Annex 2

Nonclinical and clinical evaluation of monoclonal antibodies and related products intended for the prevention or treatment of COVID-19

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Guidelines and their addenda published by the World Health Organization (WHO) are intended to be scientific and advisory in nature. Each of the following sections constitutes guidance for national regulatory authorities (NRAs) and for manufacturers of biological products. If an NRA so desires, the parent WHO Guidelines and this addendum may be adopted as definitive national requirements, or modifications may be justified and made by the NRA. It is recommended that modifications to the Guidelines and/or this addendum are made only on condition that such modifications ensure that the product is at least as safe and efficacious as that prepared in accordance with the guidance set out.

Abbreviations

ACE2	angiotensin-converting enzyme 2 (receptor)
ADA	anti-drug antibody
ADE	antibody-dependent enhancement (of disease)
AE	adverse event
AESI	adverse event of special interest
COVID-19	coronavirus disease 2019
Fc	fragment crystallizable (region)
FcγR	Fc gamma receptor
GMT	geometric mean titre
IMP	investigational medicinal product
MAAE	medically attended adverse event
mAb	monoclonal antibody
PD	pharmacodynamics
PK	pharmacokinetics
NRA	national regulatory authority
RBD	receptor binding domain
RT-PCR	reverse transcription-polymerase chain reaction
S	spike (glycoprotein)
SAE	serious adverse event
SARS-CoV-2	severe acute respiratory syndrome coronavirus 2

1. Introduction

Evaluating the safety and efficacy of monoclonal antibodies (mAbs) and related products intended for the prevention or treatment of infectious diseases requires different considerations than mAb products that target endogenous proteins, such as those intended for the treatment of noncommunicable diseases. To help address such differences, the WHO Guidelines on the nonclinical and clinical evaluation of monoclonal antibodies and related products intended for the prevention or treatment of infectious diseases (*1*) was adopted in 2023 on the recommendation of the WHO Expert Committee on Biological Standardization. These Guidelines outline the general principles applicable to the evaluation of mAbs for use against infectious diseases. However, although the document provides guidance on evaluating the safety and efficacy of mAb products regardless of the targeted pathogen, it was recognized that pathogen-specific considerations would potentially affect the interpretation and application of the guidance provided.

2. Purpose and scope

The current addendum is intended to provide supplementary considerations when evaluating the safety and efficacy of mAb products directed specifically against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) antigens. This includes mAb products intended for pre- and post-exposure prophylaxis as well as for the treatment of coronavirus disease 2019 (COVID-19). These considerations are applicable to mAbs and related products, including single and co-formulated mAbs against SARS-CoV-2. However, some mAb products (for example, bispecific mAbs or those with a different mechanism of action) may require additional nonclinical studies and the NRA should be consulted on the need for such studies. Unless otherwise indicated, the guidance applies to products that are administered parenterally.

It should be noted that mAbs and related products that target endogenous human antigens (for example, those which block the angiotensin-converting enzyme 2 (ACE2) receptor or cytokines) are not within the scope of this addendum as these require different considerations for evaluating their safety and efficacy.

Separate and detailed guidance on the production and quality control of mAbs is provided in the WHO Guidelines for the production and quality control of monoclonal antibodies and related products intended for medicinal use (*2*).

3. Terminology

The following definitions apply to the terms as used in this addendum. These terms may have different meanings in other contexts. It should be noted that additional terms relevant to this addendum are defined in the WHO Guidelines on the nonclinical and clinical evaluation of monoclonal antibodies and related products intended for the prevention or treatment of infectious diseases (*1*).

COVID-19: the disease caused by infection with SARS-CoV-2.

Long COVID: health problems that persist or develop after infection with SARS-CoV-2, and which may last from several weeks to years.

SARS-CoV-2: the virus that causes COVID-19.

Variant: a virus that possesses mutations which may confer altered transmissibility, receptor binding affinity, virulence, morbidity or mortality. A number of SARS-CoV-2 variants have been labelled as "variants of interest" (VOI), "variants of concern" (VOC) or variants under monitoring (VUM) depending on their emerging dominance among actively circulating strains.

4. General considerations

SARS-CoV-2 is an enveloped positive-sense single-stranded RNA virus belonging to the genus *Betacoronavirus*. The virus first emerged in Wuhan, China in 2019, with sustained human-to-human transmission confirmed shortly afterwards, followed by its rapid spread worldwide. Evidence of sustained global transmission led WHO to declare COVID-19 a pandemic in March 2020. COVID-19 was subsequently officially declared to no longer be a public health emergency of international concern by WHO on 5 May 2023. Nevertheless, the disease remains a major threat, with SARS-CoV-2 still in circulation in most regions of the world.

SARS-CoV-2 is transmitted primarily by the respiratory route producing a mucosal infection after a short incubation period that results in a range of disease symptoms and outcomes – from asymptomatic to severe disease leading to hospitalization and death, as well as long COVID in some cases (*3*–*7*). Moreover, there is a possibility that SARS-CoV-2 will become endemic and will continue to cause substantial levels of hospitalization and death due to the emergence of new variants. This is of particular concern among vulnerable groups such as the immunocompromised and those with underlying comorbidities (*8*, *9*).

