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*Review*

# Monoclonal Antibodies as Potential COVID-19 Therapeutic Agents

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**Abstract:** The COVID-19 pandemic continues to cause tremendous loss of life and put massive strain on the functioning of societies worldwide. Despite the cataclysmic proportions of this viral outbreak, as of yet, no effective curative treatment is available. COVID-19 vaccines, while effective and a scientific achievement of historical proportions, can only be utilized in prophylaxis and require vaccination of the majority of a given population. Convalescent plasma therapies require blood group testing and patient hospitalization and are difficult to put into place in the scale of a population. Monoclonal antibodies can be mass produced with hybridoma cell culture and are highly specific to viral antigens. What is more, monoclonal antibodies produce far more reproducible effects than other approaches to active immunization and can be further enhanced through engineering. Currently, there exist two approaches to COVID-19 treatment with use of monoclonal antibodies, each with several antibodies currently under development or in clinical testing. The first of the approaches utilizes monoclonal antibodies, which target viral spike proteins to block viral entry into host cell and mark viral particles for destruction by host immune cells. The second approach utilizes antibodies that neutralize cytokines, which take part in cytokine release syndrome, which is responsible for many of the most damaging symptoms associated with COVID-19, thus reducing systemic inflammation and ultimately—patient morbidity and mortality. There yet remain several challenges to overcome if monoclonal antibodies are to become mainstream therapeutic agents in the treatment of COVID-19. Despite this, this field of research is experiencing a massive forward leap and the exceptional amount of clinical data gathered so far can serve as groundwork for the development of effective and widely available antiviral monoclonal antibody treatments.

**Keywords:** monoclonal antibodies; immunotherapy; active immunisation; COVID-19; anti-spike mAb; anti-CD6 mAb; anti-IL6 mAb



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## 1. Introduction

A novel coronavirus disease (COVID-19) caused by the coronavirus SARS-CoV-2 broke out in Wuhan, China at the end of 2019, most likely through zoonosis from an unknown animal host. After several months of rapid spread, the outbreak has grown to a pandemic. By January 2022, over 370 million infections have been confirmed worldwide, with over 5.6 million cases proving fatal.

Coronaviruses (CoVs) infect a range of mammalian and avian hosts, including humans, and cause diseases ranging from the common cold to more severe conditions such as SARS and MERS. CoVs bear strong resemblance to one another in terms of morphology and chemical structure, and are all spherical or pleomorphic enveloped viruses containing positive-sense ssRNA. All CoVs express distinctive spike proteins on the capsid surface.

Despite numerous efforts directed at the development of COVID-19 curatives, none have proven sufficient so far. Vaccination, while effective, can only be used in prophylaxis and requires administration to the majority of a population to be successful. Much of the attention has been focused on utilizing vaccines, convalescent plasma (CP) infusions and antiviral agents. Despite being a critical component of the adaptive immune reaction, monoclonal antibodies have received comparatively less consideration. Anti-SARS-CoV-2

neutralizing monoclonal antibodies (mAbs) have the potential to become both therapeutic and prophylactic agents. Several mAbs are currently being researched as candidates for treatment of COVID-19 [1].

## 2. Monoclonal Antibodies as Therapeutic Agents

Antibodies are key players in humoral immune reactions to infections. Binding to toxins or pathogen surface antigens allows mAbs to either neutralize deleterious effects, or when binding to cells—identify them as targets for destruction by white blood cells. There are several ways for antibody-tagged pathogens to be eliminated—antibody-dependent cellular phagocytosis by white blood cells, antibody-dependent cellular cytotoxicity, or complement activation [2]. Antibodies are made up of antigen-binding fragments (Fab), which provide antigen specificity and crystallisable fragments (Fc), which mediate interactions with biological effectors. The variability of these two fragments is responsible for the narrow specificity and immense range of antibody function [3].

Hybridoma cell culture, a novel technique resulting in the immortalization of B lymphocyte clones, allows mass production of identical (monoclonal) antibodies—mAbs. The usage of mAbs allows researchers to design therapeutics against very narrowly defined pathogens, with high specificity, low off-target action, and reproducible effects. These traits are significant advantages over the traditional serum infusion approach to passive immunization [1]. Monoclonal antibodies can also be engineered to influence specific effectors or modulate other characteristics. What is more, mAbs can be used in conjunction with other therapeutics or other mAbs targeting different epitopes to achieve synergistic effects [4].

Over the course of millennia, pathogenic microbes have evolved traits which allow them to avoid antibodies, making anti-pathogen mAb design severely challenging [5]. Putting even effective mAbs to clinical use remains a challenge, as reaching adequate concentrations requires patient hospitalization and precisely dosed IV infusions. Costs of production, and thus treatment, remain high. These and several other issues afflicting the development and implementation of mAbs for the clinical environment have resulted in but a handful of mAbs achieving widespread therapeutic usage.

However, change is on the horizon as the ongoing pandemic has rekindled interest in therapeutic mAbs. Coupled with recent advances in the understanding of molecular form-function relationships in antibodies and hybridoma cell culture, this renewed interest has the potential to bring about a revolution in mAb therapies. In this review, several novel monoclonal antibodies with the potential of becoming COVID-19 therapeutics are discussed [6].

## 3. Immunotherapy in the Treatment of COVID-19

During the COVID-19 pandemic, convalescent plasma (CP) therapy, which involves the transfusion of plasma from patients recovered from a SARS-CoV-2 infection to infected patients or patients at high risk of infection has been utilized to some effect. During previous coronavirus outbreaks passive immunization, especially achieved through CP therapy has proven to be an effective treatment. Thus, the treatment has been suggested as a potential therapeutic approach for COVID patients [7].

While the treatment has been approved by the U.S. FDA by way of an Emergency Use Authorization (EUA), many subsequent studies have either shown no significant improvements upon administering the treatment, or have only shown improvements in severely or critically ill patients [8–10]. RECOVERY, CONCOR-1, and REMAP-CAP15, three largest randomized clinical trials evaluating the efficacy of CP in the treatment of COVID-19 were stopped due to perceived futility [11–13]. Thus, considering the lack of definitive data in support of using CP in treatment of hospitalized COVID-19 patients, the U.S. NIH does not recommend its use [14]. The collaborative RECOVERY group concluded that convalescent plasma treatment provides no benefit overall, and found no significant difference across subgroups, including seronegative and seropositive patients, as specified

by anti-SARS-CoV-2 antibody tests. This led the UK Medicines and Healthcare products Regulatory Agency (MHRA) to recommend against using CP for treatment of COVID-19 patients, resulting in the removal of this treatment from NHS standard practice [15].

What is more, there are several limitations which significantly decrease the viability of CP therapy as an approach to COVID-19 treatment. Binding and neutralizing antibody titers vary significantly between samples, and blood types must be matched between donor and recipient to minimize the risk of transfusion reactions. Furthermore, plasma samples must undergo screening for blood-borne pathogens [16,17].

However, CP therapy is not without advantages, as for example CP can easily be collected in high volume via apheresis, without impacting patient hemoglobin levels significantly [18]. As such, some researchers are not as quick to dismiss CP therapy as the FDA or MHRA. In a re-analysis of the RECOVERY trial, Hamilton et al. point towards the importance of recognizing patients likely to benefit from CP therapy, before accepting its inefficacy. The basis of CP is passive immunization—in the case of CP, an infusion of a mixture of antibodies into the system of a patient. In most cases, antibodies develop about seven days post-infection as part of the immune response. Thus, if CP is to be beneficial, it should be administered to seronegative or otherwise immunologically impaired patients. In this re-analysis, upon comparing patients presenting early to those presenting late (based on patient history), a chance of benefit upon CP treatment with a NNT (Number Needed to Treat) of 100 was found to be ~7% for the group presenting late, and ~90% for the group presenting early, strongly underlining the need of identifying potential candidates for CP therapy early [19].

