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MONOCLONAL ANTIBODIES IN THERAPY OF COVID-19

Specialist thesis

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Postgraduate Specialist Study: Drug development

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The work presented in this specialist thesis was under supervision of Gordana Maravić Vlahoviček, Ph. D., Associate Professor, as a part of the Postgraduate Specialist Study "Drug development" at the Faculty of Pharmacy and Biochemistry, University of Zagreb.

I am sincerely grateful to my supervisor, Gordana Maravić Vlahoviček, Ph. D., Associate Professor, who made this work possible.

I want to thank my family, friends and colleagues for all the support that I get throughout this journey.

SUMMARY

OBJECTIVES:

The aim of this research is to present an overview of current knowledge about the possibilities and challenges of mAbs therapy in treating COVID-19 in patients with high risk to develop severe form of the disease.

The hypotheses of the research are:

1. Neutralizing mAbs could be beneficial for vulnerable populations, such as the unvaccinated or recently vaccinated high-risk patients, before or after exposure to SARS-CoV-2;

2. Monoclonal antibody therapy for COVID-19 is well tolerated with minimal risks;

3. Transitioning from monotherapies towards combination therapies of mAbs will lead to lower viral escape, longevity of the therapies and reducing the potential rate of treatment failure.

MATERIALS AND METHODS

(SYSTEMATIC REVIEW OF KNOWLEDGE ON THE TOPIC):

General overview of mAbs and SARS-CoV-2 was presented using scientific literature.

For presenting evidence of the use of new mAbs with activity against SARS-CoV-2, all publication types, including peer-reviewed manuscripts, were included in this review.

Publications were identified primarily through a search including the following key search terms: monoclonal antibody, antibody treatment, COVID-19, Regdanvimab, Sotrovimab, Tocilizumab, Anakinra, Bebtelovimab, Tixagevimab, Cilgavimab, Casirivimab/Imdevimab, from January, 2020, to January 2023. PubMed, Scopus, Google Scholar, and ClinicalTrials.gov were used for this purpose.

Relevant articles were studied in analytical and critical way with regard to the definition of scientific and/or professional problem, research of existing knowledge on a defined problem, design of the working hypothesis, selection of methods for examining the hypothesis, presentation and analysis of the results and the conclusions drawn.

An overview of the clinical trials addressing this topic was performed by searching on ClinicalTrials.gov using the following condition: “SARS-CoV-2, COVID-19”. Studies published in non-English languages were excluded. This search was limited to review articles focused on mAbs with specific activity against SARS-CoV-2. Articles focusing on mAbs used in the treatment of COVID-19 but without specific activity against SARS-CoV-2 were excluded.

DISCUSSION:

The proposed research provides an overview of the currently approved mAbs for treatment of COVID-19, development and methods for obtaining them, their mechanism of action against virus of COVID-19, problems with efficacy and benefits from their use.

The paper will be a useful source of information for additional education of pharmacists and other medical professionals, as well as to all members of the general population.

CONCLUSION:

The urgent need for treatment for COVID-19 lead to development of novel neutralizing mAbs or repurposed existing ones, which can neutralize the virus in patients.

Neutralizing mAb regimens have been given Conditional Marketing Authorization (CMA) in the EU and UK and Emergency Use Authorization (EUA) for treatment of COVID-19.

To date studies have demonstrated the scientific and clinical validity of the use of neutralizing mAbs in patients with high risk of developing severe form of the disease, in order to prevent fatal outcomes. Efficacy of the treatment with mAbs was decreased by the mutations of the new circulating strains.

It is therefore justifiable to continue research in this field to improve the efficacy of mAbs against new variants of SARS-CoV-2 and come out with new knowledge about treatment of COVID-19, especially in patients with high risk to develop severe form of the disease.

SAŽETAK

CILJ ISTRAŽIVANJA:

Cilj ovog istraživanja je prikazati pregled dosadašnjih spoznaja o mogućnostima i izazovima terapije monoklonskim protutijelima u liječenju COVID-19 u bolesnika s visokim rizikom za razvoj teškog oblika bolesti.

Hipoteze istraživanja su:

1. Neutralizirajuća monoklonska protutijela mogla bi biti korisna za ranjivu populaciju, kao što su necijepljeni ili nedavno cijepljeni visokorizični pacijenti, prije ili nakon izlaganja SARS-CoV-2;

2. Liječenje monoklonskim protutijelima za COVID-19 dobro se podnosi uz minimalne rizike;

3. Prijelaz s monoterapija na kombinirane terapije monoklonskim protutijelima dovest će do smanjenja virusnog imunosnog bijega, dugotrajnosti liječenja i smanjenja potencijalne stope neuspjeha liječenja.

MATERIJALI I METODE (SUSTAVNI PREGLED SAZNANJA O TEMI):

Opći pregled monoklonskih protutijela i SARS-CoV-2 prikazan je korištenjem znanstvene literature. Za predstavljanje dokaza o upotrebi novih monoklonskih protutijela s djelovanjem protiv SARS-CoV-2, pregledane su sve vrste publikacija, uključujući recenzirane znanstvene radove. Publikacije su identificirane prvenstveno pretraživanjem koje uključuje sljedeće ključne pojmove za pretraživanje: monoklonska protutijela, liječenje protutijelima, COVID-19, Regdanvimab, Sotrovimab, Tocilizumab, Anakinra, Bebtelovimab, Tixagevimab, Cilgavimab, Casirivimab/Imdevimab, od siječnja 2020. do siječnja 2023. U tu svrhu korišteni su PubMed, Scopus, Google Scholar i ClinicalTrials.gov.

Relevantni članci proučeni su na analitički i kritički način s obzirom na definiranje znanstvenog i/ili stručnog problema, istraživanje postojećih spoznaja o definiranom problemu, oblikovanje radne hipoteze, izbor metoda za ispitivanje hipoteze, prikaz i analizu rezultata i izvedene zaključke.

Pregled kliničkih ispitivanja koja se bave ovom temom proveden je pretraživanjem na ClinicalTrials.gov uz korištenje sljedećeg uvjeta: "SARS-CoV-2, COVID-19". Isključene su studije objavljene na jezicima koji nisu engleski. Ovo pretraživanje bilo je ograničeno na pregledne članke usredotočene na monoklonska protutijela sa specifičnim djelovanjem protiv SARS-CoV-2. Članci koji se fokusiraju na monoklonska protutijela koja se koriste u liječenju bolesti COVID-19, ali bez specifične aktivnosti protiv SARS-CoV-2, isključeni su.

RASPRAVA:

Predloženo istraživanje daje pregled trenutno odobrenih monoklonskih protutijela za liječenje bolesti COVID-19, razvoj i metode za njihovo dobivanje, njihov mehanizam djelovanja protiv SARS-CoV-2, problemi s ućinkovitošću i koristi od njihove uporabe.

Rad će biti koristan izvor informacija za dodatnu edukaciju ljekarnika i drugih medicinskih djelatnika, kao i svih pripadnika opće populacije.

ZAKLJUČAK:

Hitna potreba za liječenjem bolesti COVID-19 dovela je do razvoja novih neutralizirajućih monoklonskih protutijela ili do prenamjene onih postojećih, koja mogu neutralizirati virus kod pacijenata.

Režimi liječenja neutralizirajućim monoklonskim protutijelima dobili su uvjetno odobrenje za stavljanje u promet (CMA) u EU i Velikoj Britaniji i odobrenje za hitnu uporabu (EUA) za liječenje bolesti COVID-19.

Do danas su istraživanja pokazala znanstvenu i kliničku valjanost primjene neutralizirajućih protutijela u bolesnika s visokim rizikom za razvoj teškog oblika bolesti, kako bi se spriječili smrtni ishodi. Budući da je učinkovitost liječenja monoklonskim protutijelima smanjena mutacijama novih varijanti SARS-CoV-2, opravdano je nastaviti istraživanja kako bi se poboljšala učinkovitost i stekle nove spoznaje o liječenju bolesti COVID-19, posebno u bolesnika s visokim rizikom za razvoj teškog oblika bolesti.

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1. INTRODUCTION AND REVIEW OF THE RESEARCH

1.1 MONOCLONAL ANTIBODIES

The immune system acts as a defense against various infectious agents that cause different forms of diseases. Two major components are the humoral (antibody-mediated) and cellular (cell-mediated) immune responses. The humoral immune system which comprises B-lymphocytes recognizes the type of foreign invading antigens and produces specific antibodies against them, thus allowing the organism to fight off disease. Monoclonal antibodies (mAbs) are immunoglobulins designed to target a specific epitope on an antigen. They are generally well-tolerated drugs because of their target selectivity, thus avoiding unnecessary exposure to, and consequently activity in non-target organs. Their production and storage are relatively easy (as compared to cellular immunotherapies) and they have long *in vivo* half-life (as compared to small molecules).

The increasing demand for mAbs used for diagnostic and therapeutic applications has led to the development of large-scale manufacturing processes, with improvements in production, achieved through continuous optimization of the inherent systems. The number of mAbs that have already been approved for therapeutic applications and for use in clinical trials have significantly increased in the past few years. In view of the side effects and limitations of mAbs, several improvements and modifications to mAbs have been developed. These modifications have facilitated the use of mAbs in various forms of therapeutic applications, such as treatment of infectious diseases caused by bacterial, viral, fungal and parasitic organisms.

Monoclonal antibodies have also been applied in the treatment of non-infectious diseases such as cancer, immune diseases, arthritis and other disorders resulting from organ transplantation (14).

1.1.1 ANTIBODY STRUCTURE AND CLASSES

Antibodies, or immunoglobulins (Igs), are roughly Y-shaped molecules or combinations of such molecules. There are five major classes of immunoglobulins: IgG, IgA, IgD, IgE, and IgM.

Table 1 summarizes the characteristics of these molecules, their structure, molecular weight, and functions.

Table 1. Important properties of endogenous immunoglobulin subclasses
(adapted from 13)

Property		IgA		IgG				IgM	IgD	IgE
Serum concentration in adult (mg/ml)		IgA1	IgA2	IgG1	IgG2	IgG3	IgG4			
		1.4–4.2	0.2–0.5	5–12	2–6	0.5–1	0.2–1	0.25–3.1	0.03–0.4	0.0001–0.0002
Molecular form		Monomer, dimer		Monomer				Pentamer, hexamer	Monomer	Monomer
Functional valency		2 or 4		2				5 or 10	2	2
Molecular weight (kDa)		160 (m), 300 (d)	160 (m), 350 (d)	150	150	160	150	950 (p)	175	190
Serum half-life (days)		5-7	4-6	21-24	21-24	7-8	21-24	5-10	2-8	1-5
% total IgG in adult serum		11-14	1-4	45-53	11-15	3-6	1-4	10	0.2	50
Function	Activate classical complement pathway	-		+	±	++	-	+++	-	-
	Activate alternative complement pathway	+	-	-	-	-	-	-	-	-
	Cross placenta	-		+	±	+	+	-	-	-
	Present on membrane of mature B cel	-		-	-	-	-	+	-	+
	Bind to Fc receptors of phagocytes	-		++	±	++	+	+	-	-
	Mucosal transport	++		-	-	-	-	+	-	-
	Induces mast cell degranulation	-		-	-	-	-	-	+	-
Biological properties		Secretory Ig, binds to polymeric Ig recepto		Placental transfer, secondary antibody for most response to pathogen, binds macrophage and other phagocytic cells by Fcγ recepto				Primary antibody response, some binding to polymeric Ig receptor, some binding to phagocytes	Mature B cell marker	Allergy and parasite reactivity, binds FcεR on mast cells and basophiles

Among these classes, IgGs and their derivatives form the framework for the development of therapeutic antibodies.

An IgG molecule has four peptide chains, including two identical heavy (H) chains (50–55 kDa) and two identical light (L) chains (25 kDa), which are linked via disulfide (S–S) bonds at the hinge region.

The first ~110 amino acids of both chains form the variable regions (VH and VL) and are also the antigen-binding regions. Each V domain contains three short stretches of peptide with hypervariable sequences (HV1, HV2, and HV3), known as complementarity determining regions (CDRs), the regions that bind antigen. The remaining sequences of each light chain consist of a single constant domain (CL). The remainder of each heavy chain contains three constant regions (CH1, CH2, and CH3) (Figure 1). Constant regions are responsible for effector recognition and binding.

IgGs can be further divided into four subclasses (IgG1, IgG2, IgG3, and IgG4), according to their abundance in human serum, with IgG1 being most abundant. The four IgG subclasses differ in their constant region, particularly in their hinges and upper CH2 domains (13).

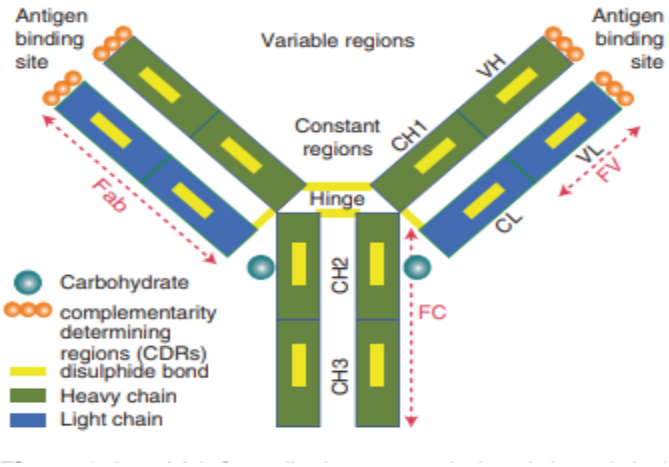

Figure 1 IgG Antibody structure *(adapted from 13)*

1.1.2 Monoclonal antibodies types and their production

There are four types of mAbs according to their origin and modifications in parts of their sequence, due to obtaining mAbs less immunogenic and more similar to human Abs:

- Murine mAbs with 0% sequence similarity to human mAbs;
- Chimeric mAbs with ~60–70%, sequence similarity to human mAbs;
- Humanized mAbs with ~90–95%, sequence similarity to human mAbs;
- Fully human mAbs with ~100% sequence similarity to human mAbs.

Decreasing the xenogenic portion of the mAb potentially reduces the immunogenic risks of generating anti-drug antibodies (ADAs) (Figure 2).

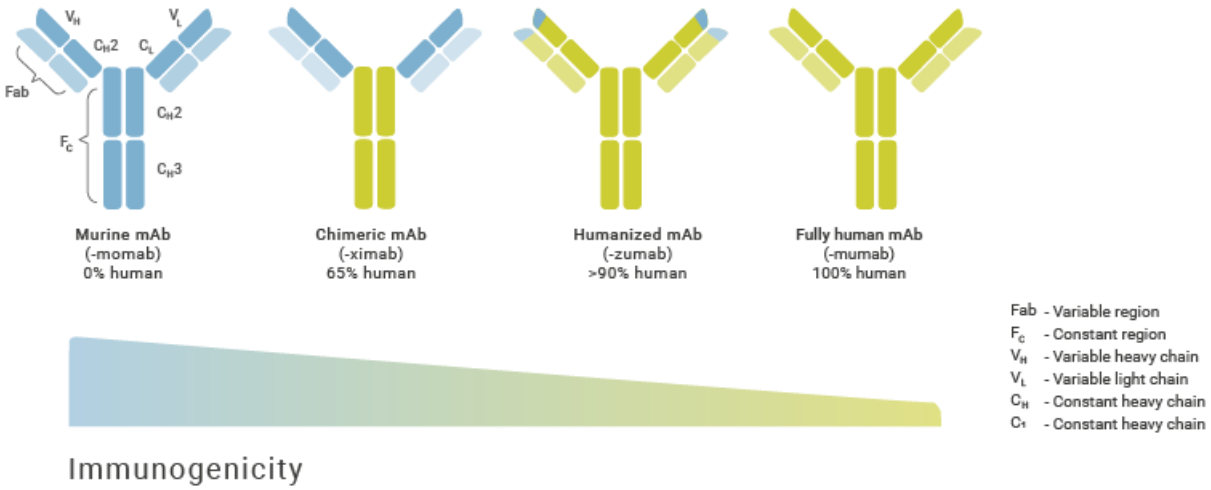

Figure 2 Types of mAb and their immunogenicity *(adapted from 25)*

The classic way to produce monoclonal antibody starts by immunizing a laboratory animal with a purified human protein against which the antibody should be directed. In most cases, mice are used (Figure 3a). The immunization process includes a number of injections with the antigens and an adjuvant and usually takes several weeks. Then the spleens of these mice are removed

and lymphocytes are isolated. Subsequently, the lymphocytes are fused with a myeloma cell using polyethylene glycol (PEG). The resulting hybridoma cell inherited from the lymphocytes the ability to produce antibodies and from the myeloma cell line the ability to divide indefinitely. Polyethylene glycol is used to fuse adjacent plasma membranes, but the success rate is low, so a selective medium in which only fused cells can grow is used. To select hybridoma cells from the excess of non-fused lymphocytes and myeloma cells, the cells are grown in HAT selection medium. This culture medium contains hypoxanthine, aminopterin, and thymidine.

Selection against the unfused lymphocytes is not necessary, since these cells, like most primary cells, do not survive for a long time in cell culture.