Monoclonal antibodies, vaccines and other therapeutics against SARS-CoV-2 were developed rapidly and authorized for use, initially under emergency procedures. These early mAbs (*10*–*12*) and other products were all based on the genetic sequence of the ancestral Wuhan strain and provided protection against severe disease, hospitalization and death. However, to date, no correlates of protection or threshold of protection have been established and

challenges remain in directly comparing the neutralizing antibody activity of different products (*13*). It is clear that some of these early products now exhibit reduced virus neutralization activity against SARS-CoV-2 variants, and a number have been withdrawn from use, especially in regions with high levels of Omicron circulation (*10*, *11*, *14*, *15*). Although most early authorized mAbs have to varying degrees lost their ability to neutralize variant strains, this finding is primarily based on in vitro testing and does not necessarily reflect clinical experience (*10*, *13*). Protection against COVID-19 was initially provided both through SARS-CoV-2 neutralizing antibodies and through the induction of broader cellular immunity following infection and/or vaccination (*16*). To date, although SARS-CoV-2 variants have evolved to evade neutralizing antibodies, with consequences for infection, virus shedding and transmission, these have not significantly affected the longer lasting and broader cellular immune responses (*10*, *11*, *16*).

Nevertheless, it is clear that the emergence of variant strains of SARS-CoV-2 poses a major challenge to product development and evaluation of efficacy in clinical use (*9*, *17–19*). In response to this challenge, considerable efforts are now under way to develop SARS-CoV-2 mAbs and vaccines that are escape resistant (*10*, *11*, *15*, *20*). For example, progress in B-cell technologies has accelerated the identification and rapid isolation of candidate antibodies which can overcome variants arising through antigenic shift (*10*, *11*, *21*).

The principal target of both mAb and vaccine development has been the virus trimeric transmembrane spike (S) glycoprotein which protrudes from the virus surface and mediates its entry into host cells by binding to the ACE2 receptor (*10*, *22*). Entry requires cleavage of the S transmembrane glycoprotein generating S1 and S2 subunits to initiate fusion of the viral and cell membranes upon entry. The SARS-CoV-2 S glycoprotein contains a furin-like cleavage site for host cell proteases (*23*). Neutralizing mAbs targeting the receptor binding domain (RBD) of the S glycoprotein, the S2 subunit or the S1/S2 proteolytic cleavage site have been variously affected by the emergence of SARS-CoV-2 variants (*10*, *11*, *14*, *20*, *24*, *25*). To expedite the development of new mAbs, consideration is being given by some but not all regulatory authorities to immunobridging studies in support of licensure (see section 7.4.2 below). However, it is important that such an approach be discussed directly with the relevant NRA.

Clearly, the ongoing evolution of SARS-CoV-2 requires continuous monitoring for significant changes in local circulating variant strains which might impact the performance of mAbs, both in clinical studies and in use (*26*). Similarly, careful attention needs to be given to the virus strains used in nonclinical and clinical evaluation studies to ensure that the virus preparation used is well characterized and standardized with respect to variant strains (*27*).

Although the emergence of resistant variants of SARS-CoV-2 is an issue of concern with regard to efficacy, no major safety signals have been identified regarding the use of mAbs to prevent or treat COVID-19. However, the potential

for antibody-dependent enhancement (ADE) of disease is always a possibility, and is an important aspect to consider as part of nonclinical and clinical evaluation programmes (*28*, *29*). The complexities of assessing and predicting mAb-induced clinical ADE of disease in humans, including the poor predictability of both in vitro systems and animal models, are discussed in detail elsewhere (*28*). Particular attention should be given to the effects of any engineered modifications of the fragment crystallizable (Fc)-mediated effector functions of mAbs that may, for example, have been made to increase the half-life of the antibody (*10*, *11*).

5. International reference materials

WHO international reference standards are the primary reference materials used worldwide and such standards are available for SARS-CoV-2 antibodies to support the development of serological assays and to increase the comparability of results obtained by different laboratories. The WHO international reference standards related to SARS-CoV-2 antibodies available at the time of publication of the current document are:

- Second WHO International Standard for anti-SARS-CoV-2 immunoglobulin (*30*);
- First WHO International Standard for antibodies to SARS-CoV-2 variants of concern (*31*); and
- First WHO International Reference Panel for antibodies to SARS-CoV-2 variants of concern (*32*), and subsequent panel expansion to include Gamma and Omicron variants (*33*).

Although the above standards are likely to be useful for laboratories characterizing SARS-CoV-2 mAbs, further studies are needed to determine whether these polyclonal plasma standards can effectively harmonize the measurement of mAb neutralizing activity between laboratories.

Furthermore, it should be noted that when stocks become exhausted or new variants emerge, new or replacement standards are established on the recommendation of the WHO Expert Committee on Biological Standardization. Users should take steps to ensure use of the most recent and appropriate WHO international standard, international reference panel or other international reference standard.

6. Nonclinical evaluation

There are several important factors to consider when designing nonclinical studies for mAbs intended to prevent or treat SARS-CoV-2 infection. Such studies should characterize the targeted SARS-CoV-2 binding site/epitope and

the ability of the mAb to neutralize virus variants. The primary pharmacological effector functions of the mAb should be considered, especially if the Fc region of the mAb has been engineered. Any potential risk of unwanted or unexpected cross-reactivity with human cells or tissues, ADE or viral resistance should also be explored.

For the assessment of inhaled or intranasally administered mAbs, the selection of an animal model should take into consideration the differences in the anatomy and physiology of human and animal respiratory systems. The animal model selected should be justified when designing proof-of-concept studies for demonstrating mAb antiviral activity. If a delivery device (for example, nebulizer or dry powder inhaler) is required, its mechanism of delivery should be similar to the device intended for clinical use in humans. In some cases, additional studies may be required to ensure optimal conditions for mAb delivery in the animal model to be used.

6.1 Pharmacodynamics studies

The pharmacodynamics (PD) of the mAb should be characterized using in vitro assays.