Furthermore, analysis of immunological data shows that a lack of early antibody responses correlates strongly with a poorer patient prognosis and more severe symptoms, or even persistent disease in patients' fully deficient in antibodies [20]. Data from 20,000 U.S. patients transfused with CP demonstrates low incidence of adverse transfusion reactions—less than 1% of patients. This indicates CP transfusions carry no risk of complications beyond the standard risk expected for plasma transfusions in severely ill patients [21]. These data paint a picture of convalescent plasma therapy, which leaves much to be desired in the context of a general COVID-19 treatment, however this approach should not be discounted altogether, as there is a population of patients, which, if identified early and precisely, can benefit from CP therapy.

Monoclonal antibody treatments on the other hand, potentially offer a two-pronged approach to combating SARS-CoV-2 infections. Firstly, mAbs can be utilized to reduce cytokine storm intensity in COVID-19 patients and alleviate symptoms. SARS-CoV-2 infection is characterized by a severe upregulation of inflammatory cytokines. This suggests that the cytokine storm, also termed hypercytokinemia, plays a key role in COVID-19 pathogenesis. Notably, elevated levels of the proinflammatory IL-1 and IL-6 cytokines correlate with more severe symptoms in patients. This elevation of cytokine levels is also seen in cytokine release syndrome (CRS), which may point towards a common mechanism between CRS and COVID-19. Thus, mAbs designed to bind IL-1 or IL-6 may prove to be viable therapeutics for SARS-CoV-2 infection treatment [22].

Secondly, an option considered from the very beginning of the SARS-CoV-2 outbreak [23] is to utilize neutralizing monoclonal antibodies, which target viral surface spike glycoproteins, thus preventing the virus from entering host cells. Viral penetration of host cell membranes is initiated by an interaction between the host angiotensin-converting enzyme 2 (ACE2) receptor and the viral spike protein [16]. Neutralizing mAbs can block this interaction. Most mAbs isolated so far target the receptor binding domain (RBD) of the viral spike protein. This domain mediates spike-ACE2 receptor binding [24,25]. Based on the current understanding of MERS-CoV and SARS-CoV however, neutralizing antibodies that target other spike protein regions should also exist [26]. Figure 1 shows a diagram of the mechanism of action of anti-SARS-CoV-2 Spike protein neutralizing antibodies.

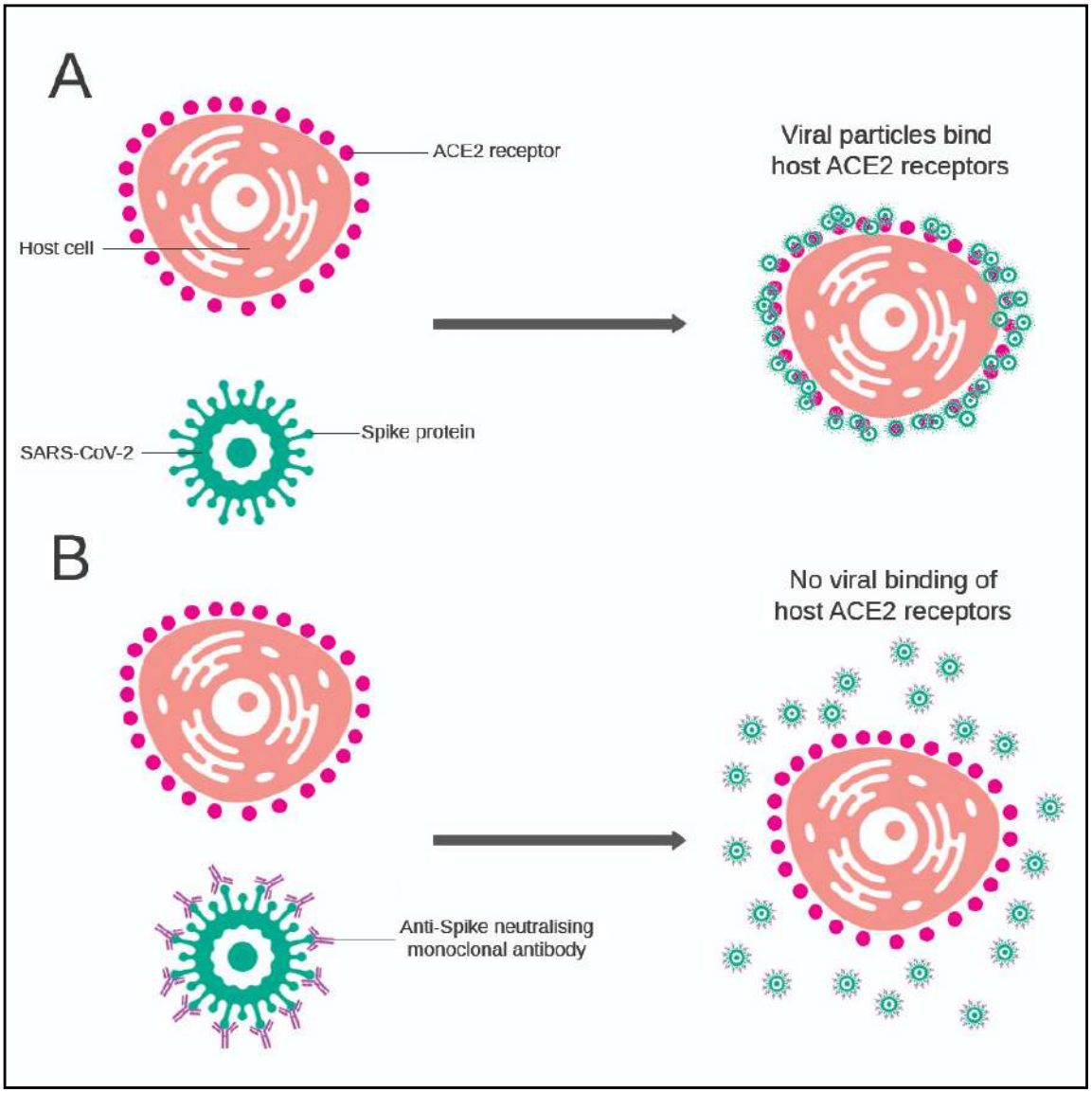


**Figure 1.** (**A**)—In immunologically naive hosts, SARS-CoV-2 particles invade host cells via membrane fusion following the binding of viral spike protein receptor binding domains with host ACE2 receptors. (**B**)—In COVID-19 convalescent hosts or patients receiving immunotherapy, SARS-CoV-2 spike proteins are recognized and bound by a range of anti-spike monoclonal antibodies, which prevent viral binding of host ACE2 receptors and cell invasion.

## 4. Anti-SARS-CoV-2 Spike Protein Monoclonal Antibodies as COVID-19 Therapeutics

CR3022 is a monoclonal antibody derived from the serum of a COVID-19 patient. The antibody binds a conserved epitope of the SARS-CoV-2 RBD [23]. CR3022 is remarkable in that it binds viral RBD and activates host effector cells even in the presence of human ACE2 [23]. Thus, the mAb has the potential to achieve infected cell eradication despite considerable amounts of ACE2 being secreted from infected cells [27]. What is more, CR3022 has been described to be able to trigger several immune reactions—antibody-dependent NK cell activation, antibody-dependent neutrophil phagocytosis, antibody dependent monocyte phagocytosis and antibody-dependent complement deposition. Most other neutralizing mAbs compete with ACE2 for RBD binding. CR3022 on the other hand may still achieve removal of infected cells or viral particles even following the infection-related upsurge of ACE2 expression [27].

CR3022 production was carried out through expression in *E. coli* HB2151 cells, the resulting antibodies were purified using Ni-NTA, a nickel-charged affinity resin that can be used to purify recombinant proteins [23]. CR3022 was found to bind the SARS-CoV-2 RBD at a KD of 6.3 nM, as determined by ELISA and BLI [23].

Recently, CR3022 has been found to block SARS-CoV-2 interaction with vimentin, which is a protein expressed in human endothelial cells. Vimentin is thought to interact with SARS-CoV-2 spike proteins and facilitate host cell entry. This interaction is thought to promote the development of infection and contribute to COVID-19 progression, in particular to the vascular complications caused by the disease [28].

