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Human neutralizing antibodies for SARS-CoV-2 prevention and immunotherapy

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Summary

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is the causative agent of coronavirus disease 2019 (COVID-19). SARS-CoV-2 has been spreading worldwide since December 2019, resulting in the ongoing COVID-19 pandemic with 237 million infections and 4.8 million deaths by 11 October 2021. While there are great efforts of global vaccination, ending this pandemic has been challenged by issues of exceptionally high viral transmissibility, re-infection, vaccine-breakthrough infection, and immune escape variants of concern. Besides the record-breaking speed of vaccine research and development, antiviral drugs including SARS-CoV-2-specific human neutralizing antibodies (HuNAbs) have been actively explored for passive immunization. In support of HuNAb-based immunotherapy, passive immunization using convalescent patients' plasma has generated promising evidence on clinical benefits for both mild and severe COVID-19 patients. Since the source of convalescent plasma is limited, the discovery of broadly reactive HuNAbs may have significant impacts on the fight against the COVID-19 pandemic. In this review, therefore, we discuss the current technologies of gene cloning, modes of action, *in vitro* and *in vivo* potency and breadth, and clinical development for potent SARS-CoV-2-specific HuNAbs.

Keywords: SARS-CoV-2, neutralizing antibody, vaccine, immunotherapy, passive immunization

Abbreviations: AAV-hACE2: Adeno-associated virus-vectored human ACE2; ACE2: Angiotensin-converting enzyme 2; BAM: Bamlanivimab; BCR: B cell receptor; CAS: Casirivimab; CDR: Complementarity-determining regions; COVID-19: Coronavirus disease 2019; EBV: Epstein-Barr virus; ETE: Etesevimab; EUA: Emergency Use Authorization; FACS: Fluorescence-activated cell sorting; FDA: The U.S. Food and Drug Administration; GISAID: Global initiative on sharing all influenza data; H: Heavy chain; HIV: Human immunodeficiency virus; HuNAbs: human neutralizing antibodies; Ig: Immunoglobulin; IMD: Imdevimab; L: Light chain; mAb: Monoclonal antibody; NTD: N-terminal domain; PBMC: Peripheral blood mononuclear cell; RBD: Receptor-binding domain; RBS: Receptor binding site; S: Spike; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2; TMPRSS2: transmembrane serine protease 2; VOC: Variants of concern; VSV: Vesicular stomatitis virus.

Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) is the causative agent of coronavirus disease 2019 (COVID-19) [1]. SARS-CoV-2 infects target cells mainly through the interaction between its spike (S) glycoprotein and the host cellular angiotensin-converting enzyme 2 (ACE2) receptor. The S protein is comprised of the S1 subunit and the S2 subunit. Within the S1 subunit, the receptor-binding domain (RBD) of SARS-CoV-2 contains the key functional determinant for binding with ACE2, whereas the S2 subunit engages the subsequent virus-cell membrane fusion for viral entry [2]. After the COVID-19 outbreak, both soluble RBD and trimeric S proteins have been extensively used as the bait for fishing out RBD- and S-specific human neutralizing antibodies (HuNAbs) during the processes of single B cell-, deep-sequencing- and phage display-based antibody gene cloning. The majority of HuNAbs obtained, therefore, block primarily the interaction between RBD/S and ACE2 for viral neutralization. Besides vaccine development, antiviral drugs including SARS-CoV-2-specific HuNAbs have also been actively explored for passive immunization. To support HuNAb-based

immunotherapy, passive immunization using convalescent patients' plasma has generated promising evidence on clinical benefits for both mild and severe COVID-19 patients [3, 4]. Recently, significant progresses in the discovery of HuNAb have resulted in HuNAb-based clinical immunotherapy for COVID-19 patients. This review aims to highlight these progresses to facilitate the knowledge exchange and the fight against COVID-19.

