immunodominance of the RBD compared with the other glycan-coated domains of the spike. The emergence of variants of concern (VOCs) by the continuous evolution of the virus is of explicit relevance to the efficacy of COVID-19 therapeutics, as mutations in the prototype spike, RBD in particular, have rendered antibodies incapable of neutralization and substantially dropped the effectiveness of vaccines. Although debatable, all VOCs are in general characterized as rapidly transmissible, immune evading, and more pathogenic in some cases, compared with the wild-type strain. The trend of increasing host fitness by the VOCs is well set to this end; however, the mechanism by which positively selected mutations in the spike modulate the viral function and ditch the immune response require critical assessments.

ALL VOCs ENHANCE ACE2 BINDING, BUT NOT ALL MUTATIONS IN RBD AUGMENT ACE2 BINDING

Collectively, three mutations, K417N, E484K, and N501Y, were mainly responsible for the increased ACE2 binding in the Beta strain that caused the second wave of COVID-19 in 2020. The Beta and Gamma variants differ by K417N/T mutations in the spike and share similar ACE2-binding affinity (Figure 1B). The Delta variant accounted for the deadliest second wave of COVID-19 in April 2021 and harbors ten mutations in its spike, i.e., T19R, G142D* (associated with frequent back mutations, increased viral loads, and immune evasion), Δ156, Δ157, R158G, L452R, T478K, D614G, P681R, and D950N, where L452R and T478K substitutions were responsible for increased ACE2 binding. The Omicron variant appeared with the highest number of mutations in its spike (Figure 1A), which not only dramatically enhanced its ACE2 binding, infectivity, and transmission but also escaped most of the FDA-approved nAbs and vaccines. Cataloging mutations in the spike protein \( (N = 303,250) \) demonstrate that all 195 amino acids in the RBD are associated with mutations in the spike; however, not all suggested mutations involve ACE2 binding. During early COVID-19 pandemic, Starr et al. conducted a deep mutational analysis and identified mutations in the RBD that alter ACE2 binding and/or are suitable for antibody-based therapeutics. Surprisingly, among the 16 suggested RBD mutations participating in the immune escape of VOCs, only six, i.e., G339D, I452R, S477N, T478K, E484K, and N501Y, are involved in increased ACE2 binding. Alternatively, the positive selection of the VOCs can be attributed to the rise in resistance to the post-vaccinated and convalescent sera by favoring best-fitting mutations within the antigenic epitopes. Besides, due to its immunodominant nature, ~40% of the anti-SARS-CoV2 antibodies bind RBD, whereas many RBD mutations create conformational alterations by changing the charges and hydrophobicity within the epitopes and break the bond network of antigenic residues. An example of this phenomenon was found during our investigation of Omicron immune escape, where N440K substitution, distant from the ACE2 interface, impaired the RBD\(^{1\text{b}}\)-immdevimab (REGN10987) binding. Our molecular modeling study demonstrated that Omicron binds ACE2 ~2.5 times stronger than ancestral strain, facilitated by T478K, Q493K, and Q498R substitutions; however, K417N and E484A played opposing roles. Hence, the idea of mutations driving increased ACE2 binding is not sufficient to explain the complexity of viral host adaptation. The rising mutations in SARS-CoV-2 are not just due to selective pressure, but probably the outcomes of net-positive mutations that emerge under atypical conditions, such as host immune response, possible events of zoonosis, and viral adaptation.

MUTATIONS IN THE ACE2 COMPETING RBD EPITOPES

As RBD is the predominantly immunogenic domain of the spike, most of the FDA-approved nAbs were developed conventionally or isolated from the convalescent plasma of COVID-19 patients that bind to the fully/partially/non-conserved ACE2-competing or non-competing epitopes on RBD. Three mutations, N501Y, E484K, and K417N/T, which

Masaud Shah\(^1\) and Hyun Goo Woo\(^{1,2}\)

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\(^{1}\)Department of Physiology, Ajou University School of Medicine, Suwon 16499, Republic of Korea; \(^{2}\)Department of Biomedical Science, Graduate School, Ajou University, Suwon 16499, Republic of Korea

Correspondence: Hyun Goo Woo, MD, PhD, Department of Physiology, Ajou University School of Medicine, Suwon 16499, Republic of Korea

E-mail: hg@ajou.ac.kr
increase the receptor binding of Beta and Gamma variants, substantially reduced the neutralization efficacy of casirivimab, etesevimab, and bamlanivimab nAbs. To take an in-depth look at the lost interface, we modeled the 3D structure of these mAbs with Beta and Gamma RBDs and found that E484K abolished the electrostatic contact with complementarity-determining region H2 (CDRH2) and CDRL3 of bamlanivimab, whereas K417N dissociated contacts with CDRH2 of etesevimab (Figure 1B). As casirivimab and imdevimab bind two non-overlapping epitopes on RBD, the latter retained its Beta neutralization, whereas casirivimab had a substantial reduction in its neutralization due to E484K substitution. The L452R and T478K substitution that augmented the RBD\textsubscript{Delta}.ACE2 interaction completely abolished the neutralization of the Delta variant by regdanvimab (CT-p59).10

