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Review Article

# Neutralizing antibodies against SARS-CoV-2: current understanding, challenge and perspective

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## Abstract

**The rapid emergence of Coronavirus disease-2019 (COVID-19) caused by severe acute respiratory syndrome 2 coronavirus (SARS-CoV-2) as a pandemic that presents an urgent human health crisis. Many SARS-CoV-2 neutralizing antibodies (NAbs) were developed with efficient therapeutic potential. NAbs-based therapeutics against SARS-CoV-2 are being expedited to preclinical and clinical studies with two antibody drugs, LY3819253 (LY-CoV555) and REGN-COV2 (REGN10933 and REGN10987), approved by the US Food and Drug Administration for emergency use authorization for treating COVID-19. In this review, we provide a systemic overview of SARS-CoV-2 specific or cross-reactive NAbs and discuss their structures, functions and neutralization mechanisms. We provide insight into how these NAbs specific recognize the spike protein of SARS-CoV-2 or cross-react to other CoVs. We also summarize the challenges of NAbs therapeutics such as antibody-dependent enhancement and viral escape mutations. Such evidence is urgently needed to the development of antibody therapeutic interventions that are likely required to reduce the global burden of COVID-19.**

**Statement of Significance: The development of SARS-CoV-2 neutralizing antibodies (NAbs) has showed efficacy for the treatment of COVID-19. We discuss in this review the current understanding of NAbs for their structures, functions and neutralization mechanisms, and the potential of cocktail NAb therapy against COVID-19.**

**KEYWORDS: SARS-CoV-2; COVID-19; spike protein; neutralizing antibodies; hACE2**

## INTRODUCTION

The rapid emergence of Coronavirus disease-2019 (COVID-19) caused by severe acute respiratory syndrome 2 coronavirus (SARS-CoV-2) as a pandemic that has led to over 43 million cases and over 1 150 000 deaths worldwide [1]. Many vaccines are currently under clinical or preclinical studies [2, 3]. Most of the developing SARS-CoV-2 vaccines target the trimeric spike (S) glycoproteins and have the capacity to elicit high level of neutralizing antibodies (NAbs) that targeting S protein or its receptor binding domain (RBD). However, there is currently no approved vaccines or specific therapeutics against COVID-19. Considering the long term of clinical trials and the uncertainty of vaccine efficacy in human, the development of SARS-CoV-2 NAbs with desired efficacy and safety profile is also a critical part of the strategy for the treatment of COVID-19. We provide here a systemic overview of the development of SARS-CoV-2 specific or cross-reactive NAbs, and discuss their structures, functions and neutralization mechanisms.

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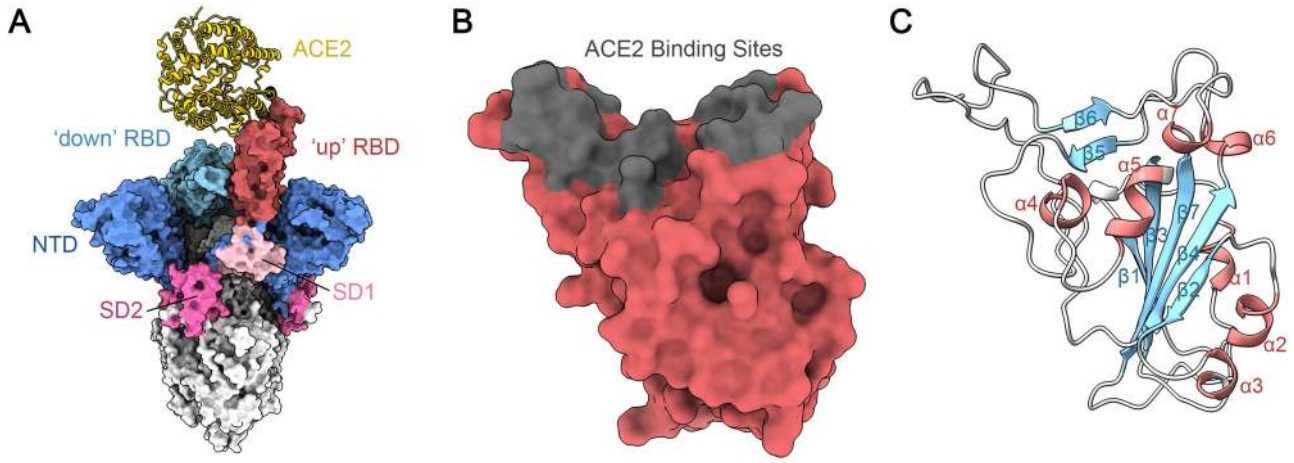


**Figure 1.** Structures of SARS-CoV-2 S trimer and its receptor-binding domain (RBD). (A) Structural diagram of SARS-CoV-2 S trimers (PDB: 6VSB) with one RBD in complex with hACE2 (PDB: 6ACG). The trimeric protein of SARS-CoV-2 is shown in molecular surface with the 'up' RBD, 'down' RBD, NTD, SD1 and SD2 colored in red, blue, deep blue, misty rose and hot pink, respectively. The model of hACE2 is represented in gold. (B) A molecular surface representation of RBD (PDB: 7C01) with the hACE2 binding sites rendered in dark grey. (C) Cartoon representation of RBD with numbered alpha-helices and beta-sheets colored in red and blue, respectively.

## SARS-COV-2 SPIKE PROTEIN AND RECEPTOR

The transmembrane S glycoprotein of coronaviruses (CoVs) which serves as the machinery to fuse the viral and host cell membranes [4] is the primary immunogenic target for virus neutralization and vaccine design. The trimeric S protein is a type I fusion protein that comprises two functional subunits, with S1 (N-terminal) responsible for mediating attachment to host cells and S2 (C-terminal) for membrane fusion [5, 6]. Two subunits remain noncovalently bound after proteolytic cleavage of the S protein [7–14]. The S1 subunit contains a RBD and an N-terminal domain (NTD) and the former exhibits two discrete conformational states, including the closed 'down' state that shield the receptor binding regions, and the 'up' state, which, in the case of SARS-CoV and SARS-CoV-2, can recognize the human angiotensin-converting enzyme 2 (hACE2) receptor on the host cell [5, 6, 15–17] (Fig. 1). The NTD may recognize sugar moieties upon initial attachment and might play a significant role in the transition of S protein from prefusion to postfusion in some CoVs [18–21]. It can also provide immunogenic epitopes for antibody targeting as indicated by that of MERS-CoV S protein [21]. The S2 subunit contains a fusion peptide near which an additional protease cleavage site, refer to as S2', locates [22].

