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Review Article

Emerging antibody-based therapeutics against SARS-CoV-2 during the global pandemic

Yaping Sun[1] and Mitchell Ho[1,2,*]

[1]Antibody Engineering Program, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892, USA, and [2]Laboratory of Molecular Biology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892, USA

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Abstract

SARS-CoV-2 antibody therapeutics are being evaluated in clinical and preclinical stages. As of 11 October 2020, 13 human monoclonal antibodies targeting the SARS-CoV-2 spike protein have entered clinical trials with three (REGN-COV2, LY3819253/LY-CoV555, and VIR-7831/VIR-7832) in phase 3. On 9 November 2020, the US Food and Drug Administration issued an emergency use authorization for bamlanivimab (LY3819253/LY-CoV555) for the treatment of mild-to-moderate COVID-19. This review outlines the development of neutralizing antibodies against SARS-CoV-2, with a focus on discussing various antibody discovery strategies (animal immunization, phage display and B cell cloning), describing binding epitopes and comparing neutralizing activities. Broad-neutralizing antibodies targeting the spike proteins of SARS-CoV-2 and SARS-CoV might be helpful for treating COVID-19 and future infections. VIR-7831/7832 based on S309 is the only antibody in late clinical development, which can neutralize both SARS-CoV-2 and SARS-CoV although it does not directly block virus receptor binding. Thus far, the only cross-neutralizing antibody that is also a receptor binding blocker is nanobody VHH-72. The feasibility of developing nanobodies as inhaled drugs for treating COVID-19 and other respiratory diseases is an attractive idea that is worth exploring and testing. A cocktail strategy such as REGN-COV2, or engineered multivalent and multispecific molecules, combining two or more antibodies might improve the efficacy and protect against resistance due to virus escape mutants. Besides the receptor-binding domain, other viral antigens such as the S2 subunit of the spike protein and the viral attachment sites such as heparan sulfate proteoglycans that are on the host cells are worth investigating.

Statement of Significance: This review summarizes ongoing efforts to develop neutralizing antibodies against SARS-CoV-2 with a focus on targets, neutralizing activities and screening strategies, including phage display, animal immunization and B cell cloning. A cocktail strategy combining two or more antibodies, including nanobodies, targeting different epitopes might protect against mutant resistance.

KEYWORDS: SARS-CoV-2; spike or S protein; human antibody; cocktail therapy; single domain antibody or nanobody

INTRODUCTION

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) first appeared in late 2019 and caused the Coronavirus Disease commonly known as COVID-19. In some cases, this coronavirus results in a syndrome leading to a critical care condition that requires specialized care in an intensive care unit (1–6). As of 12 November 2020, there are 53 001 867 confirmed cases and 1 289 231 deaths worldwide, with 203 countries/regions affected (https://coronavirus.jhu.edu/map.html). Global efforts are ongoing to treat COVID-19 and to flatten the pandemic curve. This review aims to summarize our current knowledge on

*To whom correspondence should be addressed: National Cancer Institute, Bethesda, MD 20892, USA. Tel: +1 (240) 760-7848; Web: https://ccr.cancer.gov/mitchell-ho; Tel: (240) 760-7848; Fax: (240) 541-4501; Email: homi@mail.nih.gov

antibody-based therapeutics against SARS-CoV-2 by providing an overview of neutralizing antibody development mainly targeting the spike (S) protein.

CORONAVIRUS OUTBREAK HISTORY

Coronaviruses (CoVs) are potentially lethal pathogens, with seven strains having emerged to infect humans in recent years. Human coronavirus-229E (HCoV-229E) and HCoV-OC43 were identified in the 1960s and reported to cause symptoms similar to that of a mild common cold, except in infants, the elderly and the immunocompromised (7–9). Decades later, in 2002–3, outbreak of SARS-CoV infection became a global pandemic (10, 11). SARS-CoV is thought to be an animal virus from its natural reservoir, perhaps bats, that spread to other animals (civet cats) as an intermediate host in animal-to-human transmission (12, 13). Patients infected with SARS-CoV exhibited atypical pneumonia that had the potential to progress to acute respiratory distress syndrome (14). As of 13 July 2003, when the last new probable case was reported, there was a total of 8096 probable cases and 774 deaths (case-fatality rate: 9.56%) (15). Two more coronaviruses, HCoV-NL63 and HCoV-HKU1, were found in 2004–5 from archived nasopharyngeal aspirates and caused mild to serious lower respiratory tract infections (16–18). In 2012, almost a decade after the first SARS-CoV outbreak, the Middle East respiratory syndrome coronavirus (MERS-CoV) caused a total of 2494 laboratory-confirmed cases, including 858 associated deaths (case-fatality rate: 34.4%) globally (19). It was reported that MERS-CoV has the same receptor usage and cell entry as bat coronavirus HKU4, which provides an insight into bat-to-human transmission of MERS-CoV (20, 21). In December 2019, cases of mysterious pneumonia were reported in Wuhan, Hubei Province, China, which were later confirmed to be caused by a new coronavirus named SARS-CoV-2. Although bats are probable reservoir hosts for the new coronavirus (22), any intermediate host that may facilitate transfer to humans has not been identified. While the researchers have isolated a coronavirus from a Malayan pangolin, the S protein receptor-binding domain (RBD) of pangolin-CoV is similar to that of SARS-CoV-2 (23, 24), indicating that pangolin could be a potential intermediate host. It has been speculated that SARS-CoV-2 might be the result of a recombination between bat (RaTG13) and pangolin coronaviruses based on the analysis of the S protein sequences (25). The SARS-CoV-2 S protein contains a few residues (e.g., F486 and N501) for stronger contacts with human angiotensin converting enzyme 2 (ACE2) (26). These residues are also found in the sequence of pangolin coronavirus (27).

