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Neutralizing monoclonal antibodies against highly pathogenic coronaviruses

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The pandemic of Coronavirus Disease 2019 (COVID-19) caused by severe acute respiratory syndrome 2 coronavirus (SARS-CoV-2) is a continuing worldwide threat to human health and social economy. Historically, SARS-CoV-2 follows SARS and MERS as the third coronavirus spreading across borders and continents, but far more dangerous with long-lasting symptomatic consequences. The current situation is strong evidence that coronaviruses will continue to be pathogens of consequence in the future, thus calling for the development of neutralizing antibody-based prophylactics and therapeutics for prevention and treatment of COVID-19 and other human coronavirus diseases. This review summarized the progresses of developing neutralizing monoclonal antibodies against infection of SARS-CoV-2, SARS-CoV, and MERS-CoV, and discussed their potential applications in prevention and treatment of COVID-19 and other human coronavirus diseases.

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Introduction

By the time SARS-CoV-2 (originally named 2019-nCoV by WHO) (https://www.who.int/emergencies/diseases/novel-coronavirus-2019) first emerged in late 2019 [1], seven human coronaviruses, including SARS-CoV in 2002/2003 (https://www.who.int/publications/m/item/summary-of-probable-sars-cases-with-onset-of-illness-from-1-november-2002-to-31-july-2003) and MERS-CoV in 2012 (https://www.who.int/emergencies/disease-outbreak-news/item/2021-DON317), had caused the outbreaks of severe coronavirus diseases worldwide. However, COVID-19, caused by SARS-CoV-2 infection, has posed more serious threat to public health, social stability and economy development. Presently, many vaccines against COVID-19 are in the clinical trials (https://clinicaltrials.gov/ct2/results?term=vaccine&cond=Covid-19&age_v=&gndr=&type=&rslt=&phase=2&phase=3&Search=Apply), and some have already applied for and obtained emergency use authorization. Cases of side effects after vaccination have been reported. This means that safety and efficacy, particularly in view of the growing number of mutant strains diverging from wild type [2\(^\text{nd}\)], and length of immunization still need further study with more data. Beyond vaccine development, antibody cocktails have shown some efficacy against viral mutants [2\(^\text{nd}\)]. Fully human antibodies can accurately and efficiently identify antigens with few side effects in humans. Some neutralizing monoclonal antibodies (NMAbs) have also entered clinical trials (https://clinicaltrials.gov/ct2/results?term=antibody&cond=Covid19&aige_v=&gndr=&type=&rsrlt=&phase=2&phase=3&Search=Apply). In view of the importance of NMAbs in the prevention and treatment of coronavirus diseases, this review summarizes the progresses of developing NMAbs against SARS-CoV, MERS-CoV, and SARS-CoV-2, providing scientific knowledge about these NMAbs to combat the current COVID-19 pandemic and future emerging and re-emerging coronavirus diseases.

Key targets of coronavirus NMAbs

The coronavirus spike (S) glycoprotein is the primary immunogenic target for the design of neutralizing antibodies. The trimeric S protein is a type I fusion transmembrane protein which mediates virus binding to corresponding receptors and finally entry into host cells. In the case of SARS-CoV and SARS-CoV-2, they recognize the same receptor angiotensin-converting enzyme 2 (ACE2), whereas MERS-CoV S protein binds to dipeptidyl peptidase-4 (DPP4). The S protein trimer comprises three copies of an S1 subunit that contains the N-terminal domain (NTD) and receptor binding domain (RBD) and three copies of S2 [3–6,7*8]. The RBD has two conformational states, the closed ‘down’ state, which hides the receptor-binding regions, and the open ‘up’ state, which
exposes the determinants of receptor binding (Figure 1). Finally, the S2 subunit mediates the fusion of coronavirus and host cell membrane [9**,10].

**NMAbs against SARS-CoV**

**Human NMAbs against SARS-CoV**

*NMAbs identified by screening of antibody libraries*

As the SARS outbreak during 2002/2003, some fully human-derived NMAbs targeting the RBD were identified from nonimmune phage libraries of human antibodies [11–16], such as 80R, CR3014, and m396 (Figure 2a) (Table 1). The S protein of SARS-CoV continued to mutate during transmission, but researchers found that CR3014 did not neutralize all mutant strains. However, researchers also discovered that the combination of CR3022 and CR3014, now known as an antibody cocktail, could effectively neutralize multiple mutant strains [17]. B1 is the first S2-targeting mAb screened from an antibody library of SARS-CoV convalescent patients [18] (Table 1).