The reported cryogenic electron microscopy (cryo-EM) or crystal structures of ectodomain of SARS-CoV-2 S protein presented the high similarity to that of SARS-CoV, as well as high flexibility of RBDs in either the 'down' or 'up' states that similar to other CoVs [11, 15]. When an RBD stochastically swings upwards, the binding of hACE2 to RBD can lock the RBD in the 'up' conformation and trigger S1 dissociation, further drive a large irreversible conformational arrangement of S2 [6, 8, 9, 12–14, 23–25]. During this change, the S2' cleavage site is cleaved by host proteases (e.g. TMPRSS2 for SARS-CoV and SARS-CoV-2 [26–28]) followed by the fusion peptide exposure and insertion into the host-cell membrane. Two heptad repeats (HR) including HR1 and HR2 associated from each protomer form a six-helix bundle that brings together the viral and host cellular membranes [12, 22, 29].

## SARS-COV-2 NAbs: STRUCTURES, FUNCTIONS AND MECHENISMS

The clinical spectrum of the outcome of COVID-19 is highly variable from mild flu-like symptoms to severe pneumonia [30]. NAbs play an important role in virus clearance for patients with COVID-19. Vaccine induced NAbs in healthy individuals are important for the prevention of COVID-19, which is also a significant indicator for vaccine efficacy [31–33]. In recent months, many SARS-CoV-2-derived NAbs have been emerging reported with excellent neutralizing and treatment potential, which promote the application of NAbs-based immune-therapy for the treatment of COVID-19.

At the early beginning of the pandemic, the information about the immune responses elicited in COVID-19 patients has been collected, which provides us not only the preliminary understanding of virus induced host response, but also the possibility to screening human NAbs against SARS-CoV-2. A study on antibody responses in 30 COVID-19 patients from Chongqing, China indicated that NAbs against SARS-CoV-2 were detectable at the early stage of COVID-19, peaked around 4–5 weeks, and gradually decreased within 3 months after the onset of symptoms [34]. Furthermore, NAb titer among intensive care unit (ICU) patients were apparently higher and peaked earlier than that of non-ICU patients [35]. Two independent studies showed that NAbs titers in COVID-19 patients were strongly correlated with the concentration of anti-RBD IgGs, which indicate the RBD may contain the dominant neutralizing epitopes on the spike protein [36, 37]. Series of subsequent studies on the screening and characterization

of SARS-CoV-2 NAbs confirmed that RBD is the target of the most efficient NAbs (Table 1).

## NAbs TARGETING SARS-CoV-2 RBD

The structures of the S protein or its subunit RBD in complex with NAbs have also been determined, which revealed the existence of several regions on RBD that can stimulate immune system to elicit a considerable antibody response to SARS-CoV-2. Furthermore, antibodies against different epitopes of RBD may lead to the neutralization of virus through different mechanisms. Here, we categorized these NAbs into at least four types (Type-I to Type-IV) (Table 1), pursuant to the conformations of bound RBD on S trimer and therefore lead to four distinct conformations of S trimers (All 'down' RBDs; one 'up' RBD; two 'up' RBDs and three 'up' RBDs) Interestingly, those NAbs that appear in the same type seem to prevent the viral infection with a similar mechanism.

The NAbs in **Type-I** can only bind to the 'up' RBD since the epitopes are sheltered or partially sheltered when RBD was in the down state (Fig. 2A). The binding region that Type-I NAbs target to is the flat surface on one side of the top saddle-like surface of RBD, which comprised three short alpha-helices ($\alpha$4, $\alpha$5 and $\alpha$7), two beta-strands ($\beta$5 and $\beta$6) and part of a flexible ridge-like loop (aa. 474–488) [38–44]. Epitopes of Type-I NAbs are extensively overlapping with the binding site of hACE2, and further superimposition of the complex structures of NAbs-RBD and hACE2-RBD exhibited obvious steric hindrance or direct binding-sites competition between NAbs and hACE2. The analysis implies that the neutralization mechanism of Type-I NAbs depends on blocking the binding of hACE2 to SARS-CoV-2 RBD. Interestingly, most of NAbs (C105, CV30, B38, CC12.1 and CC12.3) share the same germline of heavy chain V-genes (IGHV3–53) [39, 41, 43–45], and the structures of the NAb-RBD complexes show that these nAbs attach to RBD with almost identical pose (Fig. 2A).

Yuan *et al.* [46] have revealed the key motifs of the IGHV3–53 germline-derived NAbs for binding to RBD, which include the $_{32}$NY$_{33}$ motif from heavy-chain complementarity-determining region 1 (HCDR1) and the $_{53}$SGGS$_{56}$ motif from HCDR2. This information may help to investigate the specific binding mode of IGHV3-53 germline-derived antibodies and the corresponding epitopes. All NAbs exhibit exquisite potency in neutralizing SARS-CoV-2 and have promising therapeutic effect. For example, CC12.1 have an $IC_{50}$ value of 0.019 μg/mL against pseudovirus in vitro [45]. Administering a single 25 mg/kg dose of NAb B38 at 12 hours after viral challenge could protect hACE2 transgenic mice against SARS-CoV-2 infection with viral RNA copies in the lung significantly declined [43]. For CB6, a single dose of 50 mg/kg LALA mutant antibody before viral challenge could prophylactically prevent the rhesus macaque from SARS-CoV-2 infection [42]. These results indicated that the blocking of hACE2 binding, either by direct binding site competition or by steric hindrance, is an effective strategy for antibody-mediated neutralization of SARS-CoV-2.