CORONAVIRUS SPIKE PROTEIN IS IMPORTANT FOR VIRUS ENTRY

CoVs are enveloped viruses containing single-stranded positive-sense RNA that belongs to the *Coronaviridae* family of the *Orthocoronavirinae* subfamily, which can cause illness in animals and humans. CoVs are a large family that is genotypically and phenotypically diverse. CoVs can be divided into four distinct groups based on the genomic sequence alignment phylogenetically, defined as α, β, γ and δ. β-Coronaviruses may further be subgrouped as lineage a, b, c, and d in classical taxonomy. Both SARS-CoV and SARS-CoV-2 belong to β-genus lineage b, whereas MERS-CoV belongs to β-genus lineage c. HCoV-OC43 and HCoV-HKU1 are β-genus lineage a, whereas HCoV-229E and HCoV-NL63 are α-genus (28, 29).

The SARS-CoV-2 viral genome of about 27–32 kb encodes for structural and non-structural proteins. The structural proteins include membrane (M) protein, envelope (E) protein, nucleocapsid (N) protein and spike (S) protein. The S protein plays a role in viral entry and is crucial for determining host tropism and transmission capacity (30–32). The S protein mainly consists of two functional subunits, S1 and S2. S1 is responsible for host cell receptor binding, while S2 is responsible for viral and cellular membrane fusion (33). For many CoVs, the S protein is cleaved between the S1 and S2 subunits, which can activate the protein for membrane fusion (34–38). CoVs entry into susceptible cells is a complex process that requires the process of receptor-binding and proteolytic processing of the S protein to cause the virus-cell fusion. The structure of the S protein allows extensive conformational flexibility as it modulates its ACE2 receptor binding and later undergoes dramatic conformational change to facilitate the fusion of viral and cellular membranes (39, 40). Using cryo-electron microscopy and tomography, Ke *et al*. determined the high-resolution structure of S trimers on the virion surface (41). Each virion is a spherical with a diameter of 91 ± 11 nm. Each individual virion contains only 24 ± 9 S trimers, lower than previously estimated. Notably, the trimers do not all protrude straight from the viral surface (41). In fact, they can tilt by up to 90° toward the membrane, though tilts over 50° are decreasingly favored.

A recent report about the molecular assembly of the authentic SARS-CoV-2 virus at average resolutions of 8.7–11 Å largely confirms previous observations using recombinant S proteins (42). The biological explanation for the tilted S trimer on the virion is unclear. It might be possible that they represent different prefusion stages of the S protein. Based on the S protein sequence alignment, the overall similarities between SARS-CoV-2 S and SARS-CoV S (isolated from human, civet or bat) are ~76–78% for the whole protein and 73–76% for the receptor binding domain (RBD) (22, 39, 43). The sequence similarity may partly explain why SARS-CoV-2 and SARS-CoV share the receptor ACE2 on host cells. Additionally, this shared characteristic may provide the rationale or possibility to develop cross-neutralizing antibodies to both of CoVs (27, 39, 44, 45).

ANTIBODY THERAPEUTICS FOR COVID-19

The US Food and Drug Administration (FDA) has approved the repurposing of some drugs as emergency treatment for severe COVID-19 patients (46). However, major ongoing preclinical and clinical studies have focused on identifying anti-SARS-CoV-2 antibodies targeting its spike protein, thereby blocking virus entry effectively (27, 47). Three antibody drugs specific for the S protein of SARS-CoV-2 are being evaluated in phase 3 clinical trials: REGN-COV2 (REGN10933 + REGN10987; Regeneron/-NIAID), LY3819253 (LY-CoV555; AbCellera/Eli Lilly/NIAID) and VIR-7831/VIR-7832 (Vir biotechnology/GSK). Meanwhile, antibodies targeting other antigens are also under investigation and will be discussed later in this review.

Antibodies targeting the S protein

Neutralizing monoclonal antibodies against the S protein may block virus entry. The RBD located in the S protein is responsible for host cell receptor binding, making it a primary target of neutralizing antibody development (27). There are two conformations, prefusion and postfusion, for the S trimer structure (40). It has been experimentally shown that ~97% of S trimers are in the prefusion form, and only 3% in the postfusion form (41). A previously reported SARS-CoV monoclonal antibody, CR3022 (48, 49), was demonstrated for the first time to also bind potently with SARS-CoV-2 RBD at nanomolar affinity (50); however, it does not show cross-neutralizing ability with SARS-CoV-2. The most promising preclinical studies of antibodies targeting the spike protein are summarized in Table 1.

