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REVIEW

Engineered Therapeutic Antibody Against SARS-CoV-2

Monrat Chulanetra[1]

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Abstract

Purpose of Review The success and failure of therapeutic antibodies against SARS-CoV-2 offer a lesson on therapeutic antibody design and development.

Recent Findings Therapeutic antibody against SARS-CoV-2 facing challenging antibody escape mutation. A paratope design strategy targeting pancoronavirus conserved epitope(s) and combining two antibodies as antibody cocktails or bispecific antibodies may overcome antibody escape mutations of the SARS-CoV-2 spike. Instead of designing broadly neutralizing antibodies, repurposing antibodies can target viral or host molecules to inhibit the virus and alleviate dysregulation of the host immune response.

Summary Detailed strategies for engineering therapeutic antibodies, including antibody format, are reviewed in this article.

Keywords Engineered antibody · Therapeutic antibody · SARS-CoV-2 · Coronaviruses

Introduction

In December 2019, a severe acute respiratory syndrome (SARS)-like disease of unknown etiology emerged in individuals directly exposed to China's Huanan Wholesale Seafood Market [1, 2]. Subsequently, the disease, caused by a new coronavirus named Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), spread rapidly and became a pandemic known as COVID-19. The World Health Organization announced the COVID-19 outbreak as a Public Health Emergency of International Concern and has been maintaining this status for over 3 years, with over 760 million confirmed cases and over 6.9 million deaths worldwide (data on July 28, 2023) [3]. Currently, the pandemic is under control, mainly due to population immunity, and the disease is fading to an endemic [4, 5]. Since the virus is circulated in the environment and the population and is unlikely to be eradicated, infection remains a public health concern regarding long-term effects on individual health, human and animal reservoir and transmission, immune-escaping mutation of the virus, and morbidity and mortality of the disease in susceptible individuals.

Besides the role of population immunity in COVID-19 protection, therapeutic agents are required, even after the pandemic, to prevent and reduce disease severity, especially in susceptible individuals. Numerous new and repurposed drugs and herbal medicines have been developed and tested for COVID-19 [6–8]. Remdesivir, which inhibits viral RNA synthesis, and Paxlovid, the Mpro inhibitor, are FDA-approved drugs to treat COVID-19 [9]. However, drugs are limited in specific populations, such as pregnancy, breastfeeding, and renal impairment [10]. In addition, high viral mutation rates and drug selection pressure might introduce a drug escape mutation. Therefore, alternative treatments for COVID-19 are required.

Passive immunization with convalescent plasma from recovered patients becomes first-line therapy during the pathogenicity of unrevealed disease. Subsequently, monoclonal antibodies against COVID-19 have been developed and used as part of the therapeutic options for COVID-19, especially in susceptible populations [11–13]. The FDA approves some of them, and some are in the development pipeline.

Strategies for developing therapeutic antibodies against SAR-CoV-2 are to reduce viral load or replication by targeting virus proteins that function in the viral life cycle, such as attachment or viral replication. Another strategy is to target

✉ Monrat Chulanetra
monrat.chl@mahidol.edu

[1] Center of Research Excellence in Therapeutic Proteins and Antibody Engineering, Department of Parasitology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand

host molecules to mitigate the host's hyperimmune response and disease severity. Another factor that promotes antibody efficiency is the design of the antibody format. Details of strategies for engineering therapeutic antibodies, including antibody format, are reviewed in this article.

Antibody Targeting the SARS-CoV-2 Proteins

SARS-CoV-2, a causative agent of COVID-19, is a betacoronavirus subgroup B in the *Coronaviridae* family. Coronavirus is an enveloped, nonsegmented, positive-sense, single-stranded RNA virus [14]. The viruses in this family, including SARS-CoV-2, have been reported to infect various animal species, including humans [14–16]. Along with SARS-CoV and MERS-CoV, SARS-CoV-2 is one of the three human coronaviruses that cause severe acute respiratory syndrome (SARS) [17, 18].

Antibodies against viral infection usually target the proteins essential in the viral replication cycle. Most antibodies against SARS-CoV-2 targeted a spike (S) protein to block the viral attachment or entry into host cells [19]. Besides the spike protein, other proteins that play an essential role in viral replication are also therapeutic targets, such as Nsp3 (papain-like protease), Nsp5 (main protease, Mpro, 3CLpro), Nsp9, and Nsp12 (RNA-dependent RNA polymerase, RdRp) [20].

Spike (S) Protein

The S protein, composed of two subunits: S1 and S2, forms a homotrimer on the virion surface. The S1 subunit of the virus contains the N-terminal domain (NTD) and receptor-binding domain (RBD), which binds to angiotensin-converting enzyme 2 (ACE2) expressing on the surface of various cell types, including alveolar epithelial cells and oral, nasal, and nasopharynx epithelial cells [21, 22]. The RBD conformation of the spike is interchangeable between the upward (open) and down (close) conformations wherein the ACE2 binding site is exposed and hidden, respectively [23, 24]. S1–ACE2 receptor binding induces a spike conformation change, reveals the S2′ site on the S2 subunit, which is cleaved by host TMRRSS2, resulting in shedding of the S1 subunit, and subsequently exposes the fusion loop of the S2 subunit to create the fusion pore-mediated viral genome releases into the host's cytoplasm [25, 26].

Therapeutic conventional (full-length) antibodies against spike protein constitute a significant group of FDA-approved SARS-CoV-2 antibodies [27]. The aim of targeting spike protein is to protect the virus from entering the cell by directly or indirectly blocking the binding of spike protein to the ACE2. NTD and RBD were reported as therapeutic antibody targets [28–32], although the primary focus was on RBD [27, 28, 32]. The mechanism of antibodies to spike protein includes directly interfering with the ACE2 interaction by occlusion of the ACE2 binding site [32–35] or acting as a receptor mimic to induce premature S2 fusion loop exposure [36, 37, 38••]. Antibodies can indirectly block RBD-ACE2 interactions by a steric hindrance [28, 39]. RBD-antibody binding in the upward or down conformation or NTD can cause conformational trapping, preventing spike conformational change and hindering viral entry [28, 39–42]. Antibodies also target NTD by interrupting the trimer formation of spike protein [43]. The S2 subunit is highly conserved across different betacoronavirus lineages [44–46]. The antibodies to S2 are divided into two classes: the antibody-targeting fusion peptide and the S′ cleavage site [45]. Conformational trapping also occurs in the S2 subunit, preventing fusion loop exposure [41]. S2 subunit targeting is limited by the accessibility of antibodies depending on spike dynamics and disclosure of the epitope [45].

The challenge in developing the antibody-targeting spike protein is the high mutation of the spike in the SARS-CoV-2 variants [19]. The emerging SARS-CoV-2 Omicron variant contains > 30 mutations in the spike protein, especially in the RBD [19]. These mutations caused many available therapeutic antibodies obsolete due to loss or massive reduction of protection against new mutated variants [19, 46]. Amino acid substitutions at positions S477N, T478K, F486V, and E484A decrease the activity of the available anti-spike antibody by 272–10,000-fold [24]. The receptor binding motif (RBM) on the RBD is the most efficient antibody target [30, 38••, 47]. However, RBM is a mutation hotspot that causes loss of antibody activity, especially in Omicron variants [48]. Conversely, although the non-RBM part of the RBD is conserved, the antibody's efficiency is less than that of targeting the RBM, whereas it tolerated viral escape [30].

Some antibodies endured spike mutations and demonstrated cross-variant protection [32, 38••, 49], which applies to therapeutic antibody design. Bebtelovimab (LY-CoV1404 or 1404), which binds to conserved RBD epitopes, demonstrated cross-variant protection, including Omicron B.1.1.529 and BA.2 [32]. However, the protectivity of the Omicron BQ.1 and BQ.1.1 subvariants is diminished [50]. Anti-RBD spike antibody S2H97 interacted with the spike protein from subgenus *Sarbecoviruses* and demonstrated broad neutralization across the SARS-CoV-2 variants [38••, 49]. Antibodies developed against SARS-CoV or MERS-CoV have been tested for protection against SARS-CoV-2 [39, 41, 51]. Most of the amino acid residues essential for ACE2 binding by SARS-CoV were conserved in SARS-CoV-2 [52]. Sotrovimab (VIR-7831) is derived from memory B cells of SARS-CoV survivor bound and neutralized SARS-CoV-2 variants [51] and has demonstrated efficacy in early waves of Omicron [53, 54]. Other cross-variant protection antibodies were reported in an antibody that shared the

18 binding-epitope residues and electrostatic contacts on the RBD-binding interface with ACE2 [33] and an antibody to S2 of spike that targeted the highly conserved epitope across different betacoronavirus lineages [44]. Combining two neutralizing antibodies (antibody cocktails), for example, tixagevimab and cilgavimab, bamlanivimab and etesevimab, and casirivimab and imdevimab, demonstrated improved activity/efficiency against mutation escape variants [11, 27, 55••]. Therefore, selecting antibodies that bind to the highly conserved epitope(s) or protect across different lineages of coronaviruses, competing with ACE2 with high similarity, and formulating antibody cocktails can develop as strategies to overcome antibody escape mutations of the SARS-CoV-2 spike. If the critical mutated amino acid responsible for therapeutic resistance in the circulated variant [24] is defined, for example, R436X of the Omicron, designing antibodies targeting the mutant will be another option to develop the broadly neutralizing antibody [56]. However, reevaluation of antibody efficacy is required whenever a new variant emerges [11]. Antibody treatment should be considered to introduce antibody-selected mutations, as reported in high-risk patients treated with sotrovimab [57•, 58]. A single-dose sotrovimab treatment induced E340K/A/V/G and/or P337L/R mutations of Omicron variants, reducing susceptibility to sotrovimab [57•].

Nonstructural Proteins (Nsps)

The viral genome contains 13 open reading frames (ORFs). ORF1a and ORF1b are translated into polyprotein precursors, pp1a and pp1ab. The precursor is cleaved by the viral protease, i.e., Nsp3 and Nsp5, resulting in 16 Nsps that function in viral genome replication and modulation of host immune responses [20, 59]. Nsp12 assembles with Nsp7 and Nsp8 to form a holo-RdRp complex, an essential component for viral RNA synthesis [60, 61]. The holo-RdRp complex coordinates with other accessory subunits, known as replication and transcription complexes (RTC), and promotes the fidelity of RNA synthesis [60, 62]. SARS-CoV-2 Nsps shared structural homology or conserved amino acids/motifs with SARS-CoV and/or other betacoronaviruses [20, 63–66]. This review focuses on Nsps reported as antibody targets: nsp3, 5, 9, and 12; other SARS-CoV-2 proteins as therapeutic targets were reviewed in [20].