Famous monoclonal antibody (mAb) CR3022 as representatively, the binding of **Type-II** NAbs to spike protein would be sterically hindered unless at least two RBDs are in the 'up' state (Fig. 2B) [47, 48]. In some cases, the conditions for the binding may be harsher, requiring a certain deflection of the RBD to avoid the collision between Fab and S protein [48, 49]. Therefore, the epitopes are inaccessible and more hidden comparing to those of Type-I, which may account for the relatively less frequent report for this type of antibodies. Three NAbs including CR3022, EY6A and a single domain antibody VHH-72 that belong to Type-II were reported. All of them bind to spike protein by leaning on the bottom of RBD, with the interaction interface distal from the receptor-binding site and mainly comprising $\beta$2 strand, $\alpha$2 helices, $\alpha$3 helices and the loops between them. Significantly, the binding sites of Type-II NAbs are highly conserved between SARS-CoV and SARS-CoV-2. For example, 24 out of 28 residues in the epitope of CR3022 are conserved between two viruses, which enable the cross binding of NAbs to these two viruses [48, 50].

CR3022, which was isolated from a SARS patient, was initially verified to neutralize SARS-CoV but not SARS-CoV-2 [48]. However, a recent research investigated that CR3022 can neutralize SARS-CoV-2 in a plaque-reduction neutralization assay [47]. The binding of CR3022 could promote the release of hACE2 from RBD and further reduce the stability of the prefusion state of spike protein through locking RBD in the up state, which may present an uncommon neutralization mechanism [47]. Similarly, EY6A trapped essentially the RBDs in the 'up' state with an extra rotation outwards by $\sim$25°, and can also make the premature prefusion-to-postfusion transition of spike protein [49]. In contrast, nanobody VHH-72, which was isolated from a llama immunized with both SARS-CoV spike protein and MERS-CoV spike protein, can easily engage the 'up' RBD without extra rotation of RBD due to its small size [51]. The peculiar binding characters of VHH-72 to RBD, with an 834 Å$^2$ of contact area in the vertical direction of RBD, confer VHH-72 two different neutralizing mechanism [51]. The VHH-72 can disrupt the RBD dynamics and gives rise to the anticipatory triggering of S protein by trapping the 'up' configuration just like how CR3022 and EY6A work [51]. On the other hand, the distal framework that opposite to VHH-72 CDRs would crash with the N-glycan occupancy at N322 as well as the segment (aa 300–324) of hACE2, which means that VHH-72 could also neutralize the virus by directly interfering with the hACE2 binding [51]. In brief, the neutralization mechanisms of Type-II NAbs against SARS-CoV-2 can be attributed to the interference of the receptor-binding, or to triggering the premature transition of S protein from prefusion to postfusion by functionally mimicking the receptor binding and further trapping the RBD in the unstable 'up' conformation [52–55]..

Contrary to the Type-I NAbs, antibodies from Type-III, such as NAbs Fab 2–4, Fab 2–43 and BD23, can only bind to the 'down' conformation of RBDs (Fig. 2C) [50, 56]. All of them would attach to the saddle-like surface of closed RBD from the top direction. As the fact that the dynamic conformational rearrangement of RBD is not accompanied by internal conformational changes of RBD, NAbs that

**Table 1.** NAbs targeting SARS-CoV-2 RBD

| Type | Antibody Name | Antibody type | Origin | PDB ID | Epitopes | Neutralizing mechanism | Cross-neutralizing activity | Protective efficacy | Ref |
|---|---|---|---|---|---|---|---|---|---|
| I | C105 | Human IgG | COVID-19-convalescent patient | 6XCN, 6XCM | R403, D405, R408, T415-K417, D420-Y421, Y453, L455-N460, Y473, A475-G476, F486-N487, G502, Y505 | Block hACE2-RBD interaction | no | Neutralizing SARS-CoV-2 pseudovirus with $IC_{50}$ value of 26.1 ng/mL | [39, 93] |
| | REGN10933 | Recombinant full-human antibodies | Humanized mice and COVID-19-convalescent patients | 6XDG | R403, K417, Y421, Y453, L455-F456, A475-G476, E484-Y489, Q493 | Block hACE2-RBD interaction, ADCC & ADCP | no | Neutralizing SARS-CoV-2 live virus with $IC_{50}$ value of 37.4 pM | [40] |
| | CB6 | Human IgG | COVID-19-convalescent patient | 7C01 | R403, D405-E406, R408-Q409, T415-K417, D420-Y421, L455-N460, Y473-S477, F486-N487, Y489, Q493, Y495, N501-G502, G504-Y505 | Block hACE2-RBD interaction | no | A single dose of CB6-LALA (50 mg/kg) protected the animal from SARS-CoV-2 infection. | [42] |
| | B38 | Human IgG | COVID-19-convalescent patient | 7BZ5 | R403, D405-E406, Q409, T415-K417, D420-Y421, Y452, L454-N460, Y473-S477, F486-N487, Y489-F490, Q493-G496, Q498, T500-V503, Y505 | Block hACE2-RBD interaction | no | A single dose of B38 (25 mg/kg) B38 protected the hACE2 transgenic mice from SARS-CoV-2 infection. | [43] |
| | CV30 | Human IgG | Infected COVID-19 patients | 6XE1 | R403, T415-K417, D420-Y421, Y453, L455-N460, Y473-S477, F486-N487, Y489, Q493, T500, G502, Y505 | Block hACE2-RBD interaction | no | Neutralizing SARS-CoV-2 live virus with $IC_{50}$ value of 0.03 μg/mL | [41] |
| | CC12.3 | Human IgG | COVID-19-convalescent patient | 6XC7 | R403, D405, T415-K417, D420-Y421, Y453, L455-N460, Y473-S477, F486-N487, Y489, Q493, G496, N501, Y505 | Block hACE2-RBD interaction | no | Neutralizing SARS-CoV-2 pseudovirus with $IC_{50}$ value of 0.018 μg/mL | [44, 45] |
| | CC12.1 | Human IgG | COVID-19-convalescent patient | 6XC3 | R403, D405-E406, R408-Q409, T415-K417, D420-Y421, Y453, L455-N460, Y473-S477, F486-N487, Y489, Q493-G496, Q498, T500-V503, Y505 | Block hACE2-RBD interaction | no | Neutralizing SARS-CoV-2 pseudovirus with $IC_{50}$ value of 0.019 μg/mL | |