*NMAbs identified by use of Epstein–Barr virus (EBV) transformation technology*

Similar to the use of hybridoma technology, researchers used EBV to infect antibody-secreting B cells in order to construct immortal cell lines that stably express antibodies. In this way, a pool of human NMAbs was screened out, such as S3.1 and S230.15 [16,19] (Table 1).

*NMAbs identified from transgenic mice*

Fully humanized NMAbs have been developed from the human immunoglobulin G (IgG) transgenic mouse, XenoMouse®. immunized with the SARS-CoV S protein [20]. The NMAbs 68 and 201 targeting the NTD and RBD, respectively, identified from the immunized transgenic mice. Mice receiving 40 mg/kg of either NMAb before SARS-CoV challenge were completely protected [21] (Table 1).

*NMAbs against SARS-CoV from other sources*

*NMAbs identified by use of hybridoma technology*

Owing to limited human trials, the development of animal immunization and hybridoma technology has substantially enriched SARS-CoV antibody research. A large number of animal-derived NMAbs were screened out, such as F26G18, and the corresponding chimeric antibodies were obtained by antibody humanization. These chimeric NMAbs were shown to target RBD and exert antiviral effects by inhibiting ACE2 binding to RBD [22–24]. Similarly, many NMAbs with strong neutralizing activity against SARS-CoV were identified, including 1A5, 2C5, and 341C, all targeting RBD [25,26]. To explore effective targets, researchers immunized mice with different regions of the S protein as antigens and obtained S34 and S84 with correspondingly different targets [27]. The mutation of the S2 region was much slower, compared to S1, resulting in the development of more
broad-spectrum S2-targeting antibodies against SARS-CoV mutant strains [28,29]. Accordingly, researchers immunized mice with S2 as the antigen and screened a number of NMAbs targeting S2, among which 1A9 was the most potent [30–32].

**NMAbs against MERS-CoV**

**Human NMAbs against MERS-CoV**

NMAbs identified by screening of antibody libraries

NMAbs m336, m337, and m338 that were identified from a phage-displayed Fab library from healthy donors...
### Table 1