Unfortunately, early testing of CR3022 in mouse and hamster models of COVID-19 have shown considerable amounts of inflammation-related morbidity in subjects treated with CR3022. Authors of the initial CR3022 study emphasize the importance of creating engineered Fc variants of the antibody to limit treatment-induced pathology and maximize protective effects before the antibody can be studied in a clinical setting [27,29].

ADG-2 is an engineered neutralizing monoclonal antibody, which targets an epitope highly conserved between all clade 1 sarbecoviruses, including SARS-CoV-2 [30]. ADG2 production is carried out through expression in CHO cells as full-length IgG1 proteins. Gene fragments encoding the VH and VL domains of the antibody are subcloned into vectors, which are used to transfect CHO cells. The antibodies are purified using a protein A purification protocol [30]. ADG-2 was found to bind the RBD of every sarbecovirus, which exhibited ACE2 binding, including SARS-CoV-2 with high affinity, exhibiting KD values ranging from 0.24 to 1.12 nM [30]. ADG-2 binds to the viral spike protein RBD of almost all wild type variants of SARS-CoV-2, excluding four which as of December 2020 constituted less than 0.001% of the global viral strain distribution [30]. As many other neutralizing mAbs recognize epitopes that vary highly between SARS-CoV-2 strains and other sarbecoviruses, their action is susceptible to antibody escape mutations.

ADG-2 on the other hand, due to its broad effectiveness is a promising candidate for protection not only against SARS-CoV-2 but even future respiratory diseases caused by sarbecoviruses. The antibody triggers Fc-mediated effector cells robustly. In particular, it recruits phagocytes and activates NK cells. Compared to sham-treated mice, mice treated with ADG-2 in a therapeutic setting had lower levels of weight loss, respiratory function changes and lung pathology. What is more, these curative effects were intensified when ADG-2 was administered to mice prophylactically. These results point towards the mAb being a potential candidate for robust COVID-19 treatment [30]. ADG-2 has since been modified and renamed to ADG20, and is currently undergoing a large-scale international Phase 2/3 clinical trial [31].

Two anti-SARS-CoV-2-spike mAbs—bamlanivimab (formerly known as LY-CoV555) and etesevimab (formerly known as CB6), were isolated from the plasma of convalescent patients from the U.S. and China, respectively. Bamlanivimab had previously been accepted as an emergency use single-agent treatment by the U.S. FDA, but this authorization has been withdrawn due to a lack of efficacy against emerging variants. As in preclinical studies, etesevimab was proven to bind an epitope other than the one bound by bamlanivimab, the idea of a combination therapy emerged. Subsequently, the antibodies were tested clinically as a combination therapy [32,33]. Etesevimab was also shown to neutralize SARS-CoV-2 variants with mutations conferring resistance to bamlanivimab thanks to differences in epitopes targeted by the mAbs [34]. Large scale production of the antibodies utilized expression vectors for both chains, which were transfected into CHO cells for expression. Expressed antibodies were purified using a protein A-based purification protocol [34]. The KD equilibrium values against the SARS-CoV-2 RBD were established as 2.49 nM for etesevimab and 3.5 nM for bamlanivimab [32,33].

BLAZE-1 was a phase 2/3 clinical trial conducted at 49 health centers in the US, which included 613 ambulatory patients suffering from light to moderate COVID-19 symptoms, who have tested positive for SARS-CoV-2 presence. The patients were randomized to receive either a single dose of bamlanivimab, either 700 mg, 2800 mg, or 2800 mg, both

bamlanivimab and etesevimab 2800 mg or placebo [34]. In the trial, changes in patient viral loads within monotherapy groups did not differ significantly from the placebo group. Patients in the combination therapy group however, were observed to have statistically significantly lowered viral loads as compared to the placebo group. The numbers of patients hospitalized due to COVID-19 have dropped for the monotherapy groups and the combination therapy group, as compared to the placebo group. However, this difference was statistically significant only for the combination therapy group. To ascertain whether the viral load reduction seen in the combination therapy group would translate to tangible clinical results, further clinical studies must be performed with these mAbs [34].

The mAbs as a combination therapy, have received an Emergency Use Authorization for patients suffering from severe COVID-19 by the U.S. FDA, following the successful clinical trial results. Eli Lilly, the manufacturer of the two antibodies was contracted by the U.S. government to supply 614,000 doses of the antibodies by January 2022 at the latest, for a price of USD 1.29 billion, which amounts to USD 2100 per dose [35]. Unfortunately, due to the mAb cocktail being ineffective against the Omicron variant, in January 2022 the FDA authorization for use of bamlanivimab and etesevimab against COVID-19 has been limited to patients infected with a COVID variant known to be treatable with these drugs [36].

Casirivimab and imdevimab (used clinically as a cocktail known as REGEN-COV) are recombinant neutralizing human IgG1 mAbs, which bind the SARS-CoV-2 spike protein RBD with high affinities, with KD values of 45.8 and 46.7 pmol/L, respectively. Each of the mAbs blocks RBD-ACE2 interactions almost completely in vitro with an efficacy of over 95% [37]. The epitopes for each mAb do not overlap, which can lead to a decrease in viral mutation development. REGEN-COV was found to retain activity against every SARS-CoV-2 variant of concern aside from the Omicron variant [38]. Casirivimab and Imdevimab are produced in CHO cells. Purification is performed with a series of steps including chromatography, diafiltration, viral inactivation and filtration. The active substances are subsequently formulated with excipients [39].

In COVID-19 patients, REGEN-COV treatment has not been found to select for immune escape variants or affect viral evolution. What is more, in SARS-CoV-2 hamster model, no resistant variants have been detected in any of the 20 animals in the group, which received the full combination of antibodies. In the groups receiving casirivimab/imdevimab as a single agent, immune escape variants were found in 18 out of 40 animals [40].

In a double-blind, phase 1–3 clinical trial conducted by Regeneron, the effects of REGEN-COV were tested in 275 non-hospitalized symptomatic COVID-19 patients. The antibody combination was found to successfully reduce viral load, especially in patients who at trial entry have not yet exhibited a detectable immune response. Higher viral load in COVID-19 is related with poorer clinical outcomes. Consistently, more rapid viral clearance within the trial correlated with more favorable clinical outcomes [41].

REGEN-COV has received emergency use authorizations for use in treatment of COVID-19 in a range of countries, including the USA, UK, India, Canada, Switzerland, Japan, and the European Union [42]. Regeneron agreed to supply 1.4 million doses of REGEN-COV to the U.S. government at USD 2100 per dose by the end of January 2022 [43].

Unfortunately, due to the mAb cocktail being ineffective against the Omicron variant, the authorization for use of REGEN-COV2 against COVID-19 has been limited to patients infected with a COVID variant known to be treatable with REGEN-COV by the U.S. FDA in January 2022 [44]. Importantly, the EU EMA has not rescinded its authorization, and REGEN-COV remains widely used within the European Union.

Sotrovimab (formerly known as VIR-7831) is another anti-spike mAb, which has received an Emergency Use Authorization (EUA) for treatment of COVID-19 by the U.S. FDA. Sotrovimab is priced at USD 2100 per dose in the U.S. [45].

The mAb is authorized for use in patients with mild to moderate COVID-19 at high risk of progression to severe disease.

Sotrovimab is a human IgG1 monoclonal antibody consisting of two duplicate light chain polypeptides composed of 214 amino acids and two duplicate heavy chain polypep-

tides, each composed of 457 amino acids. Sotrovimab is produced using a Chinese Hamster Ovary cell line [46]. The Fc region of Sotrovimab has been engineered for enhanced bioavailability and a longer half-life in the lungs, allowing the sustaining of therapeutic concentrations for longer periods [47].

Sotrovimab binds a conserved epitope of the SARS-CoV-2 spike protein and through an unknown mechanism prevents membrane fusion after viral RBD-host ACE2 binding occurs [48]. Sotrovimab was shown to bind the SARS-CoV-2 SB domain with sub-picomolar avidity, and the S glycoprotein ectodomain trimer with picomolar avidity [25].