Different screening strategies such as phage display, animal immunization or single B cell cloning, were used to isolate neutralizing antibodies in these studies. By phage library panning, Wrapp *et al.* isolated single-domain camelid antibodies, V_HHs including VHH-72, from a llama immunized with prefusion-stabilized coronavirus spikes (45). These V_HHs could neutralize MERS-CoV or SARS-CoV pseudoviruses. After V_HH engineered into a bivalent format with human IgG1 Fc-fusion (VHH-72-Fc), it obtained the cross-neutralization ability with IC_{50} of 0.2 μg/mL (2.7 nM) on pseudotyped SARS-CoV, as well as SARS-CoV-2, suggesting a strategy using a nanobody to engineer cross-neutralizing antibodies for future study. Its activities on live virus are unknown. Additionally, Huo *et al.* has isolated H11-D4 from a naïve llama single-domain antibody library using the RBD of SARS-CoV-2 as an antigen for phage panning (51). They improved the affinity maturation of H11-D4 via affinity maturation by phage display and obtained the high affinity mutant H11-H4. These two V_HH nanobodies, H11-D4 and H11-H4, were capable of binding the RBD with *K*D of 39 and 12 nM, respectively, and blocked the attachment of S protein to ACE2 *in vitro*. After fused to Fc, both nanobodies could neutralize SARS-CoV-2 live virus, with H11-H4-Fc showing a particularly high potency (IC_{50}: 4–6 nM) after affinity maturation. Hanke *et al.* reported the isolation and characterization of an alpaca-derived single domain antibody Ty1 by immunizing one alpaca with SARS-CoV-2 S1-Fc and RBD (52). Ty1 showed the neutralization on SARS-CoV-2 pseudotyped viruses at an IC_{50} of 0.77 μg/mL (64 nM). A cryo-electron microscopy structure demonstrated that Ty1 binds to an epitope on the RBD accessible in both the 'up' and 'down' conformations, sterically blocking RBD-ACE2 binding. In another study, Wu *et al.* isolated two human VH single domain antibodies from an engineered VH library by panning on S1 subunit protein (53), since the VH library panning on RBD protein was unable to get neutralizing antibodies as mentioned in the study. The two human VH antibodies, n3130 and n3088, were identified to bind to the cryptic epitope located in the spike trimeric interface. The study reported that both antibodies had neutralizing ability against SARS-CoV-2 with an IC_{50} of ~2.6 μg/mL (17.3 nM). Overall, the antibodies isolated from phage libraries have relatively low neutralizing activities against SARS-CoV-2 without affinity maturation. Further improvement on the library size and screening strategies might be necessary to isolate potent neutralizing antibodies by phage display technology. Nevertheless, phage display might have an advantage over other screening strategies to isolate cross-reactive antibodies against multiple SARS-related coronaviruses or multiple variants/mutants of SARS-CoV-2. Further affinity maturation using phage display (51, 54), yeast display (55, 56) or mammalian cell display (57) might be needed to improve their neutralizing activities.

Animal immunization has also been used to isolate antibodies targeting SARS-CoV-2 S protein. Wang *et al.* identified SARS-CoV-2 reactive antibodies from S protein immunized transgenic mice (H2L2) that encode chimeric immunoglobulins with human antibody variable regions and rat antibody constant regions. Of all the hybridoma supernatant, one antibody (47D11) exhibited cross-neutralizing activity of SARS-CoV and SARS-CoV-2 pseudotyped virus infection. The chimeric 47D11 antibody was humanized by cloning of the human variable regions into a human IgG1 framework (58). Taken together, animal immunization with S protein from multiple CoVs is an efficient way to identify cross-neutralizing antibodies.

As the most popular strategy so far, single B-cell cloning allows for the rapid generation of antigen-specific monoclonal antibodies in a matter of several weeks, which is highly efficient for antibody development against emerging infectious virus (59). Pinto *et al.* reported human monoclonal antibodies targeting SARS-CoV-2 S protein isolated from memory B cells of an individual who was infected with SARS-CoV in 2003. One of these antibodies, named S309 (the antibody used as the basis for developing VIR-7831/7832), neutralizes both SARS-CoV-2 and SARS-CoV pseudoviruses, as well as authentic SARS-CoV-2 by binding the RBD (60). Interestingly, S309 recognizes an epitope containing the N343 glycan (N330 in SARS-CoV S glycoprotein) conserved within SARS-related coronavirus spike proteins without competing with ACE2 binding. Like 47D11 (58), S309 is an ACE2 non-blocker although both human antibodies 47D11 and S309 are SARS-CoV-2 and SARS-CoV cross-neutralizing antibodies (27). Up to date, the only cross-neutralizing antibody that is also an ACE2 blocker is nanobody VHH-72 (45). Nevertheless, VIR-7831/7832 based on S309 is currently being tested in phase 3 clinical trials and it is the only antibody in late clinical development that can neutralize both SARS-CoV-2 and SARS-CoV viruses. It would be interesting to examine the potential clinical benefits of this novel cross-neutralizing antibody for treating current COVID-19 patients and potential SARS-related CoV infections in the future.

Table 1. Preclinical studies of antibodies targeting the spike protein of SARS-CoV-2