6.1.1 Target antigen or epitope

To date, all neutralizing mAbs against COVID-19 target the SARS-CoV-2 S protein and exhibit both antigen binding and neutralizing activity. The ability of such a mAb to recognize the S protein should be demonstrated and its binding affinity measured. In addition, inhibition of binding of the S protein RBD to the human target ACE2 receptor should be demonstrated.

The epitope on the S protein targeted by the mAb should be identified for single-formulated mAbs. In the case of co-formulated mAbs (where there are two or more mAbs within a final product) or bispecific mAbs that target two binding epitopes, each targeted binding epitope should be identified. This is to ensure that the co-formulated or bispecific mAbs do not compete for the same epitope or have overlapping epitopes that could lead to antagonism.

In future, mAbs that target relevant SARS-CoV-2 antigens other than the S protein may be developed. In such cases, the ability of the mAb to recognize the targeted virus epitope should be demonstrated. If the mAb is intended to inhibit virus binding to a human cell then its ability to prevent virus binding and subsequent infection should be demonstrated.

6.1.2 Virus neutralization assays

The primary antiviral mechanism of mAbs is direct virus neutralization. The in vitro virus neutralization activity of the mAb should be assessed against historical,

currently dominant and emerging variants. Virus neutralization activity can be demonstrated using a live virus assay (for example, focus-reduction neutralization test, plaque-reduction neutralization test or surrogate neutralization assay) and/or a pseudovirus neutralization assay (*34*, *35*).

For co-formulated mAbs, the virus neutralization activity of each constituent mAb should be tested and any synergistic activity reported. For bispecific mAbs, the virus neutralization activity of each independent targeted epitope should be tested and reported.

6.1.3 Effector function assays

The need for effector function assays should be justified and is predominately important in the assessment of immunoglobulin G1 products. The secondary antiviral mechanism of mAbs is the effector functions driven by Fc gamma receptor (FcγR) interactions. The effector properties of the mAb, such as antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP) and complement-dependent cytotoxicity (CDC), should be assessed.

If the Fc region of the mAb has been engineered, the engineered pharmacological effects, such as extending mAb half-life or attenuation of Fc binding activity to Fc receptors, should be assessed and reported.

6.1.4 Assessment of antibody-dependent enhancement of disease

Clinical evidence of ADE of COVID-19 is limited and this has not been observed with currently approved mAb therapies or COVID-19 vaccines in humans. However, mAbs can mediate ADE via FcγR interaction or complement component C1q (*36*, *37*), and so the potential risk of ADE cannot be ruled out. Therefore, evaluation of the potential for ADE of disease should be carried out and the results reported. The selection of an in vitro assay(s) for assessing ADE should take into consideration current understanding of SARS-CoV-2 infection and ADE, and the reliability of the assay(s) in predicting mAb-induced clinical ADE of disease in humans. Although in vitro assays have limitations, they may provide useful information relevant to the potential risk of ADE (for example, information on virus neutralization, virus uptake and infectivity, or cytokine production) (*28*).

6.1.5 Virus resistance assessment

The effectiveness of mAbs has been threatened by the emergence of resistant SARS-CoV-2 variants, with reductions in the magnitude of neutralization of such variants using existing treatments reported (*38*). Therefore, the neutralization activity of the mAb against these variants should be evaluated in

virus neutralization assays using emerging variants from clinical surveillance, experimentally derived viral escape mutants and/or modelled predicted escape mutants. In addition, the risk of emergence of resistant viruses to the investigational mAb under treatment due to the use of suboptimal doses should be investigated in vitro before initiation of clinical studies. Where resistance is observed, genotyping, phenotyping and cross-resistance analyses of the potential escape mutants should be conducted.

6.2 In vivo studies

For animal proof-of-concept studies demonstrating antiviral activity, preference should be given to animal models in which the SARS-CoV-2 infection is reflective of the human infection and of the anticipated mechanism of action of the mAb. The similarity of SARS-CoV-2 infection in the chosen animal model to human infection and disease should be described and justified. With the ongoing detection of SARS-CoV-2 variants, consideration should also be given to assessing the similarity of infection across different variants in the animal model and in humans.

Several animal models for COVID-19 have been developed for the testing of mAbs and vaccines.[7] Each of the following models are able to reproduce some aspects of the clinical and pathological features of COVID-19 in humans (*39*, *40*).

- Syrian hamsters have been established as an animal model for COVID-19 due to the similarity of hamster and human ACE2. Viral replication is observed in the respiratory and gastrointestinal tracts following infection. Laboured breathing and weight loss are observed clinical symptoms. Severe interstitial pneumonia with inflammation is observed more in aged or male hamsters compared to young or female hamsters. The induction of serum neutralization antibodies has been observed following infection. In addition, hamsters have been shown to transmit SARS-CoV-2 by both close contact and non-contact routes.
- Normal mice are not a relevant model for COVID-19 as SARS-CoV-2 does not bind effectively to mouse ACE2. However, transgenic mice expressing human ACE2 or the use of mouse-adapted SARS-CoV-2 strains have made the mouse a useful model. Mice display a range of clinical symptoms (such as weight loss) and pathological disease symptoms (such as lung inflammation)

[7] Any animal species used for in vivo studies should be chosen carefully and thoroughly justified. For scientific and ethical reasons, it is desirable to apply the 3Rs principles of "Replace, Reduce, Refine".

that vary in severity. The use of mouse-adapted virus models or human ACE2 transgenic mice can also be a useful tool for studying infection with SARS-CoV-2 variants.