The nsp3 of SARS-CoV-2 is a multidomain protein; among them, the PLpro domain contains cysteine proteolytic (PLpro), deubiquitinating, and deISGylating activities [20]. The protease activity of nsp3 cleaves the pp1a polypeptide to separate nsp1, nsp2, and nsp3 [20]. Additionally, nsp3 suppresses the antiviral immune response by deubiquitination and deISGylation of interferon-stimulating gene 15 (ISG15) [64]. The PLpro domain is the main target for antiviral drug development. In nanobody format, antibodies targeting nsp3 demonstrated inhibition of hydrolytic activity to interfere with deubiquitination, deISGylation, and polyprotein cleavage activities in vitro [64, 67]. However, the ability of these nanobodies to inhibit the authentic virus and interfere with viral replication remains to be investigated.

Mpro, a chymotrypsin-like protease, is a unique protein without human homologs; it is critical in viral replication because it cleaves nsp12 from the polyprotein precursor. Its activity requires homodimerization of the proteins [68]. The Mpro consists of three domains. Domain III functions in homodimerization, allowing domains I and II to form a substrate-binding pocket with the embedded catalytic site [69]. Thus, dimerization inhibition interrupts the enzymatic activity of Mpro. There are three transitional stages during dimerization formation: extended monomeric, compact, and dimeric [69]. Antibody fragments targeting Mpro disrupted dimerization by conformationally trapping Mpro in the predimeric stages [70] or interacting with residues responsible for homodimerization [70]. Cell-penetrating antibodies bound and inhibited the catalytic surface of Mpro and demonstrated the cross-variant protective effect against authentic viruses in cell cultures [70].

Nsp9, an accessory protein in the RTC, undergoes dimerization, RNA binding, and protein recruitment for 5′-mRNA capping, which is essential for viral replication [71–73]. Nsp9-bound nanobodies have been reported to induce a topological change [71] or stabilize Nsp9 in a tetrameric form [74], which can interfere with viral replication.

Nsp12, a core component of the RTC [20], is crucial for viral replication and is a target of nucleoside analogs already approved for treating COVID-19 [8]. An antibody to the hepatitis C virus (HCV)-RdRp broadly inhibited viral RNA replication in SARS-CoV-2 variants of concern and other RNA viruses [75].

Antibody to Host Molecules

Cytokine Storms

Inducing uncontrolled inflammation, known as cytokine storms, is a life-threatening complication of COVID-19. During infection, the immune system is evoked to fight the pathogen. However, over-triggering the immune system also results in immunopathology. Hyperinflammation from COVID-19 might be triggered by the innate immune cells: macrophages, dendritic cells, and neutrophils, which are the first responders to infection, viral-induced pyroptosis, and decrease in type 1 interferon function or antibody-Fc receptor (FcR) interaction (reviewed in [76, 77]).

Anti-SAR-CoV-2 spike antibodies are involved in the activation of other immune cells or immune components through the Fc functions of antibodies, resulting in

antibody-dependent cellular cytotoxicity, antibody-dependent cellular phagocytosis, complement-dependent cytotoxicity, and antibody-dependent cellular trogocytosis [78], which, if dysregulated, may progress to hyperinflammation [79]. Furthermore, the Fc of antibodies at suboptimal neutralizing concentration can introduce an extrinsic (classical) antibody-dependent enhancement (ADE) [80], another cause of uncontrolled inflammation. The neonatal Fc receptor (FcRn) retains immunoglobulin in the bloodstream, resulting in prolonged antibody responses. Therefore, antibodies targeting FcR and FcRn could be a therapeutic strategy to reduce the immunopathology of COVID-19 [81].

Interleukin-6 (IL-6) is a critical cytokine involved in this hyperactive immune response. It is a marker for COVID-19 progression and severity prognosis [82–84]; therefore, it is a target for controlling hyperinflammation. Several FDA-approved antibodies that block IL-6 and IL-6 receptor (IL-6R) interaction have been repurposed to treat COVID-19. Tocilizumab, a humanized anti-IL-6 receptor IgG1, was initially used to treat rheumatoid arthritis [85] and is the first monoclonal antibody approved for treating COVID-19 in hospitalized adults with severe COVID-19 [86]. Its effectiveness in improving the treatment outcome of COVID-19 treatment is controversial [84, 87]. Although no significant outcomes of tocilizumab were reported [88, 89], it was found to reduce disease severity and hospitalization time [84, 90–92]. However, no effect of tocilizumab on reducing COVID-19 mortality is inconclusive [90, 91, 93, 94]. Like tocilizumab, the role of sarilumab, the FDA-approved human anti-IL6R IgG1, in treating COVID-19 is controversial [95, 96] and requires further investigation.

Other proinflammatory molecules have been proposed as therapeutic targets [97–101]. Secukinumab, a monoclonal antibody against IL-17, the upstream IL-1 and IL-6 pathways, has been tested in a phase 2 clinical trial. There has been no improvement in the outcome of COVID-19 treatment, but a reduction of thromboembolism by secukinumab has been reported [98].

Combining the antibody with the inhibitor of the cytokine signaling molecule is another strategy to control hyperinflammation. A combination of secukinumab with baricitinib, a Janus kinase (JAK) 1 and 2 inhibitors, has shown benefits of reduction of ICU support and intubation, hospital stay, and lower mortality than treatment with baricitinib alone [102].

Besides directly targeting cytokines, nasal administration of anti-CD3 suppressed T cell function, reduced lung inflammation and serum IL-6, and increased *TGFB1* expression [103].

Immunomodulation by inhibiting proinflammatory cytokines raises concerns about increased susceptibility to secondary infection [97, 104]. Treatment results are controversial [105], with either no effect reported on increasing the secondary infection [98, 102, 106, 108] or increasing the risk of secondary infection [107–109].

The effectiveness of immunomodulatory treatment for the recovery of dysregulated immune function in COVID-19 is multifactorial. The first is the administration time [84, 110], which may require calibration before dysregulation occurs to prevent the development of irreversible organ dysfunction [84, 111]. Individual factors and patient conditions also affect treatment outcomes (reviewed in [111]). Further research may focus on finding the best use of the treatment [110].

CD147

CD147 (EMMPRIN or basigin), a transmembrane glycoprotein in the immunoglobulin superfamily [109], has multiple binding partners to drive normal physiological functions and is involved in cancers and infectious diseases [112–116]. CD147 was reported as a receptor for SARS-CoV-2, which binds to the viral spike protein and facilitates viral entry of the cell lacking ACE2 [117•]. A humanized anti-CD147 antibody, meplazumab, was approved for phase I clinical trials for prophylaxis treatment for malaria and has been repurposed for the treatment of COVID-19 [117•]. Meplazumab reached the preclinical trial phase 2/3, effectively improving disease severity and mortality while reducing viral load and multiple cytokine levels [112, 118]. CD147 is involved in the viral entry process and plays a role in the inflammatory process [116, 119] and pulmonary fibrosis [120]. Therefore, blocking CD147 would help control infection and may mitigate the effect of cytokine-induced immunopathology and COVID-19 tissue fibrosis.

Engineered Antibody Format

Antibodies against COVID-19 were developed in different formats (Fig. 1), primarily as full-length antibodies with or without fragment crystallizable (Fc) engineering. Antigen-binding fragments (nanobody, single-chain antibody (scFv), Fab) can avoid Fc-induced ADE. They can be linked with other peptide/protein domains to improve the efficacy of antibodies or add an advantageous characteristic to the antibodies. Details of the format and designs of the engineered antibodies are described below.

Engineered Fc Antibodies

Knowledge of the interaction of the modified Fc has been long investigated with the in vitro and in vivo data and has already been approved for therapeutic products [121–127]. The engineered Fc for therapeutic antibodies for COVID-19 aims to (1) increase the half-life of the antibody and (2)

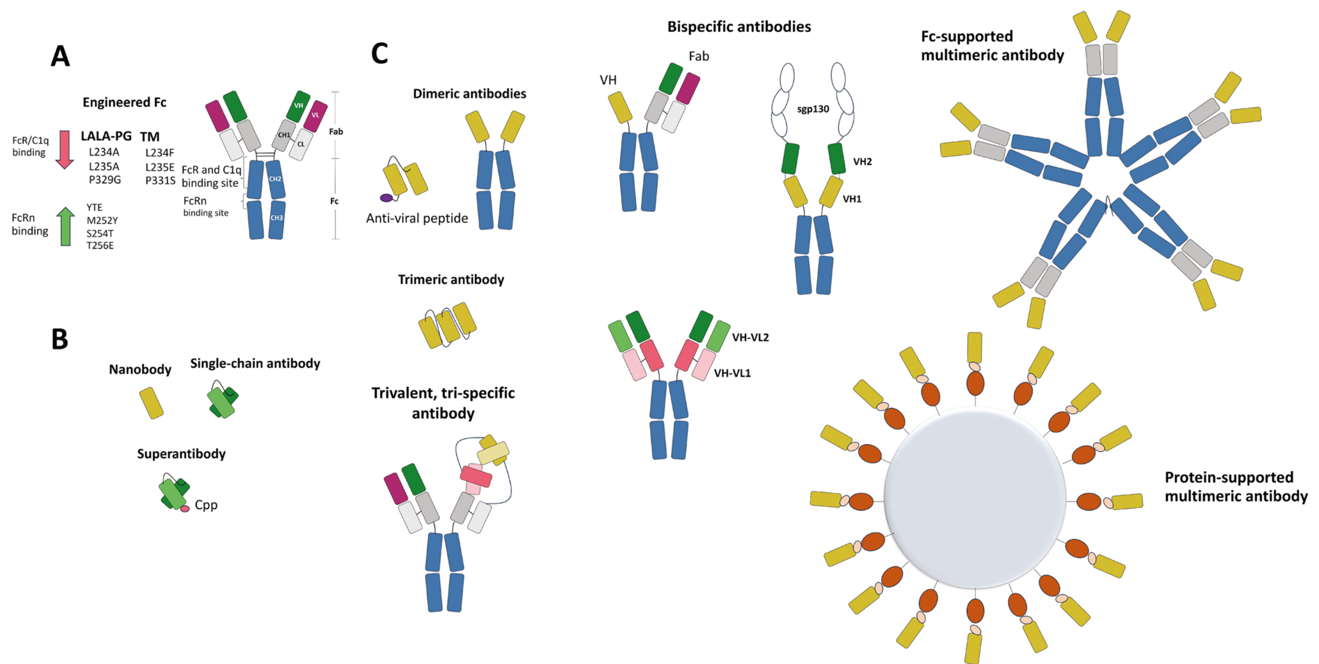

Fig. 1 Designs of SARS-Co-V2 therapeutic antibody format. **A** Conventional antibody with engineered Fc functions. **B** Antigen-binding fragments and antibody-fusion protein. **C** Multivalent/multispecific antibody

decrease the immune activation and tissue injury caused by antibodies.

