Neutralizing monoclonal antibodies for treatment of COVID-19

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Abstract

Numerous neutralizing monoclonal antibodies (mAbs) against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) have been developed, with several now under evaluation in clinical trials. With the US Food and Drug Administration recently granting emergency use authorizations for neutralizing mAbs in non-hospitalized patients with mild-to-moderate COVID-19, there is urgency to discuss the broader potential of these novel therapies and to develop strategies to deploy them effectively in clinical practice, given limited initial availability. Here, we review the precedent for passive immunization and lessons learned in the prevention and treatment of viral infections such as respiratory syncytial virus, Ebola virus, and SARS-CoV-1. We then discuss the specific cases of SARS-CoV-2, with initial deployment of convalescent plasma and subsequent efforts to identify, purify, and mass produce specific neutralizing mAbs. We review specific clinical questions including rationale for stratification of patients, potential biomarkers, known risk factors, and temporal considerations for optimal clinical use. In order to answer these questions, there is a need to understand factors such as the kinetics of viral load and its correlation with clinical outcomes, endogenous antibody responses, pharmacokinetic properties of neutralizing mAbs, and the potential benefit of combining antibodies to defend against putative resistant viral variants.

Introduction

In the midst of the 2020 Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV)-2 pandemic, a variety of prophylactic and therapeutic treatments are being developed or repurposed to combat COVID-19. Monoclonal antibodies (mAbs), that can bind to and 'neutralize' the virus in infected patients, represent a novel class of anti-viral intervention[1,2]. Neutralizing mAbs are recombinant proteins that can be derived from the B cells of convalescent patients or humanized mice (Figure 1). High-throughput screening of these B cells permits the identification of antibodies with the necessary specificity and affinity to bind to virus and block viral entry, therefore abrogating pathology associated with productive infection. These mAbs are termed neutralizing and can ultimately be used as a type of passive immunotherapy (detailed below) to minimize virulence. In this Review, we highlight the relative value that neutralizing mAbs can provide for patients and physicians and go on to examine the role of these agents among the spectrum of potential treatments for COVID-19 (for example, vaccinations, anti-virals and immunomodulatory drugs).

In the United States, three anti-SARS-CoV-2 mAb therapies have been granted emergency use authorization (EUA) for treatment of non-hospitalized patients with mild-to-moderate COVID-19 — bamlanivimab as a monotherapy, bamlanivimab together with etesevimab and casirivimab and imdevimab as a combination therapy[3-5]. As such, several questions need to be addressed about the potential clinical use of neutralizing SARS-CoV-2 mAbs: who should get them; what is the best dose and frequency; when in the course of the infection will they be most effective; what is the duration of the protection they provide; and what is their associated benefit-to-risk ratio? In addition,

neutralizing mAbs may have a prophylactic role in subjects deemed to be at high risk for severe COVID-19. Preliminary non-peer reviewed data suggest that mAbs prevent COVID-19 in high-risk individuals potentially exposed to SARS-COV-2 at nursing homes and within households[6,7].

Data, though, is still emerging. While vaccines remain the first line to prevent COVID-19, mAbs could potentially benefit certain subpopulations before and after exposure to SARS-COV-2, such as unvaccinated or recently vaccinated high-risk patients. The antiviral activity seen with neutralizing mAbs treatment emphasizes the importance of early intervention to help counter the devasting impact the virus has had in such vulnerable populations and other high-risk patients. However, mAbs are complicated to produce and may be limited in initial supply. Furthermore, any protection offered would be temporary, and the duration of effective protection remains to be determined. Answers to these questions will allow the most efficacious use of these novel and potentially life-saving treatments.

Passive immunization

Over 125 years ago, the first major success in modern immunological intervention was developed: a therapeutic serum from animals actively immunized against diphtheria toxin[8,9]. Paul Ehrlich later produced a seminal paper tying the curative antiserum to neutralizing antibodies[10]. Today, passive immunization involves infusion of antigen-specific monoclonal or polyclonal antibodies derived from non-human or human blood products. While polyclonal antibodies collected from immunized animals are the primary

source of anti-sera, there is a risk of ‘serum sickness’, especially after repeated exposures, as the recipient may generate an immune response against antibodies of non-human origin. These risks are mitigated with the use of convalescent plasma from human patients. With careful screening (for example, to assess for the presence of infectious agents and to establish antibody titre and neutralizing capacity), convalescent plasma therapy (CPT) can be effective with minimal safety risks.

Prior to the current pandemic, CPT has been used to treat infections with influenza virus[11,12], respiratory syncytial virus (RSV)[13], Ebola virus[14] and coronaviruses[12,15-17]. CPT appears most efficacious when used early after the onset of disease symptoms rather than during severe or prolonged infection[12,15,18]. It also has the potential to provide protection for the immunocompromised or unvaccinated high-risk individuals recently exposed to infection[13,15]. Administration of plasma with higher titres of neutralizing antibodies is associated with improved clinical outcomes[17]; however, the antibody titres of convalescent plasma vary considerably[19]. CPT can be convenient for use and adaptable for utilization in resource-poor settings[14] and can be rapidly deployed to combat novel virus outbreaks.

The anti-pathogen antibodies from convalescent plasma can mitigate infection by two mechanisms: antibody effector activity and neutralization. However, in rare cases, pathogen-specific antibodies can augment virulence in a process termed **antibody dependent enhancement** (ADE; Figure 2). ADE can occur via two distinct mechanisms. Firstly, pathogen-specific antibodies could increase infection via viral uptake and replication in Fc gamma receptor expressing immune cells (for example dengue hemorrhagic virus infection of macrophages). With SARS-CoV-1 and -CoV-2[20],

in vitro evidence indicates these non-lymphotropic coronaviruses are unable to productively replicate within hematopoietic cells[21]. Alternatively, ADE can be mediated via increased immune activation by Fc-mediated effector functions or immune complex formation[22]. In the case of respiratory viral infections, the resulting immune cascade can contribute to lung pathology. While the hallmarks of severe COVID-19 have overlapping features with this type of ADE, there has been no definitive evidence with SARS-CoV-2 infection currently[22].