Continued

**Table 1.** Continued

| Type | Antibody Name | Antibody type | Origin | PDB ID | Epitopes | Neutralizing mechanism | Cross-neutralizing activity | Protective efficacy | Ref |
|---|---|---|---|---|---|---|---|---|---|
| II | CR3022 | Human IgG | SARS-convalescent patient | 6YOR, 6 W41 | Y369-N370, F374-K386, L390, F392, D428, T430, F515-L517 | Trapping RBD in the less stable up conformation while leading to destabilization of S | SARS-CoV, SARS-CoV-2 | In the plaque-reduction neutralization test, CR3022 and SARS-CoV-2 showed a probit midpoint $PRNT_{50}$ of 1:11966, corresponding to $ND_{50}$ value of 0.114 μg/mL | [47, 48] |
| | EY6A | Human IgG | Late-stage COVID-19 patient | 6ZDH, 6ZER, 6ZCZ | Y369, F374-S375, F377-K386, N388, L390, P412-G413, D427-F429, L517 | Trapping RBD in the less stable up conformation while leading to destabilization of S | SARS-CoV, SARS-CoV-2 | Neutralizing SARS-CoV-2 live virus with $ND_{50}$ value of ~ 10.8 μg/mL | [49] |
| | VHH-72 | Llama single domain antibody | llama immunized with prefusion-stabilized betacoronavirus spikes | 6WAQ | Y356-T359, F361-C366, A371-T372, G391-D392, R395, N424, I489, Y494 | Trapping RBD in the less stable up conformation while leading to destabilization of S, Block hACE2_RBD interaction | SARS-CoV, SARS-Cov-2 | Neutralizing pseudotyped SARS-CoV S and SARS-CoV-2 with $IC_{50}$ values of 0.14 μg/mL and 0.2 mg/mL. | [51] |
| III | Fab 2–4 | Human IgG | Infected COVID-19 patients | 6XEY | Y449, Y453, L455-F456, E484-F486, Y489-F490, L492-S494, G496 | Locking RBD in the down conformation while occluding access to ACE2 | no | Neutralizing SARS-CoV-2 live virus with $IC_{50}$ value of 0.057 μg/mL | [56] |
| | BD23 | Human IgG | COVID-19-convalescent patient | 7BYR | G446, Y449, L452, T470, E484-F486, Y489-F490, L492-S494, G496, Q498, T500-N501, Y505 | Block hACE-RBD2 interaction | no | Neutralizing SARS-CoV-2 authentic virus with $IC_{50}$ value of 8.5 μg/mL | [50] |

Continued

**Table 1.** Continued

| Type | Antibody Name | Antibody type | Origin | PDB ID | Epitopes | Neutralizing mechanism | Cross-neutralizing activity | Protective efficacy | Ref |
|---|---|---|---|---|---|---|---|---|---|
| IV | S309 | Human IgG | Infected SARS patients | 6WPT, 6WPS | T333-L335, P337, G339-V341, N343-T345, K356-C361 | ADCC & ADCP | SARS-CoV, SARS-Cov-2 | Neutralizing authentic SARS-CoV-2 (2019n-CoV/USA_WA1/2020) with an $IC_{50}$ of 79 ng/ml | [60] |
| | H11-H4 | Llama single domain antibody | Naïve llama single-domain antibody library | 6ZHD | K444, Y449-N450, L452, L455-F456, T470, G482-E484, Y489-F490, L492-S494 | Block hACE2-RBD interaction | no | Neutralizing live SARS-CoV-2 with an $ND_{50}$ of 6 nM | [58] |
| | H11-D4 | | | 6Z43, 6Z2M | K444, Y449-N450, L452, L455-F456, T470, G482-E484, Y489-F490, L492-S494 | Block hACE2-RBD interaction | no | Neutralizing live SARS-CoV-2 with an $ND_{50}$ of 18 nM | |
| | P2B-2F6 | Human IgG | COVID-19 convalescent patient | 7BWJ | R346, K444, G446-N450, L452, V483-G485, F489, L491-S493 | Block hACE2-RBD interaction | no | Neutralizing SARS-CoV-2 pseudovirus with $IC_{50}$ value of 0.05 μg/mL | [61] |
| | REGN10987 | Recombinant full-human antibodies | Humanized mice and COVID-19-convalescent patients | 6XDG | R346, N439-L441, K443-N450, Q498, T500 | Block hACE2-RBD interaction, ADCC & ADCP | no | Neutralizing SARS-CoV-2 live virus with $IC_{50}$ value of 42.1 pM | [40] |