The neonatal Fc receptor (FcRn) is the first known receptor for transferring IgG from the mother to the fetus or the newborn [128]. Furthermore, FcRn plays a role in maintaining circulating immunoglobulin levels by binding and releasing IgG back into circulation. FcRn has been detected in epithelial, endothelial, and hematopoietic cells [129, 130]. The binding and release of Fc by FcRn are controlled by pH. Cells uptake IgG by pinocytosis, and IgG is entrapped into the endosome by FcRn. At low pH of the endosome, Fc binds to the FcRn and is sorted into tubules originating from the sorting endosomes to return to the plasma membrane [129, 131]. The increased pH (pH 7.4) causes a release of Fc [129, 131]. Binding to FcRn helps prevent IgG degradation and increases the serum half-life of the antibody. Several mutations increase the affinity of immunoglobulin molecules to FcRn or control the pH-dependent binding, resulting in prolonged circulating IgG levels. Additional details on the mechanisms and designs of the interaction between Fc and FcRn were reviewed in [129].

The functions of Fc are essential for viral clearance, reducing weight loss, and preventing the lethality of SARS-CoV-2 in animal models [79, 132, 133], and the defect in Fc function was related to the mortality of the COVID-19 patient [134]. However, Fc is not the only factor indicating the success of therapeutic antibodies for SARS-CoV-2 [136]. Antibodies with the Fc mutation, which affects FcR binding, demonstrated a therapeutic efficacy against COVID-19 [127, 137]. Stimulating the immune response by Fc function through FcR can induce a profound inflammatory response and ADE, leading to cytokine storms. Leucine positions 234 and 235, located in the CH2 domain of an antibody, and proline at position 329 or 331 are critical residues for Fc receptors and C1q binding. Mutations at these positions, such as LALA-PG (L234A/L235A/P329G) or TM (L234F/L235E/P331S), decreased the binding affinity of IgG1 to the Fc receptor and C1q molecule compared to the original [124, 125] and diminished the Fc effector function in vitro [125, 136]. The LALA (L234A/L235A) mutation also lowers the risk of Fc-mediated lung injury [27, 127]. Another way to reduce risk is to engineer Fc in an IgG4 isotype that cannot engage FcR [79, 138, 139].

Human anti-SARS-CoV-2 spike (RBD) antibodies, tixagevimab and cilgavimab (AZD7442), and etesevimab are examples of Fc-engineered antibodies for COVID-19. Tixagevimab and cilgavimab harbored the YTE (M252Y/S254T/T256E) and TM mutations to increase serum half-life (long-acting antibody) and reduce FcR and C1q complement binding, respectively [11, 137, 140]. Etesevimab contained the LALA mutation [141].

Besides engineered Fc, the half-life of the circulating antibody can also be prolonged by engineered variable regions of the antibody [142]. An engineered variable region with a lower molecular isoelectric point (pI) reduced antibody clearance in nonhuman primates [142]. The

pI-engineered variable regions in combining the Fc mutation, N434A, which increased affinity for FcRn, were found in tocilizumab [129, 143], which is repurposed for treating COVID-19. Engineered Fc to increase activity to FcγRIIIa induced protective CD8 + T cell response against respiratory virus [144].

Nanobody (Single-Domain Antibody (sdAb))

The camelids have a particular type of antibody, i.e., heavy chain antibodies, which harbored only the heavy chain domain without the light chain counterpart [145, 146]. A unique characteristic of the antibody is the long CDR3, which helps bind to antigens to compensate for the lack of the light chain. The nanobody or single-domain antibody (sdAb) is a variable domain of the heavy chain antibody that functions in antigen binding. The size is ten times lower than conventional antibodies, making the molecules easy to express in a prokaryotic system and easy to manipulate and modify [36, 37, 39]. The nanobody is stable in harsh environments such as acidic, ionic strength, heat, proteolysis, and pH [39, 147–150].

Nanobodies against SARS-CoV-2 were developed, mainly against the S protein [34, 36, 37, 151, 152]. Long CDR3 and the small size of the nanobody may facilitate the single-domain antibody to the epitope that is hiding or is rarely targeted by conventional human antibodies [34, 36, 39, 152]. Another benefit of the nanobody is the lack of the Fc portion, reducing the risk of Fc-associated ADE [41]. However, enhanced virus infectivity by nanobodies was reported [151]. Nanobodies bound to enhancing epitopes on the RBD might induce conformational changes that promote interaction with receptors [151]. Nanobodies were also developed against nonstructural proteins [37, 67, 75]. The long CDR3 of the nanobody supports the accession to the cavity or enzymatic groove of the target [67, 69].

Bi-, Tri- and, Multivalent (Multispecific) Antibodies

Combining two or more antigen-binding domains, i.e., Fab, scFv, and nanobody of the antibody molecule, to increase the antibody's avidity improved antibodies' efficacies. Antigen-binding domains were linked together or with different molecules, commonly the Fc of IgG, to create the bi-, tri-, and multivalent antibody formats. These antibody formats also facilitated combinations of antigen-binding domains with different specificity to become bi-, tri-, or multispecific antibodies.

Fc-Supported Bi-, Trivalent Antibody

The scFv and nanobody have a small molecular weight, which helps tissue penetration and facilitates gene manipulation and fusion protein linkage but is rapid kidney clearance [78]. Linking the scFv or nanobody to Fc helped increase the half-life [47, 153] and assisted in the purification of the fusion proteins [154]. However, in some antibodies, linking scFv-Fc fusion to IgG affects neutralizing but not binding activity [155]. Fc-supported dimerization of molecules and demonstrated increasing avidity and improved efficiency compared to monovalent [34, 39, 41, 153].

The fusion of the antigen-binding domain with Fc also supports constructing bispecific and multivalent antibodies. Antigen-binding domains targeting different antigens can be combined by Fc dimerization to create the bispecific antibody. The bispecific antibody to different epitopes of the spike protein increased neutralization potency [156] and resistance to mutational escape [36, 59, 60]. Combining a neutralizing nanobody and a nonneutralizing Fab to spike protein improved antibody efficiency [157]. One or more antigen-binding domains can be added to the Fc at the N- and/or C-terminal to create a multivalent bispecific antibody format [158, 159]. For example, an anti-RBD spike linked to an IL-6 trans-signaling inhibitor (antibody to IL-6: IL6R complexes) prevents viral entry and cytokine release syndrome [76, 82, 154]. Unlike antibody cocktails, bispecific antibodies can reduce production costs and administration doses [156, 159]. Fc fusion and bispecific antibodies can be engineered to produce silent Fc to reduce the risk of ADE [35, 157].

Fc also facilitated the construction of multispecific trivalent antibodies [160], which improved the antibody's potency and the prevention of viral escape. Interestingly, the molecules' arrangement affected the antibody's effectiveness [160]. Apart from fusion with the Fc of IgG, the decameric antibody was constructed by linking the antigen-binding domains to the Fc of the IgM, increasing the stability of the antibody for aerosolized administration to deliver the antibody directly to the lung [161].

Linker and Proteins Supported Multivalent (Multispecific) Antibody

The bivalent and trivalent antibody format can be generated by linking the molecules with the peptide linker [161–163]. The length of the linker can be a structurally guided design for the best potency [163]. The trivalent antibody format improved the stability of the antibody and is another format designed to be applied intranasally to function directly in the airway [161, 163].

Linking the antigen-binding domains of the antibody with the self-assembly protein or protein scaffold is another strategy for forming a multivalent antibody. Tetrameric antibodies are created by linking the Fab of scFv with or without Fc to the self-assembled human p53 tetramerizing domain, the best performance of the

Table 1 Benefits and difficulties of antibodies as well as engineered antibody formats

Antibody format	Benefits	Difficulties
Intact (full-length) antibody	-Fc-facilitated viral clearance -Stability of the antibody	-Fc-induced ADE -Antigenicity of nonfully human antibody
Engineered Fc antibody	-Reduced the Fc-induced ADE -Prolonged antibody half-life	-Antigenicity of nonfully human antibody
Antibody fragments: Fab, scFv, and nanobody	-High tissue penetration -Easy for manipulation and engineering -Stable in harsh environment (nanobody) -Accessibility to difficult accessible region via long CDR3 (nanobody)	-Short biological half-life -Antigenicity of nonfully human antibody
Multivalent antibody	-Improve efficacy -Improve stability -Reduce application dose: intranasal administration	-Uniformity of the multivalent molecules -A chance to disrupt antibody function
Multispecific and antibody cocktail	-Overcome the viral escape mutation	-Selection of antibody for combination -A chance to disrupt antibody function

tetrameric molecule is the Ig format, which CH3 of the full-length antibody is linked to the p53 tetramerizing domain, and the hinge region of the antibody molecules was preserved [164]. With a similar principle, a multibody antibody (multispecific, multiaffinity) was developed using apoferritin, which can create a multimerization of 24 proteins [165]. Separately linking the different specificities of Fab and the Fc to apoferritin creates the multispecificity and multivalency complex that can be purified using protein A [165]. The multibody overcomes the point mutation and improves neutralization, even in nonneutralizing monovalent antibodies [165]. The attachment of nanobodies to lumazine synthase protein scaffold from *Aquifex aeolicus*, using a spy tag/spy catcher, creates a multivalent molecule with thermostability [166].

Antibody Fragment-Fusion Proteins and Other Antibody Formats

The antibody molecule usually targets extracellular antigens. In order to enable the function of the antibody within cells, scFv against the Nsp5 was linked to the cell-penetrating peptide (Cpp) as a superantibody [70]. The superantibody passed through the plasma membrane to inhibit viral replication.

The bivalent antibody linked to an antiviral peptide that blocked ACE2 binding was developed. The linker between the antibody and the peptide can be cleaved to separate the molecules at the site of action [162]. The antibody is PEGylation, commonly used to improve biological half-life and stability [162, 167].

A combination of ^{131}I labeled antibodies for auger radiotherapy, electron energy penetrates deeply into the virus but not the nearby cells and can be applied for noninvasive imaging [168].

Conclusion and Perspective

Antibody therapy is considered an alternative treatment for COVID-19. Targeting and binding to multiple sites of the viral protein make the antibody-escaped mutation harder than the small-molecule drugs. Many therapeutic antibody design strategies have been developed to encounter SAR-COV-2 infection and complications. The challenges in antibody design are overcoming viral mutations and finding a therapeutic window for the antibody, particularly the immunomodulator [134]. Engineered antibodies with improved avidity and/or specificity were shown to be one strategy to avoid mutations. Multimeric antibody forms are stable to apply intranasally to function directly in the airway, reducing the concentration of antibodies and improving their effectiveness [169, 170]. Selection of the pancoronavirus conserved epitope(s) and using AI or computer-assisted designs of antigen–antibody interactions, intermolecular linkage, and immune escape mechanism predictions would help develop therapeutic antibodies [163, 171–173]. The benefits and difficulties of the engineered antibodies are summarized in Table 1. Apart from designing the paratope, fast isolation and efficient production of therapeutic antibodies are other factors that need cohesive development to make a successful therapeutic antibody.