Nonetheless, steps may be considered to mitigate the potential risk of ADE. When feasible (as with neutralizing mAb therapy), the Fc region can be modified to render the antibody therapy effector null. In the case of CPT, the potential risk of ADE can be reduced by administrating high amounts of pathogen-specific antibodies and utilizing plasma with high affinity neutralizing antibodies[20]. As shown in a non-primate model of SARS-CoV-1, neutralizing anti-receptor binding domain (RBD) or anti-heptad-repeat-2 antibodies provided protective immunity whereas antibodies specific for other S protein epitopes could trigger ADE[23]. Further in randomized controlled trials (RCTs), passive immunization of anti-S protein neutralizing mAbs did not provide clinical evidence of ADE in non-hospitalized COVID-19 patients[3,24,25].

The shortage of large, **randomized controlled trials (RCTs)** of CPT has limited our understanding of the relative benefit-to-risk profile of this treatment option. Further, logistical difficulties can complicate CPT's application. According to the EUA from the US Food and Drug Administration (FDA) for CPT in patients with COVID-19, convalescent patients should be at minimum two weeks symptom-free and have high titres of anti-SARS-CoV-2 antibodies; low titre donations could be used for therapy

following careful consideration by the health care provider[26,27]. Thus, widespread use of CPT is dependent on a readily available pool of recovering patients with high antibody titres who are willing to donate plasma, on sufficient local facilities to ensure adequate processing, screening and administration of the therapy, and on governmental coordination to regulate effective implementation.

Advantages of neutralizing mAbs

There is an increasing focus on replacing CPT with neutralizing mAbs, where the dosing affinity and neutralizing capacity of the antibodies can be more precise. Today, the process to mass produce recombinant mAbs has become scalable to meet demand and is cost competitive with other treatments. Neutralizing mAbs overcome limitations intrinsic to CPT (for example, the risk of blood borne diseases, time to development of detectable high affinity antibodies, risk of low antibody titres, as well as variable epitope specificity[28]). Further, a high titre of neutralizing antibodies — which current evidence indicates is necessary for the efficacy of CPT — is inherent with neutralizing mAbs. As of November 2020, at least 20 neutralizing mAb therapies were being tested in late stage clinical trials or had already been approved for use in 9 infectious diseases, including RSV and Ebola[29,30].

Palivizumab, a neutralizing mAb against the fusion protein of RSV, was initially approved in 1998 as a prophylaxis for severe infection in high risk infants[31-33]. Previously, the standard of care for prophylaxis in these patients was monthly infusions of RSV immune globulin[13,31]. When administered via monthly intramuscular injections,

palivizumab reduced the frequency of hospitalization and severity of RSV disease relative to placebo and was well-tolerated[31,32]. However, palivizumab was not demonstrated in RCTs to improve clinically meaningful outcomes in infants with severe RSV infection in advanced disease stages[34-36]. Further, monthly administration is required to maintain detectable levels of neutralizing mAb and as many as 5 doses may be needed to prevent severe or deadly infection[37]. A newer medication with a longer half-life (MEDI8897) is currently in Phase 2/3 trials[33].

During the Ebola virus disease outbreak in the Democratic Republic of Congo in 2018, the open-label RCT (PALM) investigated four intravenously administered treatments in 681 patients actively infected with Ebola virus: the anti-viral remdesivir, the triple mAb cocktail ZMapp, the single mAb MAb114, or the triple mAb REGN-EB3[38]. After an interim analysis, the first two treatments were discontinued as MAb114 monotherapy and REGN-EB3 were superior with respect to the primary outcome, patient mortality[38]. One potential factor the PALM study team proposed to explain the distinction between the therapeutics was that the full treatments for MAb114 and REGN-EB3 were administered as a single dose, thereby facilitating a rapid response, while ZMapp was given as three infusions. Indeed, patients treated with MAb114 and REGN-EB3 had faster rates of viral clearance, lending credence to this hypothesis. Overall, survival was higher in those who were treated early in symptom onset and had lower baseline viral loads. The relatively poor efficacy of the ZMapp triple cocktail also serves as a reminder that the number of mAbs is not necessarily a predictor of efficacy per se and that the specific epitopes matter as well.

Two main uncertainties persist with passive immunization, spanning both neutralizing mAbs and CPT. First, does their use as a prophylactic or treatment potentially affect natural long-term immunity? Considering the large doses used and the relative half-life of antibodies (~3 weeks for IgG molecules), there is a pertinent consideration whether the presence of circulating neutralizing mAbs could impact active immunity, whether through memory from infection or vaccination. In RSV, rodent and primate infection models indicate that the passive transfer of antibodies does diminish the development of humoral immunity in the recipient; however, long-term memory was sufficient to protect the hosts from re-infection, largely due to an intact T cell memory compartment[39,40]. Considering the limitations in translating data from animal models (where RSV replication is attenuated relative to in its human host), additional data, particularly from clinical trials, will provide critical insight with regard to this potential challenge.

Second, could resistant viral variants emerge that limit the effectiveness of the therapies? The polyclonal nature of CPT, in which a spectrum of differentiated antibodies target multiple epitopes of the pathogen, may help to reduce this risk. Nevertheless, emerging pre-clinical data suggests SARS-CoV2 spike mutations escape from polyclonal serum[41], and convalescent plasma has reduced neutralizing activity against some viral variants[42]. For mAbs however, depending on the infectious agent and the epitope targeted, combinations of mAbs may be necessary to maintain efficacy and prevent treatment failure. Experience with mAbs targeting human immunodeficiency virus (HIV), which has a very high mutation rate, suggests that it is more effective and durable to utilize multiple neutralizing antibodies (i.e. combinational

mAb therapy) rather than a single one[43-46]. These particular mAbs against HIV also need to be broadly neutralizing, that is targeting epitopes generally conserved among variants. On the other hand, infections involving pathogens with lower mutation rates and/or accessible broadly conserved epitopes may not require combinational mAb therapy, for example, MAb114 monotherapy, which targets a broadly conserved epitope on the Ebola virus's RBD, was as effective as the combination therapy REGN-EB3[38] and more effective than the triple cocktail (ZMapp). It is important to note though that the global nature of the SARS-CoV-2 pandemic presents a larger risk of pre-existing and escape variants than during the Ebola outbreak.