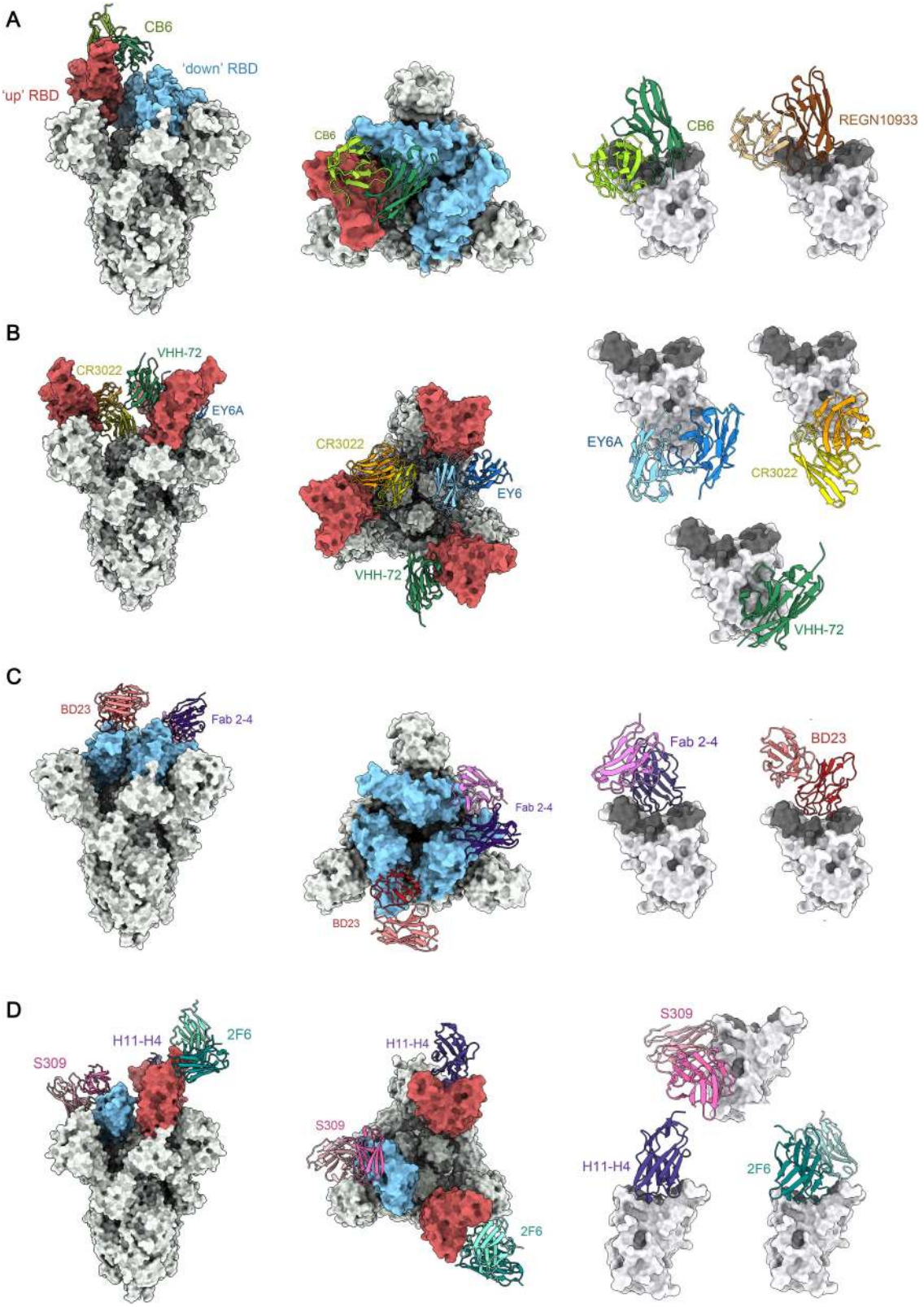


**Figure 2.** Illustrations of four different binding modes of NAbs against SARS-CoV-2 S trimers. (A) Type-I nAbs (e.g. CB6 and REGN10933) only bind the RBD in 'up' states. The binding sites of CB6 and REGN10933 partially overlap with that of hACE2. (B) Type-II NAbs (e.g. EY6A, CR3022 and VHH-72) bind S trimer when at least two RBDs are in the 'up' state. The epitopes for this type of NAbs always distal to the binding side of hACE2. (C) Type-III NAbs (e.g. BD23 and Fab 2–4) only bind to the RBD in 'down' conformation. (D) Type-IV NAbs (e.g. 2F6, S309 and H11-H4) can bind both the RBDs in 'up' and 'down' conformations.

target to the 'down' RBD are supposed can target to the 'up' RBD as the epitopes should be accessible in both states. The reason for this unusual occurrence may attribute to the existence of N-glycans from the adjacent regions or adjacent protomers participating in the interaction with NAbs, and the deflection of RBD (from 'down' to 'up' state) will lead to the loss of the contact between NAbs and the glycan chains. The two NAbs Fab 2–4 and Fab 2–43 discovered by Liu *et al*. could neutralize live viruses with $IC_{50}$ of 0.057 μg/mL and 0.003 μg/mL, respectively [56]. For Fab 2–4, The heavy chain was embedded into the saddle-like surface of RBD and the HCDR3 interacts with the ridge of RBD. The N-glycan attached to N58 of heavy chain form additional interactions with another N-glycan that attached to N481 of RBD. And crucially, the Y32 on the LCDR1 also interacts with the N-glycan which located at N343 of adjacent RBD. Fab 2–43 was observed to bind to the same glycan-containing epitopes with a different pose through low-resolution map fitting, which means that the transition of RBD from 'down' to 'up' state could push both the Fab 2–4 and Fab 2–43 away from the N343 glycan in the adjacent 'down' RBD. Analogously, antibody BD23 places the heavy chain vertically in the saddle-like surface of RBD, but leave the light chain out of interaction with RBD. Interestingly, an N-glycan at N165 from the NTD of the adjacent S protomer could facilitate the binding to BD23 and this contact will be lost when the RBD turn to an 'up' configuration [50]. Collectively, Type-III NAbs use primarily the heavy chain to interact with RBD, with footprints smaller and closer to the flexible ridge compared to those of other types, and may resist dynamic instability to some extent by target a quaternary epitope that span the RBDs and NTD. Logically, we would venture to speculate that the N-glycan chains would play a key role in stabilizing the binding of Type-III NAbs to the 'down' RBD. In other words, the neutralization mechanism of Type-III NAbs is locking RBD in the 'down' conformation and further occluding access to hACE2 [56].

The last but not least type, Type-IV NAbs, e.g. H11-D4, P2B-2F6, Ty1, S309 and REGN10987, can recognize the epitopes on both the 'up' and 'down' state of RBDs (Fig. 2D) [57–60]. The structural studies revealed two different regions that Type-IV NAbs target to, one is located on the receptor binding motif (RBM) and the other is located on the side of the RBD with no or little overlap with the RBM. In the former case, P2B-2F6 and two nanobodies H11-D4 and H11-H4 can target to the top saddle-like surface similar to NAbs from Type-III, but with a slightly rotated orientation and the key residues of their epitopes concentrated around the other side of RBM compared to that of Type-I, which are solvent accessible in the 'down' state. It looks like that the binding directions of these three antibodies and those Type-I NAbs are mirror reflection symmetry with respect to the RBD. As a result, the protruding loop (aa. 448–452) make more essential contributions to reinforce the interaction between RBD and three Type-IV NAbs. Meanwhile, three antibodies take full advantage of the hydrophobic motif on RBD raised by L492, L452 and F490 to form hydrophobic interactions compared to those of Type-III. These might explain why Type-IV antibodies can target to both the 'up' and 'down' RBD without extra N-glycan involving in interaction. Although there are only partial overlapped binding areas between Type-IV NAbs and the hACE2 (e.g. G446 and Y449 are the only overlapping residues recognized by P2B-2F6 and hACE2), the steric hindrance raised by the bound NAbs is sufficient to block the binding of hACE2 to RBD [61].