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Data Availability No datasets were generated or analyzed during the current study.

Declarations

Competing interests The authors declare no competing interests.

Conflict of Interest An author declares no conflict of interest.

Human and Animal Rights and Informed Consent No animal or human subjects by the authors were used in this study.

References

Papers of particular interest, published recently, have been highlighted as:
- • Of importance
- •• Of major importance

1. Holmes EC, Goldstein SA, Rasmussen AL, Robertson DL, Crits-Christoph A, Wertheim JO, et al. The origins of SARS-CoV-2: a critical review. Cell. 2021;184(19):4848–56. https://doi.org/10.1016/j.cell.2021.08.017.
2. Chen J. Pathogenicity and transmissibility of 2019-nCoV-A quick overview and comparison with other emerging viruses. Microbes Infect. 2020;22(2):69–71. https://doi.org/10.1016/j.micinf.2020.01.004.
3. WHO. WHO Coronavirus (COVID-19) dashboard. 2023. https://covid19.who.int/ (accessed 2023, July 28).
4. WHO. Statement on the fourteenth meeting of the International Health Regulations (2023, Jan 30). 2023. https://www.who.int/news/ (accessed 2023, July 28).
5. WHO. Statement on the fourteenth meeting of the International Health Regulations (2023, May 5). 2023 https://www.who.int/news/ (accessed 2023, July 28).
6. Al-Kuraishy HM, Al-Fakhrany OM, Elekhnawy E, Al-Gareeb AI, Alorabi M, De Waard M, et al. Traditional herbs against COVID-19: back to old weapons to combat the new pandemic. Eur J Med Res. 2022;27(1):186. https://doi.org/10.1186/s40001-022-00818-5.
7. Hwang YC, Lu RM, Su SC, Chiang PY, Ko SH, Ke FY, et al. Monoclonal antibodies for COVID-19 therapy and SARS-CoV-2 detection. J Biomed Sci. 2022;29(1):1. https://doi.org/10.1186/s12929-021-00784-w.
8. Lei S, Chen X, Wu J, Duan X, Men K. Small molecules in the treatment of COVID-19. Signal Transduct Target Ther. 2022;7(1):387. https://doi.org/10.1038/s41392-022-01249-8.
9. Puhl AC, Lane TR, Urbina F, Ekins S. The need for speed and efficiency: a brief review of small molecule antivirals for COVID-19. Front Drug Discov. 2022;2:837587. https://doi.org/10.3389/fddsv.2022.837587.
10. Zhong L, Zhao Z, Peng X, Zou J, Yang S. Recent advances in small-molecular therapeutics for COVID-19. Precis Clin Med. 2022;5(4):pbac024. https://doi.org/10.1093/pcmedi/pbac024.
11. Yang J, Won G, Baek JY, Lee YH, Kim H, Huh K, et al. Neutralizing activity against Omicron BA.5 after tixagevimab/cilgavimab administration comparable to those after Omicron BA.1/BA.2 breakthrough infections. Front Immunol. 2023;14:1139980. https://doi.org/10.3389/fimmu.2023.1139980.
12. Jakimovski D, Eckert SP, Mirmosayyeb O, Thapa S, Pennington P, Hojnacki D, et al. Tixagevimab and cilgavimab (Evusheld™) prophylaxis prevents breakthrough COVID-19 infections in immunosuppressed population: 6-month prospective study. Vaccines (Basel). 2023;11(2):350. https://doi.org/10.3390/vaccines11020350.
13. Kauer V, Totschnig D, Waldenberger F, Augustin M, Karolyi M, Nägeli M, et al. Efficacy of sotrovimab (SOT), molnupiravir (MOL), and nirmatrelvir/ritponavir (N/R) and tolerability of molnupiravir in outpatients at high risk for severe COVID-19. Viruses. 2023;15(5):1181. https://doi.org/10.3390/v15051181.
14. Brant AC, Tian W, Majerciak V, Yang W, Zheng ZM. SARS-CoV-2: from its discovery to genome structure, transcription, and replication. Cell Biosci. 2021;11(1):136. https://doi.org/10.1186/s13578-021-00643-z.
15. Rodriguez-Morales AJ, Bonilla-Aldana DK, Balbin-Ramon GJ, Rabaan AA, Sah R, Paniz-Mondolfi A, et al. History is repeating itself: probable zoonotic spillover as the cause of the 2019 novel Coronavirus Epidemic. Infez Med. 2020;28(1):3–5.
16. Haider N, Rothman-Ostrow P, Osman AY, Arruda LB, Macfarlane-Berry L, Elton L, et al. COVID-19-zoonosis or emerging infectious disease? Front Public Health. 2020;8:596944. https://doi.org/10.3389/fpubh.2020.596944.
17. Zhu Z, Lian X, Su X, Wu W, Marraro GA, Zeng Y. From SARS and MERS to COVID-19: a brief summary and comparison of severe acute respiratory infections caused by three highly pathogenic human coronaviruses. Respir Res. 2020;21(1):224. https://doi.org/10.1186/s12931-020-01479-w.
18. Pustake M, Tambolkar I, Giri P, Gandhi C. SARS, MERS and CoVID-19: an overview and comparison of clinical, laboratory and radiological features. J Family Med Prim Care. 2022;11(1):10–7. https://doi.org/10.4103/jfmpc.jfmpc_839_21.
19. Wang L, Møhlenberg M, Wang P, Zhou H. Immune evasion of neutralizing antibodies by SARS-CoV-2 Omicron. Cytokine Growth Factor Rev. 2023;70:13–25. https://doi.org/10.1016/j.cytogfr.2023.03.001.
20. Yan W, Zheng Y, Zeng X, He B, Cheng W. Structural biology of SARS-CoV-2: open the door for novel therapies. Signal Transduct Target Ther. 2022;7(1):26. https://doi.org/10.1038/s41392-022-00884-5.
21. Huang Y, Yang C, Xu XF, Xu W, Liu SW. Structural and functional properties of SARS-CoV-2 spike protein: potential antivirus drug development for COVID-19. Acta Pharmacol Sin. 2020;41(9):1141–9. https://doi.org/10.1038/s41401-020-0485-4.
22. Liu XH, Cheng T, Liu BY, Chi J, Shu T, Wang T. Structures of the SARS-CoV-2 spike glycoprotein and applications for novel drug development. Front Pharmacol. 2022;13:955648. https://doi.org/10.3389/fphar.2022.955648.
23. Zhou Y, Lu X, Wang X, Ying T, Tan X. Potent therapeutic strategies for COVID-19 with single-domain antibody immunoliposomes neutralizing SARS-CoV-2 and Lip/cGAMP enhancing protective immunity. Int J Mol Sci. 2023;24(4):4068. https://doi.org/10.3390/ijms24044068.
24. VanBlargan LA, Errico JM, Halfmann PJ, Zost SJ, Crowe JE Jr, Purcell LA, et al. An infectious SARS-CoV-2 B.1.1.529 Omicron virus escapes neutralization by therapeutic monoclonal antibodies. Nat Med. 2022;28(3):490–5. https://doi.org/10.1038/s41591-021-01678-y.
25. Zhang L, Jackson CB, Mou H, Ojha A, Peng H, Quinlan BD, et al. SARS-CoV-2 spike-protein D614G mutation increases virion spike density and infectivity. Nat Commun. 2020;11(1):6013. https://doi.org/10.1038/s41467-020-19808-4.
26. Jackson CB, Farzan M, Chen B, Choe H. Mechanisms of SARS-CoV-2 entry into cells. Nat Rev Mol Cell Biol. 2022;23(1):3–20. https://doi.org/10.1038/s41580-021-00418-x.
27. Tuccori M, Ferraro S, Convertino I, Cappello E, Valdiserra G, Blandizzi C, et al. Anti-SARS-CoV-2 neutralizing monoclonal antibodies: clinical pipeline. MAbs. 2020;12(1):1854149. https://doi.org/10.1080/19420862.2020.1854149.
28. Miljanovic D, Cirkovic A, Lazarevic I, Knezevic A, Cupic M, Banko A. Clinical efficacy of anti-SARS-CoV-2 monoclonal antibodies in preventing hospitalisation and mortality among patients infected with Omicron variants: a systematic review and meta-analysis. Rev Med Virol. 2023;33(4):e2439. https://doi.org/10.1002/rmv.2439.