VHH-72-Fc	Llama V_HH, fused to hIgG1 Fc	SARS-CoV RBD was used for phage panning by an immunized llama library	RBD	Pseudovirus SARS-CoV, SARS-CoV-2 IC_{50} 0.2 μg/mL (2.7 nM)	Daniel Wrapp *et al.*, Cell, 2020 (45)
H11-H4-Fc	Llama V_HH, fused to hIgG1 Fc	SARS-CoV-2 RBD was used for phage panning by a naïve llama library	RBD	Live SARS-CoV-2 IC_{50} 4–6 nM	Jiangdong Huo *et al.*, Nature Structural & Molecular Biology, 2020 (48)
Ty1	Alpaca V_HH	Immunized one alpaca with SARS-CoV-2 S1-Fc and RBD on a 60-day immunization schedule	RBD	Pseudoviruse SARS-CoV-2	
IC_{50} of 0.77 μg/mL (64 nM)	Leo Hanke *et al.*, Nature Communications, (52)				
n3130, n3088	Human VH	SARS-CoV-2 S1 was used for phage panning by an engineered human VH library	cryptic epitope located		
in the spike trimeric interface	Live SARS-CoV-2 IC_{50} ~ 2.6 μg/mL (17.3 nM)	Yanling Wu *et al.*, Cell Host&Microbe, 2020 (53)			
47D11	Reformat to human IgG1	Immunized transgenic H2L2 mice with SARS-CoV-1 S protein	a conserved epitope in RBD	Live SARS-CoV IC_{50} 0.19 μg/mL (1.2 nM)	
Live SARS-CoV-2 IC_{50} 0.57 μg/mL (3.8 nM)	Chunyan Wang *et al.*, Nature Communications, 2020 (58)				
P2C-1F11, P2B-2F6, P2C-1A3	Human IgG1	Single B cell antibody isolation of 8 SARS-CoV-2 infected individuals	RBD	live SARS-CoV-2 IC_{50} ~ 0.1 μg/mL (0.7 nM)	Bin Ju *et al.*, Nature, 2020 (68)
CB6	Human IgG1	Utilized SARS-CoV-2 RBD as the bait to sort specific memory B cells PBMCs of a convalescent COVID-19 patient	Overlapping with ACE2-binding sites in SARS-CoV-2 RBD	Live virus IC_{50} 36 ng/mL (0.24 nM)	
CB6 (50 mg/kg) inhibited SARS-CoV-2 infection in rhesus monkeys at both prophylactic and treatment settings	Rui Shi *et al.*, Nature, 2020 (69)				
C121, C144, C135	Human IgG1	Single B cell antibody isolation from 6 convalescent individuals	Different binding epitope from CR3022	Live SARS-CoV-2 IC_{50} 1.64, 2.55 and 2.98 ng/mL (10.9 pM, 17 pM, 19.8 pM)	Davide F. Robbiani *et al.*, Nature, 2020 (70)
CC12.1	Human IgG1	Single B cell antibody isolation from 3 convalescent individuals	RBD	Live SARS-CoV-2 IC_{100} 22 ng/mL (0.14 nM) CC12.1 (4 mg/kg) inhibited SARS-CoV-2 infection in Syrian hamsters in prophylaxis setting	Thomas F. Rogers *et al.*, Science, 2020 (71)
COVA1–18 COVA2–15	Human IgG1	Single B cell antibody isolation from 3 SARS-CoV-2 infected individuals	Competition with ACE2 binding site to RBD	Live SARS-CoV-2 IC_{50} of 7 and 9 ng/mL (46 and 60 pM)	Philip J. M. Brouwer *et al.*, Science, 2020 (72)
4A8	Human IgG1	Single B cells antibody isolation of 10 COVID-19 recovered patients with different ages and different infection phase	NTD	Live SARS-CoV-2 IC_{50} 0.6ug/mL (4 nM)	Xiangyang Chi *et al.*, Science, 2020 (73)

Jones *et al*. recently reported the isolation of LY3819253/LY-CoV555 (bamlanivimab) to the RBD of the SARS-CoV-2 spike protein using two single B cell screening methods, multiplexed bead-based assay and live cell-based assay, from a patient hospitalized with COVID-19 in mid-February 2020 (61). Next-generation sequencing of antibody genes from selected single B-cells shows that of the 440 unique antibodies identified, only 4% are cross-reactive to both full-length SARS-CoV-2 and SARS-CoV spike proteins. Notably, the neutralization potency of Ab169 (later called LY3819253/LY-CoV555 or bamlanivimab), an RBD binder and ACE2 blocker, exhibits the greatest activity with the IC_{50} value of 100 pM in live virus assay among all the antibodies. In a rhesus macaque challenge model, prophylaxis doses as low as 2.5 mg/kg reduce viral replication in the upper and lower respiratory tract. Mechanistically, LY-CoV555 binds the spike protein RBD in both up and down conformations such as mAb114 that binds the Ebola virus glycoprotein RBD in both the pre-activation and activated states for treating Ebola infection (62, 63). LY-CoV555 (bamlanivimab) is being evaluated in phase 3 clinical trials and has been recently approved as an emergency use authorization for the treatment of mild-to-moderate COVID-19.

In order to overcome virus escape mutation, Regeneron has described parallel efforts utilizing both animal immunization (genetically humanized mice) and B cell cloning from convalescent humans to generate a large collection of highly potent human neutralizing antibodies targeting the RBD of the spike protein of SARS-CoV-2 (64). Genetically humanized mice were immunized with a DNA plasmid that expresses SARS-CoV-2 S protein and boosted with a recombinant RBD protein. The most potent antibodies with IC_{50} values of low pM (e.g., 37 pM for REGN10933, 42 pM for REGN10987) might be isolated from humanized mice, suggesting that animal immunization induced high affinity antibodies to the virus spike protein. The antibody cocktail to SARS-CoV-2, REGN-COV2 (REGN10933 + REGN10987), could prevent rapid mutational escape of virus variants that have arisen in the human population (65). Genomics analysis of SARS-CoV-2 from the same individual with re-infection shows genetically significant differences between the variants associated with early infection and re-infection (66). The second infection is symptomatically even more severe than the first one. REGN-COV2 and other cocktail or multispecific therapeutics might be useful to overcome potential epitope escape variants in re-infection. In addition, REGN-COV2 appears highly potent therapeutic antibodies against SARS-CoV-2 S protein with low pM activities on live virus. REGN-COV2 cocktail therapy is being evaluated in phase 3 clinical trial.

Researchers from Astrazeneca have isolated 389 SARS-CoV-2 S-protein-reactive human monoclonal antibodies from the B cells of two convalescent individuals who had been infected with SARS-CoV-2 in Wuhan, China. Among these human antibodies, COV2–2196 and COV2–2130 bound simultaneously to the S protein and neutralized wild-type SARS-CoV-2 virus in a synergistic manner (67).