Selection against unfused myeloma cells is done with the drug aminopterin. The myeloma cell lines used for the production of mAbs contain an inactive hypoxanthine-guanine phosphoribosyltransferase (HGPRT), an enzyme necessary for the salvage synthesis of nucleic acids. The lack of HGPRT activity is not a problem for the myeloma cells because they can still synthesize purines *de novo*. By exposing the myeloma cells to the drug aminopterin synthesis of purines is blocked and these cells will not survive anymore.

Selected hybridoma cells are diluted and divided over several dishes. After approximately 2 weeks, individual clones are visible. Each clone contains the descendants of one hybridoma cell and will produce one particular type of antibody (that is why they are called mAbs). The next step is to isolate hybridoma cells from individual clones and grow them in separate wells of a 96-well plate. The hybridomas secrete antibodies into the culture medium. Using a suitable test (e.g. Enzyme Linked Immuno Sorbent Assay, ELISA), the obtained culture media can be screened for antibody binding to the antigen. The obtained antibodies can then be further characterized using other tests. In this way a mouse monoclonal antibody is generated. These mouse mAbs cannot be

used directly for the treatment of human patients. The amino acid sequence of a mouse antibody is too different from the sequence of an antibody in humans and thus will elicit an immune response and rapid removal from the blood.

To make a mouse antibody less immunogenic, the main part of its sequence must be replaced by the corresponding human sequence. Initially, human-mouse chimeric antibodies were made. These antibodies consisted of the constant regions of the human heavy and light chain and the variable regions of the mouse antibody (Figure 2) (13).

Later, so-called humanized antibodies were generated by grafting only the complementarity-determining regions (CDRs), which are responsible for the antigen binding properties, of the selected mouse antibody onto a human framework of the variable light (VL) and heavy (VH) domains. The humanized antibodies are much less immunogenic than the previously used chimeric antibodies (Figure 2) (13).

Another way to achieve full biocompatibility of mAbs is to develop fully human antibodies. This mAbs are obtained through three techniques:

1. Phage display technique

   The phage display technique comprises a powerful method for producing antibody fragments, such as single chain fragment variable (scFv) or fragment antigen binding (Fab), that bind a variety of target molecules (proteins, cell-surface glycan and receptors) (Figure 3b). This technique was based on extracting total RNA from lymphocytes of non-immunized healthy donors. Complementary DNA is than synthetized from RNA using reverse transcriptase enzymes and repertoire of VH and VL genes are amplified by PCR and cut with restriction endonucleases. Genes coding for scFv or Fab are cloned into filamentous

bacteriophages to compose a library. The library proteins are then presented on the phage surface as fusions with a phage coat protein, allowing the selection of specific binders and affinity characteristics. A human phage-displayed human antibody library is used to select antigens of interest. After 3–5 rounds of biopanning, immuno-positive phage clones are screened by ELISA, then DNA sequences are analyzed to construct and express human IgGs (15).

2. Transgenic animals

This technology was introduced in 1994 by the publication of two transgenic mouse lines, the HuMabMouse and the XenoMouse. The lines were genetically modified such that human immunoglobulin (Ig) genes were inserted into the genome, replacing the endogenous Ig genes and making these animals capable of synthesizing fully human antibodies upon immunization. Depending on the immunization protocol, high-affinity human antibodies can be obtained through further selection of hybridoma clones generated from immunized transgenic mice (Figure 3c) (15).

3. Human B-cells

Generation of neutralizing human antibodies from human B cells has also yielded promising results for infectious disease therapeutics (Figure 3d).

Neutralizing mAbs are recombinant proteins that can be derived from the B cells of convalescent patients or humanized mice.

High-throughput screening of these B cells permits the identification of antibodies with the necessary specificity and affinity to bind to a virus and block entry of the virus, therefore abrogating pathology associated with productive infection (15).

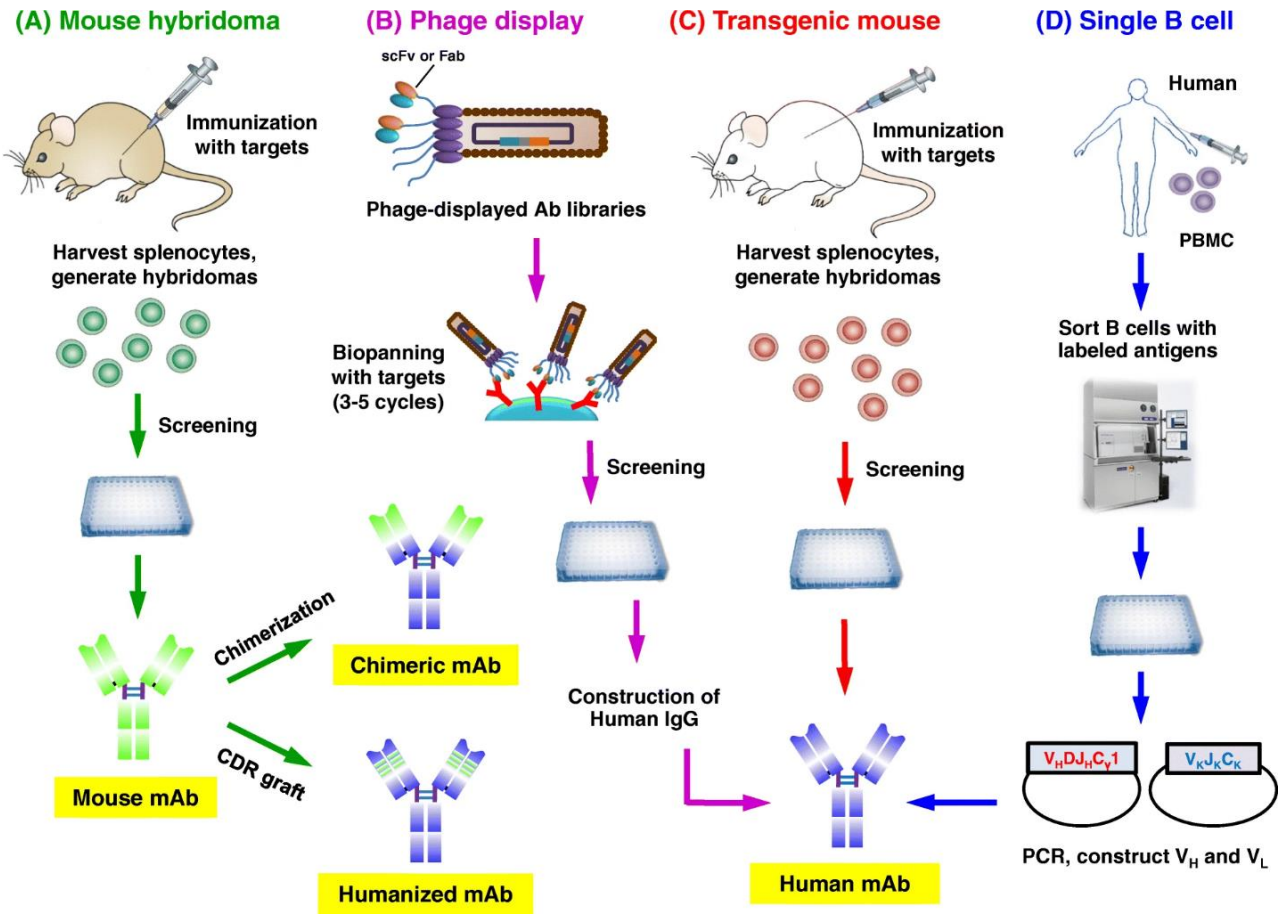

Figure 3 Monoclonal antibody production *(adapted from 15)*

1.1.3 Pharmacological mechanism of action of monoclonal antibodies

The pharmacological effects of antibodies are first initiated by the specific interaction between antibody and antigen. Monoclonal antibodies generally exhibit exquisite specificity for the target antigen. The binding site on the antigen, called the epitope, can be linear or conformational and may comprise continuous or discontinuous amino acid sequences. The epitope is the primary

determinant of the antibody's modulatory functions, and depending on the epitope, the antibody may exert antagonist or agonist effects. Monoclonal antibodies exert their pharmacological effects via multiple mechanisms that include:

- **Direct modulation of target antigen**

  This mechanism involves blocking and removal of the target antigen. Most mAbs act through multiple mechanisms and may exhibit cooperativity with concurrent therapies.

- **Complement-dependent cytotoxicity**

  The complement system is an important part of the nonadoptive immune system. It consists of many enzymes that form a cascade with each enzyme acting as a catalyst for the next. Complement-dependent cytotoxicity (CDC) results from interaction of cell-bound mAbs with proteins of the complement system. CDC is initiated by binding of the complement protein, C1q, to the Fc domain. The IgG1 and IgG3 isotypes have the highest CDC activity, while the IgG4 isotype lacks C1q binding and complement activation. Upon binding to immune complexes, C1q undergoes a conformational change, and the resulting activated complex initiates an enzymatic cascade involving complement proteins C2 to C9 and several other factors. This cascade spreads rapidly and ends in the formation of the membrane attack complex (MAC), which inserts into the membrane of the target cell and causes osmotic disruption and lysis of the target (Figure 4) (13,22).

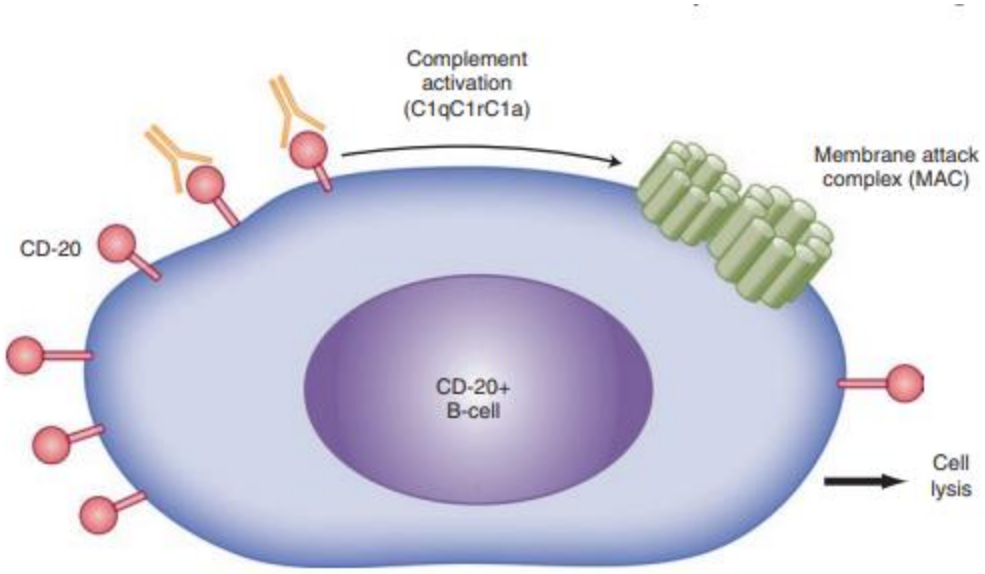

Figure 4 Complement-dependent cytotoxicity mechanism of action *(adapted from 13)*

- **Antibody-dependent cellular cytotoxicity**

  Antibody-dependent cellular cytotoxicity (ADCC) is a mechanism of cell-mediated immunity whereby an effector cell of the immune system actively lyses a target cell that has been bound by specific antibodies. It is one of the mechanisms through which antibodies, as part of the humoral immune response, can act to limit and contain infection. Classical ADCC is mediated by natural killer (NK) cells, monocytes, or macrophages, but an alternate ADCC is used by eosinophils to kill certain parasitic worms known as helminths. ADCC is part of the adaptive immune response due to its dependence on a prior antibody response. The typical ADCC involves activation of NK cells, monocytes, or macrophages and is dependent on the recognition of antibody-coated infected cells by Fc receptors on the surface of these cells. The Fc receptors recognize the Fc portion of antibodies such as IgG, which bind to the surface of a pathogen-infected target cell. The Fc receptor that exists on the surface of NK cell is called CD16 or

FcγRIII. Once bound to the Fc receptor of IgG, the NK cell releases cytokines such as IFN-γ and cytotoxic granules like perforin and granzyme that enter the target cell and promote cell death (Figure 5) (13,22).

- **Antibody-dependent cellular phagocytosis (ADCP)**

  Antibody-dependent cellular phagocytosis (ADCP) is an immune effector function in which cells or particles opsonized with antibodies are engulfed by phagocytic effector cells, such as macrophages, following interactions between the Fc region of antibodies and Fcγ receptors on effector cells (Figure 5). *In vivo,* ADCP can be mediated by monocytes, macrophages, neutrophils and dendritic cells via all three types of activating Fcγ receptors: FcγRI, FcγRIIa, and FcγRIIIa.

  ADCP is an important mechanism of action of several antibody therapies for cancer, such as rituximab, obinutuzumab, and ocrelizumab. Engagement of Fcγ receptors expressed on phagocytic effector cells with antibodies bound to target cells triggers a signaling cascade leading to the engulfment of the antibody-opsonized tumor cells. Upon full engulfment, a phagosome is formed, which fuses with lysosomes, leading to acidification and digestion of the tumor cells (13, 22).

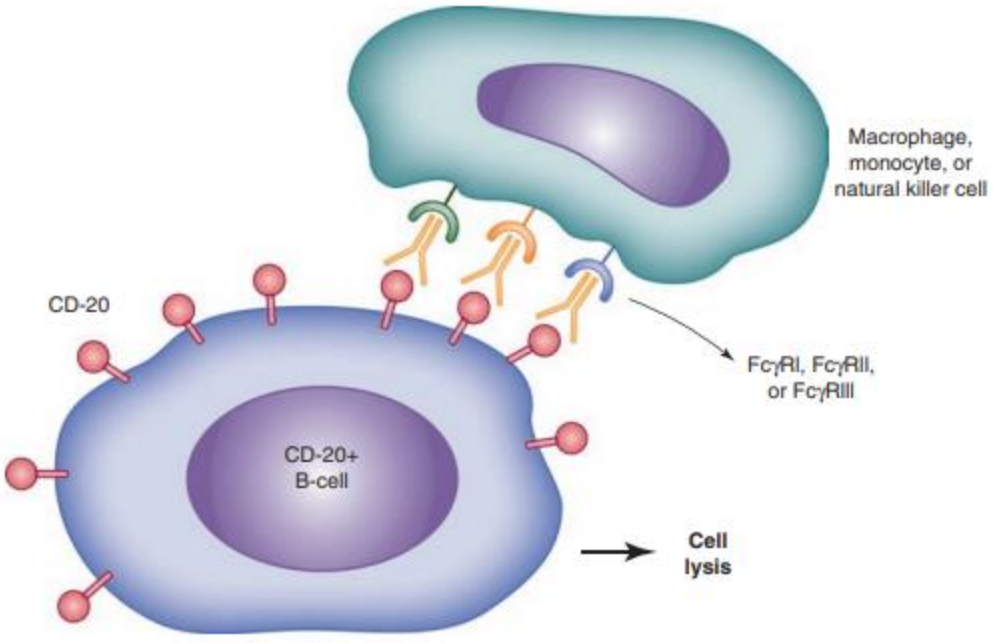

Figure 5 An example of antibody-dependent cellular cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis (ADCP)

The mAb targets the CD20 antigen, which is expressed on a significant number of B cell malignancies. The Fc fragment of the mAb binds the Fc receptors found on effector cells such as monocytes, macrophages, and NK cells. These cells in turn either engulf the mAb bound tumor cell (ADCP) or release cytotoxic agents such as perforin and granzymes, leading to destruction of the tumor cell (ADCC) *(adapted from 13)*

- **Apoptosis**

  mAbs can have direct effects in producing apoptosis or programmed cell death, which is characterized by nuclear DNA degradation, nuclear degeneration and condensation, and the phagocytosis of cell remains (13).

- **Targeted delivery of cytotoxic drugs via antibody-drug conjugates**

  Antibody-drug conjugates (ADCs) achieve their therapeutic effect through selectively delivering a potent cytotoxic agent to target cells. The mAb component enables the ADC to specifically bind to targeted cell surface antigens overexpressed on the target cells.

After binding to the cell surface antigen, the ADC is internalized by the cell, where it undergoes lysosomal degradation, leading to the release of the cytotoxic agent. Targeted delivery of cytotoxic drugs minimizes their impact on normal tissues, thereby enhancing the benefit-risk profile (13).

- **CD3+ T cell activation using bispecific constructs**

  CD3 bispecific constructs achieve their therapeutic effects through activating a patient's own CD3+ T cells to attack target-positive tumor cells. CD3 bispecific constructs have one arm directed against the CD3 receptor on T cells and the other arm directed against a target cell surface antigen overexpressed by tumor cells. Simultaneous engagement of both arms results in formation of an immunologic synapse between a target tumor cell and a CD3+ T cell, which leads to killing of the target tumor cells, either through direct killing by granzyme- and perforin-induced cell lysis or through cytokine release caused by T-cell activation (13).

1.2 SARS-COVID-19

1.2.1 COVID-19

Coronavirus disease 2019 (COVID-19) is an infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The first known case was identified in Wuhan, China, in December 2019. The disease has since spread worldwide, leading to an ongoing pandemic.