From the collective clinical data with MAb114, REGN-EB3 and palivizumab, the general benefits and risks associated with neutralizing mAbs are similar to those observed with traditional passive immunization against infectious agents. The agents themselves are relatively tolerable for patients, efficacious during the early onset of disease/symptoms and in certain cases as a prophylactic, but with limited efficacy once infections are severe. The distinctions between these therapies are largely logistical; CPT is more rapidly implemented during an emerging pandemic when few therapeutic options are yet available, while neutralizing mAbs take time to discover, obtain regulatory approval, and scale up manufacturing capacity. The use and promise of passive immunization during the coronavirus outbreaks of the 21st century (that is, with SARS-CoV-1, MERS and SARS-CoV-2) have re-emphasized these past lessons while also highlighting additional insights.

Passive immunization for coronavirus infection

During the SARS epidemic in 2003, immune system kinetics following SARS-CoV-1 infection were different in patients who recovered compared with in those who finally succumbed to the viral infection and sequelae. In patients with fatal outcomes, endogenous neutralizing antibodies peaked at 15 days from symptom onset then decreased drastically until the time of death. In contrast, in patients who went on to recover, peak neutralizing antibody responses were observed at 20 days from symptom onset[47,48]. Those who recovered tended to develop antibody responses with diverse isotypes (IgM, IgG, IgA) against two proteins on the virus, the nucleocapsid (N) and spike (S) proteins, while patients with fatal outcomes had restricted antibody responses to the N protein only. In a serological survey of confirmed convalescent serum samples, 88% had anti-SARS-CoV-1 antibodies 31-180 days after the onset of symptoms; the geometric mean of neutralizing antibody titre was 1:61[19].

Due to the brief duration of the SARS epidemic in 2003, few observational trials examining CPT were conducted. The largest involved 80 patients at Prince of Wales Hospital in Hong Kong[15]. Those given CPT prior to day 14 following onset of symptoms had a better outcome than those given CPT after day 14 ($p<0.001$); mortality was also lower in the former group (6.3% versus 21.9%). Patients who tested positive for SARS-CoV-1 by PCR had better outcomes if they were seronegative when given CPT than those who were already seropositive (66.7% versus 20%; $p<0.001$). A summary of eight observational studies using CPT during the SARS 2003 outbreak (including the aforementioned Prince of Wales Hospital Study) suggested CPT was associated with improved mortality rates, shorter hospital stays, and reduced overall viral loads in the

respiratory tract[12]. The treatments were considered tolerable[12]; however, information on minor complications may have been underreported. Importantly, robust studies on the effectiveness and safety of CPT were not completed, and thus these results must be interpreted with caution, particularly as patients treated at later times with CPT therapy may demonstrate selection bias for an already refractory pathophysiology.

In SARS-CoV-2 infection, plasma collected from 175 patients who had recovered from mild COVID-19 demonstrated neutralizing antibody and S-binding antibody titres that correlated with increased age, greater inflammation (i.e. higher C-reactive protein levels) and lower lymphocyte counts; the vast majority of the SARS-CoV-2 neutralizing antibodies were not cross-reactive to SARS-CoV-1[49]. Several reports indicate convalescent patients can maintain high titres of neutralizing antibodies several weeks after infection[49-51].

Observational studies have reported that CPT has been associated with improved outcomes in COVID-19[52]. For example, in a small case series in China, five critically ill patients with acute respiratory distress syndrome showed improved clinical status following treatment with CPT. Thirty-seven days post transfusion, three patients had been discharged and two were in a stable condition[53]. In a cohort analysis of 35,222 patients hospitalized with COVID-19 from the United States Convalescent Plasma Early Access Program, reduced mortality was associated with earlier time to transfusion (after diagnosis) and convalescent plasma with higher antibody levels[18]. Because these studies were observational, there was limited procedural control including standardization of the level of neutralizing antibodies.

In an open-label RCT with CPT for patients (n=103) with severe or life-threatening COVID-19 in China, donors were required to have high levels of antibodies specific to the RBD of the S protein[54]. However, the study was terminated early; the hazard ratio for time to clinical improvement within 28 days in the CPT group versus the standard treatment control group was 1.4 (favoring CPT) but was not statistically significant. The proportion of patients with severe disease who achieved the primary endpoint was significantly higher in the CPT group (21 of 23 patients) versus the standard treatment group (15 of 22 patients; p=0.03), but no distinction was noted in patients with life-threatening COVID-19 [53]. In a blinded RCT in Argentina, CPT (with a median titre of 1:3200 anti-SARS-CoV-2 antibodies) also failed to demonstrate benefit in patients with COVID-19-associated severe pneumonia[55].Further, in an open-label RCT in India (PLACID) in hospitalized COVID-19 patients with moderate disease severity, CPT did not demonstrate clinical benefit in patient mortality or transition to severe disease[56]. Similarly, preliminary data from the RECOVERY RCT among 10,406 hospitalized patients showed no proof of mortality benefit in the primary endpoint of 28-day mortality in the CPT treated group versus the standard treatment group[57,58].

Despite inconsistent clinical efficacy, there is evidence that CPT was associated with greater viral clearance than standard of care treatment[54,56]; collectively, this indicates improvement in viral clearance alone was not sufficient to clearly improve the clinical outcomes in patients with established COVID-19 (that is, in patients hospitalized with COVID-19). Because the currently available data on CPT is derived predominantly from inpatient (severe or critical) COVID-19 RCTs, the suitability of CPT as prophylaxis or

treatment at the onset of COVID-19 symptoms remains to be determined by appropriately controlled clinical trials.