29. McCallum M, De Marco A, Lempp FA, Tortorici MA, Pinto D, Walls AC, et al. N-terminal domain antigenic mapping reveals a site of vulnerability for SARS-CoV-2. Cell. 2021;184(9):2332-2347.e16. https://doi.org/10.1016/j.cell.2021.03.028.
30. Hastie KM, Li H, Bedinger D, Schendel SL, Dennison SM, Li K, et al. Defining variant-resistant epitopes targeted by SARS-CoV-2 antibodies: a global consortium study. Science. 2021;374(6566):472–8. https://doi.org/10.1126/science.abh2315.
31. Suryadevara N, Shrihari S, Gilchuk P, VanBlargan LA, Binshtein E, Zost SJ, et al. Neutralizing and protective human monoclonal antibodies recognizing the N-terminal domain of the SARS-CoV-2 spike protein. Cell. 2021;184(9):2316-2331.e15. https://doi.org/10.1016/j.cell.2021.03.029.
32. Westendorf K, Žentelis S, Wang L, Foster D, Vaillancourt P, Wiggin M, et al. LY-CoV1404 (bebtelovimab) potently neutralizes SARS-CoV-2 variants. Cell Rep. 2022;39(7):110812. https://doi.org/10.1016/j.celrep.2022.110812.
33. Zhang H, Lv P, Jiang J, Liu Y, Yan R, Shu S, et al. Advances in developing ACE2 derivatives against SARS-CoV-2. Lancet Microbe. 2023;4(5):e369–78. https://doi.org/10.1016/S2666-5247(23)00011-3.
34. Xu J, Xu K, Jung S, Conte A, Lieberman J, Muecksch F, et al. Nanobodies from camelid mice and llamas neutralize SARS-CoV-2 variants. Nature. 2021;595(7866):278–82. https://doi.org/10.1038/s41586-021-03676-z.
35. Bertoglio F, Fühner V, Ruschig M, Heine PA, Abassi L, Klünemann T, et al. A SARS-CoV-2 neutralizing antibody selected from COVID-19 patients binds to the ACE2-RBD interface and is tolerant to most known RBD mutations. Cell Rep. 2021;36(4):109433. https://doi.org/10.1016/j.celrep.2021.109433.
36. Shi Z, Li X, Wang L, Sun Z, Zhang H, Chen X, et al. Structural basis of nanobodies neutralizing SARS-CoV-2 variants. Structure. 2022;30(5):707-720.e5. https://doi.org/10.1016/j.str.2022.02.011.
37. Wang W, Hu Y, Li B, Wang H, Shen J. Applications of nanobodies in the prevention, detection, and treatment of the evolving SARS-CoV-2. Biochem Pharmacol. 2023;208:115401. https://doi.org/10.1016/j.bcp.2022.115401.
38.•• Starr TN, Czudnochowski N, Liu Z, Zatta F, Park YJ, Addetia A, et al. SARS-CoV-2 RBD antibodies that maximize breadth and resistance to escape. Nature. 2021;597(7874):97–102. https://doi.org/10.1038/s41586-021-03807-6. (**The antibody that interacted with spike protein across subgenus *Sarbecoviruses* demonstrated cross-variant SARS-CoV-2 protection.**)
39. Wrapp D, De Vlieger D, Corbett KS, Torres GM, Wang N, Van Breedam W, et al. Structural basis for potent neutralization of betacoronaviruses by single-domain camelid antibodies. Cell. 2020;181(5):1004-1015.e15. https://doi.org/10.1016/j.cell.2020.04.031.
40. Tortorici MA, Beltramello M, Lempp FA, Pinto D, Dang HV, Rosen LE, et al. Ultrapotent human antibodies protect against SARS-CoV-2 challenge via multiple mechanisms. Science. 2020;370(6519):950–7. https://doi.org/10.1126/science.abe3354.
41. Xiang Y, Nambulli S, Xiao Z, Liu H, Sang Z, Duprex WP, et al. Versatile and multivalent nanobodies efficiently neutralize SARS-CoV-2. Science. 2020;370(6523):1479–84. https://doi.org/10.1126/science.abe4747.
42. Chi X, Yan R, Zhang J, Zhang G, Zhang Y, Hao M, et al. A neutralizing human antibody binds to the N-terminal domain of the Spike protein of SARS-CoV-2. Science. 2020;369(6504):650–5. https://doi.org/10.1126/science.abc6952.
43. Suryadevara N, Shiakolas AR, VanBlargan LA, Binshtein E, Chen RE, Case JB, et al. An antibody targeting the N-terminal domain of SARS-CoV-2 disrupts the spike trimer. J Clin Invest. 2022;132(11):e159062. https://doi.org/10.1172/JCI159062.
44. Kan Q, Lin X, Li T, Ke X, Jian X, Hou L, et al. A novel mAb broadly neutralizes SARS-CoV-2 VOCs in vitro and in vivo, including the Omicron variants. J Med Virol. 2023;95(3):e28657. https://doi.org/10.1002/jmv.28657.
45. Silva RP, Huang Y, Nguyen AW, Hsieh CL, Olaluwoye OS, Kaoud TS, et al. Identification of a conserved S2 epitope present on spike proteins from all highly pathogenic coronaviruses. Elife. 2023;12:e83710. https://doi.org/10.7554/eLife.83710.
46. Ryu DK, Song R, Kim M, Kim YI, Kim C, Kim JI, et al. Therapeutic effect of CT-P59 against SARS-CoV-2 South African variant. Biochem Biophys Res Commun. 2021;566:135–40. https://doi.org/10.1016/j.bbrc.2021.06.016.
47. Li W, Chen C, Drelich A, Martinez DR, Gralinski LE, Sun Z, et al. Rapid identification of a human antibody with high prophylactic and therapeutic efficacy in three animal models of SARS-CoV-2 infection. Proc Natl Acad Sci U S A. 2020;117(47):29832–8. https://doi.org/10.1073/pnas.2010197117.
48 Fang Y, Sun P, Xie X, Du M, Du F, Ye J, et al. An antibody that neutralizes SARS-CoV-1 and SARS-CoV-2 by binding to a conserved spike epitope outside the receptor binding motif. Sci Immunol. 2022;7(76):eabp9962. https://doi.org/10.1126/sciimmunol.abp9962.
49. Cameroni E, Bowen JE, Rosen LE, Saliba C, Zepeda SK, Culap K, et al. Broadly neutralizing antibodies overcome SARS-CoV-2 Omicron antigenic shift. Nature. 2022;602(7898):664–70. https://doi.org/10.1038/s41586-021-04386-2.
50. U.S. Food and drug administration. FDA announces Bebtelovimab is not currently authorized in any US region. 2022. https://www.fda.gov/drugs/drug-safety-and-availability/fda-announces-bebtelovimab-not-currently-authorized-any-us-region (assessed 2023, July 28).
51. Cathcart AL, Havenar-Daughton C, Lempp FA, Ma D, Schmid MA, Agostini ML. The dual function monoclonal antibodies VIR-7831 and VIR-7832 demonstrate potent in vitro and in vivo activity against SARS-CoV-2. bioRxiv [Preprint] 2021.03.09.434607. https://doi.org/10.1101/2021.03.09.434607
52. Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S, et al. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. Cell. 2020;181(2):271-280.e8. https://doi.org/10.1016/j.cell.2020.02.052.
53. Cicchitto G, Cardillo L, Sequino D, Sabatini P, Adamo L, Marchitiello R, et al. Effectiveness of sotrovimab in the omicron storm time: a case series. Viruses. 2022;15(1):102. https://doi.org/10.3390/v15010102.
54. Cheng MM, Reyes C, Satram S, Birch H, Gibbons DC, Drysdale M, et al. Real-world effectiveness of sotrovimab for the early treatment of COVID-19 during SARS-CoV-2 delta and omicron waves in the USA. Infect Dis Ther. 2023;12(2):607–21. https://doi.org/10.1007/s40121-022-00755-0.
55.•• Copin R, Baum A, Wloga E, Pascal KE, Giordano S, Fulton BO, et al. The monoclonal antibody combination REGEN-COV protects against SARS-CoV-2 mutational escape in preclinical and human studies. Cell. 2021;184(15):3949-3961.e11. https://doi.org/10.1016/j.cell.2021.06.002. (**This study demonstrated the combination of antibodies reduced antibody-escape variants.**)
56. Chatterjee S, Bhattacharya M, Dhama K, Lee SS, Chakraborty C. Can the RBD mutation R346X provide an additional fitness to the "variant soup", including offspring of BQ and XBB of SARS-CoV-2 Omicron for the antibody resistance? Mol Ther Nucleic Acids. 2023;32:61–3. https://doi.org/10.1016/j.omtn.2023.02.030.
57.• Birnie E, Biemond JJ, Appelman B, de Bree GJ, Jonges M, Welkers MRA, et al. Development of resistance-associated mutations after sotrovimab administration in high-risk