NAb S309, reported to bind to both the open and closed S protein, was isolated from the individual suffered from SARS-CoV in 2003 [60]. It potently cross-neutralize authentic SARS-CoV and SARS-CoV-2 as 17 of 22 residues within the epitopes and a glycan at position N343 (N330 in SARS-CoV) are highly conserved. The glycan-containing epitope is distinct from the hACE2 binding site and comprises mainly $\alpha$1 helices, partial $\beta$1 strand and two loops spanning residues 358–361 and 333–335. These residues interact with S309 primarily through electrostatic and hydrophobic interactions. N-glycan of N343 in SARS-CoV-2 S is a core fucose moiety, which can extend the contact area for ~300 Å$^2$ by inserting into the interspace between the HCDR3 and LCDR2 of S309.

Another antibody REGN10987, which has developed as a therapeutic cocktail together with Type-I antibody REGN10933, is also classified as Type-IV NAbs. Only the crystal structure of REGN10987 in complex with RBD was determined, the structure superposition reveals that REGN10987 can bind to both 'up' and 'down' RBDs and the binding of REGN10987 would lead to the steric hindrance of hACE2 binding. In the meantime, both REGN10987 and REGN10933 could mediate significant levels of antibody-dependent cellular phagocytosis (ADCP) and antibody-dependent cellular cytotoxicity (ADCC), which may account for their superior antiviral potency [40]. The peculiar epitopes recognized by S309 and REGN10987 seems to raise another fascinating neutralizing mechanism which may cause stronger ADCP and ADCC response, S trimer cross-linking, steric hindrance or aggregation of virions [40, 60].

In brief, as the target of hACE2 receptor, RBD can serve as an effective immunogen to stimulate the response of NAbs. Most of NAbs were found targeting around the top saddle-like surface of RBD and directly interfere with hACE2 binding. Some other NAbs could prevent from viral infection by trapping the RBD in 'up' state and destabilize the S protein, or lock the RBD in 'down' state and make the RBM in a receptor inaccessible conformation.

## NAbs TARGETING SARS-CoV-2 NTD or S2

Although RBD is the core target for NAbs against CoVs, non-RBD-targeted NAbs were also reported and may benefit to the strategy of NAbs cocktail therapeutics. For SARS-CoV-2, some NAbs were reported targeting the NTD of spike protein, such as NAbs 4A8, COV57, 2–17, 5–24, and 4–8 [39, 56, 62]. Specially, NAb 4A8, which was isolated from Chinese convalescent patient with COVID-19, exhibits potent neutralizing activity against authentic SARS-CoV-2 virus. Structural investigation revealed that NAb 4A8 binding could not block the interaction between hACE2 and spike protein, which is distinguished from another NAb 7D10 that targeting NTD of MERS spike

protein. 7D10 can inhibit the binding of S protein to its cell receptor DPP4 [63, 64]. All above mentioned SARS-CoV-2 NTD-targeting NAbs showed high potency of viral neutralization, although the detailed mechanisms are still not clear.

On the other hand, antibodies targeting the S2 subunit of SARS-CoV-2 spike protein have rarely reported. An mAb named 1A9, generated by immunization with SARS-CoV antigen, was proved to cross-react with SARS-CoV-2 S2 subunit, but have no neutralizing activity against SARS-CoV-2 virus [65]. Another NAb COV57 which derived from SARS-CoV-2 immunization, was showed to recognize the MERS-CoV S protein [39]. Like other CoVs including SARS-CoV and MERS-CoV, SARS-Co-2 S2 subunit mediate the fusion of viral and host cell membranes and sequentially more conserved than S1 subunit, which raise the possibilities for screening broad S2 specific NAbs cross-react to different CoVs not only SARS-CoV and SARS-CoV-2.

## BETACORONAVIRUSES CROSS-REACTIVE NAbs

The CoVs which can infect humans (HCoVs) were classed into alpha and beta CoVs, especially the latter, such as HCoV-HKU1, HCoV-OC43, MERS-CoV, SARS-CoV, and SARS-CoV-2, pose serious health threats to human beings [66]. For these HCoVs, the RBDs from SARS-CoV and SARS-CoV-2 have a relatively higher amino acid identity of ∼75%, and both viruses use ACE2 as their cell entry receptor, raising the possibility for the screening of cross-neutralizing NAbs against both viruses [67, 68]. However, many reported SARS-CoV and SARS-CoV-2 cross-reactive antibodies can only neutralize one of this two viruses but lost the neutralization to the other one, which was first demonstrated by the above-mentioned antibody CR3022 [48]. Another antibody NAb, 515–5, originating from COVID-19 patient, which can effectively neutralize SARS-CoV-2 virus but only exhibits relative weaker but detectable neutralization against SARS-CoV [69]. Another unexpected phenomenon is that many SARS-CoV and SARS-CoV-2 cross-neutralizing mAbs were demonstrated with no competition with hACE2, such as NAbs ADI-55689/ADI-56046, 47D11 and above-mentioned S309, indicating the existence of conserve epitope besides hACE2 binding sites among SARS-CoV-2 and SARS-CoV [60, 70, 71]. S309 recognizes an epitope containing a conserved glycan within sarbecovirus subgenus but fails to compete with hACE2 attachment. Furthermore, a recent reported humanized antibody H014, which binds a novel conformational epitope of RBD, efficiently neutralizes both SARS-CoV and SARS-CoV-2. The cryo-EM structure showed that H104 recognizes three open RBDs and critical residues involved in interaction are mostly conserved and locate mainly on one side of the open RBD distinct from the RBM [68]. Similarly, other cross-NAbs, e.g. VHH-72, and ADI-56046, CC6.33 and COV21, most recognize the core domain (aa 318–424) of RBD other than the RBM and neutralize both viruses [45, 51, 70]. The above information together suggests that the core domain of RBD may exhibits extensive conservation and more promising cross-reactive epitopes when comparing to the RBM. Therefore, tempting to elicit NAbs targeting this region might provide broad and potent neutralizing activity against CoVs.