Cloning of monoclonal HuNAb

B cell receptor (BCR) repertoires exhibit high sequence diversity due to the somatic recombination and hypermutation during the B cell development. BCR is defined as a transmembrane receptor located on the B cell surface and interacts with a specific antigen epitope through its variable region to initiate antibody response. This variable region, therefore, shares the identical gene sequence with the antibody that is produced by this B cell. The somatic recombination of three gene segments of the heavy (H) chain locus (V, D, J) and two

gene segments of the light (L) chain locus (V, J) to diversify the variable region gene. The variable region of an antibody immunoglobulin (Ig) determines the specificity for interaction with a corresponding viral antigenic epitope. The somatic hypermutation involves the B cell proliferation in the germinal center with random mutations in the genes encoding the variable region of individual monoclonal antibody (mAb), essential for high-affinity binding to an antigenic epitope, so-called the antibody affinity maturation process. There are no identical BCRs between two different B cells. To ensure native pairing of antibody H and L chains, it is necessary to analyze one B cell at a time for cloning an mAb [5]. With the advancement of antibody gene cloning techniques, such as hybridoma technology, human B cell immortalization, antibody phage display, human immunoglobulin transgenic mice and single B cell antibody technology [6], cloning of a functional HuNAb is no longer a search of a needle in the ocean. During the ongoing COVID-19 pandemic, most of the SARS-CoV-2-specific antibodies have been obtained through the single B cell technology.

Single B cell-based antibody cloning technique

Single B cell-based antibody cloning is a technique to obtain the variable region of mAb H/L genes from individual memory B cell that has the capacity to produce the high-affinity antibody. It involves the amplification of auto-paired Ig H/L chain RNA sequences from the heterogeneous memory B cell population and *in vitro* construction into functional mAbs [7, 8]. This technique has been successfully used for isolating neutralizing mAbs from convalescent patients against various viral infections such as the Epstein-Barr virus (EBV), human immunodeficiency virus (HIV), dengue, and SARS-CoV-2 [9–12]. Since 2009, the combination of single-cell RT-PCR and single B-cell sorting has improved the success rate of antibody gene cloning greatly. The acquirement of single antigen-binding memory B cell by fluorescence-activated cell sorting (FACS) or optofluidics platform is the major technical improvement, allowing subsequent nested RT-PCR using primers to amplify naturally paired antibody H/L gene sequences from individual memory B cells [13–16]. After the COVID-19 outbreak, this method was quickly utilized to isolate HuNAb from SARS-CoV-2-infected patients [11, 17]. Moreover, there is the development of high-throughput single-cell RNA and VDJ deep sequencing of BCR repertoires accompanied by the bioinformatics analysis [18, 19]. This technique has outcompeted the single-cell RT-PCR in terms of high-throughput screening of a large pool of diverse memory B cells. Interestingly, immunization using RBD/S proteins or direct infection of mice with the genetically humanized immune system can also generate complete HuNAbs against SARS-CoV-2 [20]. This murine platform may simplify the source of antigen-specific human B cells although the antibody affinity maturation process remains to be improved in this model. Nevertheless, using this platform, some SARS-CoV-2-specific HuNAbs have been successfully cloned and screened [20]. The deep sequencing of variable regions and BCR repertoire has revealed H/L pairing of human antibody characteristics [20]. In addition, the combination of microfluidic-based technique and bioinformatics analysis can further improve the efficiency of identifying highly potent HuNAbs against specific viral antigens, which has implications for the fight against COVID-19 and other emerging infectious diseases.

Phage display-based antibody cloning technique

Phage display has been one of the widely used methods for cloning human antibodies. The two key steps of the phage display technique include the construction of an antibody gene library and the screening of antigen-specific antibodies. The human Fab library can be generated from peripheral blood mononuclear cells (PBMCs) derived from COVID-19 patients. A set of primers targeting the variable H/L chain regions are used to amplify the total antibody gene pool for the construction of the phage library [21]. In solution panning, SARS-CoV-2 RBD can be used as the bait to fish out the RBD-specific human Fabs, followed by construction of the Fabs into full-length IgG1 for subsequent biochemical and functional testing. Alternatively, the human Fab antibody library can also be synthesized using the selected human germline immunoglobulin variable segments. The diversity in the complementarity-determining regions (CDR) 3 of H/L chains (CDR-L3 and CDR-H3) can be introduced by well-designed mutagenic oligonucleotides. A competitive phage screening strategy has been successfully used to obtain RBD-specific HuNAbs against SARS-CoV-2 [22]. Interestingly, to obtain a cross-reactive HuNAb against both SARS-CoV and SARS-CoV-2. Mice immunized with SARS-CoV RBD were used for the establishment of the phage display library. Then, this library was screened using the SARS-CoV-2 RBD as the bait. Since these two RBDs share 75% similarity in their amino acid sequence, the cross-reactive murine mAbs were successfully obtained for subsequent humanization against both SARS-CoV and SARS-CoV-2 [23]. The drawbacks of the phage display-based antibody cloning technique include the unnatural pairing of VH/VL and a time-consuming panning procedure. Nevertheless, this technique remains very useful for obtaining antigen-specific antibodies including potent SARS-CoV-2-specific HuNAbs.