Symptoms of COVID-19 are variable, but often include fever (26), cough, headache, fatigue (28), breathing difficulties, loss of smell, and loss of taste (29). Symptoms may begin one to fourteen days after exposure to the virus. At least a third of people who are infected do not develop noticeable symptoms (30). Of those people who develop symptoms noticeable enough to be classified as patients, most (81%) develop mild to moderate symptoms (up to mild pneumonia), while 14% develop severe symptoms (dyspnea, hypoxia, or more than 50% lung involvement on imaging), and 5% suffer critical symptoms (respiratory failure, shock, or multi-organ dysfunction). Older people and people with chronic illness are at a higher risk of developing severe symptoms. Some people continue to experience a range of effects for months after recovery, and damage to organs has been observed (31).

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a novel severe acute respiratory syndrome coronavirus. It was first isolated from three people with pneumonia connected to the cluster of acute respiratory illness cases in Wuhan (32). All structural features of the novel SARS-CoV-2 virus particle occur in related coronaviruses in nature (33).

Coronavirus is a family of the virus and can cause illness such as the common cold, severe acute respiratory syndrome (SARS) and the Middle East respiratory syndrome (MERS) (34).

COVID-19 transmits when people breathe in air contaminated by droplets and small airborne particles containing the virus. The risk of breathing these in is highest when people are in close proximity, but they can be inhaled over longer distances, particularly indoors. Transmission can also occur if splashed or sprayed with contaminated fluids in the eyes, nose or mouth, and, rarely, via contaminated surfaces (35-38). The incubation period of COVID-19 is, on average, five to six days, with most studies reporting a range of two to 14 days (39-43).

The period during which people can transmit SARS-CoV-2 to others vary between virus variants and individuals. An infected person can transmit the virus 2 to 3 days before they experience symptoms and within the first 5 days after symptoms onset (44, 45). It is dependent on numerous factors, such as disease severity and pre-existing immunity through vaccination or prior infection (46-48). Patients with severe COVID-19 and immunocompromised patients have been shown to be infectious for a longer period (46, 49, 50).

SARS-CoV-2 virus is closely related to the original SARS-CoV virus. It is thought to have an animal (zoonotic) origin (Figure 6) (34).

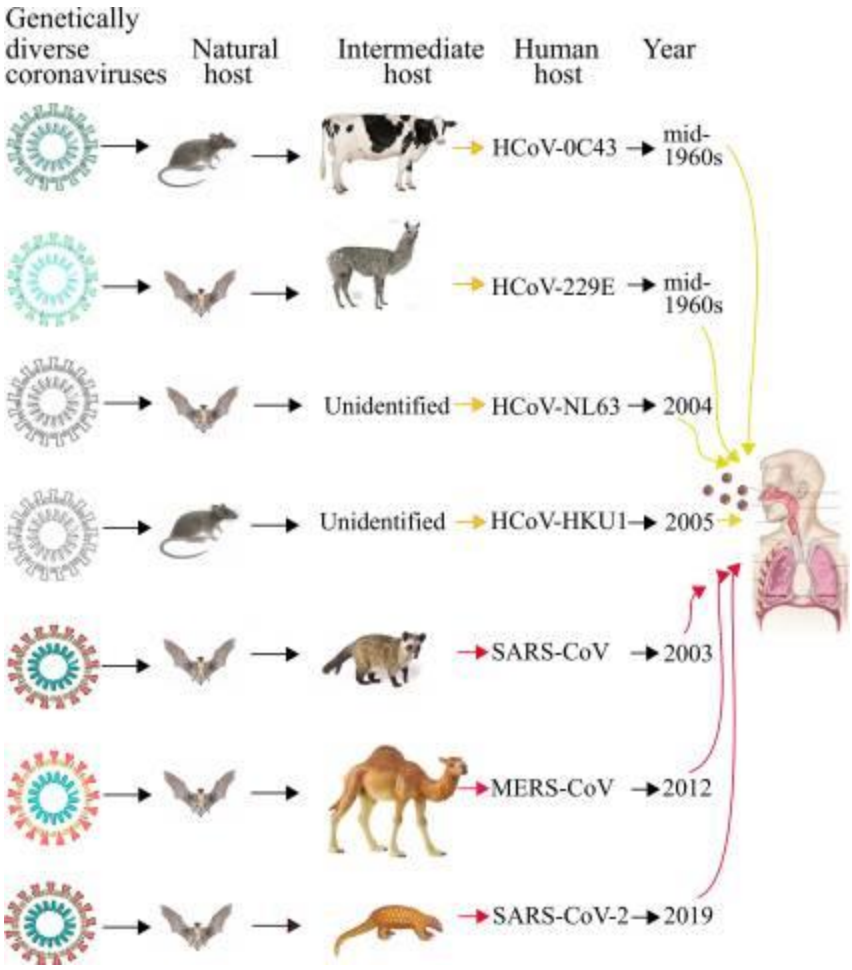

Figure 6 Family coronaviruses and their origin *(adapted from 27)*

1.2.2 SARS-CoV-2 variants

The many thousands of SARS-CoV-2 variants are grouped into either clades or lineages. The expert group convened by WHO has recommended the labeling of variants using letters of the Greek Alphabet, for example, Alpha, Beta, Delta, and Gamma.

As of December 2021, there are five dominant variants of SARS-CoV-2 spreading among global populations:

- the Alpha variant (B.1.1.7, formerly called the UK variant), first found in London and Kent,
- the Beta variant (B.1.351, formerly called the South Africa variant),
- the Gamma variant (P.1, formerly called the Brazil variant),
- the Delta variant (B.1.617.2, formerly called the India variant),
- the Omicron variant (B.1.1.529; BA.1, BA.2, BA.3, BA.4, BA.5; BA.1/BA.2, XBB) (122).

1.2.3 Coronavirus structure

The coronavirus genome is comprised of ~30000 nucleotides. It encodes for four structural proteins:

- Nucleocapsid (N) protein
- Membrane (M) protein
- Spike (S) protein
- Envelop (E) protein and several non-structural proteins (12, 17).

The capsid is the protein shell, inside the capsid there is a nuclear capsid or N-protein, which is bound to the virus single positive strand RNA that allows the virus to invade human cells and easily replicate. The N protein coats the viral RNA genome, which plays a vital role in its replication and transcription (Figure 7b) (12).

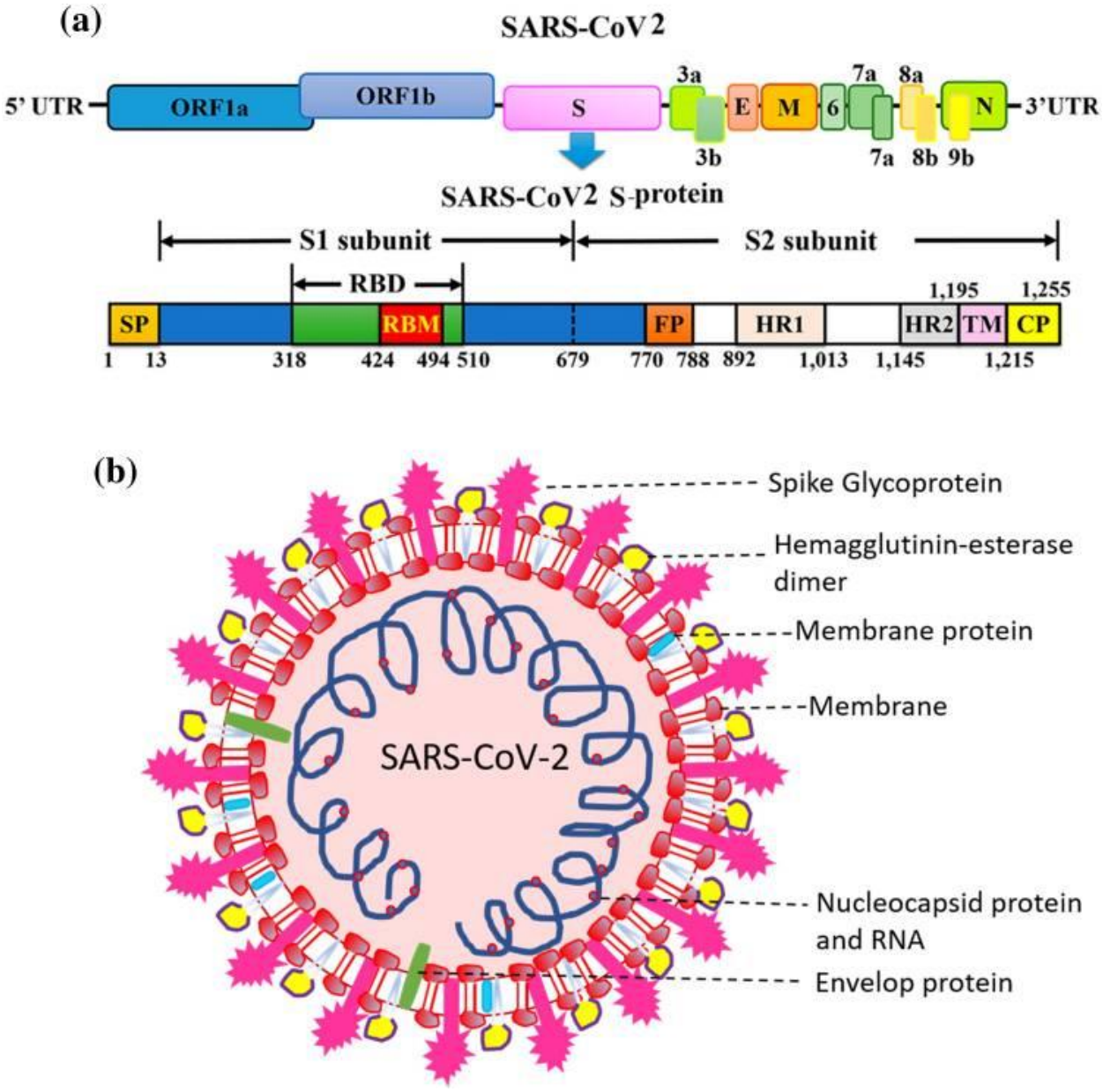

Figure 7 SARS-Cov-2 structure *(adapted from 12)*

a) Schematic representation of the genome organization and functional domains of S protein for COVID-19. The single-stranded RNA genomes of COVID-19 encode two large genes, the ORF1a and ORF1b genes, which encode 16 non-structural proteins (nsp1–nsp16). The structural genes encode the structural proteins, spike (S), envelope (E), membrane (M), and nucleocapsid (N). The accessory genes are denoted in shades of green. The structure of S protein is shown beneath the genome organization. The S protein is consisting of the S1 and S2 subunits. In the S-protein, cytoplasm domain (CP); fusion peptide (FP); heptad repeat (HR); receptor-binding domain (RBD); signal peptide (SP); transmembrane domain (TM) are shown (12).

b) The viral surface proteins - spike, envelope and membrane proteins - are embedded in a lipid bilayer. The single-stranded positive-sense viral RNA is associated with the nucleocapsid protein (12).

The M-protein is most abundant in the viral surface and it is believed to be the central organizer for the coronavirus assembly (Figure 7b) (12).

The S-protein is integrated over the surface of the virus and mediates attachment of the virus to the host cell surface receptors and fusion between the viral and host cell membranes to facilitate viral entry into the host cell (Figure 7b) (12). Its domain is composed of the N-terminal S1 region that harbors the receptor binding domain (RBD) and the C-terminal S2 region that mediates membrane fusion. S protein is a size of 180-200 kDa and normally exists in a metastable, pre-fusion conformation. Once the virus interacts with the host cell, extensive structural rearrangement of the S protein occurs, allowing the virus to fuse with the host cell membrane. The spikes are coated with polysaccharide molecules to camouflage them, evading surveillance of the host immune system during entry. The total length of SARS-CoV-2 S protein is 1273 amino acids and consists of a signal peptide (amino acids 1–13) located at the N-terminus, the S1 subunit (14–685 residues), and the S2 subunit (686–1273 residues). The last two regions are responsible for receptor binding and membrane fusion, respectively. In the S1 subunit, there is an N-terminal domain (14–305 residues) and a receptor-binding domain (RBD, 319–541 residues); the fusion peptide (FP) (788–806 residues), heptapeptide repeat sequence 1 (HR1) (912–984 residues), HR2 (1163–1213 residues), TM domain (1213–1237 residues), and cytoplasm domain (1237–1273 residues) comprise the S2 subunit (Figure 7a). Based on the structure of coronavirus S protein monomers, the S1 and S2 subunits form the bulbous head and stalk region (17).

The E-protein is a small membrane protein composed from ∼76 to 109 amino-acid and minor component of the virus particle, it plays an important role in virus assembly, membrane permeability of the host cell and virus-host cell interaction (12).

A lipid envelop encapsulates the genetic material (Figure 7b).

Hemagglutinin-esterase dimer has been located on the surface of the viral particle. The hemagglutinin-esterases (HEs) are a family of viral envelope glycoproteins that mediate reversible attachment to *O*-acetylated sialic acids by acting both as lectins and as receptor-destroying enzymes (RDEs). The HE protein may be involved in virus entry, is not required for replication, but appears to be important for infection of the natural host-cell. Such glycoprotein is made of three identical chains with 1273 amino acid each and it is composed of two well-defined protein domain regions: S1 and S2 subunits which are associated to cell recognition and the fusion of viral and cellular membranes respectively. The latter process occurs through different protein conformational changes that still remain uncharacterized (16).

1.2.4 Mechanism of SARS-CoV-2 entering the cell and replication

SARS-CoV-2 is entering the host cell via its S-protein. The S protein attaches to angiotensin converting enzyme 2 (ACE2) receptors that are found on the surface of many human cells, including those in the lungs, allowing virus entry. The coronavirus S protein is subjected to proteolytic cleavages by host proteases (trypsin and furin), in two sites located at the boundary between the S1 and S2 subunits (S1/S2 site). After that cleavage of the S2 domain (S2′ site) starts, the fusion peptide is released and the membrane fusion mechanism is activated. Typically, human cell ingests the virus in a process called endocytosis. Once entered the cytoplasm, it has been suggested that most likely COVID-19 employs a unique three-step method for membrane fusion, involving receptor-binding and induced conformational changes in S glycoprotein followed by cathepsin L proteolysis through intracellular proteases and further activation of membrane fusion mechanism within endosomes. Then the endosome opens to release virus to the cytoplasm, and uncoating of viral nucleocapsid is started via proteasomes, which typically can

hydrolyse endogenous proteins, but they are also capable of degrading exogenous proteins such as the SARS nucleocapsid protein.

A different two-step mechanism has been suggested and in this case the virion binds to a receptor on the target host cell surface through its S1 subunit and the spike is cleaved by host proteases and then the fusion at low pH is expected between viral and host target membranes via S2 subunit. Finally, the viral genetic material, a single stranded RNA, is fully released into the cytoplasm. Then the replication and transcription processes starts, mediated by the so-called replication/transcription complex (RTC). RTC is encoded in the viral genome and it is made of non-structural proteins. The RTC is believed to induce double-membrane structures in the cytoplasm of the infected cell. RNA genome is translated to generate replicase proteins from open reading frame 1a/b (ORF 1a/b). These proteins use the genome as a template to generate full-length negative sense RNAs, which subsequently serve as templates in generating additional full-length genomes. Structural viral proteins, M, S and E are synthesized in the cytoplasm and then inserted into the endoplasmic reticulum (ER), and transferred to endoplasmic reticulum-Golgi intermediate compartment (ERGIC). Also, in the cytoplasm nucleocapsids are formed from the encapsidation of replicated genomes by N protein, and as a result they coalesce within the ERGIC membrane to self-assembly into new virions. Finally, novel virions are exported from infected cells by transport to the cell membrane in smooth-walled vesicles and then secreted via exocytosis, so that they can infect other cells. In the meantime, the stress of viral production on the endoplasmic reticulum eventually leads to cell death (Figure 8). However, the exact details of complete COVID-19 mechanisms of action are still unknown (12).