The EUA was issued based on results from COMET-ICE, a double-blind clinical trial investigating the effects of sotrovimab in 583 patients at least 55 years of age affected by mild to moderate COVID-19 and suffering from one or more comorbidities. By the 29th day of the trial, 7% of patients in the placebo group experienced COVID-19 progression defined by hospitalization or death. In the sotrovimab group, only 1% of patients experienced COVID-19 progression. Thus far, Sotrovimab has retained full efficacy against all SARS-CoV-2 variants of concern, including the recently emerged Omicron variant [49].

This broad efficacy independent of viral mutations may be owed to Sotrovimab being an engineered mAb descended from S309, an antibody isolated from a SARS-CoV-1 patient, which targeted a highly conserved epitope, which was preserved throughout the course of SARS-CoV-2 evolution. In contrast, RBD-binding anti-SARS-CoV-2 antibodies frequently target highly mutable epitopes, which renders their efficacy vulnerable to viral immune escape [25].

BGB-DXP593 (formerly known as BD-368-2) is a neutralizing mAb developed in collaboration between two Chinese biotechnology companies, Beigene and Singlomics. The structure of BGB-DXP593, as observed by cryo-EM, shows that the mAb can fully block viral binding of ACE2 by completely covering the viral RBD trimer and changing its conformation. The KD constant against the SARS-CoV-2 RBD for BGB-DXP593 was measured as 0.54 nM [50]. The mAb was discovered through single-cell RNA sequencing and VDJ sequencing of convalescent patient B cells. In an in vivo experiment on a human ACE2 mouse model, BGB-DXP593 was shown to prevent infection altogether if administered pre-infection, or limit virus load significantly if administered two hours post infection.

BGB-DXP593 is currently undergoing double-blind randomized placebo-controlled trials as part of a phase II clinical trial. 180 adult outpatients exhibiting COVID-19 symptoms were recruited for the trial and assigned to either a control group or one of two treatment groups. Following treatment, the participants were observed over a period of 85 days to evaluate the safety and efficacy of BGB-DXP593 as a COVID-19 therapeutic. The primary outcome will be viral load at day eight post infection as measured by RT-qPCR testing [51,52].

CT-P59 (regdanvimab) is a fully human neutralizing anti-SARS-CoV-2 mAb isolated from the plasma of a convalescent Korean patient by the Celltrion Group acting for the Korea Center for Disease Control. The mAb is produced through recombinant DNA technology in a CHO cell line [53]. CT-P59 blocks SARS-CoV-2 particles from entering host cells by binding the viral Spike protein RBD region, thus stopping the virus from interacting with host ACE2 receptors [54]. According to crystallographic data, CT-P59 does not bind the RBD at the highly mutable positions 367, 364 and 436, which may improve its efficacy against future SARS-CoV-2 variants. Through surface Plasmon resonance analysis, CT-P59 was demonstrated to have a high affinity against the SARS-CoV-2 RBD, with a KD constant of 27 pM [55]. In vitro, CT-P59 retained efficacy against the variants of concern Gamma, Delta, Epsilon and Kappa [56]. Following favorable phase II/III trial outcomes [57], CT-P59 has been approved as a therapeutic for COVID-19 in the European Union under the trade name Regikrona in November, 2021 [58].

The neutralizing mAb 47D11, of the IgG1 subclass, which binds to the SARS-CoV-2 spike protein, does so in particular to a conserved epitope of the spike RBD domain. To produce 47D11, cDNA fragments encoding the 47D11 heavy and light chain variable regions were sub-cloned into expression plasmids. Then, 47D11 was expressed in HEK-293T cells transiently transfected expression plasmids. The expressed antibodies were purified

from cell culture supernatants through Protein-A affinity chromatography [59]. As such, 47D11 was found to bind SARS-CoV-2 S1B at a KD value of 9.2 nM [59]. Interestingly, this mAb achieves virus neutralization despite binding away from the receptor-binding motif and thus acting through a mechanism different than inhibition of RBD-ACE2 binding. Moreover, 47D11 can cross-neutralize both SARS-CoV and SARS-CoV-2, making it a possible therapeutic agent against Sarbecovirus-related diseases of the future. As 47D11 neutralizes viral particles through a mechanism independent from receptor-spike binding interference, it may be used in combination with other mAbs that target the viral RBD domain. Targeting non-overlapping epitopes with a combination therapy may result in synergistic action, in turn lowering the required mAb dosage and reducing the risk of immune escape mutations [59,60].

Recently, new insights into the mechanism of 47D11 action were achieved. The mAb has been found to stabilize SARS spike proteins in a semi-open conformation. Other RBD-binding mAbs, which do not compete with ACE2-spike interactions, such as C144 or S2M11 [61,62] are known to lock the spike proteins in a closed conformation. This mechanism of action grants 47D11 the ability to render spike proteins vulnerable to the action of mAbs, which target areas of the protein only reachable in the open conformation, such as CR3022. This further underlines the potential of 47D11 as part of a combination therapy. What is more, 47D11 activity was found to be unaffected by recently arisen SARS-CoV-2 RBD mutants, namely N501Y, K417K and E484K [63].

Furthermore, H4 and B38 are another pair of mAbs targeting non-overlapping epitopes of the SARS-CoV-2 RBD. These mAbs compete with SARS-CoV-2 binding to host ACE2 receptors. The KD values for the two mAbs against the SARS-CoV-2 RBD were measured using surface Plasmon resonance (SPR) and established as 70.1 nM for B38, and 4.48 nM for H4 [64]. To test the mAbs as protective agents against COVID-19 in vivo, mice engineered to express human ACE2 were administered a single dose of either B38 or H4. At three days post infection, viral RNA load in the lungs of both the B38 and H4 groups were found to be significantly lower than those in the control group. Severe lung pathologies were observed in the lungs of control group mice, including bronchopneumonia, interstitial pneumonia, and alveolar edema. Infection-related lesions were absent in the B38 group, and only cases of mild bronchopneumonia were found in the H4 group [64].

As of yet, no results have been published for combination therapies utilizing this pair or mAbs, and no clinical trials are underway, despite promising animal model results.

Evusheld (AZD7442) is a combination of two long-acting antibodies (LAAbs)—cilgavimab (AZD1061), and tixagevimab (AZD8895) derived from SARS-CoV-2 convalescent patients. The antibodies bind to distinct sites of the viral spike protein [65]. The antibodies have been engineered by AstraZeneca to extend half-life and reduce off-site binding. Fab regions of the two antibody components of Evusheld were expressed in CHO cells through transient transfection with an expression plasmid. The recombinant antibodies were purified from cell culture supernatants utilizing anti-CH1 capture columns [65]. Evusheld has been proposed as a pre-exposure prophylactic treatment for patients unable to receive the SARS-CoV-2 vaccines, or unlikely to benefit from them—such as cancer patients undergoing chemotherapy, or patients receiving immunosuppressive treatments for multiple sclerosis or rheumatoid arthritis or following a transplant.

Evusheld is administered as two separate sequential intramuscular injections—cilgavimab 150 mg and tixagevimab 150 mg [66]. Removing the requirement of an intravenous infusion allows the administration of this treatment by a general practitioner, removing the need for patient hospitalization.

The introduction of Evusheld treatment into clinical practice is primarily supported by data from PROVENT, an ongoing phase III pre-exposure prophylaxis trial. PROVENT showed a reduction of 77% in the risk of patients developing COVID-19 symptoms, as compared to placebo. The protective effects have been shown to persist for at least six months. Further studies are underway to establish the full duration of protective effects [67].

The efficacy of Evusheld against the SARS-CoV-2 Omicron variant is currently being researched. According to AstraZeneca, to date, none of the Omicron binding site mutations tested in vitro have resulted in immune escape and Evusheld is capable of neutralizing other emergent SARS-CoV-2 variants, including Delta and Mu [67]. However, in a December 2021 study, Evusheld was shown to have a 58-fold reduction in neutralizing activity against the Omicron variant, as compared to the Delta variant [68].