Ju *et al.* reported the isolation and characterization of 206 RBD-specific monoclonal antibodies derived from single B cells of eight SARS-CoV-2 infected individuals (68). The most potent antibodies, P2C-1F11, P2B-2F6, and P2C-1A3, neutralize live SARS-CoV-2 with an IC_{50}s of 0.03, 0.41, and 0.28 μg/mL (200 pM, 2.7 nM, 1.8 nM) respectively. These antibodies are most competitive with ACE2, indicating that blocking the RBD and ACE2 interaction is a useful surrogate for neutralization. However, none of the anti-SARS-CoV-2 antibodies cross-react with SARS-CoV RBD. Similarly, Shi *et al.* reported a human monoclonal antibody CB6 utilizing SARS-CoV-2 RBD as the bait to sort specific memory B cells PBMCs of a convalescent COVID-19 patient (69). CB6 exhibits strong neutralizing activity against live SARS-CoV-2 infection of Vero E6 cells, with an observed IC_{50} of 0.036 μg/mL (240 pM). In addition, CB6 inhibits SARS-CoV-2 infection in rhesus monkeys in both prophylactic and treatment settings. At present, CB6 is in phase 1 clinical trials in China and the USA. However, CB6 is not a cross-neutralizing antibody and cannot cross-bind to SARS-CoV S either. Robbiani *et al.* reported antibody isolation on 149 COVID-19 convalescent individuals (70). Plasma samples binding to the SARS-CoV-2 RBD and trimeric spike proteins were collected, followed by neutralization activity testing on SARS-CoV-2 pseudovirus. Lastly, 534 paired IgG heavy and light chain sequences were obtained by reverse transcription PCR from individual RBD-binding B cells from six convalescent individuals. Potent neutralizing antibodies, C121, C144, and C135 with an IC_{50}s of 1.64, 2.55, and 2.98 ng/mL (10.9 pM, 17 pM, 19.8 pM), against authentic SARS-CoV-2 were identified. The bilayer interferometry result has shown that these three antibodies can bind with different epitopes from CR3022. Negative stain electron microscopy imaging has confirmed the different binding epitope. Using similar methodology, Rogers *et al.* reported a rapid screening platform to generate over 2045 antibodies from a cohort of SARS-CoV-2 recovered participants in 2 weeks (71). CC12.1 isolated by single B cell cloning from recovery patient donors was able to show the 100% neutralization of live SARS-CoV-2 at a concentration of 22 ng/mL (146 pM). Most importantly, CC12.1 at a dose of 500 μg/animal (on average 4 mg/kg) could protect against weight loss and lung viral replication in Syrian hamsters challenged intranasally with 1×10^6 PFU of SARS-CoV-2. Another study, Brouwer *et al.* isolated 19 neutralizing antibodies from single B cell derived from three SARS-CoV-2 infected individuals (72). Two of them, COVA1–18 and COVA2–15, showed picomolar neutralizing activities against authentic SARS-CoV-2 with an IC_{50} of 7 and 9 ng/mL (46 and 60 pM), respectively. Through large-scale SPR-based competition assay and electron microscopy studies, antibodies with different binding epitopes to the spike protein were demonstrated, including RBD and non-RBD epitopes. However, these antibodies targeting non-RBD epitope are not able to neutralize SARS-CoV-2. The above two most potent antibodies can compete with ACE2 binding site to RBD.

Currently, most of the antibodies developed are targeting RBD in the spike protein. However, Chi *et al.* isolated monoclonal antibodies derived from 10 patients that have recovered from SARS-CoV-2 viral infection, the patient's age ranging from 25 to 53 years, and memory B cells were collected from different infection phase. 4A8 is a human monoclonal antibody that targets the N-terminal domain (NTD) of the SARS-CoV-2 S protein and exhibits high neutralization potency against SARS-CoV-2 although it does not directly inhibit the interaction between RBD and ACE2 (73). Liu *et al.* reported the isolation of 19 antibodies from five patients infected with SARS-CoV-2, which could neutralize SARS-CoV-2 *in vitro*. Epitope mapping showed that this collection of 19 human antibodies was about evenly divided against the RBD and NTD, indicating that these two regions at the top of the viral spike are immunogenic (74).

Interestingly, Ma *et al.* reported a strategy using cell-based chimeric antigen receptor (CAR) technology. They have developed a novel approach for the generation of CAR-NK cells using the scFv fragment of CR3022 (henceforth, CR3022-CAR-NK) for targeting SARS-CoV-2, which showed specifically killing to pseudo-SARS-CoV-2 infected target cells *in vitro* (75). While it could be a complimentary strategy worth exploring, many questions should be addressed in more biologically relevant assays, including animal testing. In particular, how biologically and therapeutically this cell-based therapy could stop SARS-CoV-2 virus proliferation and spread is unclear. The potential side-effects induced by CAR-based cell therapies need to be carefully evaluated in proof-of-concept animal studies before they can be used in humans.

Antibodies targeting the host derived proteins

Some studies have investigated the changes of several cytokines in serum of the COVID-19 patients that generates a series of immune responses, and the cytokine storm syndrome was proportional to the severity of disease (3, 4, 76). The pro-inflammatory cytokine IL-6 may have a prominent role, leading to the inflammatory cascade, which may result in increased alveolar-capillary blood-gas exchange dysfunction (77, 78). Antibodies targeting IL-6, such as Olokizumab and Siltuximab, are in phase 3 trial at present. Clinically, Stoclin *et al.* reported the case of a patient with a respiratory failure linked to COVID-19 who had a rapid favorable outcome after two infusions of Tocilizumab, an anti-IL-6 receptor antibody (79). However, Stone *et al.* reported a randomized, double-blind, placebo-controlled phase 3 trial involving 243 patients with confirmed SARS-CoV-2 infection and found that Tocilizumab was not effective for preventing intubation or death in moderately ill hospitalized patients with COVID-19 (80).