Neutralizing mAbs in development for COVID-19

The primary antigenic epitope on the coronaviruses SARS-CoV-1 and SARS-CoV-2 is the S protein, which facilitates target cell binding and fusion upon engaging the cell surface angiotensin-converting enzyme 2 (ACE2) receptor, which is found on cells in the respiratory system, gastrointestinal tract, and endothelium[59-63]. Thus, antibodies directed to the S protein can neutralize the ability of the virus to bind and fuse with the target host cell. Humanized murine technology or convalescent plasma from recovered patients has been used to derive neutralizing mAbs targeted to the RBD of the S protein (Figure 3)[64-66]. To date, most advanced research efforts for therapeutic use of neutralizing mAbs are focusing on a handful of products in clinical development, some of which are already authorized based on Phase 1/2 and Phase 2 data for emergency use (EUA) (Table 1).

REGN-COV2 is a combination of two potent neutralizing mAbs — namely casirivimab and imdevimab, which are IgG1 mAbs with unmodified Fc regions. These two mAbs were chosen from a pool of >200 neutralizing mAbs, present in the initial isolation of thousands of antibodies, derived from parallel efforts using humanized mice and the sera of convalescent COVID-19 patients[67,68]. The antibodies bind two distinct and nonoverlapping sites on the RBD[3,67]. The rationale for this antibody combination is that it is unlikely that a mutation in the S protein of SAR-CoV-2 will simultaneously render

both antibodies ineffective. In extensive *in vitro* testing, this combination retained its ability to neutralize all known S protein mutations[67]. Further, casirivimab and imdevimab combination therapy initiated antibody-mediated cytotoxicity and cellular phagocytosis in virally-infected cells *in vitro*[3]. This product was tested in rhesus macaques and golden hamsters infected with SARS-CoV-2, which serve as models for mild and severe disease, respectively[69]. In both models, prophylactic and therapeutic treatment with casirivimab and imdevimab resulted in not only a reduction in viral load but also diminished the incidence and severity of lung disease relative to a placebo.

An ongoing Phase 1/2/3 placebo-controlled trial (NCT04425629) is investigating the safety and efficacy of a single infusion of casirivimab and imdevimab — 2400 mg (n = 266, interim), 8000 mg (n =267, interim) or matching placebo (n =266) — for symptomatic adults who have not previously been hospitalized within 3 days of a positive active SARS-COV-2 diagnosis (and within 7 days of first symptoms)[3]. Median age was 42 years (7% ≥65 years), 85% were white, 9% were black, and 34% of patients were considered high risk (for example, were obese, elderly or had underlying chronic medical conditions). Pooled treatment achieved the primary endpoint of time weighted average (TWA) change from baseline in viral load (log_{10} copies/mL), collected from a nasopharyngeal swab, in patients with a positive baseline for viral RNA (N=665). The difference in TWA from Day 1 through Day 7 for the pooled doses of casirivimab and imdevimab compared with placebo was -0.36 log_{10} copies/mL ($p<0.0001$). The combination was reported to reduce viral load particularly in patients with higher viral loads who were seronegative at baseline[3,70]. On a key clinical endpoint, a lower proportion of patients treated with casirivimab and imdevimab had COVID-19-related

medically attended visits (2.8% for pooled doses vs 6.5% placebo). In post-hoc analyses, a lower proportion of patients treated with casirivimab and imdevimab had COVID-19-related hospitalizations or emergency room visits compared to placebo (2% versus 4%). The absolute risk reduction for casirivimab and imdevimab compared to placebo was greater for patients at high risk for progression to severe COVID-19 and/or hospitalization (3% vs 9%). Collectively, these results supported the Regeneron EUA of casirivimab and imdevimab in the US in November 2020[71].

Bamlanivimab is a potent neutralizing mAb (IgG1 with unmodified Fc region) against the S protein that was derived from the convalescent plasma of a patient who had COVID-19[24,66]. Bamlanivimab binds the S protein's RBD, engaging its cognate epitope in both up and down conformations, which makes this antibody potentially useful as a monotherapy. There have been historical precedents for the effectiveness of neutralizing mAbs as a monotherapy (for example, MAb114 for Ebola)[38]. To assess theoretical risk of ADE, bamlanivimab was studied in primary human macrophages and immune cell lines exposed to SARS-CoV-2 at concentrations down to 100-fold below the EC_{50} value and in these studies it did not demonstrate productive viral infection[4]. Prophylactic efficacy was tested in rhesus macaques given bamlanivimab 24 hours prior to a virus challenge[66]. The symptoms in this model were mild overall, but the treatment significantly decreased viral load and replication in the respiratory tract following inoculation, supporting its antiviral efficacy.

In the phase 2 portion of the ongoing phase 2/3 BLAZE-1 trial, ambulatory adults with mild-to-moderate symptoms of COVID-19 within 3 days of a first-positive nasopharyngeal swab positive for SARS-CoV-2 received a single infusion of one of

three doses of bamlanivimab (700, 2800, or 7000 mg) or placebo in an outpatient setting[24]. A pre-planned interim analysis was conducted of 452 patients who had reached day 11 following infusion (median age 45-46 years (12% ≥65 years), 88% white, 6% black, and 68% high risk [for example, obesity, elderly, underlying chronic medical conditions])[24]. The study revealed the viral clearance time course via the intrinsic immune response and the enhanced clearance with neutralizing mAb infusion, concomitant with improved clinical response. Viral loads were assayed from serial nasopharyngeal swabs, with post-infusion measurements enabled by a novel partnership with home-health research. Following infusion, log viral load had begun decreasing relative to baseline as early as the first post-infusion assessment on day 3 (-0.85 for placebo versus -1.35 for pooled bamlanivimab doses), continued to decrease on day 7 (-2.56 for placebo versus -2.90 for pooled bamlanivimab doses), and further decreased by day 11 (-3.47 for placebo versus -3.70 for pooled bamlanivimab doses). In a post hoc analysis, patients with early **persistent high viral load** (described as log viral load ≥5.27, at trial day 7) had a higher risk of hospitalization, and risk was further increased for elderly and obese patients. Clinical evidence demonstrating the efficacy of bamlanivimab came from two predefined secondary endpoints. First, at day 29 the percentage of patients who were hospitalized with COVID-19 was 6.3% for the placebo group and reduced to 1.6% for the pooled bamlanivimab doses. In post-hoc analyses, hospitalization among elderly (≥65 years) or obese (BMI ≥35 kg/m^2) patients was 15% for the placebo group and 4% for the pooled bamlanivimab doses. Absolute risk reduction for hospitalizations was more evident for patients with risk factors. Second, improvements in baseline symptoms was greater for the pooled bamlanivimab doses

compared to placebo from day 2 through to day 11. Collectively, these results supported the EUA of bamlanivimab monotherapy in the U.S. and Canada in November 2020[26].