Mode of action and structural basis of SARS-CoV-2-specific HuNAbs

The S glycoprotein of SARS-CoV-2 exists in a prefusion trimer conformation that may rearrange during the fusion of the virus-cell membrane. This viral entry is a dynamic process beginning with the binding of the viral S1 portion to the host cell receptor ACE2. The interaction between S1 and ACE2 makes the prefusion trimer unstable, leading to the cleavage of S into S1 and S2 by cellular proteases (e.g., Furin) with the transition into a stable post-fusion conformation. The RBD in S1 transiently hides or exposes the determinants of receptor binding, due to its hinge-like conformational movement, displaying two structural states with 'up' and 'down' conformations. The 'up' conformation is an active state for ACE2 binding [24]. Once in the 'down' conformation, RBD is usually stable but is inaccessible to ACE2. Because of this unique property, the 'up' conformation serves as the major target for SARS-CoV-2 NAbs. Interestingly, two major conformational structural regions within the S1 have been identified as SARS-CoV-2 neutralizing domains. Besides RBD, the N-terminal domain (NTD) also possesses binding sites for HuNAbs.

According to the CoV-AbDab, the coronavirus antibody database, there are amazingly a total number of 3152 SARS-CoV-2-specific antibodies by 23 September 2021, including 2693 mAbs and 459 nanobodies. One thousand hundred and forty-eight of the 2639 mAbs have neutralizing activities. In

a separate report, among a total of 1584 RBD-specific antibodies, 902 have the neutralization ability [25]. By mapping the binding sites of a collection of mAbs against the S protein, NAbs bind to multiple S epitopes including ones in RBD, NTD, and quaternary regions [26, 27]. While RBD is the primary target for NAb, these antibodies can be classified into three main groups according to the distinct binding sites at the molecular level [28]. The first group of NAbs has epitopes within the RBD overlapping largely with the binding site of ACE2. These NAbs are described as receptor binding site (RBS) antibodies. Furthermore, due to different interacting angles with viral RBD, RBS NAbs are divided into three in-depth subclasses including RBS-A, RBS-B, and RBS-C [28]. As demonstrated in the crystal structures (Fig. 1), the binding sites and interaction direction of RBS-A antibodies with viral RBD shared the highest similarity with those of ACE2 binding. RBS-A antibodies bind to RBD like ACE2 mainly on the 'left side' of the ridge. Typically, both Fab-RBD and ACE2-RBD interactions show on one side of the ridge in the crystal complex. Compared to RBS-A antibodies, the epitope of RBS-B antibodies shows relatively less overlapping with the ACE2 binding site. RBS-B antibodies are positioned more upright and straddle the central of the ridge [28]. The RBS-C antibodies are the subclass of antibodies that have the least overlapping epitopes with ACE2 binding sites as they bind to viral RBD on the opposite side of the RBS-A antibodies. It should be emphasized that all three subclasses of RBS antibodies can compete with human ACE2 for SARS-CoV-2 neutralization. While several RBS-C antibodies can bind to the 'up' or 'down' RBD, the RBS-A antibodies can only interact with the 'up' RBD. The binding mode of RBS-B antibodies is in between as some can only bind to RBD in the 'up' state while others show in the 'down' state [28, 29]. The second