- Ferrets have long been a model for studying human respiratory viruses such as influenza and respiratory syncytial virus, and are now being used to investigate SARS-CoV-2 transmission and COVID-19. Ferrets display mild clinical disease that includes fever, wheezing and nasal discharge. Virus replication is observed in the respiratory and gastrointestinal tracts. Histopathological studies have shown pneumonia with lung inflammation. Transmission studies have demonstrated virus transmission by both close contact and non-contact routes, suggesting that airborne transmission of SARS-CoV-2 among ferrets is possible, making them a useful model for such studies.
- Non-human primates have been infected with SARS-CoV-2 variants and studies have shown high levels of viral replication in both the upper and lower respiratory tract. Non-human primates display mild clinical disease but with notable histopathology findings of pneumonia. Severe disease has been observed in aged non-human primates. The induction of natural protective immunity through innate, humoral and cellular immune responses following infection has also been observed. Non-human primates should only be considered as a last resort option, and the selection of this model should be extensively justified.

Based on the above differences in clinical and pathological aspects, the selection of animal models for characterizing the potential clinical use of the mAb (for prophylaxis or treatment, or both) should be justified. Furthermore, the design of the proof-of-concept study should also reflect the intended clinical use(s) of the mAb (that is, for pre-exposure prophylaxis and/or post-exposure prophylaxis, and/or for treatment).

The characteristics of SARS-CoV-2 infection and COVID-19 outcomes in the above animal models are summarized in Table 1. This summary table is provided here for information only and the more detailed information available in the scientific literature on the use of selected animal models for SARS-CoV-2 and COVID-19 (*39–41*) should be taken into consideration when designing proof-of-concept studies.

Table 1
SARS-CoV-2 infection characteristics and disease outcomes in animal models

Relevant animal models	Infection characteristics and disease outcome
Rodent	
Syrian hamster	• Susceptible to SARS-CoV-2 as hamster ACE2 is similar to human ACE2 • Main clinical disease symptom is weight loss • High levels of viral replication in lungs at early stage after infection followed by rapid decline in virus levels, and no virus detected 1 week after challenge • Lung histopathological changes observed (for example, pneumonia, inflammatory cell infiltration) • Severe clinical disease observed more often in males than in females and aged hamsters • Naturally clear infection
Transgenic mouse	• Expressed human ACE2 (hACE2) allows SARS-CoV-2 infection • Main clinical disease symptom is weight loss but some models may also show respiratory distress • Respiratory tract infection following virus challenge but other organs (for example, brain, heart) may be infected due to secondary infection or expression of hACE2 • Lung histopathological changes observed (for example, pneumonia, diffuse alveolar damage, inflammatory cell infiltration) • Severe disease observed when challenged with higher viral load
Mouse-adapted SARS-CoV-2 mouse	• Mutations (N501Y or Q498T and P499Y) in RBD region of SARS-CoV-2 makes it adaptive to mouse ACE2 • Clinical signs typically limited to mild weight loss but loss of pulmonary function also observed with mouse-adapted virus carrying Q498T and P499Y mutations • Mild to moderate pneumonia observed with mouse-adapted virus carrying N501Y mutation • Respiratory tract infection observed

Table 1 *continued*

Relevant animal models	Infection characteristics and disease outcome
Other	
Ferret	• Naturally susceptible to SARS-CoV-2 • Clinical symptoms similar to humans (fever and mild respiratory symptoms) • Viral replication observed in respiratory tract (nasal wash, lungs) • Interstitial pneumonia observed • No deaths from infection observed
Non-human primate – non-human primates should only be considered as a last resort option, and the selection of this model should be extensively justified	
Rhesus macaque	• Mild clinical disease (for example, fever, weight loss) • High levels of viral replication in respiratory tract (detected by nasal swab, bronchoalveolar lavage, lung tissue examination); lung histopathological changes (for example, pneumonia, pulmonary discoloration) similar to humans • Severe disease not observed • Naturally cleared infection
Cynomolgus macaque	• Mild clinical disease (for example, fever, weight loss) • High levels of viral replication in respiratory tract (detected by nasal swab, bronchoalveolar lavage, lung tissue examination); lung histopathological changes (for example, diffuse alveolar damage, pulmonary discoloration) similar to humans • Severe disease not observed • Naturally cleared infection
African green monkey	• Clinical disease and histopathological changes similar to rhesus or cynomolgus macaques • Severe disease (acute respiratory distress syndrome) observed in aged monkeys

Although the use of animal models has advanced the development of several COVID-19 therapeutics, there is no specific animal model optimized to mimic human SARS-CoV-2 infection and COVID-19. The selection of appropriate animal models for proof-of-concept studies should take into consideration the disease outcome of each animal model with regard to the intended study end-points.

The design of proof-of-concept studies should also ensure the use of a well-characterized virus challenge strain and acceptable route of inoculation.

The minimum anticipated biological effect level (MABEL) or biological effective dose (BED) should be used to select dose levels and to optimize the anticipated therapeutic effect.

7. Clinical evaluation

There are several factors to be considered in the clinical development programmes of anti-SARS-CoV-2 mAbs as they impact the clinical trial design and end-points to be used. One important consideration is whether the product is intended to be used as a prophylactic (for pre-exposure prophylaxis and/or post-exposure prophylaxis), as a therapeutic, or both.

For treatment indications, the timing of administration of the mAb is especially relevant. Current anti-SARS-CoV-2 mAbs were generally found to be more effective when administered early to patients with symptomatic COVID-19 and prior to hospitalization (*42*, *43*). Some, but not all, studies suggest that such mAbs may be associated with worse outcomes for patients requiring high-flow oxygen or mechanical ventilation (*44–46*).