Other treatment arms of the BLAZE-1 trial studied, bamlanivimab together with etesevimab (an S protein-binding IgG1 with a modified Fc region, resulting in null effector function)[25,72]. Bamlanivimab and etesevimab together significantly decreased viral load (mean changes from baseline and percent patients with persistent high viral load) compared with placebo at day 3 to day 11[25]. Bamlanivimab and etesevimab treated patients had fewer COVID-19 related hospitalizations relative to the placebo group (5.8% with placebo reduced to 0.9% with bamlanivimab together with etesevimab). Recently released placebo-controlled Phase III data from 1035 patients randomized, 1:1 to bamlanivimab together with etesevimab versus placebo, demonstrated that in high-risk ambulatory (including patients 12-17 years with specific risk factors and ≥ 18 years of age with specific adult risk factors) bamlanivimab and etesevimab together was associated with a 70% reduction in COVID-19 related hospitalizations and deaths relative to placebo treatment (7.0 % in placebo reduced to 2.1 % with bamlanivimab together with etesevimab)[25,73]. Based on these data, an additional EUA of bamlanivimab together with etesevimab, has been authorized[5].

There are concurrent studies investigating neutralizing mAbs for patients hospitalized with severe COVID-19. The REGN-COV2 trial in hospitalized patients enrolls patients with or without supplemental oxygen and is still ongoing[74,75]. In prospectively designed analysis of REGN-COV2, there may be clinical benefit in patients treated with casirivimab and imdevimab and who were seronegative at the time of treatment[76]. In the RCT ACTIV-3 (n=326, 1:1 randomization), bamlanivimab added to standard of care

(typically including remdesivir) did not demonstrate additional clinical benefit in hospitalized patients[77]. In line with similar studies investigating CPT or neutralizing mAbs for patients with severe viral disease (including COVID-19)[15,18,34-36,54,56], the evidence indicates that rapid viral clearance, in itself, is insufficient. Rather, additional factors such as an excessive immune response are the primary drivers for continued disease in this particular patient population. Thus, early disease seen in outpatients is likely virally driven, whereas the pathophysiology for inpatient advanced disease is predominantly a post-viral or peri-viral phenomenon, with clinical status uncoupled from viral load.

In terms of risk associated with mAb treatment for COVID-19, treatment-emergent adverse events were comparable to placebo. The most frequent treatment-emergent side effects observed in RCT include nausea, diarrhoea, dizziness, headache, and vomiting[24,25,78]. One percent of patients receiving casirivimab and imdevimab reported a grade 2 or higher infusion related reaction within four days of administration (comparable to 1% reported for placebo treatment)[78]. In the phase 2 portion of BLAZE-1, nine patients reported an infusion related reaction [1.9 % (6/309) with bamlanivimab monotherapy, 1.8 % (2/112) with bamlanivimab and etesevimab together, and 0.6 % (1/156) with placebo]. Most reactions occurred during infusion, these were mild in severity, and not dose related[25]. Regarding evidence of ADE, *in vitro* data indicate neutralizing mAbs do not enhance productive infection of immune cells with SARS-COV-2[3,4]. From clinical data available to date, there is no clear evidence these therapies result in enhanced immune responses consistent with ADE[24,25,78]. Further, the

safety profiles of modified and modified + unmodified mAbs to treat SARS-CoV-2 are similar suggesting that ADE may not play a role in clinical outcomes[25].

For patients with COVID-19 that receive neutralizing mAbs, there is potential for the development of drug-resistant variants, which become more obvious when selective pressure is applied in the setting of drug treatment [79]. For bamlanivimab, non-clinical studies using serial passage of SARS-CoV-2 and directed evolution of the SARS-CoV-2 S protein, identified viral variants (E484K, F490S, Q493R and S494P, amino acid substitutions in the S protein RBD) which had increased resistance to this drug[4].

In clinical trials of bamlanivimab, genotypic and phenotypic testing are monitoring for potential bamlanivimab-resistance-associated S protein variations. In bamlanivimab clinical trials, viral sequencing is being performed for all patients, regardless of treatment status/progression. In other studies where only treatment failures are sampled, the selective pressure exerted by the anti-viral activity cannot be assessed. In the BLAZE-1 RCT, which was limited to US investigative sites, known bamlanivimab-resistant variants at baseline were observed at a frequency of 0.27% to date[4]. In the same trial, treatment-emergent variants were detected at S protein amino acid positions E484, F490 and S494 (including E484K, F490S and S494P); considering all variants at these positions, 9.2% and 6.1% of participants in the 700 mg bamlanivimab arm (the EUA dose) harbored such a variant post-baseline at ≥15% and ≥50% allele fractions, respectively, compared with 8.2% and 4.1%, respectively, of participants in the placebo arm. Most of these variants were first detected on Day 7 following infusion and were detected at only a single time point. The clinical impact of these variants is currently unknown[4].