| Name of NMAb | Type | Source | Preparation | Target | Mechanisms of neutralization | Developing stage | Refs |
|--------------|------|--------|-------------|--------|------------------------------|-----------------|------|
| NMAbs against SARS-CoV-2 |     |        |             |        |                              |                 |      |
| m336         | Fab  | Human  | Non-immune phage libraries of human antibodies | RBD    | Competition with ACE2 in binding with RBD | Preclinical | [11,12] |
| CR3014       | scFv | Human  | Non-immune phage libraries of human antibodies | RBD    | Competition with ACE2 in binding with RBD | Preclinical | [13,14] |
| CR3022       | scFv | Human  | A scFv phage display library generated from cells of a convalescent SARS patient | RBD    | Blocking conformational changes of S proteins | Preclinical | [17] |
| m396         | Fab  | Human  | Antibody library derived from cells of healthy volunteers | RBD    | Competition with ACE2 in binding with RBD | Preclinical | [15,16] |
| B1           | scFv | Human  | A scFv phage display library generated from cells of a convalescent SARS patient | S2     | –                            | Preclinical | [18] |
| S3.1         | IgG  | Human  | Epstein-Barr virus transformation of human B cells of a convalescent SARS patient | RBD    | Competition with ACE2 in binding with RBD | Preclinical | [19] |
| S230.15      | IgG  | Human  | Epstein-Barr virus transformation of human B cells of a convalescent SARS patient | RBD    | Competition with ACE2 in binding with RBD | Preclinical | [16] |
| 68           | IgG  | Human  | Transgenic mice | NTD    | –                            | Preclinical | [20] |
| 201          | IgG  | Human  | Transgenic mice | RBD    | Competition with ACE2 in binding with RBD | Preclinical | [21] |
| F26G18       | IgG  | Mouse  | Animal immunization and hybridoma technology | RBD    | Competition with ACE2 in binding with RBD | Preclinical | [22–24] |
| 1A5          | IgG  | Mouse  | Animal immunization and hybridoma technology | RBD    | Competition with ACE2 in binding with RBD | Preclinical | [25] |
| 2C5          | IgG  | Mouse  | Animal immunization and hybridoma technology | RBD    | Competition with ACE2 in binding with RBD | Preclinical | [26] |
| 341C         | IgG  | Mouse  | Animal immunization and hybridoma technology | 548 to 567 of S protein S2 | –                            | Preclinical | [27] |
| 1A9          | IgG  | Mouse  | Animal immunization and hybridoma technology | RBD    | –                            | Preclinical | [30–32] |
| NMAbs against MERS-CoV |     |        |             |        |                              |                 |      |
| m336         | Fab  | Human  | A phage-displayed antibody Fab library generated from B cells of healthy donors | RBD    | Competition with DPP4 in binding with RBD | Preclinical | [33,34] |
| m337         | Fab  | Human  | A phage-displayed antibody Fab library generated from B cells of healthy donors | RBD    | Blocking the binding of DPP4 and RBD | Preclinical | [35] |
| m338         | scFv | Human  | A non-immune phage-displayed scFv library | RBD    | Competition with DPP4 in binding with RBD | Preclinical | [36] |
| MERS-27      | scFv | Human  | A non-immune yeast-displayed scFv library | RBD    | Interfering with the binding of RBD to cell receptor DPP4 | Preclinical | [37] |
| LCA60        | IgG  | Human  | Epstein-Barr virus transformation of B cells of a convalescent SARS patient | RBD    | –                            | Preclinical | [38] |
| MCA1         | Fab  | Human  | A phage-displayed antibody library from a MERS-CoV survivor | RBD    | Interfering with the binding of RBD to cell receptor DPP4 | Preclinical | [39] |
| CDC2-C2      | IgG  | Human  | Antibody gene cloning of memory B cells from a MERS patient | RBD    | Interfering with the binding of RBD to cell receptor DPP4 | Preclinical | [40] |