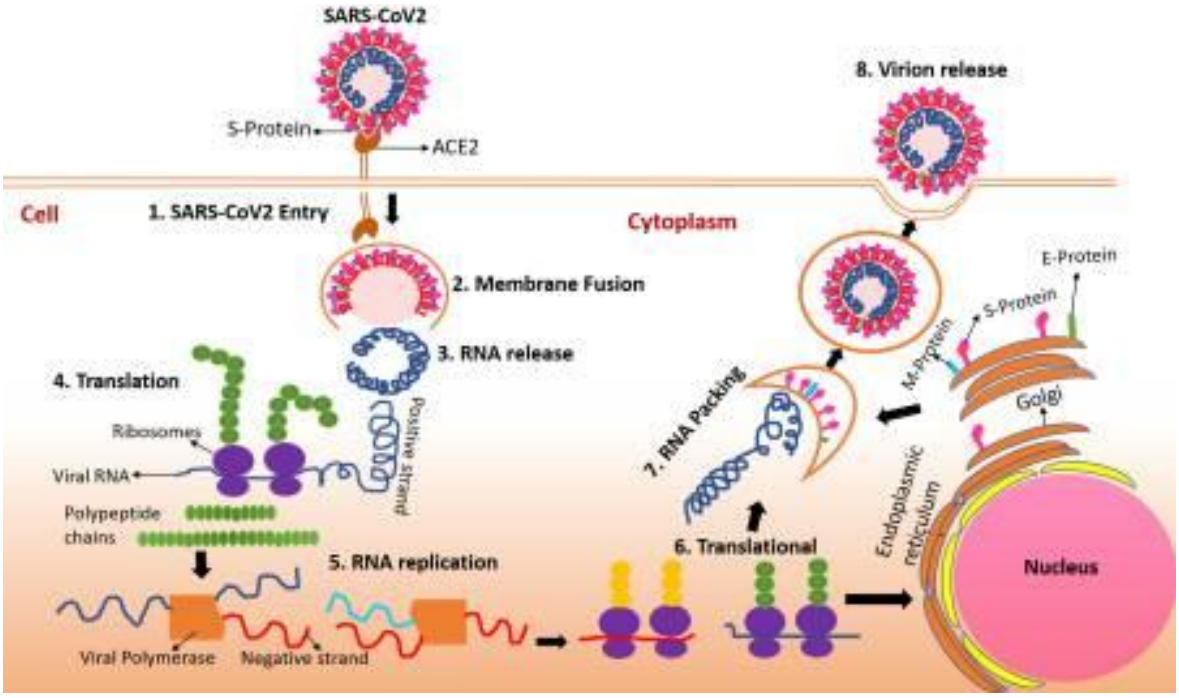

Figure 8 Mechanism of COVID-19 entry, viral replication and viral RNA packaging in the human cell

(adapted from 12)

1.2.5 Host factors for COVID-19

Human angiotensin converting enzyme 2 (hACE2) is the host factor that SARS-COV2 virus targets causing COVID-19. The effect of the virus on ACE2 cell surfaces leads to leukocyte infiltration, increased blood vessel permeability, alveolar wall permeability, as well as decreased secretion of lung surfactants. These effects cause the majority of the respiratory symptoms.

In some cases, the aggravation of local inflammation can cause a cytokine storm eventually leading to a systemic inflammatory response syndrome (26).

Upon virus entry inside the host, SARS-COV2 infects macrophages by attaching to DPP4 on the host cell through spike protein, which leads to releasing of genomic RNA in the cytoplasm. The

macrophages present CoV antigens to T cells, process which has a key role in anti-viral immune system. Afterward, antigen presentation stimulates T-cell activation and differentiation, and leads to generation and massive release of large quantity of cytokines for immune response reinforcement (Figure 9) (26).

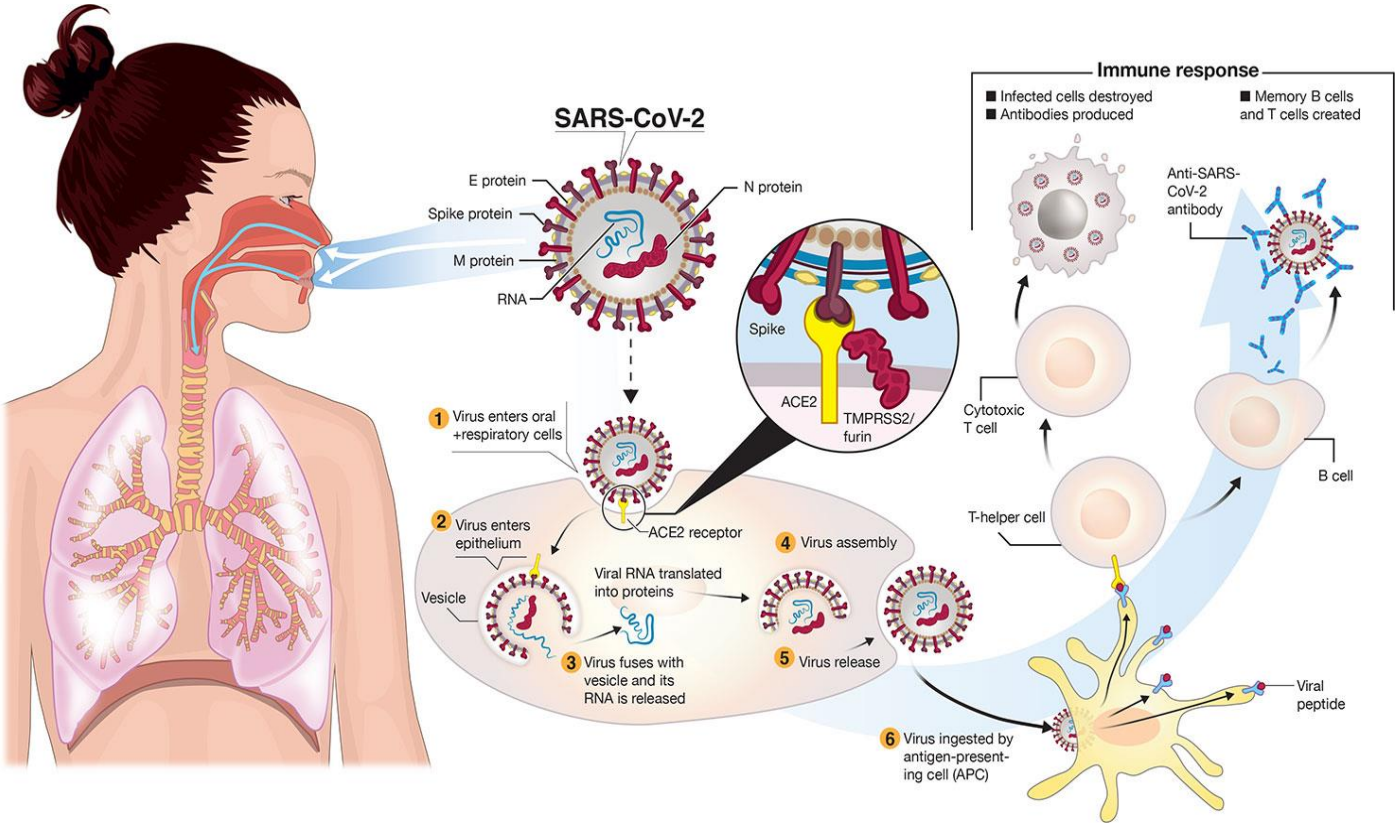

Figure 9 SARS-CoV 2 lifecycle and host immune respone *(adapted from 51)*

1.2.6 Host cytokine response

In reaction to SARS-CoV-2 infection, macrophages (53) and dendritic cells trigger an initial immune response, including lymphocytosis and cytokine release (Figure 10).

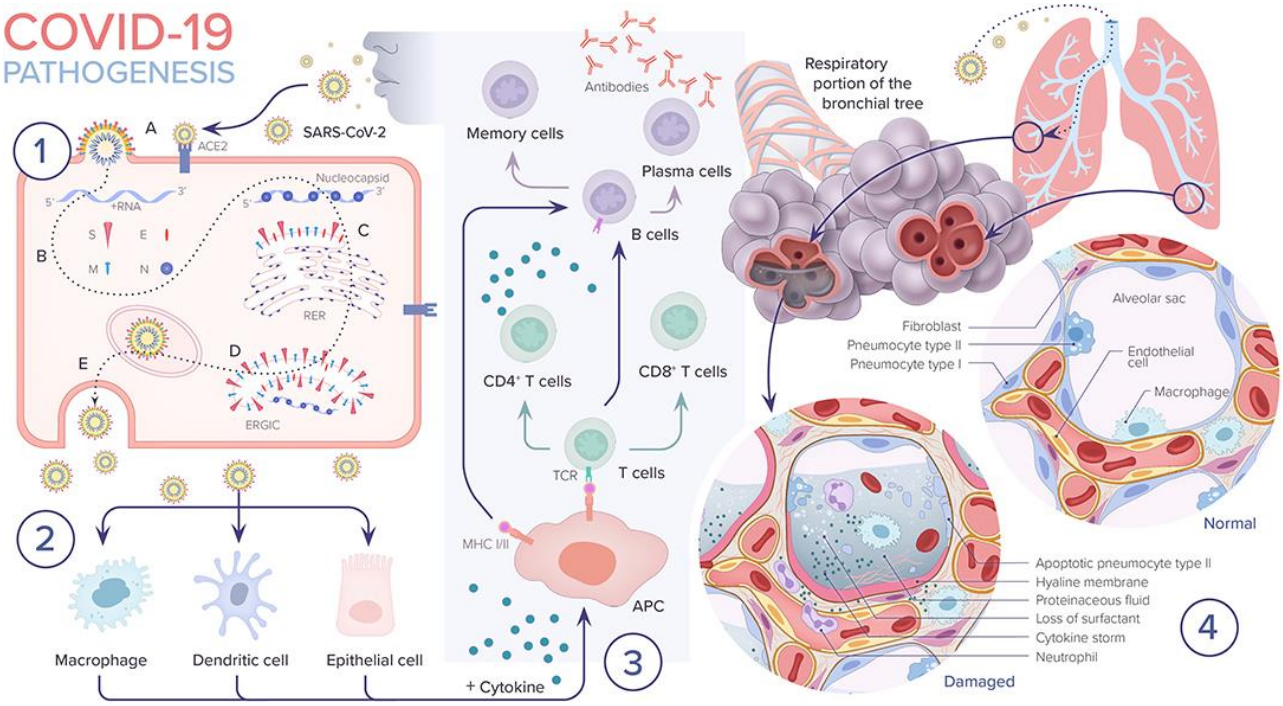

Figure 10 COVID-19 pathogenesis *(adapted from* 54*)*

In normal conditions, virus-infected cells are destroyed by NK cells of the innate immunity and CD8 positive cytolytic T cells of the adaptive immunity. This leads to apoptosis of antigen-presenting cells and relevant cytotoxic T cells to avoid unnecessary activation after the antigenic activity is over. If a defect occurs in lymphocyte cytolytic activity, whether due to genetic problems or acquired conditions, this may lead to the inability of NK and cytolytic CD8 T cells to lyse infected cells and activated antigen-presenting cells, resulting in prolonged and exaggerated interactions between innate and adaptive immune cells. In this case, many pro-inflammatory cytokines, including TNF, interferon-γ, IL-1, IL-6, IL-18, and IL-33, are secreted in an unrestrained way causing a cytokine storm (55) (Figure 11).

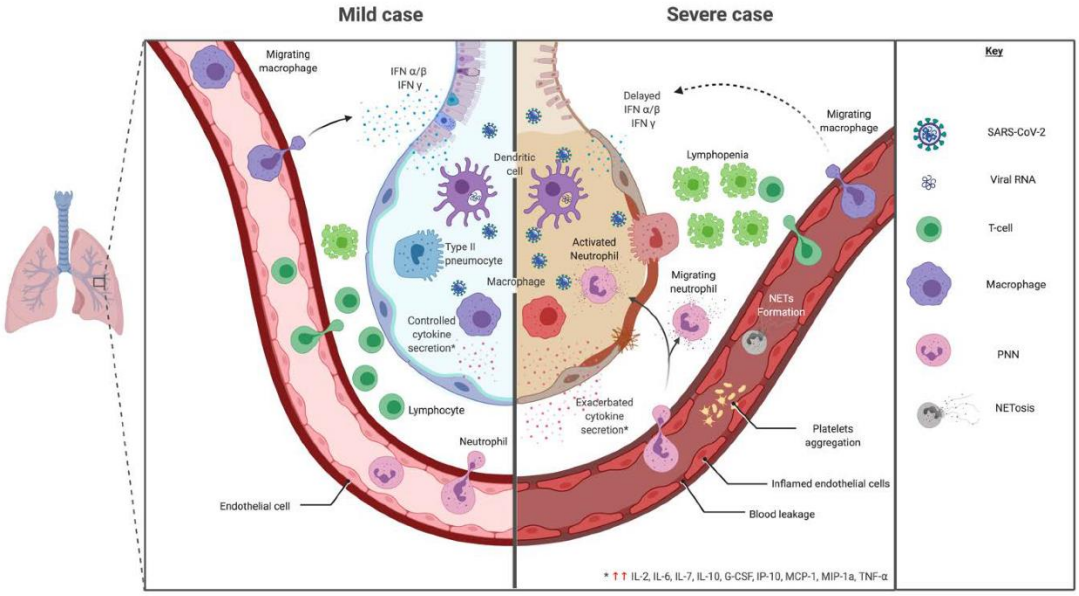

Figure 11 Mild versus severe immune response during SARS-CoV-2 infection *(adapted from 56)*

The inflammatory response results in the destruction of lymphocytes attempting to stop SARS-CoV-2 infection. (57). Cytokine production becomes rapidly dysregulated, damaging healthy cells typically first in the lungs but potentially spreading to other organs including the kidneys, heart, blood vessels, and brain. The cascade of cytokine storm-associated damage begins with disruption of the epithelial barrier in the lungs. The epithelial barrier disruption exposes the lungs and other tissues to bacterial infection.

The cytokine storm causes acute respiratory distress syndrome, blood clotting events such as strokes, myocardial infarction, encephalitis, acute kidney injury, and vasculitis.

Immune system cytokine network may also communicate with the central nervous system cytokine network, especially when the blood-brain-barrier is compromised. The cells of the central nervous system (CNS), the microglia, neurons, and astrocytes, are also involved in the release of pro-inflammatory cytokines affecting the nervous system, and effects of cytokine storms toward the CNS involve stroke, skeletal muscle injuries, brain inflammation (58).

1.2.7 Pathophysiology of COVID-19

The SARS-CoV-2 virus can infect a wide range of cells and systems of the body.

Respiratory tract

COVID-19 is most known for affecting the upper respiratory tract (sinuses, nose, and throat) and the lower respiratory tract (windpipe and lungs).The lungs are the organs most affected by COVID-19 because the receptor for the ACE2 is most abundant on the surface of type II alveolar cells of the lungs. Following viral entry, COVID-19 infects the ciliated epithelium of the nasopharynx and upper airways (59).

Nervous system

Viruses can enter the CNS through two distinct routes: haematogenous dissemination and neuronal retrograde dissemination. In haematogenous dissemination the virus spreads throughout the body via the bloodstream and then enters the brain by crossing the blood–brain barrier, whereas retrograde viral dissemination towards the CNS occurs when a virus infects neurons in the periphery and uses the transport machinery within those cells to gain access to the CNS (60). SARS-CoV-2 has been detected in cerebrospinal fluid of autopsies, and mechanism by which it invades the CNS first involve invasion of peripheral nerves given the low levels of ACE2 in the brain. The virus may also enter the bloodstream from the lungs and cross the blood-brain barrier to gain access to the CNS, within an infected white blood cell (61).

SARS-CoV-2 can also reach the CNS via neuronal dissemination. Following intranasal infection, SARS-Cov-2 infect the olfactory receptor neurons, pass through the neuroepithelium of the olfactory mucosa to reach the olfactory bulb, gain access to the mitral cells and the olfactory

nerve, and spread to the hippocampus and other brain structures (62) . One common symptom, loss of smell, results from infection of the support cells of the olfactory epithelium, with subsequent damage to the olfactory neurons (63). The involvement of both the central and peripheral nervous system in COVID-19 has been indicated trough following signs and symptoms: encephalopathy, ischemic and hemorrhagic stroke, seizures, cranial nerves impairment, peripheral neuropathies, and myopathies (64).

Gastrointestinal tract

The virus also affects gastrointestinal organs as ACE2 is abundantly expressed in the glandular cells of gastric, duodenal and rectal epithelium (65), as well as endothelial cells and enterocytes of the small intestine (66). Diarrhea is reported in some patients with COVID-19 infection. This is result of increased gastrointestinal wall permeability to foreign pathogens and the invaded enterocytes malabsorption (67).

Cardiovascular system

SARS-CoV-2 can cause acute myocardial injury and chronic damage to the cardiovascular system. Rates of cardiovascular symptoms are high because of systemic inflammatory response and immune system disorders during disease progression, but acute myocardial injuries are related to ACE2 receptors in the heart. ACE2 receptors are highly expressed in the heart and are involved in heart function (68). A high incidence of thrombosis and venous thromboembolism have been found in people transferred to Intensive care units with COVID-19 infections (69). Blood vessel dysfunction and clot formation (as suggested by high D-dimer levels caused by blood clots) are also reported. In people infected with SARS-CoV-2 clots lead to pulmonary embolisms and ischemic events within the brain. Infection also set off a chain of vasoconstrictive responses within the body like constriction of blood vessels within

the pulmonary circulation, in which oxygenation decreases alongside the presentation of viral pneumonia (70). Arterioles and capillaries damage has been reported in a small number of tissue samples of the brains and the olfactory bulbs from those who have died from COVID-19 (71). COVID-19 was also found to cause substantial changes to blood cells (including morphological and mechanical changes), such as increased sizes, sometimes persisting for months after hospital discharge (72).