As with bamlanivimab, casirivimab and imdevimab therapy has the potential to develop resistant viral variants. In non-clinical studies, serial passage of vesicular stomatitis virus (VSV) encoding the SARS-CoV-2 S protein in the presence of drug identified escape variants to casirivimab (K417E, Y453F, L455F, F486V and Q493K) or imdevimab (K444Q and V445A)[3]. Each viral variant showing reduced susceptibility to one mAb did retain susceptibility to the other mAb; all identified variants retained susceptibility to the combination. In a separate experiment, neutralization assays were performed using VSV pseudotyped with 37 SARS-CoV-2 S protein RBD variants that were identified as the most common variants in circulation as of late March 2020. Casirivimab had reduced susceptibility (4.5-fold) to G476S and S494P variants, and imdevimab had reduced susceptibility (463-fold) to the N439K variant. The casirivimab and imdevimab combination were active against all individual variants tested[3]. It has been reported that the combination of mutants at the 417 and 439 residues may abrogate effectiveness of the casirivimab and imdevimab combination[80].

In the casirivimab and imdevimab RCT NCT04425629, interim data indicated only one variant (G446V) detected in 4.5% of participants at the ≥15% allele fraction, each at a single time point[3]. The clinical impact is unknown. In a VSV pseudoparticle neutralization assay, the G446V variant had reduced susceptibility to imdevimab (135-fold) but retained susceptibility to both casirivimab alone and casirivimab and imdevimab combination.

However, not all variants must be considered clinically relevant mutations associated with resistance to treatment. During the Ebola outbreak in 2018, a genomic assessment of 48 viral genomes determined that this outbreak was due to a distinct viral variant. The

sequence information allowed researchers to evaluate the relevance of the distinct mutations to the available vaccine and therapeutics, and to conclude that the neutralizing antibodies MAb114 and ZMapp would likely be effective against the currently circulating variant[81]. A similar practice for SARS-CoV-2 surveillance may be prudent to determine if emergent S protein variants pose a threat to the efficacy of neutralizing mAb therapies.

Indeed, three variants of particular interest have been identified and are circulating globally. The UK identified a variant called B.1.1.7 with a large number of mutations in the fall of 2020. In South Africa, a variant called B.1.351 emerged. Originally detected in early October 2020, B.1.351 shares some mutations with B.1.1.7. In Brazil, a variant called P.1 emerged, containing a set of additional mutations that may affect its ability to be recognized by first-generation neutralizing monoclonal antibodies and vaccine-generated immunity from first-generation vaccines encoding monovalent spike sequences. Although these variants have been detected in the US, according to real-time data accessed via the GISAID COVID-19 variant tracker (https://www.gisaid.org/)[82] these COVID-19 variants do not currently represent a significant proportion of COVID-19 infections presently in the United States[82,83]. To date, the effect of these variants on the neutralizing capacity of vaccines and mAbs is unknown. A recent pre-print, pre-clinical paper, suggests that the variants identified in the UK and South Africa, are more resistant to CPT and vaccine sera[42].

Bamlanivimab and imdevimab maintain full neutralization activity against the primary SARS-CoV-2 receptor binding site variants (69-70del and N501Y) implicated in the strain originating in the UK, suggesting that these mAbs should maintain full activity

against the new strain originating in the UK[42,84]. Based on what is known about the strain that originated in South Africa and the presence of concurrent changes at residues 484 and 417, it appears that some of the first generation of antibody therapies may not be as effective[42].

Clinical use of mAbs in COVID-19

Bamlanivimab, bamlanivimab together with etesevimab, and casirivimab with imdevimab decrease viral load when given early on in the course of SARS-CoV-2 infection and favourably impact clinical outcomes for patients with mild-to-moderate COVID-19[24,70]. Although full clinical trial data is pending, topline and interim results from multiple trials suggest that the therapies may also function as prophylaxis in at-risk patients recently exposed to SARS-CoV-2[6,7]. One signal emerging from early data is that patients with persistently higher viral loads progress more frequently towards medically attended visits, emergency room visits, or hospitalization, and this effect is most pronounced for patients with pre-existing risk factors for disease progression[3,24]. It remains a tenet that anti-virals, whether small-molecules or neutralizing mAbs, work best when deployed early. Extrapolating from early viral load data, ideally patients would receive treatment as soon as possible (that is, within hours to days following a positive test or symptom onset). In the trial setting by day 7 to day 11, the majority of patients are either progressing towards clearance of the virus[24] or have experienced clinical decline and hospitalization, further emphasizing the need for early intervention. As the clinical trial timelines typically represent an offset of several days from initial

517 diagnosis, corresponding to day 10-14 of clinical illness, the actionable message
518 remains unchanged – treat as early as possible to maximize the chance of altering the
519 disease trajectory and promote recovery.

520 The SARS-CoV-2 pandemic poses logistical and medical challenges for distribution of
521 neutralizing mAbs. Up to 10% of initially asymptomatic, minimally symptomatic, and mild
522 infections progressed to more severe disease including respiratory distress[85]. While
523 approximately 78% of patients admitted to hospital have at least one documented
524 comorbidity[86], there continues to be patients lacking any identified comorbidity that
525 subsequently become critically ill. Thus, the absence of comorbidities does not
526 completely eliminate the risk for severe disease and sequalae, and there is an urgent
527 need for additional insight into a more personalized predictive algorithm to unlock as-
528 yet-unidentified risk factors. Contrary to the discussion in the lay media, COVID-19 can
529 potentially claim the lives of young adults in their prime, even in the absence of any
530 known underlying risk factors. Given that persistently high SARS-CoV-2 viral loads may
531 be associated with more severe clinical outcomes[87-89], it is possible that early re-
532 assessment of viral loads might help guide who among the ‘lower risk’ population might
533 be helped by neutralizing mAbs. RCT evidence indicates that the clinical value of
534 neutralizing mAb therapy is more pronounced in individuals seronegative at diagnosis[70].
535 Collectively, measuring viral load and serology would allow strategic deployment for
536 patients without otherwise identifiable risk factors while targeting early supply to the high
537 risk population. However, this strategy would be contingent on rapid turnaround of
538 laboratory testing. Meanwhile, it also seems reasonable to use neutralizing mAbs early

on during the disease for patients with well-identified risk factors for more severe disease evolution[90].