## NAbs FOR THE TREATMENT OF COVID-19

Although several vaccine candidates under their clinical trials and showed promising effectivities, there are no currently available vaccines or antiviral therapeutic agents to the treatment of COVID-19 [72]. A number of researches showed that NAbs are robust therapeutic potential against COVID-19. Based on the existing evidence and prior experience in treating a novel pathogen emerges, such as SARS-CoV, MERS-CoV and Influenza, convalescent plasma transfusion is an effective therapeutic approach against infectious diseases [73–76]. In the early state of SARS-CoV-2 pandemic, convalescent plasma has been reported to be applied in treatment for patients with COVID-19. In terms of safety, transfusion of convalescent plasma has been shown with excellent safety in hospitalized patients with COVID-19 [77, 78]. A recent study on the treatment of COVID-19 patients with convalescent plasma indicated that all five patients improved their clinical status accompanying viral loads decreased and became negative within 12 days after the transfusion [79]. Similarly, another research reported the convalescent plasma therapy was well tolerated in patients with severe COVID-19, and rapidly improved the clinical symptoms and characteristics of the patients [80]. In addition, this treatment could increase and maintain NAbs at a high level and show disappearance of virus RNA within 7 days [80].

On the other hand, NAbs serve as an alternative treatment approach against COVID-19 due to their excellent neutralizing efficiency and mature industrialization prospect. In the preclinical research, SARS-CoV-2 antibody CB6, the RBD-directed antibody, was reported for the first time for the nonhuman primate therapeutics [42]. CB6 administration showed strong viral inhibition in vivo in both prophylactic and treatment as a result of CB6 treatment reduced virus titers immediately after administration and the peak viral load was no more than $10^3$ RNA copies per milliliter in pre-exposure of SARS-CoV-2 virus. These data indicated that CB6 is an outstanding candidate for translation for the clinic study. To date, many NAbs showing promising therapeutic potential have been evaluated clinically (Table 2). At least 10 monoclonal antibodies and one polyclonal antibody, were reported under different states of clinical trials. Of these, LY-CoV555 is the first antibody to enter into phase 1 clinical trials in the world and the NAb CB6, termed as JS016 in the clinical trial, is in the steady progress of phase 1 in China. As single antibody treatment may rapidly cause escape mutants on spike protein, it is worth noting that cocktail antibodies were regarded as a more superior antibody therapy against SARS-CoV-2 in the preclinical study [40, 81]. For example, the REN10987 + REGN10933 antibody cocktail that bind to two nonoverlapping epitopes of RBD retained the capacity for neutralizing all identified mutants. In general, potent

**Table 2.** List of clinical-phase therapeutic NAbs candidates for COVID-19

| No | NCT number | NAbs | Clinic trail titles | Phase | Status | Sponsor/collaborators | **Location** |
|---|---|---|---|---|---|---|---|
| 1 | NCT04441918 | JS016 | Tolerability, Safety, Pharmacokinetic Profile and Immunogenicity of a Recombinant Humanized Anti-SARS-CoV-2 Monoclonal Antibody (JS016) for Injection in Chinese Health Subjects | 1 | Recruiting | Shanghai Junshi Bioscience Co., Ltd | Shanghai, China |
| 2 | NCT04425629 | REGN10933, REGN10987 | Safety, Tolerability, and Efficacy of Anti-Spike (S) SARS-CoV-2 Monoclonal Antibodies for the Treatment of Ambulatory Adult Patients With COVID-19 | 1,2 | Recruiting | Regeneron Pharmaceuticals | Beijing, China |
| 3 | NCT04426695 | | Safety, Tolerability, and Efficacy of Anti-Spike (S) SARS-CoV-2 Monoclonal Antibodies for Hospitalized Adult Patients With COVID-19 | 1,2 | Recruiting | | Beijing, China |
| 4 | NCT04519437 | | Study Assessing the Safety, Tolerability, Pharmacokinetics and Immunogenicity of Repeated Subcutaneous Doses of Anti-Spike (S)SARSCoV-2 Monoclonal Antibodies (REGN10933 + REGN10987) in Adult Volunteers as Related to COVID-19 | 1 | Active, not recruiting | | USA |

Continued

**Table 2.** Continued

| No | NCT number | NAbs | Clinic trail titles | Phase | Status | Sponsor/collaborators | **Location** |
|---|---|---|---|---|---|---|---|
| 5 | NCT04452318 | Study Assessing the Efficacy and Safety of Anti-Spike SARS CoV-2 Monoclonal Antibodies for Prevention of SARS CoV-2 Infection Asymptomatic in Healthy Adults Who Are Household Contacts to an Individual with a Positive SARSCoV-2 RT-PCR Assay | | 3 | Recruiting | | USA |
| 6 | NCT04479644 | BRII-198 | Safety, Tolerability, and Pharmacokinetics Study of Human Monoclonal Antibody BRII-198 | 1 | Recruiting | Brii Biosciences Limited<br>TSB Therapeutics (Beijing) Co., Ltd | Beijing, China |
| 7 | NCT04479631 | BRII-196 | Safety, Tolerability, and Pharmacokinetics Study of Human Monoclonal Antibody BRII-196 | 1 | Recruiting | Brii Biosciences Limited<br>TSB Therapeutics (Beijing) Co., Ltd | Beijing, China |
| 8 | NCT04483375 | SCTA01-X101 | Safety, Tolerability and Pharmacokinetics of SCTA01, an Anti-SARS-CoV-2 Monoclonal Antibody, in Healthy Chinese Subjects | 1 | Recruiting | Sinocelltech Ltd | Beijing, China |
| 9 | NCT04429529 | TY027 | Safety of TY027, a Treatment for COVID-19, in Humans | 1 | Active, not recruiting | Tychan Pte Ltd | USA |
| 10 | NCT04537910 | LY-CoV555, LY-CoV016 | A Study of LY3819253 (LY-CoV555) in Healthy Participants | 1 | Active, not yet recruiting | Eli Lilly and Company | USA |
| 11 | NCT04411628 | | A Study of LY3819253 (LY-CoV555) in Participants Hospitalized for COVID-19 | 1 | Completed | Eli Lilly and Company<br>AbCellera Biologics Inc. | |