1.2.8 Immunopathology

People with severe form of COVID-19 have symptoms of systemic hyperinflammation. This is proven with clinical laboratory findings of elevated IL-2, IL-7, IL-6, granulocyte-macrophage colony-stimulating factor (GM-CSF), interferon gamma-induced protein 10 (IP-10), monocyte chemoattractant protein 1 (MCP1), macrophage inflammatory protein 1-alpha (MIP-1-alpha), and tumour necrosis factor (TNF-α). Additionally, people with COVID-19 and acute respiratory distress syndrome (ARDS) have classical serum biomarkers of CRS, including elevated C-reactive protein (CRP), lactate dehydrogenase (LDH), D-dimer, and ferritin (73).

Systemic inflammation results in vasodilation, allowing inflammatory lymphocytic and monocytic infiltration of the lung and the heart. In particular, pathogenic GM-CSF-secreting T cells were shown to correlate with the recruitment of inflammatory IL-6-secreting monocytes and severe lung pathology in people with COVID-19. Lymphocytic infiltrates have also been reported at autopsy (74).

2. OBJECTIVES

The aim of the research is to present an overview of current knowledge about the possibilities and challenges of mAbs therapy in treating COVID-19 in patients with high risk to develop severe form of the disease.

The hypotheses of the research are:

- Neutralizing mAbs could be beneficial for vulnerable populations, such as the unvaccinated or recently vaccinated high-risk patients, before or after exposure to SARS-CoV-2;

- Monoclonal antibody therapy for COVID-19 is well tolerated with minimal risks;

- Transitioning from monotherapies towards combination therapies of mAbs will lead to lower viral escape, longevity of the therapies and reducing the potential rate of treatment failure.

3. MATERIALS AND METHODS

- SYSTEMATIC REVIEW OF KNOWLEDGE ON THE TOPIC-

3.1 Monoclonal antibodies as potential treatment in COVID-19

The S protein is the main antigen component in all structural proteins of SARS-CoV-2 and it is responsible for inducing the host immune response. The S protein consists of 2 subunits, S1 and S2 that mediate host cell attachment and invasion. Through its RBD, S1 subunit attaches to ACE2 receptor on the host cell and initiates a conformational change in S2 subunit that results in virus-host cell membrane fusion and viral entry. The fundamental role of the S protein in viral infection indicates that it is a potential target for vaccine development, antibody-blocking therapy, and small molecule inhibitors. Neutralizing mAbs were developed to bind to RBD of the S protein of SARS-CoV-2 and thus to prevent binding of the S protein to ACE2 receptor, on target host cells (Figure 12) (75).

Humanized murine technology or convalescent plasma from recovered patients have been used as methods to derive neutralizing mAbs targeted to the RBD of the S protein of SARS-CoV-2 (4).

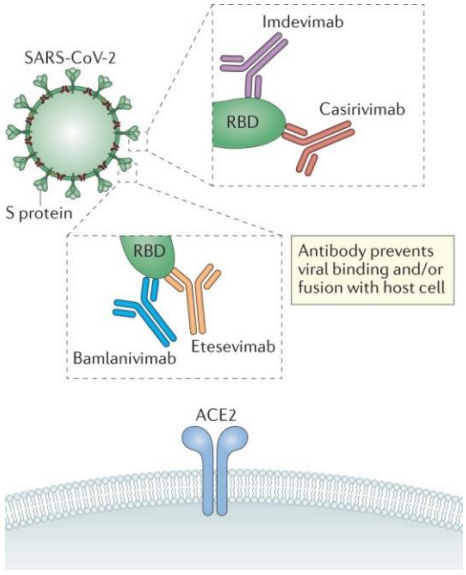

Figure 12 Inhibition of SARS-CoV-2 target cell engagement by neutralizing mAbs
(adapted from 4)

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 on the basis of phase I/II and phase II data for emergency use (Table 2) (5).

Table 2 **Anti-SARS-CoV-2 mAbs in clinical studies** *(adapted form 5)*

Anti-SARS-CoV-2 mAbs in clinical studies					
Sponsors	Drug code	Most advanced study	Trial IDs	Est. start	Est. primary completion
Memo Therapeutics	MTx-COVAB36	Phase 1 pending	NCT05351437	May 2022	Jun 2022
IGM Biosciences, Inc.	IGM-6268	Phase 1	NCT05160402; NCT05184218	Dec 2021; Jan 2022	March 2022; Nov 2022
Celltrion	CT-P63	Phase 1	NCT05017168	Sep 2021	Oct 2021
Exevir Bio BV	XVR011	Phase 1/2	NCT04884295	Aug 2021	Apr 2022
Jemincare Group	JMB2002	Phase 1	ChiCTR2100042150	NA	NA
Luye Pharma Group Ltd	LY-CovMab	Phase 1	NCT04973735	Nov 2022	May 2021
AbbVie	ABBV-47D11	Phase 1	NCT04644120	11/27/2020	Aug 2021
HiFiBiO Therapeutics	HFB30132A	Phase 1	NCT04590430	Oct 2020	Jul 2021
Immunome, Inc.	IMM-BCP-01	Phase 1 pending			
Beigene	DXP604	Phase 1	NCT04669262	12/15/2020	May 2021
Zydus Cadila	ZRC-3308	Phase 1 pending	NA	NA	NA
Hengenix Biotech Inc	HLX70	Phase 1 pending	NCT04561076	12.9.2020	Sep 2021
CORAT Therapeutics	COR-101	Phase 1 /2	NCT04674566	1/31/2021	Oct 2021
Vir Biotechnol./	VIR-7832	Phase 1 /2	NCT04746183	1/31/2021	Nov 2021
Immune Biosolutions	IBIO-123	Phase 2	NA	NA	NA
AbCellera / Eli Lilly and Company	LY3819253, LY3832479	Phase 2	NCT04634409	NA	Aug 2021
Sorrento Therapeutics, Inc.	COVI-AMG (STI-2020)	Phase 2	NCT04734860	April 2021	Sep 2021
Beigene	DXP593	Phase 2	NCT04532294; NCT04551898	8/31/2020; 10/30/2020	10/15/2020 2/28/2021
Junshi Biosciences / Eli Lilly and Company	JS016, LY3832479, LY-CoV016	Phase 2	NCT04441918; NCT04441931 NCT04427501	6/5/2020; 6/19/2020; 6/17/2020	Dec 2020; 10/2/2020; 03.11.2021
Mabwell (Shanghai) Bioscience Co., Ltd.	MW33	Pivotal Phase 2	NCT04533048; NCT04627584	8/7/2020; Nov 2020	Dec 2020; May 2021
Toscana Life Sciences Sviluppo s.r.l.	MAD0004J08	Phase 2/3	NCT04932850; NCT04952805	March 2021; June 2021	Oct 2021; March 2022

Bristol-Myers Squibb, Rockefeller University	C144-LS and C-135-LS	Phase 2/3	NCT04700163; Activ-2 study	1/11/2021; TBD	June 2021; TBD
Sinocelltech Ltd.	SCTA01 + SCTA01C	Phase 2/3	NCT04483375; NCT04644185; NCT05156645	7/24/2020; Mar 2021; Jan 2022	Nov 2020; Nov 2021; Dec 2022
Regeneron	REGN14256 + imdevimab	Phase 1/2/3	NCT05081388	Nov 2021	Nov 2022
Adagio Therapeutics	ADG20	Phase 2/3	NCT04805671 NCT04859517	Mar 2021; Apr 2021	Dec 2021 July 2022
Ology Bioservices	ADM03820	Phase 2/3	NCT05142527	Dec 2021	Apr 2022
Tychan Pte. Ltd.	TY027	Phase 3	NCT04429529; NCT04649515	6/9/2020; 12.4.2020	Oct 2020; 8/31/2020
Brii Biosciences	amubarvimab/ romlusevimab	Approved in China	NCT04479644;	7/13/2020;	Mar 2021;
		EUA requested	Activ-3 study	TBD	TBD
Eli Lilly and Company	Bebtelovimab	EUA*	NCT04634409	NA	Aug 2021
AstraZeneca	AZD7442 (AZD8895 + AZD1061); Evusheld	EUA*	NCT04507256; NCT04625725; NCT04625972	8/17/2020; 11/17/2020; 11/16/2020	Sep 2021; Feb 2022; Jan 2022
Celltrion	Regdanvimab (CT-P59)	Approved in Republic of Korea and EU	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; Sotrovimab; Xevudy	EUA* granted but withdrawn; Approved in Australia, UK, EU	NCT04545060;	8/27/2020;	Jan 2021;
			Activ-3 study	TBD	TBD
AbCellera / Eli Lilly and Company	LY-CoV555 (LY3819253); combination of LY-CoV555 with LY-CoV016 (LY3832479)	EUA* for bamlanivimab/ etesevimab combination therapy granted but withdrawn	NCT04411628 (Phase1); NCT04427501 (Phase 2); NCT04497987 (Phase 3); NCT04501978 (Activ-3 study); NCT04518410 (Phase 2/3)	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; Feb 2021
Regeneron	REGN-COV2 (REGN10933 + REGN10987): Ronapreve	EUA* granted but withdrawn; Approved in Japan, UK, EU, Australia	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

3.2 Monoclonal antibodies for COVID-19 treatment

3.2.1 Monoclonal antibodies authorized for COVID-19 treatment

3.2.1.1 Casirivimab/Imdevimab

This combination medicine contains two active substances, casirivimab and imdevimab. It was developed by Regeneron Pharmaceuticals (79). Casirivimab/Imdevimab was granted with marketing authorization from European medicines agency (EMA) on 12.11.2021 under the name Ronapreve, and emergency use authorization (EUA) from Food and drug administration (FDA) on 21.11.2020 under the name REGEN-COV (76, 77).

Casirivimab/Imdevimab is given as a single treatment by infusion into a vein or by injection under the skin. The recommended dose is 600 mg of Casirivimab and 600 mg of Imdevimab. It is indicated for treatment of COVID-19 in adults and adolescents (from 12 years of age and weighing at least 40 kilograms) who do not require supplemental oxygen and who are at increased risk of their disease becoming severe. The medicine can also be used to prevent COVID-19 in people aged 12 years and older weighing at least 40 kilograms.

When used for treatment, it should be given within 7 days of the patient developing symptoms of COVID-19. When used for prevention after contact with a person with COVID-19, it should be given as soon as possible after contact occurred. Casirivimab/Imdevimab may also be given to prevent COVID-19 when no contact has occurred. In these cases, following an initial dose of 600 mg casirivimab and 600 mg Imdevimab, a dose of 300 mg of Casirivimab and 300 mg of Imdevimab may be given every four weeks until prevention is no longer required (1,79).

Casirivimab and Imdevimab are intended to compensate/substitute for endogenous antibodies in those individuals who have yet to mount their own immune response. They are two recombinant human mAbs which are unmodified in the Fc regions.

Casirivimab is a recombinant neutralizing human immunoglobulin G1 (IgG1κ) monoclonal antibody to the spike protein of SARS-CoV-2. It binds to the S1 subunit of the spike protein receptor-binding domain (RBD), blocking the attachment of SARS-CoV-2 to the human ACE2 receptor. This prevents viral binding to the host cell, thereby preventing entry and replication of the virus, thus decreasing the viral load. Imdevimab (IgG1λ) binds to a non-overlapping portion of the spike protein RBD similar to Casirivimab (3). The combination of two monoclonal antibodies is intended to limit the development of viral mutations (78). The cocktail of the two drugs was also found to have retained activity against the Alpha (B.1.1.7; UK), Beta (B.1.351; South Africa), Gamma (P.1; Brazil), Delta (B.1.617.2; India), Epsilon (B.1.427/429; California), Iota (B.1.526; New York), and Kappa (B.1.617.1; India) variants of SARS-CoV-2. The combination drug has no activity to the Omicron variant (3).

A main study (COV-2067) involving patients with COVID-19 who did not require oxygen and were at increased risk of their illness becoming severe showed that Casirivimab/Imdevimab at the authorised dose led to fewer hospitalizations or deaths when compared with placebo (dummy treatment). Overall, 0.9% of patients treated with Casirivimab/Imdevimab (11 out of 1,192 patients) were hospitalized or died within 29 days of treatment compared with 3.4% of patients on placebo (40 out of 1,193 patients).

A main study (COV-2069) looked at the benefits of Casirivimab/Imdevimab for prevention of COVID-19 in people who had close contact with an infected household member.

Casirivimab/Imdevimab was found to be effective at preventing people from getting infected and developing symptoms after contact: amongst people who tested negative for SARS-CoV-2 following contact, fewer people given Casirivimab/Imdevimab developed symptoms within 29 days of their test results compared with people given placebo (1.5% for Casirivimab/Imdevimab compared with 7.8% for placebo). Casirivimab/Imdevimab was also found to be effective at preventing symptoms in infected people. Amongst the people who tested positive for SARS-CoV-2 after contact, 29% of people who received Casirivimab/Imdevimab developed symptoms compared with 42.3% of people who received a placebo (3). From September 2020 Casirivimab/Imdevimab is being evaluated as part of the Recovery Trial (80), and in June 2021 preliminary results from the Recovery trial showed reduced mortality from 30% to 24% in people that had produced no antibodies themselves which were 33% of the total of participants (81-83). The most common side effects with Casirivimab/Imdevimab (which may affect up to 1 in 10 people) are allergic reactions, which include infusion related reactions and injection site reactions (3).

3.2.1.2 Regdanvimab

Regdanvimab (CT-P59) is a recombinant human monoclonal immunoglobulin G1 antibody targeted against SARS-CoV-2. It is being developed by Celltrion Inc. under the trademark Regkirona for the treatment of COVID-19 (8). Regdanvimab received first full approval in South Korea in September 2021, following marketing authorization from EMA on 12.11.2021 (76, 7). It is used to treat adults with COVID-19 who do not require supplemental oxygen and who are also at increased risk of their disease becoming severe. Risk factors may include but are not limited to: advanced age, 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, immunosuppressed based on prescriber's assessment. Examples include: cancer treatment, bone marrow or organ transplantation, immune deficiencies, HIV (if poorly controlled or evidence of AIDS), sickle cell anaemia, thalassaemia, and prolonged use of immune-weakening medications. In patients with confirmed COVID-19 and mild symptoms, early treatment can reduce the severity of the disease and associated hospitalization or intensive care unit admittance. Regdanvimab contains a single N-linked glycosylation site on each heavy chain. Although IgG1 antibodies typically triggers effector mechanisms, such as ADCC, the available characterization data indicates that Regdanvimab is unable to mediate Fc-related activities (8).

Regdanvimab neutralizes SARS-CoV-2 by binding to the RBD of the virus's S protein and blockade interaction with the ACE2 receptor. This prevents subsequent viral entry into human cells and viral replication. The binding interaction between Regdanvimab and the S protein RBD is different compared to other neutralizing antibodies, because Regdanvimab binds to the RBD protein trimer exclusively in its "up" conformation and in an orientation distinct from most other ACE2-blocking antibodies (8, 84). The antibody retains antiviral activity against newer SARS-CoV-2 variants (8). It is proven that Regdanvimab neutralize D614G variant, one of the most infectious S protein's mutations (84).

Regdanvimab is developed from sera of Korean convalescent plasma, from which peripheral blood mononuclear cells were isolated, RNA extracted and converted to cDNA. Then with phage display technique scFv formats were generated and expressed in Chinese Hamster Ovary (CHO)-K1 cells (84). Regdanvimab drug product is formulated for administration by intravenous infusion as a sterile liquid solution in a 20-ml Type I borosilicate glass vial intended to deliver 960 mg of antibody per 16 ml at a concentration of 60 mg/ml. The formulation of drug product

includes L-histidine, L-histidine monohydrochloride monohydrate, polysorbate 80, L-arginine monohydrochloride and water for injection. The pH of the drug product solution is 6.0 (8).

The recommended dosage of Regdanvimab is a single intravenous infusion of 40 mg/kg. Treatment should be initiated as soon as possible after diagnosis, and not later than 7 days after the onset of symptoms. Adverse reactions reported with Regdanvimab based on experience from clinical trials in healthy subjects and mild to moderate COVID-19 patients are listed in Table 3 (8).

Table 3 **Adverse reactions of Regdanvimab** *(adapted from 8)*

Blood and lymphatic system disorders	
Common	Neutropenia
Metabolism and nutrition disorders	
Common	Hypertriglyceridemia
Uncommon	Hyperkalemia, dyslipidemia
Nervous system disorders	
Uncommon	Headache
Hepatobiliary disorders	
Uncommon	Hepatitis
Skin and subcutaneous tissue disorders	
Uncommon	Rash
Renal and urinary disorders	
Uncommon	Proteinuria
General disorders and administration site conditions	
Uncommon	Fever
Investigations	
Uncommon	Blood triglycerides increased, gama-glutamyltransferase increased, blood creatinine phosphokinase increased, blood lactat dehydrogenase increased, C-reactive protein increased
Injury, poisoning and procedural complications	
Uncommon	Infusion related reaction (e.g fever and dysopnea)

3.2.1.3 Sotrovimab

Sotrovimab is a third monoclonal antibody to treat COVID-19 in patients from 12 years of age and weighing at least 40 kilograms who do not require supplemental oxygen and who are at increased risk of the disease becoming severe (1). It was developed from GlaxoSmithKline and Vir Biotechnology (86). In European Union (EU) it is registered as Xevudy and granted marketing authorization from EMA on 17.12.2021. It was granted EUA from FDA on 26.05.2021 (76, 77). Sotrovimab is a human IgG1-kappa monoclonal antibody consisting of 2 identical light chain polypeptides composed of 214 amino acids each and 2 identical heavy chain polypeptides, each composed of 457 amino acids. It is produced by a CHO cell line and has a molecular weight of approximately 149 kDa (9). Sotrovimab is formulated as sterile injection for IV infusion. Each ml contains Sotrovimab (62.5 mg), L-histidine (1.51 mg), L-histidine monohydrochloride (2.15 mg), L-methionine (0.75 mg), polysorbate 80 (0.4 mg), and sucrose (70 mg). The solution has a pH of 6.0 (9).