| MERS-GD27    | IgG  | Human  | Antibody gene cloning of memory B cells from a convalescent MERS patient | RBD    | Interfering with the binding of RBD to cell receptor DPP4 | Preclinical | [41] |
| REGN3051     | IgG  | Human  | Transgenic mice | RBD    | Blocking the binding of RBD to DPP4 | Preclinical | [42,43] |
| REGN3048     | IgG  | Human  | Transgenic mice | RBD    | –                            | Preclinical | [44] |
| 7.7g6        | IgG  | Human  | Transgenic mice | RBD    | Preventing conformational changes in the S2 subunit | Preclinical | [45–47] |
| Name of NMAb | Type | Source | Preparation | Target | Mechanisms of neutralization | Developing stage | Refs |
|--------------|------|--------|-------------|--------|-------------------------------|------------------|------|
| 4C2 2E6 D12 F11 G2 G4 5F9 7D10 | IgG | Mouse | Animal immunization and hybridoma technology | RBD | Interfering with the binding of RBD to cell receptor DPP4 | Preclinical | [46] |
| | | | Animal immunization and hybridoma technology | RBD | Interfering with the binding of RBD to cell receptor DPP4 | Preclinical | [49] |
| | | | Animal immunization and hybridoma technology | NTD | – | Preclinical | [49] |
| | | | Animal immunization and hybridoma technology | S2 | Inhibition of membrane fusion | Preclinical | [49,50] |
| | | | Animal immunization and hybridoma technology | NTD | Precluding the conformational changes required for membrane fusion | Preclinical | [52] |
| | | | Animal immunization and hybridoma technology | NTD | Interfering with the binding of RBD to cell receptor DPP4 and precluding the conformational changes required for membrane fusion | Preclinical | [51] |
| | IgG | Mouse | Animal immunization and hybridoma technology | RBD | Blocking the binding of RBD to DPP4 | Preclinical | [48] |
| | IgG | Macaques | Animal immunization and gene cloning | RBD | Blocking the binding of RBD to DPP4 | Preclinical | [41] |
| | IgG | Macaques | Animal immunization and gene cloning | Non-RBD regions of S1 | – | Preclinical | [41] |
| | HCAbs | Camel | VHH complementary DNA library | RBD | Interfering with the binding of RBD to cell receptor DPP4 | Preclinical | [61] |
| | HCAbs | Llama | A VHH phage display library | RBD | Interfering with the binding of RBD to cell receptor DPP4 | Preclinical | [62] |
| | HCAbs | Llama | A VHH phage display library | RBD | Interfering with the binding of RBD to cell receptor DPP4 | Preclinical | [63] |
| | scFv | Human | Non-immune phage libraries of human antibodies | RBD | Competition with ACE2 in binding with RBD | Preclinical | [64] |
| | Fab | Human | A synthetic human Fab antibody library AB1 | RBD | Competition with ACE2 in binding with RBD | Preclinical | [65] |
| | HCAbs | Human | A fully human phage displayed single-domain antibody library of healthy adult donors | S1 | Non-competition with ACE2 in binding with RBD | Preclinical | [67] |
| | Fab | Human | A highly diverse naïve human Fab library | RBD | Blocking the binding of RBD to ACE2 | Preclinical | [66] |
| | IgG | Human | A scFv phage display library generated from cells of a convalvescent SARS patient | RBD | Competition with ACE2 in binding with RBD | Preclinical | [68] |
| | Fab | Human | A yeast-displayed Fab library generated from cells of a COVID-19 convalvescent patient | RBD | Competition with ACE2 in binding with RBD | Preclinical | [70] |
| | IgG | Human | A yeast-displayed library generated from cells of SARS-infected patients | RBD | Blocking receptor attachment and inducing S1 shedding | Preclinical | [71] |
| | IgG | Human | Engineered antibody | RBD | Interfering with the binding of RBD to ACE2 | Preclinical | [72] |
| | IgG | Human | Epstein-Barr virus transformation of human B cells of SARS-infected patients | RBD | S trimer cross-linking, steric hindrance or aggregation of virions | Preclinical | [73] |