Heparan sulfate proteoglycans (HSPGs) provide the attachment sites for virus such as polyomaviruses, papillomavirus, and hepatitis C virus, to make primary contact with the host cell surface (81–83). Treatment of the cells with heparinase or heparin prevents the binding of the S protein to host cells and inhibits SARS pseudovirus infection (84). Based on the findings in previous studies including ours using the HS20 human monoclonal antibody targeting heparan sulfate to inhibit viral infection (81, 84–87), we speculated that in addition to ACE2, HSPGs might be another potential target on human cells that can be blocked by therapeutic antibodies for treating COVID-19 (27). Recently, Clausen *et al*. showed that SARS-CoV-2 S protein interacted with cell surface heparan sulfate and ACE2 through its RBD. Interestingly, the S protein binding to heparan sulfate and ACE2 on the cell surface may occur co-dependently. Heparin and purified heparan sulfate can block S protein binding and infection by SARS-CoV-2 virus, suggesting using heparin as bait to attract the virus away from human cells. It would be interesting to further validate whether heparin, an approved medication to treat blood clots, might be repurposed to reduce SARS-CoV-2 infection. In another study, Zhang *et al*. also showed that heparan sulfate facilitated spike-dependent viral entry and screened approved drugs to identify inhibitors targeting the HS-dependent cell entry (88). Altogether, these studies indicate heparan sulfate as a co-receptor for viral entry and support the rationale for developing therapeutics that target heparan sulfate for inhibiting SARS-CoV-2 and other virus infections. Biochemical analysis for identification of specific binding motifs of HS (e.g., 2-O, 3-O, or 6-O sulfation (87) and N-sulfation (82)) for SARS-CoV-2 attachment would be useful for designing specific anti-viral inhibitors.

ONGOING CLINICAL TRIALS OF ANTIBODIES TARGETING THE SPIKE PROTEIN

There are 13 clinical trials ongoing related to human monoclonal antibodies targeting SARS-CoV-2 spike protein described in Table 2. According to the COVID-19 Antibody Therapeutics Tracker (https://chineseantibody.org/covid-19-track/) (46), three antibody drugs have entered into phase 3 clinical trials. Among them, REGN10933 and REGN10987 represent a non-competing pair of antibodies that can simultaneously bind to RBD and thus can be partners for a therapeutic antibody cocktail aimed at decreasing the potential for mutant viral strain escaping. Regeneron in collaboration with the National Institute of Allergy and Infectious Diseases (NIAID) at the National Institutes of Health (NIH) initiated a phase 3 clinical trial evaluating REGN-COV2 (REGN10933 + REGN10987) for the treatment and prevention of COVID-19 in late June 2020. LY3819253 (LY-CoV555) developed by AbCellera/Eli Lilly in collaboration with the NIAID/NIH also entered phase 3 clinical trial. Notably, on 9 November 2020, the US FDA issued an emergency use authorization for bamlanivimab (LY3819253/LY-CoV555) for the treatment of mild-to-moderate COVID-19 in adult and pediatric patients (https://www.fda.gov/news-events/press-announcements/coronavirus-covid-19-update-fda-authorizes-monoclonal-antibody-treatment-covid-19). Bamlanivimab has been shown in two randomized, double-blind, placebo-controlled clinical trial in 465 non-hospitalized adults with mild-to-moderate COVID-19 symptoms to reduce COVID-19-related hospitalization or emergency room visits. However, a clinical benefit of bamlanivimab treatment has not been established in hospitalized patients due to COVID-19.

Recently, GlaxoSmithKline (GSK) and Vir Biotechnology declared that they had launched the phase 2/3 study of VIR-7831, which also has the development name

Table 2. Ongoing clinical trials of antibodies targeting the spike protein of SARS-CoV-2

REGN-COV2 (REGN10933 + REGN10987)	Animal immunization using genetically-umanized mice	RBD	Phase 3	Regeneron	Live SARS-CoV-2 IC_{50} of 40 pM	Johanna Hansen *et al.*, Science, 2020 (62, 63); NCT04452318
LY3819253 (LY-CoV555; bamlanivimab)	B cell cloning from convalescent patients	RBD	Phase 3; emergency use authorization for treating mild-to-moderate COVID-19 patients	AbCellera/Eli Lilly and Company	Live SARS-CoV-2 IC_{50} of 100pM	Bryan E. Jones, et al, bioRxiv, 2020 (61); NCT04497987
VIR-7831/7832 (S309)	Single B cells antibody isolation of an individual who was infected with SARS-CoV in 2003	glycan epitope contains position N343	Phase 3	Vir Biotechnology/GlaxoSmithKline	Live SARS-CoV-2 IC_{50} of 500 pM	Dora Pinto *et al.*, Nature, 2020 (60); NCT04545060
DXP-593	High-throughput single B cell sequencing from over 60 convalescent patients	N/A	Phase 2	Beigene/Singlomics Biopharmaceuticals/Peking University	N/A	NCT04551898
JS016	Utilized SARS-CoV-2 RBD as the bait to sort specific memory B cells PBMCs of a convalescent COVID-19 patient	Overlapping with ACE2-binding sites in SARS-CoV-2 RBD	Phase 1	Junshi Biosciences/Eli Lilly and Company	Live SARS-CoV-2 IC_{50} of 240 pM	Rui Shi *et al.*, Nature, 2020 (59); NCT04441918
TY027	N/A	N/A	Phase 1	Tychan Pte. Ltd.	N/A	NCT04429529
CT-P59	N/A	N/A	Phase 1	Celltrion	N/A	NCT04525079
BRII-196	N/A	N/A	Phase 1	Brii Biosciences	N/A	NCT04479631
BRII-198	N/A	N/A	Phase 1	Brii Biosciences	N/A	NCT04479644
SCTA01	N/A	N/A	Phase 1	Sinocelltech Ltd.	N/A	NCT04483375
AZD7442 (AZD8895 + AZD1061)	B cell cloning of two convalescing individuals who had been infected with SARS-CoV-2 in Wuhan, China	Overlapping with ACE2-binding sites in SARS-CoV-2 RBD	Phase 1	AstraZeneca	Live SARS-CoV-2 IC_{50} of 100 pM	NCT04507256
MW33	N/A	N/A	Phase 1	Mabwell (Shanghai) Bioscience Co., Ltd	N/A	NCT04533048
STI-1499/COVI-SHIELD	Screening antibodies in its proprietary G-MAB™ fully human antibody library	Overlapping with ACE2-binding sites in SARS-CoV-2 RBD	Phase 1	Sorrento Therapeutics, Inc.	N/A	NCT04454398