individuals infected with the SARS-CoV-2 omicron variant. JAMA. 2022;328(11):1104–7. https://doi.org/10.1001/jama.2022.13854. (**This study reported an antibody's pressure led to the antibody-escape mutation (s).**)
58. Gliga S, Lübke N, Killer A, Gruell H, Walker A, Dilthey AT, et al. Rapid selection of sotrovimab escape variants in severe acute respiratory syndrome coronavirus 2 omicron-infected immunocompromised patients. Clin Infect Dis. 2023;76(3):408–15. https://doi.org/10.1093/cid/ciac802.
59. Yadav R, Courouble VV, Dey SK, Harrison JJEK, Timm J, Hopkins JB, et al. Biochemical and structural insights into SARS-CoV-2 polyprotein processing by Mpro. Sci Adv. 2022;8(49):eadd2191. https://doi.org/10.1126/sciadv.add2191.
60. Kirchdoerfer RN, Ward AB. Structure of the SARS-CoV nsp12 polymerase bound to nsp7 and nsp8 co-factors. Nat Commun. 2019;10(1):2342. https://doi.org/10.1038/s41467-019-10280-3.
61. Subissi L, Posthuma CC, Collet A, Zevenhoven-Dobbe JC, Gorbalenya AE, Decroly E, et al. One severe acute respiratory syndrome coronavirus protein complex integrates processive RNA polymerase and exonuclease activities. Proc Natl Acad Sci U S A. 2014;111(37):E3900–9. https://doi.org/10.1073/pnas.1323705111.
62. Malone B, Urakova N, Snijder EJ, Campbell EA. Structures and functions of coronavirus replication-transcription complexes and their relevance for SARS-CoV-2 drug design. Nat Rev Mol Cell Biol. 2022;23(1):21–39. https://doi.org/10.1038/s41580-021-00432-z.
63. Verba K, Gupta M, Azumaya C, Moritz M, Pourmal S, Diallo A, et al. CryoEM and AI reveal a structure of SARS-CoV-2 Nsp2, a multifunctional protein involved in key host processes. Res Sq [Preprint]. 2021;rs.3.rs-515215. https://doi.org/10.21203/rs.3.rs-515215/v1.
64. Armstrong LA, Lange SM, Dee Cesare V, Matthews SP, Nirujogi RS, Cole I, et al. Biochemical characterization of protease activity of Nsp3 from SARS-CoV-2 and its inhibition by nanobodies. PLoS ONE. 2021;16(7):e0253364. https://doi.org/10.1371/journal.pone.0253364.
65. Azizogli AR, Pai V, Coppola F, Jafari R, Dodd-O JB, Harish R, et al. Scalable inhibitors of the Nsp3-Nsp4 coupling in SARS-CoV-2. ACS Omega. 2023;8(6):5349–60. https://doi.org/10.1021/acsomega.2c06384.
66. Kandwal S, Fayne D. Genetic conservation across SARS-CoV-2 non-structural proteins - insights into possible targets for treatment of future viral outbreaks. Virology. 2023;581:97–115. https://doi.org/10.1016/j.virol.2023.02.011.
67. Qiao H, Li L, Wang L, Yu H, Hu F, Zhou X, et al. Preparation and characterization of nanobodies targeting SARS-CoV-2 papain-like protease. Protein Expr Purif. 2023;207:106267. https://doi.org/10.1016/j.pep.2023.106267.
68. Jin Z, Du X, Xu Y, Deng Y, Liu M, Zhao Y, et al. Structure of Mpro from SARS-CoV-2 and discovery of its inhibitors. Nature. 2020;582(7811):289–93. https://doi.org/10.1038/s41586-020-2223-y.
69. Sun Z, Wang L, Li X, Fan C, Xu J, Shi Z, et al. An extended conformation of SARS-CoV-2 main protease reveals allosteric targets. Proc Natl Acad Sci U S A. 2022;119(15):e2120913119. https://doi.org/10.1073/pnas.2120913119.
70. Glab-Ampai K, Kaewchim K, Saenlom T, Thepsawat W, Mahasongkram K, Sookrung N, et al. Human superantibodies to 3CLpro inhibit replication of SARS-CoV-2 across variants. Int J Mol Sci. 2022;23(12):6587. https://doi.org/10.3390/ijms23126587.
71. Pan Y, Chandrashekaran IR, Tennant L, Rossjohn J, Littler DR. Inside-out: antibody-binding reveals potential folding hinge-points within the SARS-CoV-2 replication co-factor nsp9. PLoS ONE. 2023;18(4):e0283194. https://doi.org/10.1371/journal.pone.0283194.
72. Slanina H, Madhugiri R, Bylapudi G, Schultheiß K, Karl N, Gulyaeva A, et al. Coronavirus replication-transcription complex: vital and selective NMPylation of a conserved site in nsp9 by the NiRAN-RdRp subunit. Proc Natl Acad Sci U S A. 2021;118(6):e2022310118. https://doi.org/10.1073/pnas.2022310118.
73. Miknis ZJ, Donaldson EF, Umland TC, Rimmer RA, Baric RS, Schultz LW. Severe acute respiratory syndrome coronavirus nsp9 dimerization is essential for efficient viral growth. J Virol. 2009;83(7):3007–18. https://doi.org/10.1128/JVI.01505-08.
74. Esposito G, Hunashal Y, Percipalle M, Venit T, Dieng MM, Fogolari F, et al. NMR-based analysis of nanobodies to SARS-CoV-2 Nsp9 reveals a possible antiviral strategy against COVID-19. Adv Biol (Weinh). 2021;5(12):e2101113. https://doi.org/10.1002/adbi.202101113.
75. Glab-Ampai K, Kaewchim K, Thavorasak T, Saenlom T, Thepsawat W, Mahasongkram K, et al. Targeting emerging RNA Viruses by engineered human superantibody to hepatitis C virus RNA-dependent RNA polymerase. Front Microbiol. 2022;13:926929. https://doi.org/10.3389/fmicb.2022.926929.
76. Montazersaheb S, Hosseiniyan Khatibi SM, Hejazi MS, Tarhriz V, Farjami A, et al. COVID-19 infection: an overview on cytokine storm and related interventions. Virol J. 2022;19(1):92. https://doi.org/10.1186/s12985-022-01814-1.
77. Wang X, Tang G, Liu Y, Zhang L, Chen B, Han Y, et al. The role of IL-6 in coronavirus, especially in COVID-19. Front Pharmacol. 2022;13:1033674. https://doi.org/10.3389/fphar.2022.1033674.
78. Grunst MW, Uchil PD. Fc effector cross-reactivity: a hidden arsenal against SARS-CoV-2's evasive maneuvering. Cell Rep Med. 2022;3(2):100540. https://doi.org/10.1016/j.xcrm.2022.100540.
79. Chan CEZ, Seah SGK, Chye H, Massey S, Torres M, Lim APC, et al. The Fc-mediated effector functions of a potent SARS-CoV-2 neutralizing antibody, SC31, isolated from an early convalescent COVID-19 patient, are essential for the optimal therapeutic efficacy of the antibody. PLoS ONE. 2021;16(6):e0253487. https://doi.org/10.1371/journal.pone.0253487.
80. Ikewaki N, Kurosawa G, Levy GA, Preethy S, Abraham SJK. Antibody dependent disease enhancement (ADE) after COVID-19 vaccination and beta glucans as a safer strategy in management. Vaccine. 2023;41(15):2427–9. https://doi.org/10.1016/j.vaccine.2023.03.005.
81. Fu Y, Cheng Y, Wu Y. Understanding SARS-CoV-2-mediated inflammatory responses: from mechanisms to potential therapeutic tools. Virol Sin. 2020;35(3):266–71. https://doi.org/10.1007/s12250-020-00207-4.
82. Abidi E, El Nekidy WS, Alefishat E, Rahman N, Petroianu GA, El-Lababidi R, et al. Tocilizumab and COVID-19: timing of administration and efficacy. Front Pharmacol. 2022;13:825749. https://doi.org/10.3389/fphar.2022.825749.
83. Yin JX, Agbana YL, Sun ZS, Fei SW, Zhao HQ, Zhou XN, et al. Increased interleukin-6 is associated with long COVID-19: a systematic review and meta-analysis. Infect Dis Poverty. 2023;12(1):43. https://doi.org/10.1186/s40249-023-01086-z.
84. Jafrin S, Aziz MA, Islam MS. Elevated levels of pleiotropic interleukin-6 (IL-6) and interleukin-10 (IL-10) are critically involved with the severity and mortality of COVID-19: an updated longitudinal meta-analysis and systematic review on 147 studies. Biomark Insights. 2022;17:11772719221106600. https://doi.org/10.1177/11772719221106600.
85. Nishimoto N, Kishimoto T. Humanized antihuman IL-6 receptor antibody, tocilizumab. Handb Exp Pharmacol. 2008;181:151–60. https://doi.org/10.1007/978-3-540-73259-4_7.

86. U.S. Food and Drug Administration. FDA roundup: December 23, 2022. 2022. https://www.fda.gov/news-events/press-announcements/fda-roundup-december-23-2022. (assessed 2023, Jul 28).
87. Gupta S, Leaf DE. Tocilizumab in COVID-19: some clarity amid controversy. Lancet. 2021;397(10285):1599–601. https://doi.org/10.1016/S0140-6736(21)00712-1.
88. Salama C, Han J, Yau L, Reiss WG, Kramer B, Neidhart JD, et al. Tocilizumab in patients hospitalized with Covid-19 pneumonia. N Engl J Med. 2021;384(1):20–30. https://doi.org/10.1056/NEJMoa2030340.
89. Stone JH, Frigault MJ, Serling-Boyd NJ, Fernandes AD, Harvey L, Foulkes AS, et al. Efficacy of tocilizumab in patients hospitalized with Covid-19. N Engl J Med. 2020;383(24):2333–44. https://doi.org/10.1056/NEJMoa2028836.
90. Kow CS, Hasan SS. The effect of tocilizumab on mortality in hospitalized patients with COVID-19: a meta-analysis of randomized controlled trials. Eur J Clin Pharmacol. 2021;77(8):1089–94. https://doi.org/10.1007/s00228-021-03087-z.
91. Lin WT, Hung SH, Lai CC, Wang CY, Chen CH. The effect of tocilizumab on COVID-19 patient mortality: a systematic review and meta-analysis of randomized controlled trials. Int Immunopharmacol. 2021;96:107602. https://doi.org/10.1016/j.intimp.2021.107602.
92. Gupta S, Padappayil RP, Bansal A, Daouk S, Brown B. Tocilizumab in patients hospitalized with COVID-19 pneumonia: systematic review and meta-analysis of randomized controlled trials. J Investig Med. 2022;70(1):55–60. https://doi.org/10.1136/jim-2021-002001.
93. Rosas IO, Bräu N, Waters M, Go RC, Malhotra A, Hunter BD, et al. Tocilizumab in patients hospitalised with COVID-19 pneumonia: efficacy, safety, viral clearance, and antibody response from a randomised controlled trial (COVACTA). EClinicalMedicine. 2022;47:101409. https://doi.org/10.1016/j.eclinm.2022.101409.
94. Selvaraj V, Khan MS, Bavishi C, Dapaah-Afriyie K, Finn A, Lal A, et al. Tocilizumab in hospitalized patients with COVID-19: a meta analysis of randomized controlled trials. Lung. 2021;199(3):239–48. https://doi.org/10.1007/s00408-021-00451-9.
95. Chamlagain R, Shah S, Sharma Paudel B, Dhital R, Kandel B. Efficacy and safety of sarilumab in COVID-19: a systematic review. Interdiscip Perspect Infect Dis. 2021;2021:8903435. https://doi.org/10.1155/2021/8903435.
96. CORIMUNO-19 Collaborative group. Sarilumab in adults hospitalised with moderate-to-severe COVID-19 pneumonia (CORIMUNO-SARI-1): an open-label randomised controlled trial. Lancet Rheumatol. 2022;4(1):e24–32. https://doi.org/10.1016/S2665-9913(21)00315-5.
97. Pacha O, Sallman MA, Evans SE. COVID-19: a case for inhibiting IL-17? Nat Rev Immunol. 2020;20(6):345–6. https://doi.org/10.1038/s41577-020-0328-z.
98. Resende GG, da Cruz Lage R, Lobê SQ, Medeiros AF, Costa E Silva AD, NogueiraSá AT, et al. Blockade of interleukin seventeen (IL-17A) with secukinumab in hospitalized COVID-19 patients - the BISHOP study. Infect Dis (Lond). 2022;54(8):591–9. https://doi.org/10.1080/23744235.2022.2066171.
99. Cavalli G, Larcher A, Tomelleri A, Campochiaro C, Della-Torre E, De Luca G, et al. Interleukin-1 and interleukin-6 inhibition compared with standard management in patients with COVID-19 and hyperinflammation: a cohort study. Lancet Rheumatol. 2021;3(4):e253–61. https://doi.org/10.1016/S2665-9913(21)00012-6.
100. Kokkotis G, Kitsou K, Xynogalas I, Spoulou V, Magiorkinis G, Trontzas I, et al. Systematic review with meta-analysis: COVID-19 outcomes in patients receiving anti-TNF treatments. Aliment Pharmacol Ther. 2022;55(2):154–67. https://doi.org/10.1111/apt.16717.
101. Pandey P, Al Rumaih Z, Kels MJT, Ng E, Kc R, Malley R, et al. Therapeutic targeting of inflammation and virus simultaneously ameliorates influenza pneumonia and protects from morbidity and mortality. Viruses. 2023;15(2):318. https://doi.org/10.3390/v15020318.
102. Hasan MJ, Rabbani R, Anam AM, Huq SMR. Secukinumab in severe COVID-19 pneumonia: does it have a clinical impact? J Infect. 2021;83(1):e11–3. https://doi.org/10.1016/j.jinf.2021.05.011.
103. Moreira TG, Gauthier CD, Murphy L, Lanser TB, Paul A, Matos KTF, et al. Nasal administration of anti-CD3 mAb (Foralumab) downregulates NKG7 and increases TGFB1 and GIMAP7 expression in T cells in subjects with COVID-19. Proc Natl Acad Sci U S A. 2023;120(11):e2220272120. https://doi.org/10.1073/pnas.2220272120.
104. Rose-John S, Winthrop K, Calabrese L. The role of IL-6 in host defence against infections: immunobiology and clinical implications. Nat Rev Rheumatol. 2017;13(7):399–409. https://doi.org/10.1038/nrrheum.2017.83.
105. Koritala T, Pattan V, Tirupathi R, Rabaan AA, Al Mutair A, Alhumaid S, et al. Infection risk with the use of interleukin inhibitors in hospitalized patients with COVID-19: a narrative review. Infez Med. 2021;29(4):495–503. https://doi.org/10.53854/liim-2904-1.
106. Belletti A, Campochiaro C, Marmiere M, Likhvantsev V, Yavorovskiy A, Dagna L, et al. Efficacy and safety of IL-6 inhibitors in patients with COVID-19 pneumonia: a systematic review and meta-analysis of multicentre, randomized trials. Ann Intensive Care. 2021;11(1):152. https://doi.org/10.1186/s13613-021-00941-2.
107. Kimmig LM, Wu D, Gold M, Pettit NN, Pitrak D, Mueller J, et al. IL-6 Inhibition in critically ill COVID-19 patients is associated with increased secondary infections. Front Med (Lausanne). 2020;7:583897. https://doi.org/10.3389/fmed.2020.583897.
108. Guaraldi G, Meschiari M, Cozzi-Lepri A, Milic J, Tonelli R, Menozzi M, et al. Tocilizumab in patients with severe COVID-19: a retrospective cohort study. Lancet Rheumatol. 2020;2(8):e474–84. https://doi.org/10.1016/S2665-9913(20)30173-9.
109. Pettit NN, Nguyen CT, Mutlu GM, Wu D, Kimmig L, Pitrak D, et al. Late onset infectious complications and safety of tocilizumab in the management of COVID-19. J Med Virol. 2021;93(3):1459–64. https://doi.org/10.1002/jmv.26429.
110. Rubin EJ, Longo DL, Baden LR. Interleukin-6 receptor inhibition in Covid-19 - cooling the inflammatory soup. N Engl J Med. 2021;384(16):1564–5. https://doi.org/10.1056/NEJMe2103108.
111. Zizzo G, Tamburello A, Castelnovo L, Laria A, Mumoli N, Faggioli PM, et al. Immunotherapy of COVID-19: inside and beyond IL-6 signalling. Front Immunol. 2022;13:795315. https://doi.org/10.3389/fimmu.2022.795315.
112. Muramatsu T. Basigin (CD147), a multifunctional transmembrane glycoprotein with various binding partners. J Biochem. 2016;159(5):481–90. https://doi.org/10.1093/jb/mvv127.
113. Knutti N, Huber O, Friedrich K. CD147 (EMMPRIN) controls malignant properties of breast cancer cells by interdependent signaling of Wnt and JAK/STAT pathways. Mol Cell Biochem. 2019;451(1–2):197–209. https://doi.org/10.1007/s11010-018-3406-9.
114. Dai L, Guinea MC, Slomiany MG, Bratoeva M, Grass GD, Tolliver LB, et al. CD147-dependent heterogeneity in malignant and chemoresistant properties of cancer cells. Am J Pathol. 2013;182(2):577–85. https://doi.org/10.1016/j.ajpath.2012.10.011.
115. Pushkarsky T, Zybarth G, Dubrovsky L, Yurchenko V, Tang H, Guo H, et al. CD147 facilitates HIV-1 infection by interacting