Inhaled or intranasally administered mAbs are currently under development and may provide some advantages due to more-localized administration and lower systemic exposure. Additional considerations may be required for these alternative routes of administration, such as compatibility of the delivery device with the mAb formulation, mAb distribution within the airways and potential for systemic exposure. In addition, robust pharmacokinetic and pharmacodynamic modelling should be performed. For efficacy evaluation, consideration may be given to measuring the prevention of infection. Sponsors should consult with the NRA to help ensure a comprehensive regulatory approach for such products.

Because of the functionality of the mAb, healthy volunteers may not be suitable candidates for therapeutic efficacy trials, but may be appropriate for prophylactic studies. Healthy volunteers may also provide useful data on product safety, preliminary pharmacokinetics (PK) and potential for anti-drug antibody (ADA) induction in Phase I studies. PK parameters may require confirmation in infected patients to highlight any differences compared to healthy volunteers. As repeated administration of the mAb may alter its safety and activity profiles, repeat-dosing studies should be conducted to support the use of additional administrations.

Clinical trial duration can vary depending on the biological half-life of the mAb. A number of anti-SARS-CoV-2 mAbs have been engineered for increased half-lives of approximately 6 months. The duration of follow-up for participants should be appropriate for the investigational product to provide information on its long-term efficacy and safety, and should be discussed with the NRA.

Participants in clinical trials should be representative of the population targeted for eventual product use. This population should include individuals who are unlikely to mount an adequate immune response to COVID-19 vaccination secondary to immunocompromised status, elderly people and/or subjects with comorbidities such as:

- obesity
- cardiovascular disease, including hypertension
- chronic lung disease, including asthma
- type 1 or type 2 diabetes mellitus
- chronic kidney disease, including those on dialysis
- chronic liver disease.

All of these have been identified as groups at high risk of severe COVID-19 and death (*47*). However, the immunocompromised population is also quite heterogeneous and the risk of progression to severe disease, even in those adequately vaccinated, can vary considerably between the different pathologies.

The COVAXID cohort study reported the 1-year follow-up immune response of 356 subjects after COVID-19 messenger RNA vaccination in a real-world setting. Subjects who had undergone solid organ transplant and who had been treated with mycophenolate mofetil, those with common variable immunodeficiency, with chronic lymphocytic leukaemia treated with ibrutinib, or with X-linked agammaglobulinemia exhibited lower vaccine responses compared to other groups of immunocompromised patients (*48*). Those with a higher risk of disease progression even after vaccination thus require alternative therapies. In this regard, several randomized trials and real-world studies have investigated the role of mAbs in reducing hospitalization and preventing progression from asymptomatic to symptomatic disease, and even death (*49*).

The complications that result from the inclusion of immunocompromised individuals in clinical studies include ethical concerns, for example with regard to the comparator used (that is, whether a placebo or active comparator is used). The extrapolation of efficacy data in low-risk patients, based on neutralizing antibody titres, may be reasonable, and should be discussed early in clinical development with the NRA and ethics committee. In addition, in immunocompromised patients, the virus can remain viable for a longer period of time which prolongs the duration of potential spreading. Studies have shown that mAbs can reduce the time needed to clear the replicating virus, which not only benefits infected immunocompromised individuals through the avoidance of longer isolation periods (*50*) but also reduces the risk of virus transmission to others.

Vaccines and mAb therapies are complementary approaches to prophylaxis and treatment of COVID-19. However, due to the specificity of

immune support provided by mAbs, certain variants of SARS-CoV-2 can evade the response. One related issue is that the early mAbs are no longer effective against more recently circulating VOC, reinforcing the belief that new mAbs with conserved efficacy across different VOC are needed. Therefore, the decision to administer mAbs should be based on factors such as the regional prevalence of resistant variants, and on individual patient health status (*51*, *52*).

The epidemiological situation, including circulating variants, should be monitored and noted in the study report as any change in circulating virus variants may have a significant impact on the clinical efficacy of the mAb product. The viral strain of infected patients should thus also be determined and recorded during the clinical study. In addition, continued monitoring of emerging viral variants and of the neutralization activity of the mAb against them is vital.

Furthermore, the risk of development of viruses resistant to the investigational mAb should be evaluated in all clinical breakthrough cases, using phenotypic, genotypic and cross-resistance analysis.

7.1 Inclusion and exclusion criteria

For considerations specific to the inclusion or exclusion of pregnant or breastfeeding women see section 7.4.4 below.

7.1.1 Prophylaxis

Inclusion criteria

- Participants who can benefit from passive immunization with antibodies.
- Medically stable participants.
- Result from SARS-CoV-2 serology and RT-PCR testing at screening.
- Able to understand and comply with study requirements/procedures based on the assessment of the investigator.

Exclusion criteria

- Significant infection or other acute illness, including fever > 37.8 °C on the day prior to or day of randomization.
- Known history of allergy or reaction to any component of the study drug formulation.
- Previous known hypersensitivity, infusion-related reaction or severe adverse reaction following administration of a mAb.
- Bleeding disorder or prior history of significant bleeding or bruising following intramuscular injections or venepuncture.

- Any other significant disease, disorder or finding that may significantly increase the risk to the participant because of participation in the study, affect the ability of the participant to participate in the study or impair interpretation of the study data.
- Receipt of any investigational medicinal product (IMP) in a set period (as defined by the sponsor) immediately prior to the study, or expected receipt of an IMP during the period of study follow-up, or concurrent participation in another interventional study.