N/A: not available.

GSK4182136. This study, named COMET-ICE, will enroll 1300 patients worldwide to test VIR-7831/GSK4182136 in early treatment of patients infected with SARS-CoV-2 who are at high risk of hospitalization.

DXP-593 was identified from peripheral blood mononuclear cells collected from convalescent patients by high-throughput single-cell sequencing by a joint research team from the Beijing Advanced Innovation Center for Genomics at Peking University. It has been demonstrated to show highly potent neutralizing antibodies against SARS-CoV-2; the phase 2 study is currently ongoing.

JS016, which is discussed above as antibody CB6, is the first SARS-CoV-2 neutralizing antibody to enter clinical trials in China. These trials are led by Junshi and Eli Lilly in China and the rest of the world, respectively. JS016 has been tested in rhesus monkeys in prophylactic and treatment settings. TY027, developed by Tychan in Singapore, is also in phase 1 clinical trial. CT-P59, developed by Celltrion, has entered a phase 1 clinical trial in mild COVID-19 patients. Celltrion previously showed its antiviral activities in neutralizing the mutated G-variant strain (D614G variant) which might be associated with the increased viral transmission of COVID-19. Brii Biosciences (Brii Bio) company, in collaboration with Tsinghua University and 3rd People's Hospital of Shenzhen, has launched the phase 1 clinical trial of BRII-196, BRII-198 for assessing safety, tolerability, and pharmacokinetics in healthy adult volunteers. Sinocelltech Ltd. has developed an anti-SARS-CoV-2 monoclonal antibody, SCTA01, and started phase 1 clinical trial in healthy subjects in China. Most recently, AstraZeneca launched the phase 1 clinical trial of AZD7442 (AZD8895 + AZD1061) in UK as a potential combination therapy for the prevention and treatment of COVID-19. Mabwell (Shanghai) Bioscience Co., Ltd., initiated a phase I clinical trial with the MW33 antibody. Sorrento Therapeutics, Inc., started a phase 1 clinical trial of STI-1499 (COVI-GUARD™) for hospitalized COVID-19 patients.

CONCLUSION AND PERSPECTIVE

Most of the therapeutic antibody development against SARS-CoV-2 are currently in the preclinical stage, with about 35% of antibodies in various stages of clinical trials. Nearly 82% of current antibodies are human monoclonal antibodies with 3, REGN-COV2, LY3819253/LY-CoV555, and VIR-7831/VIR-7832, in phase 3 clinical trials, and the second largest group is single-domain antibody (also commonly called nanobody), indicating that emerging single domain antibody development after FDA approved the first nanobody caplacizumab in 2019 (89). Single-domain antibodies can bind novel epitopes including buried cavities inaccessible by conventional antibodies (90, 91). Naturally occurring nanobodies derived from camels (45, 92–94) and sharks (90, 95–97) are stable and relatively easy to express and fold in various conditions; therefore, they can be effective building blocks for the construction of multivalent and multispecific molecules to effectively neutralize virus. When revising this review article, another paper reported nanobodies (e.g., Nab20, Nab21) isolated from an RBD-immunized llama with picomolar to femtomolar affinities that inhibit SARS-CoV-2 viral infection at sub-ng/ml concentration (98). Furthermore, multivalent nanobody constructs can achieve high neutralization potency (IC_{50}s as low as 0.058 ng/ml). For respiratory infection such as COVID-19, nanobodies are particularly attractive because they might be administered as an inhaler directly to the site of infection (99). Further studies are necessary to evaluate the feasibility of developing nanobody-based therapeutics as inhaled drugs for treating COVID-19 and other respiratory infections.

Around 85% of antibodies are developed targeting the S protein, primarily focused on targeting RBD in an effort to block the viral entry at the initial step of binding to the host cell receptor. In the perspective of cross-neutralizing antibody development for SARS related CoVs, it seems that antibody, which is derived from SARS-CoV patient or SARS-CoV immunization animals, could have the cross-neutralizing activities on SARS-CoV-2. However, antibodies derived from SARS-CoV-2 patients usually do not show cross-neutralizing ability on SARS-CoV. Although both S proteins from SARS-CoV-2 and SARS-CoV bind human ACE2 on the cells for viral entry and both S proteins have highly similar structures, hypothetically, an ACE2 blocker that can neutralize both viruses is feasible. However, it seems rare to identify such a shared epitope using existing SARS-CoV-2 antibodies. This apparent discrepancy will require further investigation on the structure and function of the S protein of these two viruses. Cross-neutralizing antibodies are being explored by using various strategies, including single domain antibodies.