**Table 1 (Continued)**

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### Table 1 (Continued)

| Name of NMAb | Type | Source | Preparation | Target | Mechanisms of neutralization | Developing stage | Refs |
|--------------|------|--------|-------------|--------|-------------------------------|------------------|------|
| BD-368-2     | IgG  | Human  | High-throughput single-cell RNA and VDJ sequencing of convalescent COVID-19 patients' B cells | RBD    | Competition with ACE2 in binding with RBD | Preclinical      | [74] |
| CB6          | IgG  | Human  | Antibody gene cloning of B cells from a COVID-19 convalescent patient | RBD    | Competition with ACE2 in binding with RBD | Preclinical      | [79] |
| B38          | IgG  | Human  | Antibody gene cloning of B cells from a COVID-19 convalescent patient | RBD    | Competition with ACE2 in binding with RBD | Preclinical      | [86]** |
| COV2-2196    | IgG  | Human  | Antibody gene cloning of B cells from COVID-19 patients | RBD    | Blocking the binding of RBD to ACE2 | Preclinical      | [87,88] |
| REGN10987    | IgG  | Human  | Antibody gene cloning of B cells from transgenic mice and SARS-CoV-2-infected patients | RBD    | Blocking the binding of ACE2 to the RBD | Clinical         | [90,92,93]** |
| P2C-1F11     | IgG  | Human  | Antibody gene cloning of B cells from a COVID-19 patient | RBD    | Competition with ACE2 in binding with RBD | Preclinical      | [82] |
| CC12.1       | IgG  | Human  | Antibody gene cloning of B cells from COVID-19 patients | RBD    | Blocking the binding of RBD to ACE2 | Preclinical      | [75] |
| COVA1-18     | IgG  | Human  | Antibody gene cloning of B cells from COVID-19 patients | RBD    | Competition with ACE2 in binding with RBD | Preclinical      | [94,100] |
| COVA2-15     | IgG  | Human  | Antibody gene cloning of B cells from COVID-19 patients | RBD    | Competition with ACE2 in binding with RBD | Preclinical      | [89] |
| COVA2-02     | IgG  | Human  | Antibody gene cloning of B cells from COVID-19 patients | RBD    | Blocking the binding of RBD to ACE2 | Preclinical      | [83] |
| S2E12        | IgG  | Human  | Antibody gene cloning of B cells from COVID-19 patients | RBD    | Blocking the binding of RBD to ACE2 | Preclinical      | [80] |
| S2M11        | IgG  | Human  | Antibody gene cloning of B cells from COVID-19 patients | RBD    | Blocking the binding of RBD to ACE2 | Preclinical      | [101] |
| CV07-209     | IgG  | Human  | Antibody gene cloning of B cells from COVID-19 patients | RBD    | Blocking the binding of RBD to ACE2 | Preclinical      | [102,104] |
| C1A-B12      | IgG  | Human  | Antibody gene cloning of B cells from COVID-19 patients | RBD    | Blocking the binding of RBD to ACE2 | Preclinical      | [103] |
| 2-17         | IgG  | Human  | B cell sorting of COVID-19 patients and V(D)J sequencing | RBD    | Blocking the binding of RBD to ACE2 | Preclinical      | [78]** |
| 5-24         | IgG  | Human  | B cell sorting of SARS patients and V(D)J sequencing | RBD    | Blocking the binding of RBD to ACE2 | Preclinical      | [78]** |
| 4-8          | IgG  | Human  | B cell sorting of COVID-19 patients and V(D)J sequencing | RBD    | Blocking the binding of RBD to ACE2 | Preclinical      | [78]** |
| MW05         | IgG  | Human  | Antibody gene cloning of B cells from COVID-19 patients | RBD    | Blocking the binding of RBD to ACE2 | Preclinical      | [76] |
| MW07         | IgG  | Human  | Antibody gene cloning of B cells from COVID-19 patients | RBD    | Blocking the binding of RBD to ACE2 | Preclinical      | [95] |
| 311mab-3185  | IgG  | Human  | Antibody gene cloning of B cells from a COVID-19 convalescent patient | RBD    | Blocking the binding of RBD to ACE2 | Preclinical      | [96] |
| 311mab-32D4  | IgG  | Human  | Antibody gene cloning of B cells from a COVID-19 convalescent patient | RBD    | Blocking the binding of RBD to ACE2 | Preclinical      | [97] |
| C121         | IgG  | Human  | Antibody gene cloning of B cells from COVID-19 patients | RBD    | Interfering with the binding of RBD to cell receptor ACE2 | Preclinical      | [98,99]** |
| C144         | IgG  | Human  | Antibody gene cloning of B cells from a COVID-19 patient | RBD    | Blocking the binding of RBD to ACE2 | Preclinical      | [100] |
| C135         | IgG  | Human  | Antibody gene cloning of B cells from a COVID-19 patient | RBD    | Blocking the binding of RBD to ACE2 | Preclinical      | [106] |
| CV30         | IgG  | Human  | Antibody gene cloning of B cells from a COVID-19 patient | RBD    | Blocking the binding of RBD to ACE2 | Preclinical      | [84]**, [85] |
| EY6A         | IgG  | Human  | Antibody gene cloning of B cells from a COVID-19 patient | RBD    | Blocking the binding of RBD to ACE2 | Preclinical      | [105] |
| LY-CoV555    | IgG  | Human  | Antibody gene cloning of B cells from a COVID-19 patient | RBD    | Interfering with the binding of RBD to cell receptor ACE2 | Clinical        | [84]**, [85] |
| S2X259       | IgG  | Human  | Antibody gene cloning of B cells from a COVID-19 patient | RBD    | Blocking the binding of RBD to ACE2 | Preclinical      | [81] |
showed potent antiviral activity against MERS-CoV pseudovirus [33, 34]. The 3B11 was screened from a nonimmune phage-displayed single chain fragment variable (scFv) library [35]. In addition, MERS-4 and MERS-27 were identified from a yeast-displayed scFv library from healthy donors [36]. These antibodies all targeted the RBD and inhibited viral invasion by blocking the binding between RBD and DPP4 (Figure 2b). Originating from MERS-CoV-infected patients, MCA1 is an RBD-targeting NMAb screened from a phage display library [37].

**NMAbs identified by use of EBV transformation technology**
In addition to constructing phage libraries, immortalized B cell-based EBV infection has also been performed in antibody studies. For MERS-CoV, LCA60 was screened in this way [38].

**NMAbs identified from transgenic mice**
REGN3051 and REGN3048 are fully humanized NMAbs screened from transgenic mice [39] (Table 1). A group of chimeric antibodies were also screened from transgenic mice [40]. Among them, 7.7g6, 1.6F9, 1.2g5 and 4.6e10 target RBD, while 1.6c7 and 3.5g6 target S2 to prevent viral invasion by inhibiting the conformational change of S2 [40] (Table 1).

**NMAbs identified by use of gene cloning technology**
Many NMAbs, such as CDC2-C2 [41] and MERS-GD27 [42, 43], have also been obtained using a fast and efficient method known as cloning and expressing antibody genes [44].