Sotrovimab binds to a conserved epitope on the S protein RBD of SARS-CoV-2, but does not compete with human ACE2 receptor binding. It inhibits an undefined step that occurs after virus attachment and prior to fusion of the viral and cell membranes. Sotrovimab has demonstrated activity via two antiviral mechanisms *in vitro*, ADCC and ADCP (9).

Sotrovimab is engineered with LS mutations on Fc domain, M428L and N434S amino acid substitutions, that results in binding to the neonatal Fc receptor. This leads to potentially enhanced drug distribution to the lungs and extended half-life. (9, 10, 86). These mutations do not impact wild-type Fc-mediated effector functions in cell culture (9).

3.2.1.4 Tocilizumab

Tocilizumab is a humanized monoclonal antibody developed by Osaka University and Chugai, and was licensed in 2003 by Hoffmann-La Roche as immunosuppressive drug, used for the treatment of rheumatoid arthritis, systemic juvenile idiopathic arthritis and cytokine release syndrome (87). Tocilizumab has extension of indication to be included in treatment of adults with COVID-19 who are receiving systemic treatment with corticosteroids and require supplemental oxygen or mechanical ventilation (11). This extension of indication was granted by EMA on 07.12.2021 under the brand name RoActemra by Roche. Tocolizumab was granted EUA for the treatment of COVID-19 by FDA on 24.06.2021 under the brand name Actemra (76, 77). Tocilizumab is a humanised recombinant monoclonal antibody of the IgG1-kappa subclass produced by grafting the CDR of mouse anti-human IL-6 receptor to human IgG1. It is composed of 2 heavy and 2 light chains with 12 intra-chain and 4 inter-chain disulphide bonds with molecular weight of 149 kDa. Tocilizumab is produced by recombinant DNA technology, where genes coding for Tocilizumab are isolated and transferred into CHO cell line. Then these cells are cloned in order to establish master and working cell banks (88). Tocilizumab mechanism of action includes targeting both soluble and membrane bound IL-6 receptors, thus preventing IL-6 binding and reducing IL-6 signaling (Figure 13) (11, 87, 89). IL-6 is the key cytokine leading to an inflammatory storm, which may result in increased alveolar-capillary blood-gas exchange dysfunction, especially impaired oxygen diffusion, and eventually lead to pulmonary fibrosis and organ failure (11). Extended indication of Tocolizumab for treatment of COVID-19 is due to observation that late stage COVID-19 lung inflammation may lead to a hyper-inflammation state and COVID-19 related acute respiratory distress syndrome where IL-6 is involved (11).

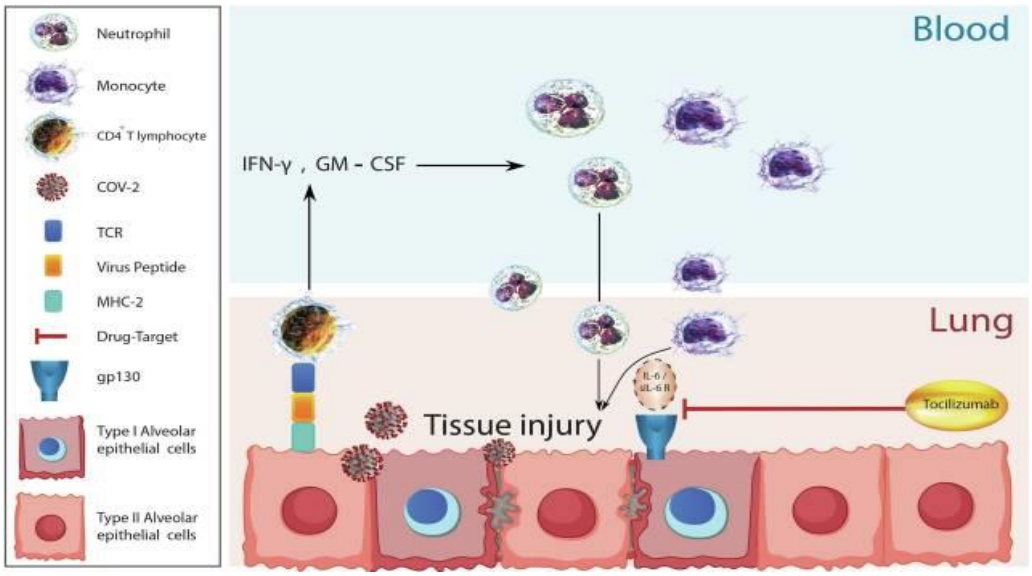

Figure 13 Mechanisam of action of Tocolizumab
(adapted from 11)

Tocolizumab is formulated as 20 mg/ml concentrate for solution for infusion. The recommended treatment of COVID-19 is a single 60-minute intravenous infusion of 8 mg/kg in patients who are receiving systemic corticosteroids and require supplemental oxygen or mechanical ventilation. If clinical signs or symptoms worsen or do not improve after the first dose, one additional infusion of 8 mg/kg may be administered. The interval between the two infusions should be at least 8 hours. For individuals whose body weight is more than 100 kg, doses exceeding 800 mg per infusion are not recommended. Administration of Tocolizumab is not recommended in patients with COVID-19 who have any of the following laboratory abnormalities: liver enzyme >10x ULN; absolute neutrophil count < 1 x 10^9/l; platelet count < 50 x 10^3 /µl (89).

3.2.1.5 Anakinra

Anakinra is recombinant modified version of the human IL-1 receptor antagonist protein (89). It is registered under the brand name Kineret by Swedish Orphan Biovitrum AB and granted

marketing authorization from EMA in 2002 (90,91). It is used to treat: signs and symptoms of rheumatoid arthritis in adults, second line treatment to manage symptoms of rheumatoid arthritis in combination with Methotrexate after treatment with Methotrexate has failed, cryopyrin-associated periodic syndrome, including neonatal-onset multisystem inflammatory disease, mediterranean fever in combination with Colhicine, Still's disease (90).

Anakinra in USA is used off label for treating: Schnitzler's syndrome (92), juvenile idiopathic arthritis, gout, calcium pyrophosphate deposition, Behçet's disease, ankylosing spondylitis, uveitis (93). Anakinra has extension of indication to treat COVID-19 in adults with pneumonia requiring supplemental oxygen (low- or high-flow oxygen) and who are at risk of progressing to severe respiratory failure (determined by plasma concentration of soluble urokinase plasminogen activator receptor (suPAR) ≥ 6ng/) (1,89). It was granted from EMA in December 2021. From FDA Kineret was granted EUA on 08.11.2022 (76, 77). Anakinra is a recombinant, non-glycosylated version of the human interleukin-1 receptor antagonist (IL-1Ra) and is identical to the natural non-glycosylated form of human IL-1Ra, except for the addition of a single methionine residue at the N-terminus. The recombinant protein consists of 153 amino acids with a molecular weight of 17.3 kilodaltons (94). Anakinra is monocyte derived IL-1 inhibitor using recombinant DNA system. Its production involves following steps:

- obtaining human leukocytes from normal donors and taken the mononuclear fraction
- mononuclear fraction is lysed and total RNA extracted from the lysate
- cDNA is prepared from total RNA and DNA sequence encoding for the IL-1 inhibitor was obtained
- cloning DNA sequence in plasmid vector

- transferring the vector containing the synthetic DNA sequence and operational elements into *Escherichia coli* host cells

- culturing *Escherichia coli* cells under conditions appropriate for amplification of the vector and expression of the IL-1 inhibitor

- detection and isolation of the IL-1 inhibitor (95).

Anakinra neutralises the biologic activity of IL-1α and IL-1β by competitively inhibiting their binding to IL-1 type I receptor (IL-1RI). IL-1 is a pivotal pro-inflammatory cytokine, mediating many cellular responses including those important in synovial inflammation (93). Anakinra is formulated as 100 mg/0.67 ml solution for injection in pre-filled syringe. The recommended dose for treating COVID-19 in adults is 100 mg administered once a day by subcutaneous injection for 10 days. Adverse reactions reported more frequently in patients with COVID-19 treated with Anakinra were neutropenia, elevation of liver function test, rash and injection site reactions (93).

3.2.1.6 Bebtelovimab

Bebtelovimab is a recombinant human IgG1 mAb. It is developed by AbCellera and Eli Lilly as a treatment for COVID-19, and was granted with EUA from FDA on 11.02.2022. Bebtelovimab is not holding marketing authorization from EMA. It is indicated for treatment of mild to moderate COVID-19 in adults and pediatric patients (12 years of age and older weighing at least 40 kilograms) with a positive COVID-19 test, and who are at high risk for progression to severe COVID-19, including hospitalization or death, and for whom alternative COVID-19 treatment options approved or authorized by the FDA are not accessible or clinically appropriate. It is not authorized for patients who are hospitalized due to COVID-19 or require oxygen therapy due to COVID-19. It may be associated with worse clinical outcomes when administered to hospitalized patients with COVID-19 requiring high flow oxygen or mechanical ventilation (77,

96, 97). Bebtelovimab is consisting of 2 identical light chain polypeptides composed of 215 amino acids each and 2 identical heavy chain polypeptides composed of 449 amino acids with molecular weight of 144 kDa (96, 97). It is isolated from a patient who has recovered from the COVID-19 (2, 98). Peripheral blood mononuclear cells were collected and IgG secreted by B-cells was isolated. Sequences encoding for VH and VL chain was identified, amplified by PCR and inserted into expression plasmids. Bebtelovimab was then recombinantly produced by transient transfection either in human-embryonic kidney (HEK293) or in to CHO cells (96-98).

Bebtelovimab is a recombinant neutralizing human IgG1λ mAb to the spike protein of SARS-CoV-2 and is unmodified in the Fc region. It binds the S protein with a dissociation constant KD = 0.046 to 0.075 nM and blocks S protein attachment to the human ACE2 receptor (96,97).

Bebtelovimab is formulated as a sterile, preservative-free, clear to opalescent and colorless to slightly yellow to slightly brown solution in a single-dose vial of 2 ml for intravenous injection. Each ml contains 87.5 mg of Bebtelovimab, L-histidine (0.4 mg), L-histidine hydrochloride monohydrate (0.6 mg), sodium chloride (2.9 mg), sucrose (60 mg), polysorbate 80 (0.5 mg), and water for injection. The Bebtelovimab solution has a pH range of 5.5-6.5. (96). The dosage in adults (18 years and older) and pediatric patients (≥12 years of age and weighing at least 40 kg) is bebtelovimab 175 mg administered as a single intravenous injection over at least 30 seconds. It should be administered as soon as possible after positive results of direct SARS-CoV-2 viral testing and within 7 days of symptom onset. Possible side effects include itching, rash, infusion-related reactions, nausea and vomiting (96, 97). The EUA for Bebtelovimab is supported by clinical and nonclinical data. Clinical data came from the BLAZE-4 trial, performed before Omicron was a dominant strain (50% of participants had Delta, and 29% had Alpha). BLAZE-4 was randomized, single-dose clinical trial that enrolled both low risk and high risk subjects with

mild to moderate COVID-19 who are not hospitalized. This trial was evaluating clinical efficacy of 175 mg Bebtelovimab alone and combined with 700 mg Bamlanivimab and 1400 mg Etesevimab. Efficacy of mAbs was compared to placebo in preventing hospitalization or death, when administered within 3 days of testing positive. The primary endpoint was persistently high viral load by day 7. A persistently high viral load was observed in 21%, 13% and 14% of participants in the placebo, combination of mAbs and Bebtelovimab groups, respectively. The secondary endpoint of COVID-19-related hospitalization or death by day 29 was observed in 1.6%, 2.4% and 1.6% of those three groups, respectively (96, 99).

3.2.1.7 Tixagevimab/Cilgavimab

Tixagevimab/Cilgavimab is a combination medicine that contains two active substances Tixagevimab and Cilgavimab. It is registered under the brand name Evusheld by Astra Zeneca (19, 20). It is granted with marketing authorization from EMA on 25.03.2022, and with EUA from FDA on 08.12.2021 (76, 77). Tixagevimab and Cilgavimab are two recombinant human IgG1κ mAbs. They have amino acid substitutions in the Fc regions in order to extend antibody half-life and to reduce antibody effector function and potential risk of antibody-dependent enhancement of disease (20).

Tixagevimab and Cilgavimab bind on different sites of the RBD of SARS-CoV-2, thus blocking its interaction with ACE2 receptor, and blockade of virus entry. They do not compete with one another for binding to the RBD, suggesting binding sites for these two antibodies are non-overlapping (20, 100). Tixagevimab and Cilgavimab are derived from B cells isolated from individuals with prior SARS-CoV-2 infection (101). Heavy- and light-chain genes from single B cells were sequenced followed by cDNA gene synthesis, cloning into immunoglobulin expression vectors and expression in CHO cells by transient transfection (102).

Tixagevimab/Cilgavimab is formulated as a solution for injection 150 mg/150 mg. It is administred as separate sequential intramuscular injections at different injection sites in two different muscles, preferably in the gluteal muscles. Its most common adverse reactions were injection site reactions (1.3%) and hypersensitivity (1.0%) (20).

Tixagevimab/Cilgavimab is used for:

1. Prevention of COVID-19 in adults and adolescents (12 years of age and older who weight at least 40 kg) who are not currently infected with SARS-CoV-2 and who have not had recent known close contact with someone who is infected with SARS-CoV-2 and:

- Who have moderate to severe immune compromise due to a medical condition or have received immunosuppressive medicines or treatments **and** may not mount an adequate immune response to COVID-19 vaccination **or**
- For whom vaccination with any available COVID-19 vaccine, according to the approved or authorized schedule, is not recommended due to a history of severe adverse reaction to a COVID-19 vaccine(s) or COVID-19 vaccine ingredient(s) (20).

2. Treatment of mild to moderate COVID-19 in adults and adolescents (≥12 years of age weighing at least 40 kg) (20).

Recommended dosage for these indications are:

1. For prevention of COVID-19 recommended dose is 300 mg of Tixagevimab/Cilgavimab, administered as two separate 1.5 mL, sequential, injections of: 150 mg of Tixagevimab and 150 mg of Cilgavimab. It is recommended to increase the dose to 600 mg in regions where BA.1 and BA.1.1 are circulating, administered as two separate 3.0 mL, sequential, injections of 300 mg of Tixagevimab and 300 mg of Cilgavimab (20).

2. For treatment of COVID-19 the recommended dose is 600 mg of Tixagevimab/Cilgavimab, administered as two separate 3.0 mL, sequential, injections of: 300 mg of Tixagevimab and 300 mg of Cilgavimab. It should be given as soon as possible after a positive viral test for SARS-CoV-2 and within 7 days after the onset of symptoms (20).

The most frequently adverse reaction of the medicine is injection site reaction (20).

To evaluate the potential Tixagevimab/Cilgavimab to prevent COVID-19 PROVENT clinical trial was conducted. Data from 5,000 people showed that Tixagevimab/Cilgavimab, given as two injections of 150 mg Tixagevimab and 150 mg Cilgavimab, reduced the risk of COVID-19 infection by 77%, with the duration of protection from the virus estimated to be at least six months. In the study, adults who had never had COVID-19 and had not received a COVID-19 vaccine or other preventative treatment received Tixagevimab/Cilgavimab or placebo. Of the people given Tixagevimab/Cilgavimab, 0.2% had lab-confirmed breakthrough COVID-19 after treatment, compared with 1.0% of the people who received placebo. The safety profile of Tixagevimab/Cilgavimab was favorable and side effects were generally mild, with a small number of people reporting reactions at the injection site or hypersensitivity (19, 20).

TACKLE is an ongoing Phase III, randomized, double-blind, placebo-controlled, multicenter study assessing Tixagevimab/Cilgavimab for the treatment of adult patients with mild to moderate COVID-19. The study enrolled individuals who had not received COVID-19 vaccination, were not hospitalized for COVID-19 treatment, and had at least 1 or more COVID-19 symptom that was at least mild in severity. The majority of participants (90%) were considered at higher risk of progressing to severe COVID-19. The primary efficacy endpoint was a composite of either severe COVID-19 or death from any cause by Day 29, in subjects who

received treatment within 7 days from symptom onset and were not hospitalized at baseline. Tixagevimab/Cilgavimab, given as two injections of 300 mg Tixagevimab and 300 mg Cilgavimab showed incidence of 4,4% for severe COVID-19 or death from any cause through Day 29, compared to 8,9% incidence from placebo group (20).