In December 2021, the U.S. FDA issued an EUA for Evusheld as a pre-exposure prophylactic treatment for SARS-CoV-2. The EUA recommends Evusheld administration for patients who have not been recently infected by or exposed to SARS-CoV-2 and are at risk of inadequate immune response or severe adverse reaction to available COVID-19 vaccines [66]. The U.S. Government ordered a million doses of Evusheld from AstraZeneca in February 2022. The costs of this contract have not yet been made publicly available [69].

In March 2021, the UK MHRA and EU EMA approved Evusheld for use in adults who are unlikely to mount an immune response following COVID-19 vaccination or at risk of adverse reactions to COVID-19 vaccination, and who are not currently infected or had recent exposure to SARS-CoV-2. The MHRA also advises that the administration of 600 mg, instead of 300 mg of Evusheld may confer a protective effect against the Omicron variant [70].

A study performed on patients recruited from Strasbourg and Lyon University Hospitals suggests that consistently with the EMA guidelines, a 300 mg dose of Evusheld was insufficient in 90% of patients to elicit an anti-RDB titer, which would confer in vivo neutralizing activity against the Omicron variant [71].

LY-CoV1404, also known as bebtelovimab is a fully human IgG1 anti-SARS-CoV-2 RBD mAb. Importantly, the mAb has been shown to bind and neutralize all currently known SARS-CoV-2 variants of concern (VOCs), including Omicron. LY-CoV1404 binds an epitope unaffected by widely circulating mutations, rendering it safe from viral escape.

The binding affinity of LY-CoV1404 against the S protein RBD was determined to be between 75 and 220 pM, depending on the assay used. No loss in affinity was detected when testing LY-CoV1404 against SARS-CoV-2 VOC S proteins, which supports the notion that LY-CoV1404 binds an epitope unaffected by prevalent VOC mutations [72].

An immunofluorescence assay was carried out to assess LY-CoV1404 in vitro neutralization activities against authentic and pseudo-typed SARS-CoV-2 subpopulations. LY-CoV1404 retained its neutralization potency against all variants, including the Omicron variant. Neutralization activities for a range of other antibodies, including Sotrovimab and ADG20 were also studied against the same viral isolates, and LY-CoV1404 was the only mAb to retain full potency against the Omicron variant.

Based on structural analysis and the GISAID EpiCoV database, frequencies of mutations in the RBD amino acids of the LY-CoV1404 binding site were established. Only two changes were detected, with 99.4% and 78.2% conservation rates. Neither of the changes is expected to affect LY-CoV1404 binding affinity if it were to emerge [72]. These data demonstrate that the LY-CoV1404 epitope has remained virtually unchanged throughout the pandemic.

LY-CoV1404 activity was also measured in the context of mutations known to limit the activity of other anti-SARS-CoV-2 mAbs. Only three very rarely occurring mutations (K444Q, V445A, and G446V) were found to impact LY-CoV1404 activity, with limited severity. This data indicates that aside from being highly effective against known VOCs, LY-CoV1404 is likely to retain its activity against prospective future VOCs [72].

LY-CoV1404 production was carried out through transfection of mammalian cell lines with pcDNA vectors and cell culturing. The antibodies were purified from cell culture supernatants using nickel-based sepharose columns and size exclusion chromatography [72].

As part of the BLAZE-4 trial, bebtelovimab was tested in a clinical environment. The study was a randomized, single dose phase 1/2 trial conducted on US-based ambulatory patients presenting with mild or moderate COVID-19 symptoms within three days after

initial positive SARS-CoV-2 test results. Patients were classified as high or low-risk, based on likelihood of progression to severe COVID-19.

In phase 1, ascending doses and IV infusion rates of bebtelovimab were tested in low-risk patients.

In phase 2, low and high-risk patients were randomized to either bebtelovimab, bebtelovimab + bamlanivimab + etesevimab, bamlanivimab + etesevimab, or (only in the case of low-risk patients) placebo arms of the trial. Assigned treatments were administered intravenously, in a single dose.

Upon examination of pre-established viral load endpoints, statistically significant reductions in viral load at days five and eleven from baseline were found in patients treated with bebtelovimab and bebtelovimab + bamlanivimab + etesevimab, compared to placebo. No statistically significant differences between the bebtelovimab or bebtelovimab + bamlanivimab + etesevimab groups were found at other examined time points, relative to placebo. What is more, the time of symptom resolution decreased significantly in patients treated with bebtelovimab, relative to placebo [73].

As part of this study, in vitro neutralization potencies of the tested mAbs against SARS-CoV-2 isolates were also tested. All mAb combinations tested succeeded in neutralizing wild type and delta variant SARS-CoV-2. Bebtelovimab was the only mAb fully successful in neutralizing the Omicron isolate, with an IC99 estimated at 2.44 ng/mL.

This clinical trial was characterized by several limitations. Patients were recruited only from the US. Only the low-risk patient group was placebo controlled. The study was conducted before the emergence of the Omicron variant. As such, there is no clinical data on the in vivo efficacy of bebtelovimab against Omicron [73].

However, non-clinical data on the neutralisation of Omicron by bebtelovimab was deemed sufficient by the U.S. FDA. The results of this study in conjunction with the in vitro virus neutralization studies resulted in the FDA issuing an EUA for bebtelovimab for the treatment of patients at high risk of severe COVID-19 in February 2022 [73].

The U.S. government ordered 600,000 doses of bebtelovimab from Eli Lilly for USD 720 million, which amounts to USD 1200 per dose [74].

## 5. Efficacy of Neutralizing Monoclonal Antibodies against SARS-CoV-2 Variants of Concern

Since the emergence of the Omicron variant (previously known as B.1.1.529) in November 2021, and its rapid designation as a Variant Of Concern, Omicron has supplanted the Delta lineage as the globally dominant SARS-CoV-2 variant. Critically, due to several mutations in the viral RBD, Omicron became capable of avoiding neutralization by a significant portion of widely used therapeutics. Thus, the ability to neutralize the Omicron variant became a key factor to be considered in the evaluation of all anti-SARS-CoV-2 mAb treatments.

The Omicron Spike protein contains 29 amino acid substitution mutations, three deletions and three insertions, as compared to the original Wuhan strain. Critically, 15 of these amino acid changes are localized in the RBD—the target of most neutralizing mAb therapeutics. Neutralizing mAbs are subdivided into four classes, depending on their mode of action. Classes 1 and 2 compete with viral binding of host ACE2, while classes 3 and 4 inhibit viral activity through other mechanisms and bind away from the viral ACE2 binding motif. Class 2 and 3 mAbs can bind viral spike proteins regardless of RBD conformation, while class 1 and 4 mAbs bind the RBD only in the up conformation. Previously described variants exhibited mutations, which affected the binding sites of class 1 and 2 mAbs only, while Omicron displays mutations, which affect the binding of all mAb classes [68].

In a study conducted in December 2021, nine mAbs approved for clinical use in COVID-19 patients were tested via the S-Fuse neutralization assay to evaluate their efficacy against Omicron. The antibodies tested were bamlanivimab and etesevimab, casirivimab and imdevimab (REGEN-COV), cilgavimab and tixagevimab (Evusheld), regdanvimab (Regikrona), sotrovimab, and ADG20.

Activities of the nine mAbs were measured in vitro against Omicron and variant Delta for comparison. Aside from bamlanivimab, all antibodies were successful in neutralising Delta. Five of the antibodies (bamlanivimab, etesevimab, casirivimab, imdevimab and regdanvimab) exhibited no neutralizing activity against Omicron whatsoever. Of the other four, sotrovimab was the only mAb which did not display a severe reduction in activity, with a 2.8-fold decrease. Importantly, the decrease seen in the neutralizing activity of sotrovimab is within the threshold of "no change in efficacy" as defined by the U.S. FDA. Combinations of mAbs mimicking clinically utilized cocktails were also studied. Bamlanivimab and etesevimab, and casirivimab and imdevimab (REGEN-COV) exhibited no activity against Omicron. Cilgavimab + tixagevimab (Evusheld) displayed a 58-fold decrease in activity against Omicron, as compared to Delta [68].