group of NAb is represented by the cross-reactive CR3022 antibody that recognizes a cryptic site in RBD. CR3022 is an antibody isolated from a phage library of a convalescent SARS patient in 2003. CR3022 binds to a cross-reactive epitope on SARS-CoV-2 RBD but it does not neutralize SARS-CoV-2. The position of the CR3022 cryptic site is highly conserved near the 'tail' part of an 'up' RBD. This binding site is common for cross-reactive antibodies against both SARS-CoV and SARS-CoV-2. Different from CR3022, however, several CR3022-like antibodies present various neutralizing activities against both SARS-CoV and SARS-CoV-2 [28]. The third group of NAb is represented by the S309 antibody that neutralizes both SARS-CoV-2 and SARS-CoV pseudoviruses as well as authentic SARS-CoV-2 by engaging the RBD of the S glycoprotein through binding to an epitope containing the N343 glycan, without competing with ACE2 interaction. The S309 antibody was identified from a SARS convalescent patient in 2003 as well. It binds to either the 'up' or the 'down' RBD. The binding site of S309 is not as conserved as the one for the CR3022 antibody [28]. Besides three groups of RBD-specific NAbs, the mode of action for NTD-directed NAbs remains incompletely revealed [26, 27]. It is possible that the binding of these NAbs to NTD may affect indirectly the conformational interaction between RBD and ACE2. Since the S2 subunit is more conserved among coronaviruses than S1, some cross-reactive neutralizing antibodies specific to S2 of the S protein have been reported [30, 31]. Interestingly, SARS survivors have developed potent cross-clade pan sarbecovirus neutralizing antibodies after immunization with the BioNTech mRNA vaccine [32]. The findings of multiple NAb targets of vulnerability on SARS-CoV-2 S protein are useful not only for designing vaccine and antiviral but also for building the basis for HuNAb-cocktail treatment.
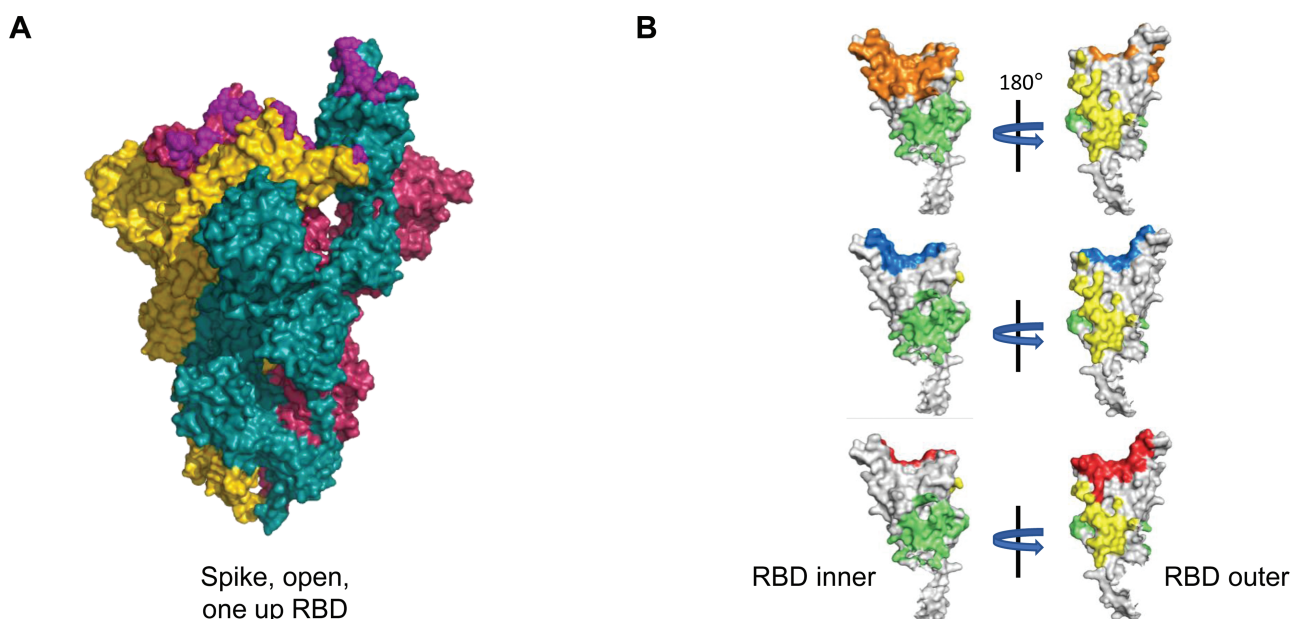

Figure 1. Categorized recognition sites of SARS-CoV-2 RBD-specific mAbs. (A) Conformational structure of SARS-CoV-2 trimer spike glycoprotein (PDB: 7BNM, 1 up RBD) with the ACE2 binding footprints (purple) on RBDs. (B) The crystal structure of RBD (white) is displayed in two orientations (PDB:7BNM, amino acid position: 320–540). The binding sites of different subclasses of mAbs are labelled in orange for mAb CB6 (RBS-A, PDB ID: 7C01), blue for mAb 2-4 (RBS-B, PDB ID: 6XEY), red for mAb P2B-2F6 (RBS-C, PDB ID: 7BWJ), green for mAb CR3022 (PDB ID: 6W41) cryptic site, and yellow for mAb S309 (PDB ID: 6WPS) proteoglycan site.