with virus-associated cyclophilin A. Proc Natl Acad Sci U S A. 2001;98(11):6360–5. https://doi.org/10.1073/pnas.111583198.
116. Zhang MY, Zhang Y, Wu XD, Zhang K, Lin P, Bian HJ, et al. Disrupting CD147-RAP2 interaction abrogates erythrocyte invasion by *Plasmodium falciparum*. Blood. 2018;131(10):1111–21. https://doi.org/10.1182/blood-2017-08-802918.
117.• Wang K, Chen W, Zhang Z, Deng Y, Lian JQ, Du P, et al. CD147-spike protein is a novel route for SARS-CoV-2 infection to host cells. Signal Transduct Target Ther. 2020;5(1):283. https://doi.org/10.1038/s41392-020-00426-x. (**This study repurposed the anti-CD147 antibody for the treatment of SARS-CoV-2.**)
118. Bian H, Zheng ZH, Wei D, Wen A, Zhang Z, Lian JQ, et al. Safety and efficacy of meplazumab in healthy volunteers and COVID-19 patients: a randomized phase 1 and an exploratory phase 2 trial. Signal Transduct Target Ther. 2021;6(1):194. https://doi.org/10.1038/s41392-021-00603-6.
119. Yurchenko V, Constant S, Eisenmesser E, Bukrinsky M. Cyclophilin-CD147 interactions: a new target for anti-inflammatory therapeutics. Clin Exp Immunol. 2010;160(3):305–17. https://doi.org/10.1111/j.1365-2249.2010.04115.x.
120. Wu J, Chen L, Qin C, Huo F, Liang X, Yang X, et al. CD147 contributes to SARS-CoV-2-induced pulmonary fibrosis. Signal Transduct Target Ther. 2022;7(1):382. https://doi.org/10.1038/s41392-022-01230-5.
121. Alegre ML, Tso JY, Sattar HA, Smith J, Desalle F, Cole M, et al. An anti-murine CD3 monoclonal antibody with a low affinity for Fc gamma receptors suppresses transplantation responses while minimizing acute toxicity and immunogenicity. J Immunol. 1995;155(3):1544–55.
122. van der Woude CJ, Stokkers P, van Bodegraven AA, Van Assche G, Hebzda Z, Paradowski L, et al. Phase I, double-blind, randomized, placebo-controlled, dose-escalation study of NI-0401 (a fully human anti-CD3 monoclonal antibody) in patients with moderate to severe active Crohn's disease. Inflamm Bowel Dis. 2010;16(10):1708–16. https://doi.org/10.1002/ibd.21252.
123. Oganesyan V, Damschroder MM, Woods RM, Cook KE, Wu H, Dall'acqua WF. Structural characterization of a human Fc fragment engineered for extended serum half-life. Mol Immunol. 2009;46(8–9):1750–5. https://doi.org/10.1016/j.molimm.2009.01.026.
124. Oganesyan V, Gao C, Shirinian L, Wu H, Dall'Acqua WF. Structural characterization of a human Fc fragment engineered for lack of effector functions. Acta Crystallogr D Biol Crystallogr. 2008;64(Pt 6):700–4. https://doi.org/10.1107/S0907444908007877.
125. Schlothauer T, Herter S, Koller CF, Grau-Richards S, Steinhart V, Spick C, et al. Novel human IgG1 and IgG4 Fc-engineered antibodies with completely abolished immune effector functions. Protein Eng Des Sel. 2016;29(10):457–66. https://doi.org/10.1093/protein/gzw040.
126. Zhang D, Goldberg MV, Chiu ML. Fc engineering approaches to enhance the agonism and effector functions of an anti-OX40 antibody. J Biol Chem. 2016;291(53):27134–46. https://doi.org/10.1074/jbc.M116.757773.
127. Shi R, Shan C, Duan X, Chen Z, Liu P, Song J, et al. A human neutralizing antibody targets the receptor-binding site of SARS-CoV-2. Nature. 2020;584(7819):120–4. https://doi.org/10.1038/s41586-020-2381-y.
128. Brambell FW. The transmission of immunity from mother to young and the catabolism of immunoglobulins. Lancet. 1966;2(7473):1087–93. https://doi.org/10.1016/s0140-6736(66)92190-8.
129. Ramdani Y, Lamamy J, Watier H, Gouilleux-Gruart V. Monoclonal antibody engineering and design to modulate FcRn activities: a comprehensive review. Int J Mol Sci. 2022;23(17):9604. https://doi.org/10.3390/ijms23179604.
130. Pyzik M, Sand KMK, Hubbard JJ, Andersen JT, Sandlie I, Blumberg RS. The neonatal Fc receptor (FcRn): a misnomer? Front Immunol. 2019;10:1540. https://doi.org/10.3389/fimmu.2019.01540.
131. Lee CH, Kang TH, Godon O, Watanabe M, Delidakis G, Gillis CM, et al. An engineered human Fc domain that behaves like a pH-toggle switch for ultra-long circulation persistence. Nat Commun. 2019;10(1):5031. https://doi.org/10.1038/s41467-019-13108-2.
132. Winkler ES, Gilchuk P, Yu J, Bailey AL, Chen RE, Chong Z, et al. Human neutralizing antibodies against SARS-CoV-2 require intact Fc effector functions for optimal therapeutic protection. Cell. 2021;184(7):1804-1820.e16. https://doi.org/10.1016/j.cell.2021.02.026.
133. Gorman MJ, Patel N, Guebre-Xabier M, Zhu AL, Atyeo C, Pullen KM, et al. Fab and Fc contribute to maximal protection against SARS-CoV-2 following NVX-CoV2373 subunit vaccine with Matrix-M vaccination. Cell Rep Med. 2021;2(9):100405. https://doi.org/10.1016/j.xcrm.2021.100405.
134. Zohar T, Loos C, Fischinger S, Atyeo C, Wang C, Slein MD, et al. Compromised humoral functional evolution tracks with SARS-CoV-2 mortality. Cell. 2020;183(6):1508-1519.e12. https://doi.org/10.1016/j.cell.2020.10.052.
135. Yamin R, Jones AT, Hoffmann HH, Schäfer A, Kao KS, Francis RL, et al. Fc-engineered antibody therapeutics with improved anti-SARS-CoV-2 efficacy. Nature. 2021;599(7885):465–70. https://doi.org/10.1038/s41586-021-04017-w.
136. Chen B, Vousden KA, Naiman B, Turman S, Sun H, Wang S, et al. Humanised effector-null FcγRIIA antibody inhibits immune complex-mediated proinflammatory responses. Ann Rheum Dis. 2019;78(2):228–37. https://doi.org/10.1136/annrheumdis-2018-213523.
137. ACTIV-3–Therapeutics for Inpatients with COVID-19 (TICO) Study Group. Tixagevimab-cilgavimab for treatment of patients hospitalised with COVID-19: a randomised, double-blind, phase 3 trial. Lancet Respir Med. 2022;10(10):972–84. https://doi.org/10.1016/S2213-2600(22)00215-6.
138. Merigeon EY, Yang D, Ihms EA, Bassit LC, Fitzpatrick EA, Jonsson CB, et al. An ACE2-IgG4 Fc fusion protein demonstrates strong binding to all tested SARS-CoV-2 variants and reduced lung inflammation in animal models of SARS-CoV-2 and influenza. Pathog Immun. 2022;7(1):104–21. https://doi.org/10.20411/pai.v7i1.491.
139. Qiang M, Ma P, Li Y, Liu H, Harding A, Min C, et al. Neutralizing antibodies to SARS-CoV-2 selected from a human antibody library constructed decades ago. Adv Sci (Weinh). 2022;9(1):e2102181. https://doi.org/10.1002/advs.202102181.
140. Loo YM, McTamney PM, Arends RH, Abram ME, Aksyuk AA, Diallo S, et al. The SARS-CoV-2 monoclonal antibody combination, AZD7442, is protective in nonhuman primates and has an extended half-life in humans. Sci Transl Med. 2022;14(635):eabl8124. https://doi.org/10.1126/scitranslmed.abl8124.
141. Wu X, Li N, Wang G, Liu W, Yu J, Cao G, et al. Pharmacokinetics, and immunogenicity of a Novel SARS-CoV-2 neutralizing antibody, Etesevimab, in Chinese healthy adults: a randomized, double-blind, placebo-controlled, first-in-human phase 1 study. Antimicrob Agents Chemother. 2021;65(8):e0035021. https://doi.org/10.1128/AAC.00350-21.
142. Igawa T, Ishii S, Tachibana T, Maeda A, Higuchi Y, Shimaoka S, et al. Antibody recycling by engineered pH-dependent antigen binding improves the duration of antigen neutralization. Nat

Biotechnol. 2010;28(11):1203–7. https://doi.org/10.1038/nbt.1691.