In the same study, flow cytometry was utilized to examine mAb binding to cells infected with either Delta or Omicron, at antibody concentrations of 1 μg/mL and 0.1 μg/mL. The five mAbs, which lost all activity against Omicron in the neutralisation assay, displayed extreme levels of decrease in binding to Omicron-infected cells, as compared to Delta-infected cells (up to 242-fold). The binding abilities of the other four antibodies were impaired to a lesser degree: Sotrovimab—6-fold at 1 μg/mL and 4-fold at 0.1 μg/mL, ADG20- 2-fold at 1 μg/mL and 2-fold at 0.1 μg/mL and Cilgavimab—3-fold at 1 μg/mL and 3-fold at 0.1 μg/mL, and tixagevimab—9-fold at 1 μg/mL and 11-fold at 0.1 μg/mL [68].

Another study examined the neutralizing activities of the same antibodies against the original Wuhan-Hu-1 SARS-CoV-2 strain and the Omicron variant [75]. Consistently with the results described by Planas et al. [68] all neutralizing mAbs aside from sotrovimab and cilgavimab + tixagevimab (Evusheld) displayed a complete loss of neutralizing activity. Sotrovimab exhibited a less than two-fold decrease in neutralizing activity against Omicron, as compared to the Wuhan strain, and Evusheld exhibited a 100-fold decrease in neutralizing activity [75].

These data strongly underline the urgent need for the discovery of neutralizing mAbs, which target RBD epitopes exhibiting low rates of mutation. Currently, the only neutralising mAb shown to exhibit strong neutralizing activity against the Omicron strain is bebtelovimab (not tested in either of the two described studies due to its recent discovery), with an IC99 of 2.44 ng/mL [73].

## 6. Anti-IL6 Monoclonal Antibodies as COVID-19 Therapeutics

Cytokine release syndrome (CRS) is characterized by an acute systemic increase in levels of proinflammatory cytokines [76]. CRS is predominantly caused by microbial infections and certain pharmaceuticals, and is also common in patients undergoing immune system therapies such as chimeric antigen receptor T-cell therapy.

Interleukin 6 (IL-6), a small polypeptide composed of four α helices, is one of the key cytokines released in CRS. The interleukin acts either as a proinflammatory cytokine if secreted by macrophages, or an anti-inflammatory myokine if produced in muscle tissues.

Virtually all stromal and immune cells are capable of IL-6 production. The expression of IL-6 is primarily activated by tumor necrosis factor alpha and IL-1β. During initial stages of infection-related inflammation, only monocytes and macrophages produce IL-6, upon stimulation by Toll-like receptors. This initial release of IL-6 is critical in the host immune response as it activates several immune cell populations. This early stage of IL-6 action is limited to cells expressing IL-6R [77].

In COVID-19, viral particles bind to epithelial cells in the host alveoli and elicit adaptive and innate immune responses. This leads to the release of cytokines, among them IL-6. The influx of proinflammatory factors, action of vascular endothelial growth factor and a decrease in E-cadherin expression seen at this stage of infection lead to an increase in vascular permeability. Heightened vascular permeability leads to an inflow of fluid into the alveoli, causing dyspnea. In severe COVID-19 cases, respiratory failure, or multiple organ dysfunction due to shock may occur [78,79].

Tocilizumab is a humanized recombinant anti-IL-6R monoclonal IgG1 antibody produced through recombinant DNA technology, cultured in CHO cells, and purified through processes including protein A chromatography, viral inactivation and nanofiltration. The antibody comprises two heavy and two light chains with a total molecular weight of 149 kDa [80].

Tocilizumab binds IL-6 receptors, both soluble and membrane-bound (sIL-6R and mIL-6R), blocking signal transduction by these receptors. The KD value for tocilizumab against human IL-6R was calculated as about 1 nM, which signifies remarkably high specificity [81].

Blocking IL-6R action inhibits transduction of both classical and trans IL-6 pathways, thus potentially inhibiting CRS [77]. Tocilizumab has been used successfully in the treatment of rheumatoid and systemic juvenile idiopathic arthritis, and to some effect in Castleman and Crohn's diseases [82].

A clinical trial of tocilizumab in the treatment of severe COVID-19 patients in China has shown promising results. All the patients included in the study were diagnosed with either severe or critical COVID-19. COVID-19 symptom intensity was measured at baseline and five days post tocilizumab administration. A statistically significant decrease in symptom intensity was reported in the tocilizumab-treated group. The body temperature of patients affected by fever (all patients) has returned to normal levels. A reduction in required mechanical oxygen intake was seen in 75% of the patients, and one of the patients did not need to be supplied oxygen after the treatment. In lung CT scans of 90.5% of the treated patients, absorption of pulmonary lesions could be seen. C-reactive protein and blood lymphocyte levels have also returned to normal levels in all patients.

However, this study is marred by two significant factors—the sample group included only 21 patients, and peripheral blood IL-6 levels were only reported before tocilizumab treatment, and not post-treatment [77].

A large-scale US-based study, including 4485 patients diagnosed with critical COVID-19, has shown a decrease in 30-day patient mortality from 37.1% in patients not treated with tocilizumab to 27.5% for patients treated with tocilizumab within two days of admission [83].

Another study, conducted by researchers across six countries, on 389 patients affected by SARS-CoV-2 pneumonia who were not receiving mechanical ventilation has shown that tocilizumab treatment reduced the likelihood of progression to mechanical ventilation, but failed to improve survival at 28 days post infection [84].

A meta-analysis of studies assessing the use of tocilizumab as an addition to COVID-19 patient standard of care (SOC) was conducted. The overall mortality of the compound tocilizumab-treated group was found to be lower than the untreated group, and this effect was enhanced for a subgroup, which included only severe and critical COVID-19 patients [85].

In December 2021, EMA authorized the use of Tocilizumab under the trade name RoActemra for use in adults suffering from COVID-19 symptoms, who are undergoing treatment with corticosteroids and require mechanical ventilation.

The decision was based on the results of a large-scale clinical study, which has shown a reduction in risk of death in patients treated with Tocilizumab in addition to standard treatment compared to standard treatment alone. Within 28 days, 31% of the patients in the Tocilizumab group died, compared to 35% in the standard treatment group. What is more, within 28 days, 57% of patients in the Tocilizumab group were able to leave the hospital, compared to 50% in the standard treatment group [86].

Unfortunately, tocilizumab prices remain prohibitively high, at USD 3625 per dose in the USA, despite significantly lower estimated manufacturing costs. This discrepancy has drawn controversy considering the lifesaving potential of Tocilizumab for COVID-19 patients [87].

## 7. Anti-CD6 Monoclonal Antibodies as COVID-19 Therapeutics

Itolizumab is a humanized IgG1 monoclonal antibody, which binds domain 1 of human CD6 [88]. CD6 is a receptor expressed by T-effector white blood cells. CD6 stimulation results in activation and differentiation of other T lymphocytes [89]. By binding the CD6 domain 1, itolizumab blocks the action of downstream Th-1 and Th-17 pathways involving STAT3, MAPK and AKT, resulting in lowered T-cell proliferation and ultimately—lower IL-2, IL-17A, INF-γ, TNFα and IL-6 levels [90]. Itolizumab modulates the immune response by T-effector cells, inhibiting their migration to inflammation sites, while leaving T-reg cells unaffected, thus having the potential to preserve the host antiviral response while reducing hyperinflammation-related morbidity [88,91,92].

Itolizumab was developed in Havana, Cuba, at the Center of Molecular immunology. Following promising clinical trial results, the mAb has been authorized for use in treatment of psoriasis in India. Due to its hyperinflammation-suppressing activity, the mAb has recently been suggested as a candidate treatment for COVID-19-induced CRS.