In vitro potency of SARS-CoV-2-specific HuNAbs

To study the neutralizing potency of HuNAbs targeting the S glycoprotein of SARS-CoV-2, both pseudovirus and authentic virus neutralization assays have been developed. For the pseudovirus assay, two systems have been established through co-transfection with the functional S glycoprotein of SARS-CoV-2. One system employs the luciferase reporter in the backbone of lentivirus (e.g., HIV-1) [33, 34]. The other system employs the luciferase reporter in the backbone of the vesicular stomatitis virus (VSV) [26, 35]. The authentic neutralization assay uses primary SARS-CoV-2 strains isolated from COVID-19 patients directly. It should be noted that different research groups may use various types of target cells for *in vitro* neutralization assays, which may generate incomparable data to compare the neutralization potency. For example, various Vero cell lines and 293T cells stably expressing human ACE2 have been extensively used for viral neutralization assays. Bearing this in mind, one may refer to some representative HuNAbs in (Table 1). All these HuNAbs are generated in the native form of IgG1. Usually, HuNAbs with the half-maximal inhibitory concentrations (IC$_{50}$) less than 0.1 μg/ml are categorized as potent neutralizers whereas those with IC$_{50}$ values of 0.1–1 μg/ml and 1–10 μg/mL are considered as moderate and weak neutralizers, respectively [36]. The most potent SARS-CoV-2 HuNAb may display IC$_{50}$ values at the single-digit ng/ml. For example, one of the most potent HuNAbs, namely 2–15, displays the IC$_{50}$ value of as low as 0.7 ng/ml [26].

Efficacy of SARS-CoV-2-specific HuNAbs in animal models

Several animal models have been established to mimic the natural course of SARS-CoV-2 infection in humans. Since ACE2 contains natural variation in different animal species, their susceptibility to live SARS-CoV-2 infection may vary significantly. Based on the structural analysis, there are 29 amino acid residues at the interface of ACE2 and SARS-CoV-2 RBD, which determines the binding affinity of these two proteins. Sequence alignment of these 29 amino acid residues reveals high similarity between human ACE2 with homologues of Syrian golden hamster, rhesus macaque and common marmoset [37]. Since hamster ACE2 exhibits a high binding affinity with the S glycoprotein of SARS-CoV-2 by in silico prediction, this animal model exhibits acute clinical and histopathological manifestations that model the upper and lower respiratory tract infection in humans [37]. Non-human primates share 100% identity of these 29 amino acid residues with human ACE2 but only display a transient and mild clinical manifestation after the live SARS-CoV-2 challenge [38–40]. The amount of ACE2 and TMPRSS2 expression in upper and lower respiratory tracts of rhesus monkeys likely affects their susceptibility to live SARS-CoV-2 infection,

Table 1. Characteristics of human neutralizing antibodies (HuNAbs)

Antibody	Pseudovirus neutralization IC$_{50}$(ng/ml)	Live virus neutralization IC$_{50}$ (ng/ml)	Cross with SARS-CoV	Subclass	Reference
REGN10933 (casirivimab, CAS)	10.4	5.61	No	RBS-A	Hansen *et al.* (2020) [20]
REGN10987 (imdevimab, IMD)	6.09	6.32	No	RBS-C	Hansen *et al.* (2020) [20]
BD-368-2	1.2	15	na*	RBS-C	Cao *et al.* (2020) [19]
P2C-1F11 (BRII196)	30	30	No	RBS-A	Ju *et al.* (2020) [68]
2-15	5	0.7	na	RBS-A	Liu *et al.* (2020) [26]
S309	na	79	Yes	S309 proteoglycan site	Pinto *et al.* (2020) [69]
CV07-250	na	3.5	No	RBS-B	Kreye *et al.* (2020) [70]
47D11	61	570	Yes	na	Wang *et al.* (2020) [71]
C105	26.1	na	No	RBS-A	Barnes *et al.* (2020) [72]
COVA1-18	8	7	No	RBS-A	Brouwer *et al.* (2020) [27]
COVA2-39	36	54	No	RBS-B	Brouwer *et al.* (2020) [27]
CC6.29	2.0	7.1	No	RBS-A	Rogers *et al.* (2020) [73]
CB6 (LY-CoV016, etesevimab, ETE)	41	36	No	RBS-A	Shi *et al.* (2020) [52]
Ab169 (LY-CoV555, bamlanivimab, BAM)	0.012	0.02	na*	RBS-B	Jones *et al.* (2021) [53]
H014	450	5700	Yes	CR3022 cryptic site	Lv *et al.* (2020) [74]
ZB8	9.5	13	No	RBS-B	Zhou *et al.* (2021) [46]