**NMAbs against MERS-CoV from other sources**

**NMAbs identified by use of hybridoma technology**
A large number of mouse-derived antibodies have been screened. Among them, Mersmab1 [45], 4C2 and 2E6 were screened for targeting RBD and subsequently produced humanized antibodies that showed potent antiviral activity in vitro and in vivo [46, 47]. RBD-23D3, RBD-2E4, and RBD-40G7, all targeting RBD, were identified with high cross-neutralizing activity among mutant isolates [48]. NMAbs D12 and F11 targeting RBD, G2 targeting NTD, and G4 targeting S2 subunit were all identified by immunized mice [49, 50]. Screened by hybridoma technology, 5F9 and 7D10 are murine NMAbs targeting the NTD [51, 52] (Table 1). In addition to murine-derived antibodies, researchers have obtained neutralizing antibodies from immunized animals of other species. For example, JC57-14, targeting RBD, JC57-14 and FIB-H1, targeting non-RBD regions of S1, were screened from macaques [41]. Furthermore, JC57-14 could protect DPP4-transgenic mice against MERS-CoV infection [41].
**Single domain antibodies (sdAbs) identified by screening of antibody libraries**

In addition to conventional antibodies, heavy-chain-only antibodies (HCAbs) produced by camelids contain a single-variable domain (VHH), instead of two variable regions on the heavy and light chains, respectively, of conventional IgG antibodies that affords the equivalent effect [53]. VHH shows affinities and specificities for antigens comparable to conventional antibodies. VHHs can be easily constructed into multivalent formats and show higher thermo-stability and chemo-stability, compared to most other antibodies [54–59]. VHHs are also less susceptible to steric hindrance during binding [60]. For MERS-CoV, VHH-83, NbMS10 and VHH-55 were screened from antibody libraries of immunized camels [61,62,63] (Table 1).

**NMAbs screened by gene cloning and sequencing techniques**

Antibody gene cloning and sequencing technologies for identification of SARS-CoV-2 NM Abs from B cells sorted from COVID-19 patients are being used more frequently, and several high-throughput screening methods have been established [74,75], considerably reducing the time required for antibody development and enriching antibody diversity. These NM Abs showed strong neutralizing activity *in vitro* or *in vivo*. Most of them target the RBD in S1 subunit, and their mechanism of action is summarized in Table 1. Also, NM Abs targeting SARS-CoV-2 NTD, for example, 4A8 and 4–8, were isolated in this way [76,77,78]. A large group of RBD-targeting NM Abs, including BD-368-2, P2C-1F11, CB6, S2H13 and C1A-B12, could interfere with the binding of RBD to the receptor ACE2, showing strong neutralizing activity *in vitro* [74,79–82]. CB6 showed potent *in vivo* efficacy, protecting rhesus macaques against SARS-CoV-2 infection in both prophylactic and treatment settings [79]. CC12.1 exhibited the most potent *in vitro* neutralizing activity and completely protected Syrian hamsters against the challenge of a Washington strain (USA-WA1/2020) *in vivo* [75]. CV07-209 could reduce lung pathology in a COVID-19 hamster model [83]. LY-CoV555 protected against SARS-CoV-2 infection in nonhuman primates and showed potent neutralization effect and good safety profiles in clinical trials [84,85] (Table 1). Notably, B38 and H4 target different neutralizing epitopes in RBD [86,87]. No competition takes place between the two NM Abs; therefore, the combination results in an ideal cocktail candidate for COVID-19 therapy, which is also effective in preventing escape mutations. Such antibody pairs are not uncommon in SARS-CoV-2 antibody studies, and their combination has shown better neutralizing activity compared to the use of each compound alone. Examples are COV-2-2196/COV-2-2130 [87,88], S2M11/S2E12 [89] and REGN10933/REGN10987 (REGN-CoV2) [90,91,92] (Figure 2c). Further, REGN-CoV2 has shown neutralization effect and safety in clinical trials [93] (Table 1). Similarly, researchers screened a large set of NM Abs with different targets against SARS-CoV-2 [94]. Among them, COVA1-18 and COVA2-15 showed the strongest antiviral activity [94]. Many NM Abs, such as MW05 [95], 311mab-31B5/311mab-32D4 [96], C121 [97] and CV30 [98,99] were identified from the sorted SARS-CoV-2 RBD-specific, IgG class-switched memory B cell of COVID-19 convalescent patients using antibody gene cloning technology. They have shown neutralizing activity against SARS-CoV-2 *in vitro* and *in vivo* through competition with ACE2 in binding with RBD (Table 1). It was found that epitopes of some NM Abs are relatively conservative in sequence (e.g. DH1047, A19-46.1, S2X259 and CV1-30), and these NM Abs show cross-neutralizing activity against SARS-CoV-2 variants and other sarbecoviruses [100,111,102–105]. Like these NM Abs, EY6A targets a conserved footprint in RBD that