To assess the therapeutic potential of itolizumab, a small clinical trial was conducted by the Cuban CECMED. Fifteen patients with severe or critical COVID-19 symptoms were recruited and administered a single intravenous dose of itolizumab. Serum IL-6 levels of the patients were measured immediately after the treatment and two days post-treatment. In 13 of the patients, the IL-6 levels did not increase or decreased. In two of the patients, the IL-6 levels increased after treatment. IL-6 serum levels of all patients with baseline levels above 28.3 pg/mL, which signifies severe disease, significantly decreased. The mean value of IL-6 serum levels for the critical group dropped from 290.2 pg/mL to 183.1 pg/mL [91].

While the primary outcome of the test was favorable, the study being single-arm—lacking a placebo group, the cohort consisting of only 15 patients, and general sparsity of the data available leave the trial sorely lacking.

Thirty patients suffering from severe and critical COVID-19 were recruited for a phase II, open-label, two arm randomized clinical trial in India. Twenty of the patients received a dose of itolizumab on top of COVID-19 standard of care (SOC), while 10 belonging to the control arm were treated according to the SOC. The primary outcome of the trial was a measurement of the 30-day mortality rate, as compared between the two arms. Secondary outcomes in this trial included plasma biomarker and CRP levels, lymphocyte counts, hospitalization durations and respiratory symptom remission. All patients in the itolizumab-treated group have recuperated successfully and were discharged, while in the control arm, three of the ten patients have died. Compared to the control group, the itolizumab-treated group has also experienced significant improvements in lung oxygen saturation and none of the itolizumab-treated patients required mechanical ventilation at 30 days post-treatment.

The secondary outcomes have also improved for the itolizumab-treated group [92]. Based on these results, Itolizumab was introduced as an emergency COVID-19 treatment in India.

Unfortunately, the scientific community has observed several worrisome inconsistencies pertaining to the study design of this clinical trial. Two patients whose condition has become more severe at initiation of the study have been considered non-randomized and excluded from the final analysis. One of these patients later died, potentially breaching the principle of intention-to-treat analysis. What is more, lung oxygen saturation levels and inflammatory marker baseline values have not been divulged in the study. Only a percentage improvement from baseline can be found in the data provided. Lastly, a higher proportion of patients were on non-invasive ventilation (NIV) in the control group, as compared to the itolizumab-treated group. All the patients who have died during the trial belonged to this NIV-supported group [93].

A guarded approach towards the evaluation and use of itolizumab is needed, as more clear data on its effects are required. A full release of the data gathered in the Cuban and Indian studies, and further, larger clinical studies are in order, before the mAb could see widespread use as an anti-COVID-19 treatment.

A summary of characteristics for the mAbs currently undergoing research discussed in this review can be found in Table 1.

**Table 1.** A summary of primary studies concerning mAbs being developed to treat or prevent COVID-19.

| Antibody | Cohort Size | Cohort Type | Observation Period | Primary Endpoints | Results | References |
|---|---|---|---|---|---|---|
| ADG20 | 6412 | Adults at high risk of SARS-CoV-2 infection | 4 days to 6 months, dependent on endpoint measured | Proportion of patients with RT-qPCR-confirmed SARS-CoV-2 infection, treatment emergent adverse effects and injection site reactions | No results posted yet | [31] |
| CR3022 | 10 mice, 15 hamsters | Balb/c mice, Syrian golden hamsters | Mice: 2 days; hamsters: 3 days. | Viral lung titre and weight loss. | Mice: viral load reduction alongside an increase in weight loss with mAb-treated mice;hamsters: increase in both viral load and weight loss upon mAb treatment | [23] |
| BGB-DXP593 | 181 | Adult outpatients with severe COVID-19 symptoms | 8 days for primary outcome, up to 85 days overall | Changes from baseline to day 8 in viral load as measured by RT-qPCR testing | No results posted yet | [51] |
| H4 and B38 | 16, 4 per group | hACE2 transgenic mice | 3 days | Viral lung titer and weight loss | Significant reduction in weight loss and viral RNA copies in combination treatment group | [64] |
| Tocilizumab | 4485 | Hospitalized patients with critical COVID-19 symptoms | 30 days | 30-day mortality | Significant reduction in in-hospital mortality in patients who received tocilizumab within 2 days of ICU admission | [83] |
| Itolizumab | 24 | Hospitalized patients with moderate to critical COVID-19 symptoms | 48 h | Serum IL-6 levels | IL-6 levels decreased or did not increase in the majority of patients | [91] |
| Evusheld | 5197 | Adults $\geq$ 60 years of age or with pre-specified comorbidities | 183 days | Incidence of SARS-CoV-2 RT-PCR-positive symptomatic illness | 77% reduction in incidence of symptomatic COVID-19 in patients treated with Evusheld | [66] |
| Bebtelovimab | 714 | Patients with mild-to-moderate COVID-19 within 3 days of positive test results | 7 days | Proportion of patients with persistently high viral load on day 7, time to sustained symptom resolution | Statistically significant reductions in viral load and time of symptom resolution in patients treated with bebtelovimab | [73] |

## 8. Challenges of Developing mAbs for Use in the Clinical Environment

The idea of utilising mAbs to combat infections did not come about only with the inception of the ongoing pandemic. In fact, over the last two decades, many groups have attempted to develop antibodies against several acute respiratory infections (ARIs). Unfortunately, most such attempts have been unsuccessful and to date, no mAbs (aside from anti-SARS-CoV-2 mAbs) have been approved as ARI therapeutics due to insufficient efficacy.

The underlying causes for this lack of success are manifold. Testing of motavizumab, a mAb designed to prevent RSV infections, was halted by the U.S. FDA due to a rate of skin reactions at injection sites higher than that seen with palivizumab, a previously developed anti-RSV mAb [94]. In the end, neither of the two anti-RSV mAbs were approved for clinical use due to lacking clinical benefit [95].

Development of other antiviral mAbs has been cancelled considering viral escape incidents, whether actual or anticipated. Such were the cases of suptavumab and CR8020 [95].

Another challenge for the development of effective antiviral mAbs is the prediction of clinical efficacy based on in vitro potency. In many cases, despite high binding affinities and neutralization potencies, mAbs fail to achieve measurably beneficial results in vivo. For example, MEDI-8897 was shown to have more than five-fold greater binding affinity for RSV than motavizumab [96]. In spite of this fact, MEDI-8897 failed to produce clinical results superior to those of motavizumab in early clinical studies [95].

Importantly, the anti-SARS-CoV-2 mAbs currently in development possess binding affinities and neutralizing potencies far higher than mAbs developed for ARI treatment previously. This may be a cause for optimism, although caution is required as mAbs are usually administered in doses far exceeding in vitro neutralizing potencies. Thus, the cause of failure for previously tested mAbs being insufficient dosage or activity is unlikely.

A principal factor, which needs to be considered in any novel immunotherapeutic method, is the risk of immune escape. Immune escape is the generation of de novo mutations, which allow a pathogenic organism to evade detection by antibodies produced by a host previously exposed to a different strain of the same pathogen. Both mAbs and CP therapies are subject to being affected by immune escape variants.

In a study by Zhang et al. the sensitivity of convalescent serum from patients infected with the original SARS-CoV-2 strain to the newly arisen Omicron variant and other variants of concern (Alpha, Beta, Gamma, Delta) was compared. The mean effective neutralization dose 50 (ED50) for the tested sera decreased over eight-fold against Omicron, and only one to four-fold against the other variants of concern. These findings indicate with the spread of Omicron, immune escape becomes an even larger issue than previously [97].

Anti-spike mAb-based therapies put selective pressure on SARS-CoV-2, and as such, it is reasonable to suspect that extensive utilization of mAb-based therapeutics could exacerbate immune escape by selecting for resistant variants.

Mutation forecasts named escape maps can be created for prospective mAb-treatment-resistant mutants. Using escape maps, cocktails of mAbs, which minimalize the risk of immune escape can be designed, such as etesevimab + bamlanivimab [98]. Due to extensive heterogeneity and the polyclonal nature of antibodies present, the identification of escape mutations cannot be conducted for convalescent plasma, thus rendering CP therapy-related immune escape risks incalculable.