*Not available.

which explains why the intratracheal viral inoculation has been used for a viral challenge in the experiments. Mouse is generally resistant to wild-type SARS-CoV-2 challenge because mouse ACE2 does not effectively bind the viral S glycoprotein [41]. However, after transduction with adeno-virus- or adeno-associated virus-vectored human ACE2 (Ad5-hACE2 or AAV-hACE2), the mouse becomes susceptible to SARS-CoV-2 infection [42]. Meantime, transgenic mice with human ACE2 expression lead to mild or lethal SARS-CoV-2 infection [43]. Subsequently, SARS-CoV-2 with the N501Y mutation can interact with murine ACE2 and infect mouse species directly [44, 45]. These animal models have been widely used to evaluate the *in vivo* efficacy of various HuNAbs and vaccines against SARS-CoV-2 (Table 2). We and others reported that most of these HuNAbs can suppress viral loads in the lungs and alleviate lung injury in animal models. We, however, found that systemic HuNAb injection or DNA vaccination has limited efficacy in preventing SARS-CoV-2 nasal infection in Syrian hamsters likely due to insufficient biodistribution of antibody at the site of viral transmission [21]. Using the same model, we recently found that potent neutralizing dimeric IgA may enhance SARS-CoV-2 nasal infection probably by engaging

alternative cellular entry and cell-to-cell transmission mechanisms [46]. In fact, few animal studies have shown significant reduction of nasal viral loads in various animal models post HuNAb injection and systemic vaccination [26, 47, 48], implicating that an exceptionally high dose is likely required for improved protection. Some published studies claim sterile protection without actual evaluation of SARS-CoV-2 nasal infection. These results explain the rising number of re-infections and over thousands of vaccine-breakthrough infections in humans [49–51]. The data generated in animal models, therefore, may have significant implications to human vaccine and passive immunization studies.

Efficacy of SARS-CoV-2-specific HuNAbs in human trials

Several anti-SARS-CoV-2 HuNAbs have been developed for clinical studies. Paired HuNAbs have been formulated to improve the breadth of neutralization due to emerged SARS-CoV-2 variants and to minimize HuNAb escape variants. Most neutralizing antibodies under Emergency Use Authorization (EUA) showed live virus neutralization IC_{50} below 0.1 μg/ml. The paired antibodies often include one

Table 2. *In vivo* efficacy of HuNAbs determined in animal models

Antibody	Dosage (mg/kg)	Animal model	Experiments	Efficacy	Reference
REGN-COV2	50, 5, 0.5	Syrian hamster	Prophylactic (–2 day)	Alter the course of infection	Baum *et al.* (2020) [75]
			Treatment (1 dpi*)	Alter the course of infection	
	50, 0.3	Rhesus macaque	Prophylactic (–3 day)	50 mg/kg could minimize virus replication, but 0.3 mg/kg could not	
	150, 25		Treatment (1 dpi)	Accelerate viral clearance at both dosage	
BD-368-2	20	hACE2 transgenic mice	Prophylactic (–1 day)	Completely inhibit infection in lung	Cao *et al.* (2020) [19]
			Therapeutic (2h after infection)	3–4 logs decrease in virus copies in lung	
H014	50	hACE2-humanized mice	Prophylactic (–12 hour) plus therapeutic (4 hours after infection)	1–2 logs decrease in virus copies in lung	Lv *et al.* (2020) [74]
			Therapeutic (4 hours after infection)	0.5–1 log decrease in virus copies in lung	
2-15	1.5	Syrian hamster	Prophylactic (–1 day)	4 logs or more decrease in virus copies in lung	Liu *et al.* (2020) [26]
1B07	10	AdV-hACE2-transducedBALB/c mice	Prophylactic (–1 day)	About 1 log decrease in virus copies in lung	Hassan *et al.* (2020) [20]
CB6	50	Rhesus macaque	Prophylactic (–1 day)	4–5 logs decrease in virus copies in lung	Shi *et al.*, (2020) [52]
			Treatment (1 dpi and 3 dpi)	3–4 logs decrease in virus copies in lung	
COV2-2196/2130	0.2mg/mice	AdV-hACE2-transducedBALB/c mice	Prophylactic (–1day)	About 1 log decrease in virus copies in lung	Zost *et al.* (2020) [76]
	50	Rhesus macaque	Prophylactic (–3 day)	Completely inhibit infection in lung	
CC12.1/C12.23	16.5, 4.2, 0.9, 0.2, 0.06	Syrian hamster	Prophylactic (–1day)	16.5, 4.2, 0.9 mg/kg could minimize virus replication, 0.2, but 0.06 mg/kg could not	Rogers *et al.* (2020) [73]