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**NM Abs screened by gene cloning and sequencing techniques**

Antibody gene cloning and sequencing technologies for identification of SARS-CoV-2 NM Abs from B cells sorted from COVID-19 patients are being used more frequently, and several high-throughput screening methods have been established [74,75], considerably reducing the time required for antibody development and enriching antibody diversity. These NM Abs showed strong neutralizing activity *in vitro* or *in vivo*. Most of them target the RBD in S1 subunit, and their mechanism of action is summarized in Table 1. Also, NM Abs targeting SARS-CoV-2 NTD, for example, 4A8 and 4–8, were isolated in this way [76,77,78]. A large group of RBD-targeting NM Abs, including BD-368-2, P2C-1F11, CB6, S2H13 and C1A-B12, could interfere with the binding of RBD to the receptor ACE2, showing strong neutralizing activity *in vitro* [74,79–82]. CB6 showed potent *in vivo* efficacy, protecting rhesus macaques against SARS-CoV-2 infection in both prophylactic and treatment settings [79]. CC12.1 exhibited the most potent *in vitro* neutralizing activity and completely protected Syrian hamsters against the challenge of a Washington strain (USA-WA1/2020) *in vivo* [75]. CV07-209 could reduce lung pathology in a COVID-19 hamster model [83]. LY-CoV555 protected against SARS-CoV-2 infection in nonhuman primates and showed potent neutralization effect and good safety profiles in clinical trials [84,85] (Table 1). Notably, B38 and H4 target different neutralizing epitopes in RBD [86,87]. No competition takes place between the two NM Abs; therefore, the combination results in an ideal cocktail candidate for COVID-19 therapy, which is also effective in preventing escape mutations. Such antibody pairs are not uncommon in SARS-CoV-2 antibody studies, and their combination has shown better neutralizing activity compared to the use of each compound alone. Examples are COV-2-2196/COV-2-2130 [87,88], S2M11/S2E12 [89] and REGN10933/REGN10987 (REGN-CoV2) [90,91,92] (Figure 2c). Further, REGN-CoV2 has shown neutralization effect and safety in clinical trials [93] (Table 1). Similarly, researchers screened a large set of NM Abs with different targets against SARS-CoV-2 [94]. Among them, COVA1-18 and COVA2-15 showed the strongest antiviral activity [94]. Many NM Abs, such as MW05 [95], 311mab-31B5/311mab-32D4 [96], C121 [97] and CV30 [98,99] were identified from the sorted SARS-CoV-2 RBD-specific, IgG class-switched memory B cell of COVID-19 convalescent patients using antibody gene cloning technology. They have shown neutralizing activity against SARS-CoV-2 *in vitro* and *in vivo* through competition with ACE2 in binding with RBD (Table 1). It was found that epitopes of some NM Abs are relatively conservative in sequence (e.g. DH1047, A19-46.1, S2X259 and CV1-30), and these NM Abs show cross-neutralizing activity against SARS-CoV-2 variants and other sarbecoviruses [100,111,102–105]. Like these NM Abs, EY6A targets a conserved footprint in RBD that

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**NM Abs identified from EBV transformed memory B cells of a recovered SARS patient**

S309 was identified from EBV-transformed memory B cells of a recovered patient who was infected by SARS-CoV in 2003 and showed strong cross-neutralizing activity against both SARS-CoV and SARS-CoV-2 [73].

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Neutralizing monoclonal antibody against coronaviruses Xiang et al. 9

is distinct from receptor binding motifs, and it inhibits viral invasion by altering the pre-fusion conformation of S proteins [106]. Moreover, it showed cross-reactivity against SARS-CoV S1 protein [106].