Next-generation sequencing of neutralizing antibodies against SARS-CoV-2 has been conducted in several studies. In Regeneron's study, over 200 antibodies that were isolated from humanized mice show predominant lineage of antibodies, which utilize VH3–53 paired with VK1–9, VK1–33, or VK1–39, while antibodies isolated from infected humans utilize VH3–66 paired with VK1–33 or VH2–70 paired with VK1–39. Interestingly, VH3–53 usage (e.g., VH3–53/VK1–9 pair (100)) has been found in other human-derived neutralizing antibody against SARS-CoV-2 spike protein (71, 100). In a recent study conducted by Eli Lilly, sequencing of about 400 antibodies shows that the VH3 germline gene family (e.g., VH3–53, VH3–66) representing 57% of total diversity. Among them, VH3–30 usage is the most common (38%). However, the selected Ab169 (LY-CoV555) has VH1–69 and VK1–39 germline framework sequences. Further sequence analysis of neutralizing antibodies against SARS-CoV-2 RBD might provide valuable insights of the usage of germline sequences for potent anti-viral neutralizing activities. Such information could be useful for not only therapeutic antibody development or optimization but also vaccine or adjuvant design.

Antibody targeting S2 subunit may also worth studying since it shows more overall sequence similarity not only between SARS-CoV and SARS-CoV-2 but also among other human coronaviruses. Up to date, antibodies targeting S2 with potent neutralization activities against SARS-CoV-2 have not been reported. It appears challenging to isolate antibodies to S2 probably due to the dynamic structure of the S2 subunit as part of the virus spike trimer and elusive conformational change at the fusion stage. Further biochemical studies of the S2 subunit and establishment of a suitable screening assay might be crucial for generating neutralizing antibodies targeting S2.

Antibodies specific for host targets including the viral attachment sites on human cells such as heparan sulfate should be explored as well. A cocktail combination therapy targeting two or more distinct sites or pathways on the viral surface or host attachment sites might be one of the most effective approaches to eliminate virus in the host. Besides cocktail combination therapies of two or more monoclonal antibodies targeting multiple epitopes on the virus, engineered multivalent or multispecific molecules using various antibody binding sites such as single chain Fvs or single domain antibodies might emerge as a new class of promising antibody therapeutics for anti-viral therapy.

The mechanisms of neutralizing antibodies in terms of protection against SARS-CoV-2 viral infection as well as additional immune functions that may have both protective and pathological consequences are being studied (101). Besides neutralization, antibodies may have additional anti-viral activities mediated by Fc, including antibody-dependent cellular phagocytosis (ADCP), complement-dependent cytotoxicity, and antibody-dependent cellular cytotoxicity (ADCC). Using primary natural killer (NK) effector cells and SARS-CoV-2 S-glycoprotein-expressing expiCHO as target cells, S309 shows the Fc-mediated ADCC of SARS-CoV-2 S-glycoprotein-transfected cells (102). In addition, using NK cells or macrophage from healthy donors, REGN10987 shows strong ADCC and ADCP activities against human Jurkat cells expressing SARS-CoV-2 spike protein (103). However, antibody responses can also cause pathological damages (101). Although sub-neutralizing antibody titers from second infections have been related to antibody-dependent enhancement (ADE) in patients with dengue. Evidence of ADE in SARS-CoV-2 patients has not been established so far (104). Further understanding how antibodies may play protective and potential pathogenic roles is important for drug design and clinical development. Besides ADE, anti-drug antibody (ADA) response might be worth evaluating as well. Since most current antibodies in the clinical trials are human antibodies, immunogenicity and ADA effect have not been reported so far. Future analysis of ADA, in particular animal-derived antibodies (e.g., camelid nanobodies), would be useful for better understanding of potential ADA in patients. Humanization of nanobodies might be necessary to reduce immunogenicity in humans.

The review is focused on discussing screening strategies for isolating neutralizing antibodies against SARS-CoV-2 and their functional properties. We are also aware that such antibodies with high affinity and specificity, including nanobodies, can also be utilized as detectors for diagnostics (105). A microfluidic device or magnetic beads using antibodies highly specific for viral proteins can be developed to capture virus for detection (106).

Addendum

When preparing this review, US President Trump and the First Lady were diagnosed with COVID-19 on 1 October 2020 (https://www.whitehouse.gov/wp-content/uploads/2020/10/MemoFromThePresdentsPhysician-3.png). On 2 October 2020, the President received a single 8-gram dose of Regeneron's REGN-COV2 as a "compassionate use" request from the President's physicians. On 5 October 2020, President Trump returned to the White House after being discharged from the Walter Reed National Military Medical Center in Bethesda, Maryland. On November 21, 2020, REGN-COV2 (casirivimab and imdevimab) received Emergency Use Authorization from the U.S. FDA for the treatment of mild to moderate COVID-19 in adults, as well as in pediatric patients at least 12 years of age and weighing at least 40 kg (https://www.fda.gov/news-events/press-announcements/coronavirus-covid-19-update-fda-authorizes-monoclonal-antibodies-treatment-covid-19).

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CONFLICT OF INTEREST STATEMENT

M.H. is the Editor-in-Chief of the journal and is blinded from reviewing or making decisions on the manuscript.

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