Another way to classify candidate patients for neutralizing mAbs would be to select patients who are expected to have poor anti-viral responses (for example, elderly or immunocompromised patients) or identifying patients with poor T and/or B cell function via experimental techniques (such as, by serology or flow cytometry). Regarding the latter, there is a lack of published evidence on humoral immune response dynamics and correlation with clinical outcomes. Further, technical difficulties in stratifying patients based on antibody production, lymphocyte function, and/or viral load might pose a significant impediment to the timely identification of the most appropriate patients for neutralizing mAb therapy.

Finally, anti-viral and antimicrobial therapies are traditionally plagued by promoting escape variants from the treatment, and sometimes combination therapy can mitigate this risk. As a first-generation approach for neutralizing mAbs, monotherapies have been developed and have been demonstrated to be efficacious, but it is expected that a greater number of combination therapies will follow. For example, in phase 2/3 trials involving patients with COVID-19, bamlanivimab and bamlanivimab and etesevimab combination had similarly improved magnitudes and timings of symptom improvement relative to the placebo[25]. However, to date, bamlanivimab and etesevimab combination does not appear to lead to the emergence of drug-resistance variants of SARS-CoV-2. This is similar to what has been observed for the other authorized neutralizing mAb combination (casirivimab and imdevimab), as already described above. At present, there remains a role for continued utilization of monotherapy while transitioning towards

combination therapies in order to mitigate the selection pressure for viral escape as manufacturing capacity becomes available, to ensure longevity of the therapies, and to reduce the potential rate of treatment failure.

Summary and conclusions

The sudden arrival and devastating spread of the SARS-CoV-2 pandemic has stimulated an accelerated programme of international research to identify effective ways to limit the spread of infection, and to reduce the morbidity and mortality associated with COVID-19[91,92]. The first data are now emerging on vaccines designed to prevent disease[93,94]. In the context of active infection, clinical trials of anti-viral medications and for sufficiently hypoxemic patients, judiciously timed authorized anti-inflammatory agents such as dexamethasone, baricitinib (in combination with remdesivir), and still under review, tocilizumab, suggest that mortality rates can be reduced[95-99]. Furthermore, many trials have been conducted or are underway with various immune-modulating medications designed to limit the tissue damage associated with the later stages of COVID-19. However, to date there have been few unequivocal successes. Neutralizing mAbs, particularly in combination with other medications, represent an attractive approach that has potential utility in both prophylactic and treatment settings. Encouraging early clinical trial data support further investigation of neutralizing mAbs to determine the optimal dosing regimen. Unanswered questions regarding this novel therapeutic approach set a pressing research agenda; we need to establish which at-risk subjects would benefit most from prophylactic neutralizing mAbs, the duration of

protection offered by these mAbs and any potential impact of mAb therapy on subsequent vaccination. It will also be important to determine the optimum timing for administration of neutralizing mAbs based on viral load, serology and other potential clinical factors.

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Figure Legends

Figure 1. Neutralizing mAb identification, selection, and production. The neutralizing mAbs given emergency authorization for treatment of COVID-19 were derived either from convalescent patients or humanized mice exposed to SARS-CoV-2 antigens. However, mAbs can be generated by multiple methods including from vaccinated subjects (not depicted here). These pathways of mAb generation depicted here converge in the process of selection and production. mAb=monoclonal antibody

Figure 2. Mechanism of action of mAbs for viral infection and antibody dependent enhancement. A. mAbs can directly interfere with viral pathogenesis in multiple ways. First, binding of a neutralizing antibody on the virion can prevent target cell binding and/or fusion. Further, antibody binding opsonizes the virions or infected cells for phagocytic uptake. If viral proteins intercalate into target cell membranes during viral egress, mAbs can facilitate target cell death via complement fixation or antibody dependent cytotoxicity. These mechanisms may result in apoptosis or necrosis of the infected cell. **B.** In some instances, opsonization of a virion can facilitate viral pathogenesis in a process termed antibody dependent enhancement (ADE). ADE can occur via two distinct mechanisms. Firstly, pathogen-specific antibodies could increase infection via viral uptake and replication in Fc gamma receptor expressing immune cells. Secondly, ADE can be mediated via increased immune activation by Fc-mediated effector functions or immune complex formation. The process of ADE and its potential impact during COVID-19 infection is expertly reviewed in Lee et al.,[22].

Figure 3. Inhibition of SARS-CoV-2 target cell engagement by neutralizing mAbs. Neutralizing mAbs being developed to combat COVID-19 are generated against the Receptor Binding Domain (RBD) of the Spike (S) protein. The anti-RBD mAbs prevent binding of the S protein to its cognate receptor, angiotension-converting enzyme (ACE) 2, on target host cells. Three neutralizing mAbs regimens have been given emergency authorization for treatment of COVID-19: (1) casirivimab+imdevimab bind distinct epitopes on the RBD with dissociation constants K_D = 46 pM and 47 pM, respectively. Imdevimab binds the spike protein RBD from the front or lower left side while casirivimab targets the spike-like loop from the top direction (overlapping with the ACE2 binding site[3,100]); (2) bamlanivimab binds an epitope on the RBD in both its open and closed confirmations with a dissociation constant K_D = 71pM , covering 7 of the approximate 25 side chains observed to form contact with ACE2[4] and (3) bamlanivimab+etesevimab bind to distinct, but overlapping, epitopes within the RBD of the spike protein of SARS-CoV-2. Etesevimab binds the up/active conformation of the RBD with a dissociation constant K_D = 6.4 nM[5], it contains the LALA mutation in the Fc region, resulting in null effector function.