7.1.2 Treatment

Inclusion criteria

- Participant has a documented laboratory-confirmed SARS-CoV-2 infection.
- WHO Clinical Progression Scale score > 1 and < 4 (*53*).
- Participant must be dosed with the IMP within a set period (as defined by the sponsor) following self-reported onset of COVID-19-related symptoms (mild to moderate COVID-19).
- One or more of the signs/symptoms relevant to COVID-19 infection (for example, cough, sore throat, shortness of breath or difficulty breathing at rest or with activity, body pain or muscle pain/aches, fatigue, headache, chills, nasal obstruction or congestion, nasal discharge, nausea or vomiting, diarrhea, new loss of taste or smell).
- Oxygenation saturation of ≥ 92% obtained at rest by study staff within 24 hours prior to Day 1 (unless participant regularly receives chronic supplementary oxygen for an underlying lung condition).

Exclusion criteria

- Current hospitalization for severe COVID-19, requiring oxygen therapy or mechanical ventilation.
- Previous known hypersensitivity, infusion-related reaction or adverse reaction to any mAb, or known allergy to components of the IMP or placebo.
- Current requirement or anticipated impending need for mechanical ventilation.
- Any significant disease, disorder or finding that may increase risk to the participant and that might affect their ability to participate in the study.

- Participant must not participate in another clinical trial for the treatment of COVID-19 or SARS-CoV-2 during the study period until reaching hospitalization or 28 days after entry into the study (whichever is earliest).
- Receipt of systemic steroids or inhaled steroids prior to study entry, unless a stable dose is being used for a chronic condition.
- Receipt of any other IMP or expected receipt of an IMP during the study follow-up period, or concurrent participation in another interventional study.

7.2 Phase I studies

Phase I and first-in-human studies are conducted to determine the initial safety and tolerability of the IMP following completion of the essential nonclinical studies. Clinical experience has demonstrated that most COVID-19 mAb products are, in general, well tolerated.

The determination of starting dose, dose escalation steps and maximum exposure for first-in-human studies should take into consideration all available nonclinical information (for example, PD, PK, toxicokinetics and toxicological profiles, and dose or exposure/effect relationships) as well as safety and toxicity information derived from testing in a relevant animal model during nonclinical evaluation. For additional information on animal models of SARS-CoV-2 infection see section 6.2 above.

Products with the same antibody scaffolding and manufacturing process used for previously authorized anti-SARS-CoV-2 mAbs (that is, a product that only differs from the authorized product in the epitope binding site) may leverage the clinical development of the authorized product to expedite certain aspects of their clinical development. However, this should be discussed with the NRA, particularly if the mechanism of action has changed. Phase I trials may be conducted in healthy volunteers to determine the mAb safety profile, PK and potential physiological responses. If the product is intended to be administered in the elderly, in children or in other specific groups, then safety and tolerability data may be required for those specific groups.

7.3 Clinical pharmacology

7.3.1 Pharmacokinetics

Multiple-dose PK studies may not be required if the mAb is intended to be given only in a single dose. However, if the product is intended to be repeatedly administered, safety and tolerability data may be required to support the dosing regimen.

7.3.2 Pharmacodynamics

The PK, combined with nonclinical PD target levels, should guide the doses to be evaluated. Such studies may involve the ex vivo assessment of the neutralizing activity of the mAb in serum collected at different timepoints following administration.

7.4 Phase II and III studies

7.4.1 Efficacy

The clinical trial design of Phase II and III studies for efficacy determination will depend on whether the mAb is intended to be a prophylactic or therapeutic product.

The efficacy of a prophylactic mAb should be evaluated in terms of its ability to prevent the disease or progression to severe disease, but may also be assessed in terms of its ability to eliminate the pathogen, reduce the viral load or reduce virus shedding.

The efficacy of a therapeutic mAb should be evaluated in terms of its ability to prevent disease progression (that is, prevent deterioration in overall clinical status, hospitalization or death) and/or reduce clinically relevant end-points, such as time to sustained alleviation of symptoms, following confirmation of infection. The efficacy of a therapeutic mAb may also include the ability to eliminate the pathogen, reduce the viral load or reduce virus shedding.

An emphasis should be placed on designing randomized controlled trials that take into account the intended target population, the selected clinical end-point(s) (Table 2) and case definitions (*53*).

The local epidemiology of circulating variants may also affect efficacy outcomes, particularly if the mAb has different binding affinities to such variants. For this reason, in vitro neutralizing assays against any identified new variant should be conducted to ensure that the mAb retains activity against this new variant and that the study can safely be continued.

The selection of an appropriate authorized product as a comparator for use in efficacy trials will also require careful consideration and may vary depending on intended use – that is, for prevention or treatment. A randomized controlled double-blind trial design should be used in efficacy studies intended to prevent or treat infections. A placebo control may be considered when there is no appropriate comparator, no known therapeutic agent is effective, or when the natural history of the untreated infectious disease is relatively benign or self-limiting (that is, of low risk to patients) and where switching to an approved treatment is ensured in case of progression to severe disease. Any other current standard of care practices for the prevention or treatment of the infection must be provided to all participants regardless of the treatment arm. It is recommended that in all cases, these issues are discussed in advance with the NRA and ethics committee.

7.4.2 Immunobridging

To accelerate initial approval of novel mAbs manufactured on the same platform technology as already approved mAbs, an immunobridging approach could be an acceptable pathway for mAbs intended for prevention (*54*). The immunobridging should be based on a cross-variant comparison in a non-inferiority study with an approved mAb with the same indication. The geometric mean titres (GMTs) of neutralizing antibodies at Day 28 of an already approved mAb product against a virus strain for which efficacy was shown (for example, Alpha) should be compared to the GMTs achieved at the same timepoint with the new investigational mAb product against circulating variants. The acceptable non-inferiority margin when using a comparison of GMT values should be discussed with the NRA. Following initial approval, post-marketing efficacy data (including data from the investigation of breakthrough cases) should be collected, neutralizing antibody concentrations monitored to determine the timing of antibody waning, and long-term efficacy and safety data generated for at least 6 months.