Continued

**Table 2.** Continued

| No | NCT number | NAbs | Clinic trail titles | Phase | Status | Sponsor/collaborators | **Location** |
|---|---|---|---|---|---|---|---|
| 12 | NCT04497987 | A Study of LY3819253 (LY-CoV555) in Preventing SARSCoV-2 Infection and COVID-19in Nursing Home Residents and Staff | | 3 | Recruiting | Eli Lilly and Company<br>National Institute of Allergy and Infectious Diseases (NIAID)<br>AbCellera Biologics Inc. | |
| 13 | NCT04427501 | A Study of LY3819253 (LY-CoV555) and LY3832479 (LY-CoV016) in Participants with Mild to Moderate COVID-19 Illness (BLAZE-1) | | 2 | Recruiting | Eli Lilly and Company<br>AbCellera Biologics Inc. | |
| 14 | NCT04634409 | A Study of Immune System Proteins in Participants With Mild to Moderate COVID-19 Illness (BLAZE-4) | | 2 | Recruiting | Eli Lilly and Company<br>AbCellera Biologics Inc.<br>Shanghai Junshi Bioscience Co., Ltd | |
| 15 | NCT04525079 | CT-P59 | To Evaluate the Safety, Tolerability and Pharmacokinetics of CT-P59 in Healthy Subjects | 1 | Recruiting | Celltrion | Korea |
| 16 | NCT04469179 | SAB-185(polyclonal antibody) | Safety, Tolerability, and Pharmacokinetics of SAB-185in Ambulatory Participants With COVID-19 | 1 | Recruiting | SAb Biotherapeutics, Inc.<br>Department of Health and Human Services Joint Program Executive Office (JPEO) | USA |
| 17 | NCT04468958 | | Safety, Tolerability, and Pharmacokinetics of SAB-185 in Healthy Participants | 1 | Recruiting | Chemical, Biological, Radiological, and Nuclear Defense (CBRND) Enabling Biotechnologies (EB) | USA |
| 18 | NCT04545060 | VIR-7831 | VIR-7831 for the Early Treatment of COVID-19 in Outpatients | 2,3 | Recruiting | Vir Biotechnology, Inc.<br>GlaxoSmithKline | USA |

Data source: https://clinicaltrials.gov/

therapeutic antibodies or rational antibodies mixture are expected to be a rapid intervention against the continuing pandemic of COVID-19 in the absence of vaccines.

## CHALLENGES OF NAbs THERAPEUTICS

Although NAbs against SARS-CoV-2 show potent therapeutic potential, antibodies can also exacerbate viral infection with elusive molecular mechanisms. Antibody-dependent enhancement (ADE) is the great obstacle in the development of vaccines and therapeutic antibody drugs against some viruses. In previous clinical studies, it was found that the administration of vaccines of respiratory syncytial virus (RSV) and dengue virus both cause ADE effects [82, 83]. For dengue virus, it is appear to worsen disease after the second infection with viruses of other serotypes because of pre-existing vaccine elicited antibodies which have low or no cross-neutralizing activity [84]. Additionally, non-NAb targeting the spike protein of the feline infectious peritonitis virus can accelerate the viral infection of macrophage in vitro [85]. For CoVs, ADE has been demonstrated occurred when antibody bound SARS-CoV binds to Fc$\gamma$RII of human macrophages and subsequently triggers the associated-downstream signal [86]. Although it is insufficient to predict the ADE phenomenon of COVID-19 due to the incomplete study evidence and fuzzy mechanisms of ADE. However, a recent study demonstrated that ADE occurs on those highly efficient NAbs against MERS-CoV RBD, indicating which may also occur on NAbs against SARS-CoV-2 [87]. Therefore, it should be necessary to optimize the animal models and further investigate the ADE risk about SARS-CoV-2.

SARS-CoV-2 is an RNA virus with higher mutation rate of its surface protein amino acids compared to those DNA viruses. It is necessary to understand the relationship between NAbs and mutant viral strains. It is well demonstrated that D614G mutation of SARS-CoV-2 spike protein, the major mutation detected to date, cause increased infectivity and case fatality [88, 89]. The mutation (D614G) is located between two protomers and could eliminate the contact of interprotomer [90]. Furthermore, a recent study showed that D614G shifts S protein conformation toward an ACE2-binding fusion-competent state, and therefore may increase the infectivity of virus [91]. Although the potency of RBD-directed NAbs against the D614G variant was not attenuated, the conformational shift toward an ACE2 binding-competent state induced by D614G could still influence the effectivity of some NAbs (e.g. Type-III NAbs which only bind the closed RBDs).

On the contrary, some naturally occurred SARS-CoV-2 S mutations might be resistant to RBD-targeting NAbs. The virus with any of the mutations on E484, F490, Q493 and S494 of spike protein shows complete or partial resistance to the potent NAbs (C121 and C144), which have been proved to be potential therapeutic antibodies [92]. To deal with the emergence, as for antibody treatment, combination treatment of two or more NAbs (cocktail) targeting distinct epitopes is an effective means to suppress the escape variants. Indeed, the cocktail antibodies (REN10987 + REGN10933) directing two independent epitopes of RBD still neutralize the variants of mutated RBD [81]. Similarly, mixture of antibodies (C121+ C135 or C144 + C135) apparently reduce the emergence of resistance strains compared to single mAb treatment [92]. Therefore, as more and more NAbs have been isolated, the more combinations of cocktail antibodies are expected to be further clinic usage.

## CONCLUSION

NAbs hold excellent potential for prophylactic and therapeutic applications against infectious diseases including COVID-19. The process of the development of NAbs-based therapeutics against SARS-CoV-2 has been expedited to the preclinical and clinical evaluation. NAbs therapy should be considered as a potential candidate intervention for COVID-19 given that the vaccine is currently unavailable and the existence of many uncertain questions that need to be addressed in the future. In this review, we summarized the SARS-CoV-2 specific NAbs and analysis their structures, functions and neutralization mechanisms. We provide insight into how these NAbs specific recognize S protein of SARS-CoV-2 or cross-react to other CoVs. We also discuss the challenges of NAbs therapeutics such as ADE and escape mutations. Such evidence is urgently needed to the future development of antibody therapeutic interventions that are required to reduce the global burden of COVID-19.

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## CONFLICTS OF INTEREST STATEMENT

None declared.

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