Omicron contains at least 16 mutations in the RBD, and due to the triple mutations at the furin cleavage site (Figure 1A), it is known to be the most rapidly spreading variant of SARS-CoV-2.12 Various RBD mutations, particularly, K417N, N440K, G466S, E484A, and Q493R, impair the neutralizing of most FDA-approved nAbs. We found that N417N and E484A that rather substantially reduced the ACE2-RBD binding affinity\textsuperscript{10} were directly involved in regdanvimab and bamlanivimab escape (Figure 1B). This finding further supports the theory that rising mutations in SARS-CoV-2 are not just due to the increased ACE2 affinity, but probably the outcome of net-positive mutations that emerge in atypical conditions. Taking the immune escape of Omicron into consideration, the FDA has now revised its authorization for the use of two monoclonal antibody (mAb)-based cocktail therapies (etesevimab/bamlanivimab cocktail and casirivimab/imdevimab cocktail) in COVID-19 due to the emergence of Omicron and its sub-variants including BA.2.\textsuperscript{13} Alternatively, other therapies such as sotrovimab recovered from SARS-CoV-1 patients\textsuperscript{14} and bebtelovimab\textsuperscript{15} that target rather conserved epitopes on spike could be used against Omicron.

REPOSITIONING VOC-ESCAPED mAbs USING CDR DIVERSIFICATION
mAbs are developed by several well-established techniques that require the exposure of antigen to a repertoire of antibodies induced by the antigen within animal models or screened in high-throughput techniques such as phage and yeast display. Selecting high-affinity mAbs during screening is one of the crucial steps in therapeutic antibody development that often require time-consuming and expensive procedures like binding kinetics, empirical affinity matura- tion, and epitope mapping (Figure 2A).16 In fact, antibody evolution is a natural process within our body to increase the affinity and neutralization potency against SARS-CoV-2 during infection. For some antibodies, maturation imposes multiple mutations that enable variants to escape, whereas certain antibodies become more effective against VOCs and other sarbecoviruses.\textsuperscript{17} Considering the swift immune escape scenario of SARS-CoV-2, CDR diversification, guided by pre-defined hotspots-mediated epitope-paratope knowledge, is vibrant for redesigning escaped antibodies that were previously matured and approved against certain variants. Such techniques have been implemented by AstraZeneca to enhance the affinity of hybridoma-derived AB1 against muCCL20.15 One practical example is the recent development of D27LEY nAb, which was computationally designed by CDR diversification of the SARS-CoV-1 nAbs against SARS-CoV-2 RBD.\textsuperscript{39}

Antibodies can be designed using state-of-the-art heuristic approaches such as Opt-MAVEN\textsuperscript{30} and AbDesign\textsuperscript{31} that utilize the dock-and-design strategy. The success of precise antigen positioning is constrained by the accuracy of scoring methods and sampling algorithms of the computational tools that often prioritize the desired binding patches on static antigens to the antibody scaffold. Conformational changes at the Epi-Para interface of the rigid-body docking procedure may lead to the reorientation of Ab-Ag poses and abominate the complex, as exemplified by the Prd-Pdar protein complex.\textsuperscript{22} This biased antigen positioning can be overcome with an induced-fit docking algorithm that allows some extent of side chain freedom of epitope residues. It is therefore important to consider the pre-defined interaction pattern of the cognate binders for CDRs restoration to ensure the favorability of desired binding mode over others and its stability in the solution state. In our ongoing study, we have applied this computational strategy (outlined in Figure 2B) and rationally redesigned mAbs targeting disoriented pre-defined epitopes of SARS-CoV-2 immune-escaped Delta and Omicron (BA.1 and BA.2) variants (data not shown). We have established an approach that entails the heuristic design strategy to some extent, as well as emulates the biased pose-prioritization binding of the docking algorithms. In addition, in-solvent simulation of the resulting poses could confirm the stability and resistance to the conformational changes and reorientation of Ab-Ag poses. As discussed above, effective nAbs development undergoes expensive and time-consuming procedures and clinical regulations; The CDR diversification strategy could be easily deployed to bypass the first four steps (Figure 2A) and redirect or design novel nAbs against emerging SARS-CoV-2 variants.

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M.S. and H.W. wrote the manuscript and discussed the proposed methodology. H.W provided funding acquisition.

DECLARATION OF INTERESTS
All authors declare that there is no competing interest.

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