Deciding upon the acceptability of an immunobridging approach, particularly for bispecific mAbs and mAbs with a different mechanism of action, will be in the remit of the NRA.

Presently, there are not sufficient data to derive a specific mAb concentration or neutralizing threshold to derive a correlate of protection for SARS-CoV-2.

7.4.3 Safety

The continual evaluation of mAb product safety is an important component within all phases of clinical studies. Although mAbs generally have a very good safety profile, each product is unique and should be considered independently.

Safety data should be obtained from a sufficient number of subjects during the clinical trials to characterize and quantify the product safety profile, which can include the type, frequency and severity of adverse drug reactions. In some cases, it may be possible to consider safety data from multiple clinical studies if both the products tested and the study conditions are sufficiently similar.

Evaluating the safety and tolerability of anti-SARS-CoV-2 mAbs should include the recording of all adverse events (AEs), serious adverse events (SAEs), medically attended adverse events (MAAEs) and adverse events of special interest (AESIs) over the duration of the study (Table 2).

Product reactogenicity should also be clearly characterized by monitoring immune responses to the mAb through ADA titres and immune system activity.

Table 2
Objectives, estimands and clinical end-points

Objectives	Estimand description/end-point
Primary	
Estimate the efficacy of the mAb	An appropriate time frame for the assessment of efficacy should be provided to the NRA based on the end-point being assessed (for example, 6 months for prophylaxis, Day 28 for treatment) For pre-exposure prophylaxis, a binary response whereby a participant is defined as a COVID-19 case if a SARS-CoV-2 RT PCR-positive[a] symptomatic illness occurs post dose(s) of the mAb and prior to the specified time frame. For post-exposure prophylaxis, a composite outcome of either hospitalization or progression of symptoms post dose(s) of the mAb during the specified time frame. For treatment, a composite outcome of medical attendance visits, hospitalization or death from any cause and/or time to sustained resolution of symptoms post dose(s) of the mAb during the specified time frame.[b]
Estimate the safety and tolerability of the mAb	AEs, SAEs, MAAEs and AESIs during the study period
Secondary	
Estimate the efficacy of the mAb in preventing severe or critical symptomatic COVID-19	Incidence of SARS-CoV-2 RT-PCR-positive severe or critical symptomatic illness occurring after dosing with the mAb
Estimate the efficacy of the mAb in preventing COVID-19-related emergency department visits	The incidence of COVID-19-related emergency department visits occurring after dosing with the mAb
Assess the PK of the mAb following administration of an appropriate dose via an appropriate route	Serum concentrations
Evaluate ADA response to the mAb in serum	Incidence of ADA to the mAb in serum

Table 2 *continued*

Objectives	Estimand description/end-point
Exploratory	
Estimate the efficacy of the mAb over a longer time frame	An appropriate time frame for assessment of efficacy for pre-exposure prophylaxis should be provided to the NRA based on the end-point being assessed. (for example, 12 months for prophylaxis) A binary response whereby a participant is defined as a COVID-19 case if a SARS-CoV-2 RT-PCR-positive symptomatic illness occurs post dose(s) of mAb and prior to the specified time frame.
Determine anti-SARS-CoV-2 mAb levels in serum following the administration of the mAb	Post-treatment GMT and geometric mean fold rise from baseline value through an extended time frame
Quantify SARS-CoV-2 viral loads in infected participants treated with the mAb	Viral genome copies in nasopharyngeal swabs at illness visits as determined by quantitative RT-PCR
Quantify the duration of viral shedding in participants with symptomatic COVID-19 treated with the mAb	Duration of SARS-CoV-2 shedding in saliva
Characterize the risk of development of resistance to the mAb in patients with virological failure	Genotypic analysis and biochemical and/or susceptibility analysis of SARS-CoV-2 variants
Assess additional immune responses following administration of the mAb	Other exploratory assays for humoral, mucosal and cellular immune responses may be performed based upon emerging safety, efficacy and PD data.
Estimate the efficacy of the mAb in preventing long COVID	The incidence of long COVID occurring after dosing with the mAb

[a] RT-PCR-positive = reverse transcription-polymerase chain reaction-positive.

[b] As the rate of progression to severe COVID-19 has significantly decreased due to the increased levels of immunization and seropositivity in the population, as well as the lower progression rates seen with Omicron variants, the primary efficacy end-point of progression to severe disease or death may no longer be appropriate. The use of an alternative end-point (such as sustained resolution of symptoms or non-progression of the clinical status) should be considered. In all cases, consultation with the NRA is recommended during trial design and end-point selection.

7.4.4 During pregnancy and breastfeeding

Some studies have shown that COVID-19 infection during pregnancy was associated with a greater probability of maternal, fetal and neonatal complications, including pre eclampsia, increased risk of admission to an intensive care unit for the mother, preterm birth and neonatal mortality compared to non-infected pregnant women (*55*, *56*). However, many studies related to SARS-CoV-2 infection in pregnancy were performed among hospitalized patients, which may have led to overestimation of the risk of severe outcomes as not all cases of SARS-CoV-2 infection in the pregnant population were included (*57*, *58*). Moreover, pregnant women who were obese and women with comorbidities were more likely to develop severe disease or to present greater risk of complications related to COVID-19 than pregnant women without such conditions (*57*, *59*, *60*).

The extensive physiological changes associated with pregnancy may alter drug PK and PD, thus directly affecting the safety and efficacy of any drug administered during pregnancy through alterations in drug absorption, distribution, metabolism and excretion (*61*).