143. Petkova SB, Akilesh S, Sproule TJ, Christianson GJ, Al Khabbaz H, Brown AC, et al. Enhanced half-life of genetically engineered human IgG1 antibodies in a humanized FcRn mouse model: potential application in humorally mediated autoimmune disease. Int Immunol. 2006;18(12):1759–69. https://doi.org/10.1093/intimm/dxl110.
144. Bournazos S, Corti D, Virgin HW, Ravetch JV. Fc-optimized antibodies elicit CD8 immunity to viral respiratory infection. Nature. 2020;588(7838):485–90. https://doi.org/10.1038/s41586-020-2838-z.
145. Hamers-Casterman C, Atarhouch T, Muyldermans S, Robinson G, Hamers C, Songa EB, et al. Naturally occurring antibodies devoid of light chains. Nature. 1993;363(6428):446–8. https://doi.org/10.1038/363446a0.
146. Muyldermans S. Nanobodies: natural single-domain antibodies. Annu Rev Biochem. 2013;82:775–97. https://doi.org/10.1146/annurev-biochem-063011-092449.
147. Kunz P, Zinner K, Mücke N, Bartoschik T, Muyldermans S, Hoheisel JD. The structural basis of nanobody unfolding reversibility and thermoresistance. Sci Rep. 2018;8(1):7934. https://doi.org/10.1038/s41598-018-26338-z.
148. Mohseni A, Molakarimi M, Taghdir M, Sajedi RH, Hasannia S. Exploring single-domain antibody thermostability by molecular dynamics simulation. J Biomol Struct Dyn. 2019;37(14):3686–96. https://doi.org/10.1080/07391102.2018.1526116.
149. Hussack G, Mackenzie CR, Tanha J. Characterization of single-domain antibodies with an engineered disulfide bond. Methods Mol Biol. 2012;911:417–29. https://doi.org/10.1007/978-1-61779-968-6_25.
150. Chen J, He QH, Xu Y, Fu JH, Li YP, Tu Z, et al. Nanobody medicated immunoassay for ultrasensitive detection of cancer biomarker alpha-fetoprotein. Talanta. 2016;147:523–30. https://doi.org/10.1016/j.talanta.2015.10.027.
151. Kaewchim K, Glab-Ampai K, Mahasongkram K, Saenlom T, Thepsawat W, Chulanetra M, et al. Neutralizing and enhancing epitopes of the SARS-CoV-2 receptor-binding domain (RBD) identified by nanobodies. Viruses. 2023;15(6):1252. https://doi.org/10.3390/v15061252.
152. Ye G, Gallant J, Zheng J, Massey C, Shi K, Tai W, et al. The development of Nanosota-1 as anti-SARS-CoV-2 nanobody drug candidates. Elife. 2021;10:e64815. https://doi.org/10.7554/eLife.64815.
153. Chi X, Zhang X, Pan S, Yu Y, Shi Y, Lin T, et al. An ultrapotent RBD-targeted biparatopic nanobody neutralizes broad SARS-CoV-2 variants. Signal Transduct Target Ther. 2022;7(1):44. https://doi.org/10.1038/s41392-022-00912-4.
154. Ettich J, Werner J, Weitz HT, Mueller E, Schwarzer R, Lang PA, et al. A hybrid soluble gp130/spike-nanobody fusion protein simultaneously blocks interleukin-6 *trans*-signaling and cellular infection with SARS-CoV-2. J Virol. 2022;96(4):e0162221. https://doi.org/10.1128/JVI.01622-21.
155. Bertoglio F, Meier D, Langreder N, Steinke S, Rand U, Simonelli L, et al. SARS-CoV-2 neutralizing human recombinant antibodies selected from pre-pandemic healthy donors binding at RBD-ACE2 interface. Nat Commun. 2021;12(1):1577. https://doi.org/10.1038/s41467-021-21609-2.
156. Dean AQ, Stauft CB, Twomey JD, Tan J, Varani L, Wang TT, et al. Comparative assessment of the binding and neutralisation activity of bispecific antibodies against SARS-CoV-2 Variants. Antib Ther. 2022;6(1):49–58. https://doi.org/10.1093/abt/tbac032.
157. De Gasparo R, Pedotti M, Simonelli L, Nickl P, Muecksch F, Cassaniti I, et al. Bispecific IgG neutralizes SARS-CoV-2 variants and prevents escape in mice. Nature. 2021;593(7859):424–8. https://doi.org/10.1038/s41586-021-03461-y.
158. Yuan M, Chen X, Zhu Y, Dong X, Liu Y, Qian Z, et al. A bispecific antibody targeting RBD and S2 potently neutralizes SARS-CoV-2 omicron and other variants of concern. J Virol. 2022;96(16):e0077522. https://doi.org/10.1128/jvi.00775-22.
159. Lim SA, Gramespacher JA, Pance K, Rettko NJ, Solomon P, Jin J, et al. Bispecific VH/Fab antibodies targeting neutralizing and non-neutralizing Spike epitopes demonstrate enhanced potency against SARS-CoV-2. MAbs. 2021;13(1):1893426. https://doi.org/10.1080/19420862.2021.1893426.
160. Misasi J, Wei RR, Wang L, Pegu A, Wei CJ, Oloniniyi OK, et al. A multispecific antibody prevents immune escape and confers pan-SARS-CoV-2 neutralization. bioRxiv [Preprint]. 2022;2022.07.29.502029. https://doi.org/10.1101/2022.07.29.502029.
161. Liu H, Wu L, Liu B, Xu K, Lei W, Deng J, et al. Two pan-SARS-CoV-2 nanobodies and their multivalent derivatives effectively prevent Omicron infections in mice. Cell Rep Med. 2023;4(2):100918. https://doi.org/10.1016/j.xcrm.2023.100918.
162. Panda M, Kalita E, Singh S, Kumar K, Prajapati VK. Nanobody-peptide-conjugate (NPC) for passive immunotherapy against SARS-CoV-2 variants of concern (VoC): a prospective pan-coronavirus therapeutics. Mol Divers. 2022;1–27. https://doi.org/10.1007/s11030-022-10570-x.
163. Schoof M, Faust B, Saunders RA, Sangwan S, Rezelj V, Hoppe N, et al. An ultrapotent synthetic nanobody neutralizes SARS-CoV-2 by stabilizing inactive Spike. Science. 2020;370(6523):1473–9. https://doi.org/10.1126/science.abe3255.
164. Leach A, Miller A, Bentley E, Mattiuzzo G, Thomas J, McAndrew C, et al. Application of a method for engineering multivalent antibodies to substantially enhance functional affinity of clinical trial anti-SARS-CoV-2 antibodies. Research Square [Preprint]. 2021. https://doi.org/10.21203/rs.3.rs-259484/v1.
165. Rujas E, Kucharska I, Tan YZ, Benlekbir S, Cui H, Zhao T, et al. Multivalency transforms SARS-CoV-2 antibodies into ultrapotent neutralizers. Nat Commun. 2021;12(1):3661. https://doi.org/10.1038/s41467-021-23825-2.
166 Lu Y, Li Q, Fan H, Liao C, Zhang J, Hu H, et al. A multivalent and thermostable nanobody neutralizing SARS-CoV-2 omicron (B.1.1.529). Int J Nanomedicine. 2023;18:353–67. https://doi.org/10.2147/IJN.S387160.
167. Ibrahim M, Ramadan E, Elsadek NE, Emam SE, Shimizu T, Ando H, et al. Polyethylene glycol (PEG): the nature, immunogenicity, and role in the hypersensitivity of PEGylated products. J Control Release. 2022;351:215–30. https://doi.org/10.1016/j.jconrel.2022.09.031.
168. Pillarsetty N, Carter LM, Lewis JS, Reiner T. Oncology-inspired treatment options for COVID-19. J Nucl Med. 2020;61(12):1720–3. https://doi.org/10.2967/jnumed.120.249748.
169. Tu B, Gao Y, An X, Wang H, Huang Y. Localized delivery of nanomedicine and antibodies for combating COVID-19. Acta Pharm Sin B. 2023;13(5):1828–46. https://doi.org/10.1016/j.apsb.2022.09.011.
170 Cruz-Teran C, Tiruthani K, McSweeney M, Ma A, Pickles R, Lai SK. Challenges and opportunities for antiviral monoclonal antibodies as COVID-19 therapy. Adv Drug Deliv Rev. 2021;169:100–17. https://doi.org/10.1016/j.addr.2020.12.004.
171 Yu J, Uzuner U, Long B, Wang Z, Yuan JS, Dai SY. Artificial intelligence-based HDX (AI-HDX) prediction reveals fundamental characteristics to protein dynamics: mechanisms on

SARS-CoV-2 immune escape. iScience. 2023;26(4):106282. https://doi.org/10.1016/j.isci.2023.106282.

172. Kapingidza B, Marston DJ, Harris C, Wrapp D, Winters K, Rhodes B, et al. Engineered immunogens to expose conserved epitopes targeted by broad coronavirus antibodies. bioRxiv [Preprint]. 2023;28:2023.02.27.530277. https://doi.org/10.1101/2023.02.27.530277.

173. Fassi EMA, Manenti M, Citarella A, Dei Cas M, Casati S, Micale N, et al. Computational design, synthesis, and biophysical evaluation of β-amido boronic acids as SARS-CoV-2 Mpro inhibitors. Molecules. 2023;28(5):2356. https://doi.org/10.3390/molecules28052356.

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