3.2.1.8 Bamlanivimab/Etesevimab

Bamlanivimab/Etesevimab is a combination of two mAbs, administered together as a treatment for COVID-19. It was developed by Eli Lilly and was granted with EUA from FDA on 09.02.2021 (77,103). Bamlanivimab is a human IgG1κ antibody unmodified in Fc region (103). It consists of 2 identical light chain polypeptides composed of 214 amino acids each and 2 identical heavy chain polypeptides composed of 455 amino acids and has molecular weight of 146 kDa (22). It is derived from VH1-69 and Vk1-39 germline families, isolated from B cells obtained from a convalescent patient and produced in CHO cell line (22,103). Bamlanivimab binds RBD of the S protein and has been shown to bind both the open (up) and closed (down) conformations of RBD, thus preventing binding of the virus to ACE2 receptor (22).

Etesevimab is a human IgG1κ antibody of molecular weight od 145 kDa consisting of 2 identical light chain polypeptides composed of 216 amino acids each and 2 identical heavy chain polypeptides composed of 449 amino acids. It is modified in the lower hinge by L235A, L236A, which significantly mutes Fc activity to reduce any chance of antibody dependent enhancement (22,103). Etesevimab was isolated from B cells of convalescent patient and derived from VH3-66/VK1-39 germline families, modified in Fc region and produced in CHO cell line (22).

Etesevimab binds the RBD of S protein and blocks S protein attachment to the human ACE2 receptor. Bamlanivimab and Etesevimab bind to different but overlapping epitopes in the receptor binding domain (RBD) of the S-protein (103).

Bamlanivimab and Etesevimab, administered together, are indicated for the treatment of mild-to-moderate COVID-19 in people aged 12 years and older weighing at least 40 kilograms with positive results of direct SARS-CoV-2 viral testing, and who are at high risk for progression to severe COVID-19, including hospitalization or death. This combination is also indicated for post-exposure prophylaxis after exposure to SARS-CoV-2 and are not authorized for pre-exposure prophylaxis to prevent COVID-19 before being exposed to the SARS-CoV-2 virus (103). Both mAbs are formulated as sterile injections for intravenous infusion, containing 35 mg Bamlanivimab and 35 mg Etesevimab per ml (103).

Recommended dosage for use of this combination is following:

- for treatment: dosage in adults (18 years and older) and pediatric patients (<18 years and weighing at least 40 kg) is Bamlanivimab 700 mg and Etesevimab 1,400 mg. The dosage for pediatric patients weighing less than 40 kg will vary depending on body weight: >20 kg to <40 kg: 350 mg Bamlanivimab and 700 mg Etesevimab; >12 kg to 20 kg: 175 mg Bamlanivimab and 350 mg Etesevimab; 1 kg to 12 kg: 12 mg/kg Bamlanivimab and 24 mg/kg Etesevimab. For treatment of COVID-19, Bamlanivimab and Etesevimab should be administered together as soon as possible after positive results of direct SARS-CoV-2 viral testing and within 10 days of symptom onset.
- post-exposure prophylaxis: dosage in adults (18 years and older) and pediatric individuals (<18 years and weighing at least 40 kg) is 700 mg Bamlanivimab and 1,400 mg Etesevimab administered together as a single intravenous infusion. The dosage for

pediatric individuals weighing less than 40 kg will vary depending on body weight: >20 kg to <40 kg: 350 mg Bamlanivimab and 700 mg Etesevimab; >12 kg to 20 kg: 175 mg Bamlanivimab and 350 mg Etesevimab; 1 kg to 12 kg: 12 mg/kg Bamlanivimab and 24 mg/kg Etesevimab. For post-exposure prophylaxis, Bamlanivimab and Etesevimab should be given together as soon as possible following exposure to SARS-CoV-2 (103).

The data supporting the EUA for treatment of COVID-19 with Bamlanivimab and Etesevimab are based on a BLAZE-1 randomized, double-blind, placebo-controlled clinical trial. It included 1,035 non-hospitalized participants with mild to moderate COVID-19 symptoms who were at high risk for progressing to severe COVID-19. Of these, 518 received a single infusion of Bamlanivimab 2,800 milligrams and Etesevimab 2,800 milligrams together, and 517 received placebo. The primary endpoint was COVID-19 related hospitalizations or death by any cause during 29 days of follow-up. Hospitalization or death occurred in 7% of participants who received placebo compared to 2% participants treated with Bamlanivimab 2,800 milligrams and Etesevimab 2,800 milligrams administered together. It was a 70% reduction in patients tested with the combination. All of deaths, which were 2%, occurred in the placebo group (103).

3.2.2 Monoclonal antibodies under investigation

3.2.2.1 Amubarvimab (BRII-196, P2C-1F11) /Romlusevimab (BRII-198, P2B-1G5)

Amubarvimab (BRII-196, P2C-1F11) and Romlusevimab (BRII-198, P2B-1G5) are two human neutralizing recombinant human IgG1 monoclonal antibodies against the SARS-CoV-2 spike protein (22,104). They are developed by Brii Biosciences, Tsinghua University and the Third People's Hospital of Shenzhen, and combination is approved in December 2021 by the National Medical Products Administration of China for the treatment of mild COVID-19 in patients aged ≥ 18 years, and those aged 12–17 years with a bodyweight of ≥ 40 kg (conditional approval) who are at high risk of progressing to severe disease, including hospitalization or death. An EUA application for Amubarvimab/Romlusevimab is currently under review in the USA (104).

The recommended dose is Amubarvimab 1000 mg plus Romlusevimab 1000 mg administered as separate sequential intravenous infusions (104).

Amubarvimab (BRII-196) and Romlusevimab (BRII-198) are human IgG1 mAbs derived from their respective precursor antibodies, P2C-1F11 and P2B-1G5, isolated directly from B cells of a convalesced COVID-19 patient. Amubarvimab binds to two regions spanning amino acids 453-505 in the RBM and amino acids residues 403-421 in the core region of RBD. Among its predicted contact residues, 11 of 23 overlap with the ACE2 binding sites on SARS-CoV-2 RBD, providing the structural basis for Amubarvimab in competing with ACE2 for binding to the RBD and blocking subsequent virus entry. In addition, the Fc region of Amubarvimab and Romlusevimab was engineered with a triple-amino-acid (M252Y/S254T/T256E) substitution to

allow an extended half-life to potentially prolong the treatment window and reduce effector functions. Romlusevimab binds to different site of RBD of S-protein, thus have additive effect when combined with Amubarvimab (105). Amubarvimab/Romlusevimab significantly reduced the risk of hospitalization or death from any cause in non-hospitalized adults with symptomatic COVID-19 who were at high risk of clinical progression in the phase III part of an ongoing, adaptive, randomized, double-blind, placebo-controlled, multinational phase II/III trial (ACTIV-2; NCT04518410) (104). To estimate the impact of current most prevalent variant Omicron BA.4/5 on clinical efficacy of the antibody combination, a detailed analysis and prediction based on BA.4/5 live virus neutralization result and PK modeling generated from the interim human population PK analysis was performed as part of the ACTIV-2/A5401 study of non-hospitalized COVID-19 patients. Based on this, combination of Amubarvimab/Romlusevimab total serum exposures were found to be effective *in vivo* against current circulating Omicron subvariant BA.4/5 during the commonly recognized 2-week treatment window post administration (105).

3.2.2.2 BMS-986413 (C144-LS) and BMS-986414 (C135-LS)

BMS-986413 (C144-LS)/BMS-986414 (C135-LS) is a combination medicine with two human mAbs, developed by Bristol-Myers Squibb and Rockefeller University (5, 22).

BMS-986413 (C144-LS) is a human IgG1λ antibody derived from B cells from a convalescing patient. It is a VH3-53/VL2-14 germline derived antibody that competes with ACE2 binding and has an affinity for SARS-CoV-2 RBD. A characteristic of C144 is the ability to bind two RBDs simultaneously, one with primary binding activity that competes with ACE2 binding, while

binding a second RBD at a distal site. This cross-linking locks the RBDs in a closed conformation adding to the overall potency of the response (22, 106).

BMS-986414 (C135-LS) is a human IgG1κ antibody derived from B cells from a convalescing patient. It is a VH3-30/VK1-5 germline derived antibody that has an affinity for SARS-CoV-2 RBD. The epitope for C135 does not overlap with the primary epitope of C144, but overlaps significantly with the C144 distal binding site (its binding site on the "second" RBD) (22).

Both C135 and C144 have been modified by insertion of the M428L/N434S ("LS") mutations, which increase the circulating half-life of the antibodies by modifying the interaction with the recycle receptor, FcRn (22,106). The C135/C144 cocktail is currently in Phase II/III clinical trials, including participation in the large ACTIV-2 trial of ambulatory patients to determinate safety and efficacy in treating COVID-19 outpatients. Recommended dosage is administered subcutaneously (SC) as 4 separate injections for one dose (two injections of C135-LS 200mg and two injections of C144-SL 200mg) (107).

3.2.2.3 Adintrevimab (ADG-20)

Adintrevimab (ADG20) is a fully human IgG1 monoclonal antibody derived from a survivor of the 2003 SARS-CoV epidemic. It is developed by Invivid (formerly Adagio Therapeutics).

Adintrivimab is developed from parent mAb ADG-2, where Fc region is modified in order to extend its half-life. Adintrevimab binds to a distinct epitope in the RBD of the spike glycoprotein of SARS-CoV-2 that partially overlaps the ACE2 binding site. *In vitro,* Adintrevimab has demonstrated potent neutralizing activity against most variants and sublineages of SARS-CoV-2

(including Alpha, Beta, Gamma, and Delta) as well as other SARS-like viruses. Adintrevimab displays reduced in vitro activity against Omicron BA.1/BA.1.1 and lacks activity against BA.2, BA.3, BA.4, and BA.5 (22,108).

Adentrevimab is currently in Phase II/III clinical trials and is being developed as an intranasal delivered antibody (22, 109, 110). For the treatment of mild to moderate COVID-19 in patients at high risk of disease progression, it is included in STAMP trial, a combined Phase 2/3 global clinical trial designed to provide a path to authorization, marketing approval and commercial launch. For the prevention of COVID-19, it is included in EVADE trial, a combined Phase 2/3 global clinical trial, in both post-exposure and pre-exposure populations (109,110).

3.2.2.4 TY027

TY027 is a fully engineered human IgG whose preliminary data from the phase 1 trial showed safety and tolerability up to 20 mg/kg. It is developed by Tychan.

TY027 is being explored for treatment of patients with COVID-19 to slow the progression of the disease and accelerate recovery, as well as potentially providing temporary protection against infection from SARS-CoV-2. The Phase 3 clinical trial is in the final phase of trials for TY027 and will involve 1,305 volunteer COVID-19-positive patients. The first 15 patients will be randomized 1:1:1 to be given either a single dose of 1,500 mg TY027, 2,000 mg TY027 or placebo for initial safety assessment. Subsequently, patients will be randomized 1:1 to receive either a single fixed dose of 2,000 mg TY027 or placebo (N = 645 per group). All individuals will be admitted to the hospital for up to one-week post-dosing and followed up on days 14 and

28. If the antibody is proven efficacious in this trial, it will be submitted for review by regulatory agencies as a new drug.

3.2.2.5 VIR 7832

VIR 7832 is human IgG1 mAb developed by VIR Biotechnology. It is designed along with Sotrovimab. Like Sotrovimab, IR-7832 is derived from antibody S309, which was isolated from B cells from a 2003 SARS-CoV-1 convalescent patient. Thus, both antibodies bind an epitope shared by both SARS-CoV-1 and -CoV-2. VIR-7832 has M428L/N434S ("LS" mutant) mutations on Fc region which ensures extended half-life and high concentration in the lungs to ensure optimal penetration into airway tissues affected by SARS-CoV-2 (22,111). VIR-7832 also has been modified in its Fc with the "GAALIE" (G236A, A330L, I332E) mutations in the Fc domain which have been associated with activation of $CD8^+$ T cells in other respiratory viral infections. The GAALIE mutations in VIR-7832 were shown to enhance binding to FcγRIIa and FcγRIIIa without a concomitant increase in binding to FcγRIIb, which reportedly activates $CD8^+$ T cells to respond to respiratory viral infections. The increased activating receptor Fc activity has not been associated with a concomitant increase in ADE. This leads to potentially enhance virus-specific T cell function, which could help treat and/or prevent COVID-19 infection (22,111). VIR-7832 is currently in AGILIE, Phase I/II clinical trials (NCT04746183) to determinate the optimal dose, activity and safety of the drug for the treatment of COVID-19 (5).

3.2.2.6 IGM-6268

IGM-6268 is a potent Immunoglobulin M (IgM) that is derived from CoV2-14, an antibody isolated from a naïve human antibody library (22). IGM-6268 specifically targets RBD of the SARS-CoV-2 S-protein. This humanized pentameric IgM antibody has 10 binding sites to the S-protein and a J-chain to enable the formation of IgM pentamers.

The primary mechanism of action of IGM-6268 is to block the binding of the SARS-CoV-2 RBD on the S-protein to hACE2 receptor. By blocking this binding, IGM 6268 neutralizes the infectivity of the virus (112). Conversion to an IgM isotype improved the binding avidity to RBD by about 14-fold through the high avidity of IgM. Improvement in neutralization activity by converting an IgG to an IgM is about 230-fold against wild-type virus. With mutant viruses such as Gamma and Beta, the IgG format exhibited approximately a 100-fold reduction in potency, whereas the IgM format retained very high potency. IGM-6268 has demonstrated significant neutralization potency against those variants thus far tested including Omicron BA.1 (22, 112).

IGM-6268 is developed by IGM Biosciences as intranasal/intraoral spray and is currently in Phase I clinical trials (112). It is being developed as a treatment for or prophylaxis of symptoms associated with mild to moderate COVID-19 (22).

3.3 Treatment with monoclonal antibodies

Regarding novel treatment with mAbs several questions need to be addressed about the potential clinical use: 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; what is their associated benefit-to-risk ratio.

3.3.1 Selection of patients for treatment

Neutralizing mAbs may have a prophylactic role in individuals who are at high risk of severe COVID-19. Preliminary non-peer-reviewed preprint data suggest that mAbs prevent COVID-19 in high-risk individuals potentially exposed to SARS-CoV-2 in nursing homes or within households. Up to 10% of initially asymptomatic, minimally symptomatic and mild infections progressed to severe disease including respiratory distress. Approximately 78% of patients admitted to hospitals have at least one documented co-morbidity, there continue to be patients lacking any identified co-morbidity who subsequently become critically ill. Thus, the absence of co-morbidities does not completely eliminate the risk of severe disease, and there is an urgent need for additional insight into a more personalized predictive algorithm to unlock unidentified risk factors.

COVID-19 can potentially claim the lives of young adults in their prime, even in the absence of any known underlying risk factors. Given that persistently high SARS-CoV-2 viral loads may be associated with severe clinical outcomes, it is possible that early reassessment of viral loads might help guide who among the ‘lower-risk’ population might be treated by neutralizing mAbs. Randomized control trials evidence indicates that clinical value of neutralizing mAbs therapy is more pronounced in individuals who are seronegative at diagnosis. Collectively, measuring viral load and serology would allow strategic deployment for patients without risk factors while targeting early supply to the high-risk population. It also seems reasonable to use neutralizing mAbs early on during the disease for patients with well-identified risk factors for severe disease evolution.

Another way to classify candidate patients, eligible for treatment with neutralizing mAbs, would be to select patients who are expected to have poor antiviral responses (for example, elderly or immunocompromised patients) or to identify patients with poor T cell and/or B cell function via experimental techniques (such as by serology or flow cytometry). Regarding the latter, it is a very complex process and there is a lack of published evidence on humoral immune response dynamics and correlation with clinical outcomes. Also, technical difficulties in stratifying patients on the basis of 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 (4). According to mAb's conditional marketing authorization from EMA and EUA from FDA, eligibility criteria for therapy in the treatment of non-hospitalized patients with mild/moderate SARS-CoV-2 infection considered at high risk of progression to severe COVID-19 includes: older age (≥65 years); obesity or being overweight (adults with BMI >25 kg/m2, or if aged 12–17 years, BMI ≥85th percentile for their age and sex); pregnancy; chronic kidney disease; diabetes; immunosuppressive disease or immunosuppressive treatment; cardiovascular disease (including congenital heart disease) or hypertension; chronic lung diseases (chronic obstructive pulmonary disease, asthma, interstitial lung disease, cystic fibrosis and pulmonary hypertension); sickle cell disease; neurodevelopmental disorders or genetic or metabolic syndromes and severe congenital anomalies; having a medical-related technological dependence (e.g. tracheostomy, gastrostomy or positive pressure ventilation not related to COVID-19) (113).

3.3.2 Emergence of resistance

Under the pressure of natural immunity and treatment with mAbs, SARS-CoV-2 has the potential to select viral variants with reduced susceptibility to mAbs and vaccines. Antiviral and antimicrobial therapies are traditionally plagued by their promoting escape variants, 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. Transitioning towards combination therapies comes in order to mitigate the viral escape, to ensure longevity of the therapies and to reduce the potential rate of treatment failure (4).
In laboratory studies, some SARS-CoV-2 variants that harbor certain mutations have markedly reduced susceptibility to several of the authorized mAbs. Clinical relevance of the reduced *in vitro* susceptibility of select variants to the mAbs is under investigation.