NMAbs against SARS-CoV-2 from other sources

NMAds identified by use of hybridoma technology

2H2 and 3C1 were identified by using animal immunization and hybridoma technology. Because the two NMAds target different epitopes in SARS-CoV-2 RBD, they can be used in combination, that is, a cocktail therapy (Figure 2c). Their combination exhibited more potent neutralizing activity against authentic SARS-CoV-2 infection in vitro [107]. Similarly, 7D6 and 6D6 were identified from mice immunized with SARS-CoV-2 S protein, and SARS-CoV-2/SARS-CoV S protein/MERS-CoV RBD, respectively, showing cross-neutralizing activity against SARS-CoV and SARS-CoV-2 as well as its variants [108]. 7B11 and 18F3, SARS-CoV neutralizing mAbs by targeting different neutralizing epitopes in RBD of SARS-CoV S protein, were identified from mice immunized with SARS-CoV S-RBD [109].

NMAds identified by screening of antibody libraries

H014, a humanized SARS-CoV-2 NMAb, was originally identified from a phage display antibody library generated from RNAs of the peripheral lymphocytes of SARS-CoV RBD-immunized mice. It exhibited potent neutralizing activity against SARS-CoV-2 infection in vitro by blocking RBD-ACE2 binding through steric hindrance [110].

NMAds identified from transgenic mice

47D11, a chimeric antibody with human variable region and rat constant region, was identified from transgenic mice, showing cross-neutralizing activity against SARS-CoV and SARS-CoV-2 [111*].

SdAds identified by screening of antibody libraries

3F11 was identified from a phage display library from nonimmune camel and was expressed by fusion with human IgG Fe fragment in order to overcome the limitations of sAds [58,112]. H11 was also identified from a naive llama phage display antibody library. Researchers obtained H11-H4 and H11-D4 with more affinity for SARS-CoV-2 RBD by random mutation of H11, both exhibiting strong antiviral activity in vitro [113].

More commonly, camels are immunized to obtain sdAds. VHH-72, was identified from a phage display library of a llama immunized with SARS-CoV and MERS-CoV S proteins multiple times showed cross-neutralizing activity against pseudotyped SARS-CoV, MERS-CoV and SARS-CoV-2 [63*]. NIi-CoVnb-112 was isolated from an immune llama phage display library [114]. W25 was identified from a VHH *Escherichia coli* (E. coli) — displayed antibody library of immune alpaca. It showed potent neutralizing activity against the D614G isolate, whether monomer or dimer [115]. Another sdAb that exhibited strong neutralizing activity in multimeric form is VHH E (Figure 2c), which was screened from an immune camel phage display library [116**]. The trimeric VHH EEE inhibits both SARS-CoV-2 pseudovirus and authentic virus infection. The combination of VHH E and VHH V, targeting different sites of the RBD, is effective in preventing escape mutations, whereas multimers could not [116**]. In a similar method, Ty1 was screened from an alpaca phage display library [117]. In addition, a large number of nanobodies have been screened as candidate drugs for the treatment of COVID-19 [118–120].

Conclusion and prospects

Coronaviruses constitute a large group in nature, and genome sequence analysis shows that many coronaviruses are highly homologous to SARS-CoV, MERS-CoV or SARS-CoV-2 [121]. Therefore, coronaviruses may continue to threaten human health. Rapid development of therapeutic and prophylactic drugs is essential, both for coronaviruses that have already emerged to infect humans and for those that may emerge in the future. With the development of high-throughput screening technology for antibodies, the cycle time for antibody development is shortening. Antibody drugs could be the antiviral drug of choice based on their advantages of high targeting and low side effects. Moreover, different species of coronaviruses have conserved loci between their genomes, and it may be possible to design and screen antibodies with broad-spectrum antiviral activity based on these loci. Many studies on the mechanism of NMAds with cross-neutralizing activity against SARS-CoV-2 variants and other sarbecoviruses have shown that the targets of these NMAds are relatively conservative [85,100–103,105]. In a recent study, 41 RBD-directed NMAds were classified into seven antibody communities with distinct footprints and competition profiles [122]. A number of NMAd cocktails consist of NNAbs from different RBD-directed antibody communities showed enhanced neutralizing potency. However, the potency of some NNAbs in the combinations is compromised by emerging SARS-CoV-2 variants. Improving the neutralizing activity of these NMAds through other means (e.g. mutation and multimeric forms) greatly enhance their application prospects [72,122]. Therefore, in addition to the combination strategy, the in vitro modification of antibodies is also crucial to improve the neutralizing activity of the antibody drugs. Of course, the acceleration of antibody drug formation, the miniaturization of effective antibody molecules and the improvement of in vivo longevity are expected.

Conflict of interest statement

Nothing declared.

Data availability

No data was used for the research described in the article.
References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

* of special interest
** of outstanding interest

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