*dpi: day post infection.

RBS-A HuNAb plus either one RBS-B or one RBS-C HuNAb. For example, the pair from the Eli Lily company included the RBS-B LY-CoV555 (bamlanivimab, BAM) and the RBS-A LY-CoV016 (etesevimab, ETE) with live virus-neutralizing IC_{50} around 0.02 µg/ml and 0.036 µg/ml, respectively [52, 53]. LY-CoV555 and LY-CoV016 were probably the first paired HuNAbs administered to COVID-19 patients in early June 2020 [54]. The randomized phase 2/3 trial evaluated the efficacy of combined LY-CoV555 (2800 mg) and LY-CoV016 (2800 mg) as compared with the LY-CoV555 monotherapy. A statistically significant reduction in viral load was found at day 11 among non-hospitalized patients with mild to moderate illness by the combination therapy but not by the monotherapy [54]. Subsequently, another clinical trial reported the REGN-CoV2 HuNAbs for prophylaxis and immunotherapy of COVID-19 patients. This phase 1/2/3 trial showed that both 2400 and 8000 mg of REGN-COV2 antibody cocktail showed an approximately 2-log reduction of viral loads in patients with baseline viral load higher than 10^7 copies/mL compared with the placebo group [55]. It should be noted that these clinical dosages are indeed much higher than 10–20 mg/kg tested in small animal models. Then, both companies have acquired permission from the U.S. Food and Drug Administration (FDA) for EUA of their HuNAbs. Now, these HuNAbs are used for emergency treatment of mild-to-moderate adult and pediatric patients (age 12 and older with body weight at least 40 kg) who have positive viral loads and are at high risk for progressing to severe COVID-19. Seven hundred milligram LY-CoV555 is recommended as monotherapy, or it can be combined with 1400 mg LY-CoV016. As for the REGN-CoV2, it consisted of paired RBS-A REGN10933 (casirivimab, CAS) and RBS-C REGN10987 (imdevimab, IMD) at a 1:1 ratio. REGN10933 and REGN10987 displayed live virus-neutralizing IC_{50} around 0.0056 µg/ml and 0.0063 µg/ml, respectively [20]. The recommended dosage of REGN-CoV2 is 2400 mg (1200 mg each) [56]. Recently, the combination of BRII-196 and BRII-198 was developed by the BRII Biosciences including one RBS-A and one RBS-C antibody, both with live virus neutralizing IC_{50} around 0.03 µg/ml. This antibody combination showed 78% efficacy in trials and has been approved for clinical use on 9 December 2021 by China FDA. Since antibody combination therapy may face reduced neutralizing abilities against SARS-CoV-2 variants of concerns (VOCs), more broadly reactive HuNAbs and next-generation antibodies, such as bispecific antibodies and engineered antibodies are on the list for clinical trials in different regions and countries.

Impact of SARS-CoV-2 VOCs on HuNAbs

SARS-CoV-2 is an RNA virus that is usually prone to mutate during the natural course of infection in humans. SARS-CoV-2, however, has a self-proof-correcting machinery system that maintains the relative genomic fidelity during viral replication [57]. Analysis of global phylogenies indicates a slow mutation rate of approximately two mutations per month in the viral genome [58]. Unlike SARS in 2003, since COVID-19 has not been eliminated till now, there is a growing concern on viral escapes due to immune pressure conferred by natural infection or vaccination. Indeed, the increasing and long-lasting COVID-19 pandemic has already resulted in several VOCs with various resistance profiles to HuNAbs.