In registration trials, immune escape events have insofar been an infrequent phenomenon. However, in clinical practice, anti-SARS-CoV-2 spike protein mAb-based therapeutics are used sparingly and reserved for immunocompromised patients. On a global scale, the risk of mAb-therapy-related immune escape becomes significant, and the generation of escape-capable variants could become the driving force behind subsequent pandemic waves. Already, several new mutations were identified in variants of concern and variants of interest, which points towards the possibility that the variants have emerged during patient treatment [99].

Another point of concern is the efficacy of mAb therapies in the context of emerging variants. The U.S. FDA has recently withdrawn its authorization for use of bamlanivimab

as a single agent, due to its lack of efficacy against SARS-CoV-2 variants carrying the E484K mutation [100]. The possibility of new variants becoming resistant to existing neutralizing mAbs needs to be a priority consideration. The inefficacy of many mAbs against the Omicron variant has already substantially reduced the number of available therapeutics. The primary approach against this issue is to use cocktails of neutralizing mAbs that bind distinct epitopes on the Spike protein, increasing efficacy and reducing the risk of iatrogenic resistant variants arising [101].

Utilizing neutralizing monoclonal antibodies, which bind conserved epitopes, and the function of which is crucial for viral activity, such as sotrovimab, is another possible approach. Thus far, published research on the efficacy of mAb therapeutics against emerging variants remains sparse and ameliorating this should be considered a priority for the development of future therapeutics [54].

## 9. Benefits and Risks of mAb Therapy for Potential Patients

The key factor in the evaluation of novel therapeutics is the risk-benefit ratio in direct relation to prospective patients. As monoclonal antibodies have seen extensive clinical use, much is known about the beneficial effects and adverse reactions they may induce.

The primary target populations for anti-SARS-CoV-2 mAb treatment include patients with increased susceptibility to comorbidities, such as those aged over 65, suffering from diabetes, chronic respiratory or cardiovascular conditions, and immunocompromised patients [102].

Patients belonging to these populations could benefit the most from mAb therapy, as the therapeutic effects of neutralizing mAbs have been shown to be strongest in patients with weak or delayed immune reactions to SARS-CoV-2 infection. Anti-SARS-CoV-2 mAb therapeutics such as bamlanivimab and etesevimab, REGEN-COV, sotrovimab, or bebtelovimab have been shown to significantly lower patient viral loads if administered in the initial stages of SARS-CoV-2 infection [103]. Viral load reduction is strongly correlated with lower mortality and morbidity rates, lower rates of hospitalization and lower risk of progression to severe COVID-19. More broadly, rapid neutralization of the virus also lessens the risks of viral escape and mutation. Many of the widely circulating SARS-CoV-2 VOCs are thought to have arisen from hospitalized patients, underlining the importance of this point [97].

Some mAbs, such as Evusheld have also been shown to be effective as pre-exposure prophylactic agents [65]. Critically, immunocompromised patients commonly respond weakly to vaccination, thus highlighting the importance of prophylactic mAb treatments, which do not rely on the immune response of the recipient in this patient population. Prophylactic mAb treatments could also serve as an alternative to vaccines for patients who react adversely to components of available vaccines or as a treatment to supplement vaccines in prophylaxis for patients especially likely to suffer from severe COVID-19. Neutralising mAbs used prophylactically can also complement vaccination in the case of severe SARS-CoV-2 antigenic drift [72].

Monoclonal antibody treatments also have several advantages over conventional low-molecular-mass drugs. Monoclonal antibodies are characterized by high specificities, which minimize the risk of off-target action and adverse patient reactions. Another advantage is the long half-life of therapeutic mAbs, which allows infrequent administration of doses. Due to the infrequent dosing, mAb treatments can be used in outpatients at high risk of severe COVID-19 to decrease hospitalization rates [104].

So far, the monoclonal antibodies used against COVID-19 have been shown to be safe and severe adverse reactions have been rare. However, therapeutic mAbs are known to cause a range of side effects, either immediate or delayed. As mAbs are highly specific and target different epitopes, risk profiles vary. Adverse reactions can be triggered through numerous mechanisms, impacted by variables such as underlying conditions and interactions with medications. Especially little is known about the full risk potential of newer mAbs.

In the case of COVID-19 mAbs, injection site reactions and infusion-related reactions are most reported. Fever, chills, flushing, nausea, pruritus, and rashes are the most frequent symptoms of infusion-related reactions. Symptoms usually arise 30 to 60 min after the infusion is initiated. Infusion-related reactions tend to be self-limiting. Treatment consists of terminating the infusion and treating symptoms. After symptom resolution, in most cases the infusion can be reinitiated, albeit at a slowed rate [103]. The risk of infusion reactions can be minimized through premedication, slow incremental increases in infusion rate, and ensuring patient hydration and diuresis [104].

In the case of sotrovimab, 1% of treated patients exhibited mild to moderate infusion-related reactions. Rashes and diarrhea were the most commonly occurring symptoms. One case of anaphylaxis resulting from sotrovimab administration was reported [49]. The symptoms of anaphylaxis include rashes, tongue and lip swelling, hypotension and respiratory compromise resulting in dyspnea and wheezing. Anaphylactic reactions can also lead to a potentially fatal systemic inflammatory response syndrome [104]. Adverse immune reactions to mAb treatments can arise due to a range of mechanisms, such as cytokine release syndrome, acute IgE-mediated anaphylactic reactions, anaphylactoid reactions, serum sickness and tumor lysis syndrome.

Monoclonal antibodies used for conditions other than COVID-19 have been known to cause cardiotoxicity, autoimmune disorders, dermatitis, and cancer [104].

These adverse reactions are highly unlikely in the case of anti-SARS-CoV-2 treatments, as most neutralizing mAbs exhibit high specificity against viral proteins and minimal off-target binding.

To ensure prompt and effective management of adverse reactions, mAb treatment should be carried out only by highly trained medical professionals experienced in administering IV infusions and prepared to respond to the emergence of adverse reactions, including potentially lethal anaphylactic reactions.

## 10. Conclusions and Future Perspectives

The COVID-19 pandemic, while immeasurably destructive and deeply tragic, has also brought about a quantum leap in medical research. The numbers of monoclonal antibodies and vaccines under active research are the highest in history. The exceptionally rapid development of novel therapeutics has been aided by innovative technologies. For example, the RNA platform technology used to develop the new generation of vaccines may soon be utilized as a tool for vector design and antibody production, simplifying the process and reducing costs [105].

COVID-19-related mAb research has also served to create significant advancements in antibody discovery and identification. A computational approach to mAb identification has recently been utilized to find members of an influenza-specific mAb class [106]. This novel method of analysis could accelerate mAb development and eventually, with use of reverse vaccinology, could lead to rapid development of more efficacious vaccines [107].

An unprecedented wealth of clinical and preclinical data centered on a single novel pathogen has been amassed over the past two years and can be used to inform research on passive and active immunization techniques. In future cases of large-scale microbial outbreaks, this leap in science will undoubtedly be an asset in diminishing the health crises. Monoclonal antibodies may take center place in these potential future developments, as they can be produced on a large scale, safely administered, and theoretically used to treat any patient, independent of blood grouping [6].

Animal model research of COVID-19 mAb treatments demonstrated the broad potential of mAb-based therapies, however clinical trials on human subjects for many mAb therapies remain inconclusive, are rife with inconsistencies or simply lacking in scale. Despite rapid advancements, significant efforts are still in order if anti-SARS-CoV-2 mAbs are to become mainstream therapeutic agents. Accessibility is another major hurdle in the way of mAb-based treatments. Even though the field of mAb manufacturing is undergoing considerable improvements, large scale production remains prohibitively expensive. Thus,

developing countries, which often are most affected by pandemic outbreaks, might be the last to benefit from mAb-based therapeutics—similarly to the case of SARS-CoV-2 vaccines.

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