Currently, information on medicinal drug use in pregnancy and breastfeeding generally is collected in the post-marketing setting, using data from observational studies such as pregnancy exposure registries and other cohort studies, case control studies and surveillance methods. However, this approach commonly results in delayed access to new medicinal products for pregnant and breastfeeding women. There are multiple reasons for considering the inclusion of pregnant women in clinical trials, including:

- Women need safe and effective treatment during pregnancy.
- Failure to establish the dose/dosing regimen, safety and efficacy of treatments during pregnancy may compromise the health of women and/or the fetus.
- In some settings, the enrolment of pregnant women in clinical trials may offer the possibility of direct benefits to such women and/or their fetus that are unavailable outside the research setting.
- The development of accessible treatment options for pregnant women is a significant public health issue.

Systematic consideration should be given to the possible use of any new medicine by pregnant and breastfeeding women and, where warranted, to planning formal investigations in these populations. Such planning should take into account different variables, such as the benefit and risk perspective, as well as the need for systematic and timely study of medicines likely to be used in this population to support dosing, use rationale and other aspects (*62*). Data obtained to date, mainly from clinical settings, suggest that COVID-19 mAb products seem to be well tolerated and likely to be safe when used during pregnancy (*63–68*).

Sponsors should consult with the NRA early in the product development phase on the requirements for specific nonclinical studies, and on the potential inclusion of pregnant women in clinical studies to promote the health of pregnant women and their fetus, and to inform prescribing decisions during pregnancy. Proper follow-up of the mother-child pair should also be considered to fully determine the impact of product administration on maternal and newborn health (*63*–*68*).

7.4.5 Human challenge studies

Human challenge studies of SARS-CoV-2 infection have been conducted to better understand COVID-19, especially during the early stages of infection, and for the potential evaluation of candidate vaccines, antiviral drugs and antibodies (*69*, *70*). The first reported study (*70*) paid careful attention to the preparation of the challenge stock and used highly characterized virus, including whole genome sequencing to confirm that the challenge virus was unaltered compared to the original isolate. Although not technically a clinical trial (no product was being investigated), regulatory oversight of the challenge strain was provided by the United Kingdom Medicines and Healthcare products Regulatory Authority, which confirmed that its manufacture would be suitable for use in future efficacy studies of an IMP. However, it may not be possible to undertake such studies in some jurisdictions and the relevant NRA should always be consulted directly. Such studies have not yet been used to assess the efficacy of mAbs but may be used in the future.

7.4.6 Paediatric considerations

In children, severe COVID-19 is uncommon. However, those with certain underlying conditions (such as cardiovascular, respiratory, neuromuscular or malignant disease, or immunocompromised individuals) are prone to unfavourable outcomes. Therefore, while most children infected with SARS-CoV-2 will recover without therapy, treatment of mild or moderate infection should be considered in paediatric patients at highest risk of progression to severe disease. This would be in alignment with the current indication for the use of mAbs against SARS-CoV-2 in adults.

None of the currently licensed mAbs against SARS-CoV-2 are authorized for use in children under 12 years of age. In addition, even for mAbs against SARS-CoV-2 that have already been commercialized, safety and efficacy data in paediatric patients are limited. Furthermore, the additional data available from observational studies are associated with limitations. With regard to post-authorization data, it is important to highlight that the generation of data in children has been greatly hampered by the loss of effectiveness of early mAbs against recently circulating VOC. Overall, the data generated so far do not suggest

an excessive risk of toxicity in children compared with adults, and mAbs seem to be well tolerated. However, the lack of a comparator group in studies makes clear estimation of the effectiveness of mAbs in preventing COVID-19 progression in children difficult. Therefore, further studies are needed to fully define the safety and efficacy of mAb therapy in the paediatric population (*71–74*).

The inclusion of children and adolescents in clinical trials should always be considered when planning a study to avoid knowledge gaps and to facilitate early access to new medicinal products (*73–77*). Sponsors are encouraged to discuss paediatric drug development with the NRA early in clinical development, including: (a) the potential for extrapolating efficacy data from studies in adults; (b) appropriate PK trials in paediatric subjects to support dose selection; (c) the recommended size of the pre-approval safety database in children; and (d) the targeted age group(s) (*78–80*).

7.4.7 Post-authorization studies

The potential risk of treatment failure due to the development of SARS-CoV-2 variants resistant to the mAb, along with the potential risks associated with any biological therapy (including mAbs) should continue to be assessed post-authorization.

Data monitoring (including systematic and proactive review of the emerging data) should be conducted using all available data sources, for example by evaluating:

- new and cumulative nonclinical data (antiviral activity and viral resistance);
- data on variants detected in clinical studies among patients who received mAbs;
- spontaneous reports related to lack of efficacy, including information for variant lineages; and
- literature reports or studies conducted by public health authorities.

The requirements for a risk-management plan, Phase IV studies and/or use of real-world evidence and data should be discussed with the NRA.

Authors and acknowledgements

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The resulting draft document was posted on the WHO Biologicals website from 1 November to 4 December 2023 for a first round of public consultation. Comments were received from Dr S. Hufton and Dr G. Mattiuzzo, Medicines and Healthcare products Regulatory Agency, United Kingdom; Dr J. Wang, National Institutes for Food and Drug Control, China; Dr S. Tognarelli, Paul-Ehrlich-Institut, Germany; Dr T. Cohen, AstraZeneca, USA; the Nonclinical working party, 3Rs working party, and Pregnancy group, European Medicines Agency, Netherlands (Kingdom of the); Dr J. Holst, Holst PharmaWorks, Norway; and Dr R. Gupta, Vir Biotechnology, USA.

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