A total number of 4,230,187 genome sequences of SARS-CoV-2 have been submitted to the hCoV-19 database of the Global initiative on sharing all influenza data (GISAID) since the outbreak of COVID-19. Several VOCs have had significant impacts on the trend of the pandemic. The top four noticeable VOCs include B.1.1.7 variant (Alpha, United Kingdom), B.1.351 (Beta, South Africa), P1 (Gamma, Japan/Brazil) and B.1.617.2 (Delta, India) [59]. Since late November 2021, a new VOC, B.1.1.529 (Omicron, South Africa) has been predicted to become another dominated strain. The Omicron variant was first discovered in South Africa with heavily mutated S glycoprotein containing 32 mutations including 15 in the RBD region, in addition to three deletions and one insertion [60]. The mutations or deletions of amino acids in the S glycoprotein of VOCs have led to not only HuNAb resistance but also probably increased the affinity binding to human ACE2 for higher viral transmissibility [61, 62]. Critically, all these five VOCs contain the D614G mutation, which enhances viral transmissibility and infectivity [62]. Moreover, the E484 position in RBD is an important binding site for many RBD-specific HuNAbs [61]. Unfortunately, VOCs of B.1.351 and B.1.1.529 have the E484K/A mutation. Moreover, some amino acid deletions in the S protein, such as the 242-244del in B.1.351 variants and the 144del in B.1.1.7 variants reduced more than the 1000-fold neutralizing activity of the NAbs that target the supersite of NTD domain [35]. To date, the impact of S mutations in Omicron variants on the ongoing pandemic remains unknown. Various combinations of these mutations and deletions in emerging VOCs, however, have substantially reduced the potency of HuNAbs elicited by vaccines and passive immunization, and therefore, becomes new challenges to public health and vaccine responses. Hopefully, the boost vaccine and immunotherapy using cocktailed HuNAbs can overcome these VOCs.

Future perspectives

With continuously emerging VOCs, vaccine-induced correlates of immune protection remain to be comprehensively investigated. Due to the urgency of the COVID-19 pandemic, huge global efforts have been placed for vaccine development. Till now, six vaccines have been approved by regulatory agencies for emergency use within just one year of the pandemic including (1) two mRNA-based vaccines, namely BNT162b2 (by Pfizer Inc. and BioNTech SE) and mRNA-1273 (by Moderna), expressing full S glycoprotein with an efficacy rate of 95% [63, 64] (2) the chimpanzee adenovirus-vectored vaccine, named ChAdOx1 nCoV-19 (by the Oxford University and AstraZeneca Inc.), encoding the full S glycoprotein with an efficacy rate of 70.4% [65] (3) the human adenovirus-vectored vaccine, namely Ad26.COV2.S (by Johnson & Johnson Inc.), encoding the full S glycoprotein with an efficacy rate of 73.1%, and (4) two inactivated vaccines CoronaVac and BIBP (by Sinovac Biotech and SinoPharm) with an efficacy rate of 83.5% and 78.1%, respectively. Since these efficacy rates were mainly determined for the prevention of severe diseases, their potency in preventing SARS-CoV-2 nasal infection and eliciting broadly reactive HuNAbs against VOCs remains to be carefully investigated. In recent studies, the B.1.351 variant was significantly resistant (10.3–12.4-fold) to neutralization by sera derived from vaccinated individuals (Moderna or BioNTech) compared to the D614G strain [35]. Although vaccinations reduced death rates, vaccine-induced attenuation

of peak viral burden has decreased for the B.1.617.2 variant (absolute difference of 10–13% for BNT162b2 and 16% for ChAdOx1) compared to the B.1.1.7 variant in the UK [66]. These results are in line with the increasing number of breakthrough infections among the fully vaccinated population [49]. Nevertheless, extensive vaccination programs among general populations have contributed greatly to reduced numbers of hospitalization, mortality, and infections in countries like Israel, the UK, and the USA even when some VOCs have displayed reduced neutralization sensitivity [35]. Future boost vaccination and HuNAb-based immunotherapy should focus on broadly reactive immune protection [67]. Since systemic HuNAb did not completely prevent SARS-CoV-2 nasal infection, the role of mucosal immunity especially tissue-resident memory T-cells in the upper respiratory tract should be studied for long-term protection. Lastly, antibody-mediated enhancement of SARS-CoV-2 infection and immunopathogenesis should still be carefully monitored in the context of human infections and clinical care.

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D.Z. and Z.C. wrote the manuscript. R.Z. contributed to the data and revision of the manuscript.

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Conflict of interest

D.Z. and Z.C. are co-inventors of the ZDY HuNAbs.

Data availability

No data are available as this is a review article.

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