942 **Table 1 Neutralizing mAbs for SARS-CoV-2 currently in development up to**
943 **Dec. 11, 2020**

Sponsors	**Drug code/ International proprietary name**	**Status**	**Trial ID**	**Est. start**	**Est. primary completion**
Junshi Biosciences / Eli Lilly and Company	JS016, etesevimab	**EUA when used in combination with bamlanivimab***	**NCT04441918; NCT04441931; NCT04427501**	6/5/2020; 6/19/2020; 6/17/2020	Dec 2020; 10/2/2020; 3/11/2021
Tychan Pte. Ltd.	TY027	Phase 1; Phase 3 pending	**NCT04429529; NCT04649515**	6/9/2020; 12/4/2020	Oct 2020; 8/31/2020
Brii Biosciences	BRII-196	Phase 1	**NCT04479631**	7/12/2020	Mar 2021
Brii Biosciences	BRII-198	Phase 1	**NCT04479644**	7/13/2020	Mar 2021
AbbVie	ABBV-47D11	Phase 1 pending	**NCT04644120**	11/27/2020	May 2021
Sorrento Therapeutics, Inc.	COVI-GUARD (STI-1499)	Phase 1	**NCT04454398**	9/17/2020	Feb 2021

Mabwell (Shanghai) Bioscience Co., Ltd.	MW33	Phase 1	**NCT04533048**	8/7/2020	Dec 2020
HiFiBiO Therapeutics	HFB30132A	Phase 1	**NCT04590430**	Oct 2020	July 2021
Ology Bioservices	ADM03820	Phase 1 pending	**NCT04592549**	11/16/2020	Aug 2021
Hengenix Biotech Inc	HLX70	Phase 1 pending	**NCT04561076**	12/9/2020	Sep 2021
U. Cologne / Boehringer Ingelheim	DZIF-10c	Phase 1 /2 pending	**NCT04631705**; **NCT04631666**	11/23/2020; 11/23/2020	6/30/2021; 6/30/2021
Sorrento Therapeutics, Inc.	COVI-AMG (STI-2020)	Phase 1 /2 pending	**NCT04584697**	Dec 2020	April 2021
Beigene	BGB DXP593	Phase 1; Phase 2 pending	**NCT04532294;** (Phase 1); **NCT04551898** (Phase 2 pending)	8/31/2020; 10/30/2020	10/15/2020; 2/28/2021

Sinocelltech Ltd.	SCTA01	Phase 1; Phase 2/3 pending	**NCT04483375; NCT04644185**	7/24/2020; 2/10/2021	Nov 2020; 5/10/2021
AstraZeneca	AZD7442 (AZD8895 + AZD1061)	Phase 1; Phase 3 pending	**NCT04507256; NCT04625725; NCT04625972**	8/17/2020; 11/17/2020; 11/16/2020	Sep 2021; 7/31/2021; 6/16/2021
Celltrion	CT-P59	Phase 1; Phase 2/3	**NCT04525079; NCT04593641**; **NCT04602000**	7/18/2020; 9/4/2020; 9/25/2020	Nov 2020; 12/23/2020; Dec 2020
Vir Biotechnol./ GlaxoSmithKline	VIR-7831/ GSK4182136	Phase 2/3	**NCT04545060**	8/27/2020	Jan 2021
AbCellera / Eli Lilly and Company	bamlanivimab; combination of bamlanivimab + etesevimab	**EUA***	**NCT04411628** (Phase 1); **NCT04427501** (Phase 2); **NCT04497987** (Phase 3); **NCT04501978** (Phase 3);	5/28/2020; 6/13/2020; 8/2/2020; 8/4/2020; Aug 2020	8/23/2020; 9/15/2020; 3/8/2021; July 2021; Nov 2020

			NCT04518410 (Phase 2/3)		
Regeneron	REGN-COV2 (casirivimab + imdevimab)	**EUA***	**NCT04425629** (Phase 1/2); **NCT04426695** (Phase 1/2); **NCT04452318** (Phase 3)	6/16/2020; 6/10/2020; 7/13/2020	12/19/2020; 1/25/2021; 6/15/2021

944 Complete list at: https://www.antibodysociety.org/covid-19-biologics-tracker/

Glossary

Passive immunotherapy

The introduction of monoclonal or polyclonal antibodies derived from non-human or human blood products to provide protection against infection or envenomation.

Emergency use authorization

(EUA). A mechanism to facilitate the availability and use of medical countermeasures during a public health emergency. U.S. Food and Drug Administration issuance of an EUA permits the use of unapproved medical products or unapproved uses of approved medical products when no adequate alternatives are available.

Convalescent plasma therapy

(CPT). The administration of donated plasma, from an individual who has had an illness and recovered from it (for example, previously infected with SARS-CoV-2 but have now recovered), to an infected individual. The recovered patient's plasma contains antibodies, which when administered to patients is thought to boost the ability to fight disease.

Antibody dependent enhancement

(ADE). The promotion of viral uptake into cells due to the presence of suboptimal antibodies. ADE can result in enhanced viral replication and/or aberrant inflammation.

Immune globulin

A sterilized solution made from plasma and containing antibodies.

Monoclonal antibody

(mAb). Clonal antibody recognizing a single epitope on an antigen. Generally used in reference to recombinant sources.

Variants

Mutations arise in viruses resulting in genetic variation and the emergence of different versions of the virus.

Nucleocapsid (N) proteins

Proteins that enclose and protect a viral genome, such as the SARS-CoV-2 genomic RNA.

Spike (S) protein

Proteins found on the coronavirus cell surface, responsible for binding of the virus to host cells and subsequent viral entry. The receptor binding domain (RBD) of the S protein is the preferred target of neutralizing mAb therapies to SARS-CoV-2.

Randomized controlled trials

(RCTs). Are studies that randomly assign participants into an experimental group or a control group, in order to measure the effectiveness of a new intervention or treatment.

Time weighted average

(TWA). An average which takes both numerical level and time of a particular variable into consideration.

Medically attended visits

(MAVs). A medical visit such as telemedicine visits, in-person outpatient visits to or from a medical provider, urgent care or emergency department visits, or hospitalization.

Absolute risk reduction

This is the difference between the risk of an event in the control group and the risk of an event in the treated group.

Persistent high viral load

The continued presence of high amounts of viral load in patients, which is associated with increased risk of hospitalization.