SARS-CoV-2 variants susceptible to mAbs are:

- *Alpha (B.1.1.7):* retains *in vitro* susceptibility to all anti-SARS-CoV-2 mAb products currently available
- *Beta (B.1.351):* has markedly reduced *in vitro* susceptibility to Bamlanivimab and Etesevimab. *In vitro* studies also suggest that the Beta variant has markedly reduced susceptibility to Casirivimab; however, the combination of Casirivimab and Imdevimab appears to retain activity against the variant. Sotrovimab also appears to retain activity against the variant.
- *Gamma (P.1):* has markedly reduced *in vitro* susceptibility to Bamlanivimab and Etesevimab. The Gamma variant also has reduced susceptibility to Casirivimab; however,

the combination of Casirivimab plus Imdevimab appears to retain activity against the variant. Sotrovimab also appears to retain activity against the Gamma variant.

- *Delta (B.1.617.2, non-AY.1/AY.2):* This variant retains *in vitro* susceptibility to all anti-SARS-CoV-2 mAbs currently available.

- *Omicron (B.1.1.529):* This variant of concern (VOC) includes the BA.1, BA.1.1, BA.2, BA.3, BA.4, BA.5 subvariants. This variant, which includes numerous mutations in the spike protein, has markedly reduced *in vitro* susceptibility to some anti-SARS-CoV-2 mAb products, as noted below:

  Bamlanivimab plus Etesevimab and Casirivimab plus Imdevimab are not expected to be active against these subvariants.

  Sotrovimab retains activity against the Omicron BA.1 and BA.1.1 subvariants but has decreased *in vitro* activity against the Omicron BA.2 subvariant, BA.4 and BA.5.

  Bebtelovimab retains *in vitro* activity against all circulating Omicron subvariants

  The originally authorized dose of Tixagevimab 150 mg plus Cilgavimab 150 mg has reduced *in vitro* activity against the Omicron BA.1 and BA.1.1 subvariants. However, the FDA updated the EUA to authorize a dose of Tixagevimab 300 mg plus Cilgavimab 300 mg, which is expected to maintain activity against these subvariants. The duration of protection against the BA.1 and BA.1.1 subvariants remains unclear. Tixagevimab plus Cilgavimab has retained *in vitro* activity against the Omicron BA.2 subvariant (23).

  On 9th December 2022 EMA issued Emergency task force (ETF) in which stated that monoclonal antibodies currently authorized for COVID-19 are unlikely to be effective against emerging strains of SARS-CoV-2. These are Evusheld (Tixagevimab /

Cilgavimab), Regkirona (Regdanvimab), Ronapreve (Casirivimab /Imdevimab) and Xevudy (Sotrovimab). The ETF statement does not refer to RoActemra (Tocilizumab), monoclonal antibody, which does not target the virus but acts as a modulator of the immune response and is used with a corticosteroid medicine in patients who require extra oxygen or mechanical ventilation (118). This comes after recent laboratory studies show that all EU-approved monoclonal antibodies targeting the spike protein are poorly effective at neutralizing Omicron strains BA.4.6, BA.2.75.2 and XBB. The data also show that these monoclonal antibodies do not significantly neutralize BQ.1 and BQ.1.1, which are expected to become the dominant strains in the EU in the beginning of 2023 (118-121). It is not known to what extent *in vitro* neutralization activity against VOC is decreased, as the relationship between viral susceptibility *in vitro* and serum concentration of the monoclonal antibodies *in vivo* is not completely understood, and there are no clinical trial data available to determine whether clinical efficacy is reduced. Also, it is unknown if the efficacy of the monoclonal antibodies against VOC can be restored by administering higher doses than those currently recommended. Consequently, the use of monoclonal antibodies for the prevention or treatment of COVID-19 in patients at increased risk for progressing to severe COVID-19 will likely not provide a clinical benefit in regions of the EU in which BQ.1.1, BQ.1, BA.4.6, BA.2.75.2, XBB and BJ.1 are spreading. It remains unknown to what extent the approved mAbs that have decreased neutralization activity against the Omicron sublineages BA.1, BA.2, BA.4 and BA.5 will be clinically effective (118). FDA released statements that circulating SARS-CoV-2 variants BQ.1 and BQ.1.1 are non-susceptible to Bebtelovimab, Sotrovimab, Bamlanivimab/Etesevimab and Casirivimab/Imdevimab (77).

4. DISCUSSION

The sudden spread of the COVID-19 has stimulated an accelerated research to identify effective ways to limit the spread of infection and to reduce the morbidity and mortality associated with it. Neutralizing mAbs were attractive approach with potential utility in both prophylactic and treatment settings. mAbs therapies are laboratory-produced proteins using humanized murine technology or convalescent plasma from recovered patients. Most of them are recombinant IgG1 mAbs. Their mechanism of action is based on binding to RBD of S-protein of SARS-CoV2 virus, and prevent it from attaching to human cells. They are formulated as sterile intravenous infusions for administration in hospital settings under supervision.

While these therapies have been used to treat COVID-19 and were granted with marketing authorizations from EMEA and FDA, some treatments have become less effective or ineffective as COVID-19 mutates. RNA viruses exhibit high mutation frequencies in the human body, and an increasing list of SARS-CoV-2 variants have been detected. To date, there have been hundreds of mutations identified in S protein and many are rapidly spreading in the population. Some of these point mutations might trigger local or global protein structure changes that enhance virulence or cause loss of efficacy for mAbs. Therefore, Ab combination/cocktail therapies were considered as a strategy to prevent the emergence of SARS-CoV-2 escape mutants. In addition to the REGN-COV2 cocktail Abs (Casirivimab and Imdevimab), the combination treatment of Bamlanivimab with Etesevimab also reduced SARS-CoV-2 log viral load at day 11 in patients with mild to moderate COVID-19. Although it has been reported that Casirivimab, Bamlanivimab, and Etesevimab lose neutralizing activity against B 1.351 (Alpha), and P.1 (Gamma) variants of SARS-CoV-2, the cocktails of Abs with non-overlapping epitopes on the RBD have been shown to exhibit great efficacy for neutralizing SARS-CoV-2 mutant escape variants (123).

A lot of clinical studies were conducted to determine efficacy and treatment guidelines of new developed mAbs or repurposed ones. The COVID-19 treatment guidelines recommendations for the use of anti-SARS-CoV-2 mAbs are based on current knowledge of the *in vitro* activities against the circulating SARS-CoV-2 variants and subvariants. At this time, anti-SARS-CoV-2 mAb recommendations are for the treatment of nonhospitalized patients with mild to moderate COVID-19 who are at high risk of progressing to severe disease (23). Additional considerations that needs to be considered following a treatment with mAbs are:

- treatment should be started as soon as possible after SARS-CoV-2 infection is confirmed by an antigen test or a nucleic acid amplification test and within 7 days of symptom onset.
- mAbs should be administered in a setting where severe hypersensitivity reactions, such as anaphylaxis, can be managed. Patients should be monitored during the infusion and observed for at least 1 hour after infusion.
- treatment with mAbs should be considered for patients with mild to moderate COVID-19 who are hospitalized for a reason other than COVID-19 if they otherwise meet the eligibility criteria for outpatient treatment.
- risk for progression to severe COVID-19 in high-risk patients is substantially greater for those who are not vaccinated or those who are vaccinated but not expected to mount an adequate immune response to the vaccine due to an underlying immunocompromising condition.
- there are no data on the combined use of antiviral agents and mAbs for the treatment of non-hospitalized patients with COVID-19. Clinical trials are needed to determine whether this combination therapy has a role in the treatment of COVID-19.

- severely immunocompromised patients may have prolonged SARS-CoV-2 replication, leading to more rapid viral evolution. There is a concern that using a mAb in these patients may result in emergence of resistant virus. Additional studies are needed to assess this risk. The role of mAbs plus antiviral therapy in the treatment of COVID-19 is not yet known (23).

mAbs available are not authorized for use in the following patients: hospitalized for COVID-19; those who require oxygen therapy or respiratory support due to COVID-19; those who are on chronic oxygen therapy due to an underlying non-COVID-19-related comorbidity and who require an increase in oxygen flow rate from baseline or respiratory support because of COVID-19.

A study of the ACTIV-3/TICO trial randomized patients who were hospitalized for COVID-19 to receive mAbs with current standard of care (including Remdesivir or glucocorticoids) or placebo with current standard of care. The TICO primary objective is to determine whether investigational agents are safe and efficacious when given with current standard of care (114,115). The TICO protocol has been amended three times, because of adding a new agent or agents. Version 1 included the Lilly neutralizing monoclonal antibody LY-CoV555 (Bamlanivimab), version 2 of the protocol included the GSK/Vir neutralizing monoclonal antibody Vir-7831(Sotrovimab) and the Brii Bioscience neutralizing monoclonal antibodies Brii-196/198 (Amubarvimab/Romlusevimab), version 3 of the protocol added the AstraZeneca neutralizing monoclonal antibody AZD7442 (Tixagevimab/Cilgavimab) and version 4 of the protocol added the Molecular Partners DARPin® molecule MP0420 (114). Of the agents that have entered the protocol so far, LY-CoV555, Vir-7831, Brii-196/198 and AZD7442 did not

pass the initial futility assessment and were discontinued, while MP0420 remain under study (114,116).

RECOVERY is a randomized, controlled, open-label platform trial comparing several possible treatments with usual care in patients admitted to hospital with COVID-19 (117). In the RECOVERY study, hospitalized patients with COVID-19 were randomized to receive usual care with Casirivimab 4,000 mg plus Imdevimab 4,000 mg IV or usual care alone. There was no difference in 28-day all-cause mortality between the Casirivimab plus Imdevimab arm and the usual care arm. In the subgroup of patients who were seronegative for the anti-spike protein antibody, there was a significant reduction in 28-day all-cause mortality in the Casirivimab plus Imdevimab arm (396 of 1,633 casirivimab plus imdevimab recipients [24%] died vs. 452 of 1,520 usual care recipients [30%]; rate ratio 0.79; 95% CI, 0.69–0.91; $P = 0.0009$). Under the current authorization, this higher dose of Casirivimab plus Imdevimab is not available, and the lower dose is only authorized for use in non-hospitalized patients with COVID-19. In addition, rapid serology testing that can identify seronegative individuals in real time is currently not widely available (23,117). mAbs may be available through expanded access programs for the treatment of immunocompromised patients who are hospitalized because of COVID-19. It is not yet known whether these mAb products provide clinical benefits in people with B-cell immunodeficiency or other immunodeficiencies (23).

To define the utility of specific mAbs in the future, ongoing population-based genomic surveillance of the types and proportions of circulating SARS-CoV-2 variants, as well as studies on the susceptibility of different variants to available anti-SARS-CoV-2 mAbs, will be important (23). Framework for the development of efficient monoclonal antibody therapies will integrate knowledge of the dynamics of mAb resistance by SARS-CoV-2 variants, epitope mutations,

epistatic effects and evolutionary dynamics. Monoclonal antibody development must be supported by epitope identification, *in vitro* studies and the monitoring of resistance mutations. Resistance monitoring is a multifaceted process, spanning individual and combinatorial mutational effects *in vitro*, as well as genomic surveillance of circulating strains and longitudinal clinical studies that track genetic changes during treatment. Combining these different approaches will allow the development of mAbs highly effective against currently circulating strains, and robust to resistance by future variants (Figure 14) (120).

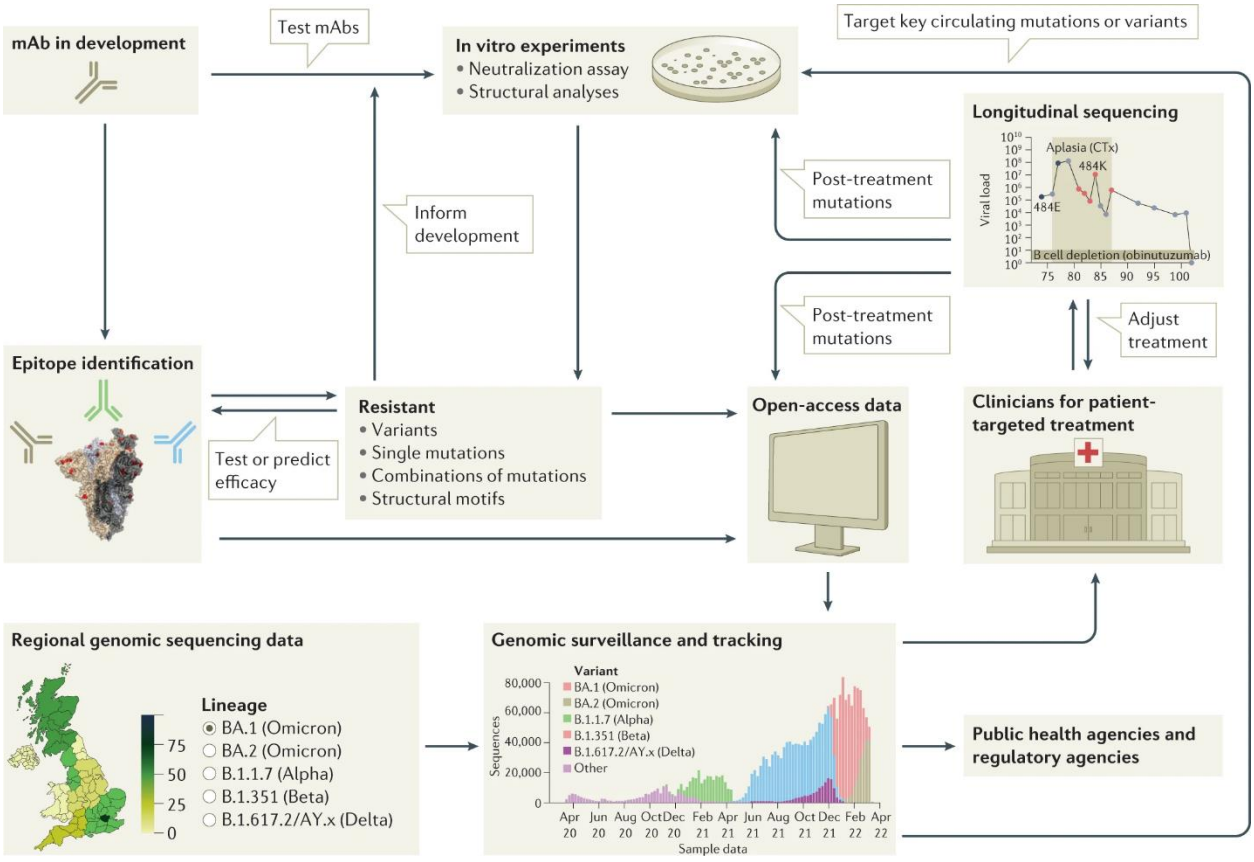

Figure 14 Framework for development effective mAbs
(adapted from 120)

5. CONCLUSION

The current global COVID-19 pandemic caused by SARS-CoV-2 has resulted in a public health crisis with more than 168 million cases reported globally and more than 4.5 million deaths. The economic impact has been significant as public health measures to reduce the spread have led to lockdowns resulting in near closure of many sectors of the economy. All of these urgent the need for an effective therapy for COVID-19 despite vaccination program. Antibodies are a principal determinant of the humoral immune response to COVID-19 infections and may have the potential to reduce disease and spread of the virus. The development of mAbs represents a therapeutic option that can be produced at large quantity and high quality. Neutralizing mAbs being developed to combat COVID-19 are generated against the receptor-binding domain (RBD) of the spike (S) protein of SARS-CoV-2. The anti-RBD mAbs prevent binding of the S protein to its cognate receptor, hACE2, on target host cells. It was belived that mAbs represents the most effective and viable therapy and/or prophylaxis option against COVID-19, because at first they have shown a reduction of the viral load, as well as lowering hospitalizations and death rates. Additional benefit was that they have no serious adverse effect and low toxic profile due to targeted delivery and mechanism of action. To date mAbs treatments which were given Conditional Marketing Authorization (CMA) in the EU and UK and Emergency Use Authorization (EUA) in the USA for treatment of Covid-19 are: Casirivimab/Imdevimab; Regdanvimab; Sotrovimab; Tocilizumab; Anakinra; Bebtelovimab; Tixagevimab/Cilgavimab Criteria for patients eligible to receive these treatments were established. Other than being aged ≥12 years, there are no longer any age criteria restricting the use of these products in patients with the following conditions: sickle cell disease, neurodevelopmental disorders, medical-related technological dependence, asthma, cardiovascular disease, hypertension, and chronic lung disease. The anti-SARS-CoV-2 mAbs available are not authorized for use in the following

patients: hospitalized for COVID-19; who require oxygen therapy or respiratory support due to COVID-19; who are on chronic oxygen therapy due to an underlying non-COVID-19-related comorbidity and who require an increase in oxygen flow rate from baseline or respiratory support because of COVID-19. Some SARS-CoV-2 variants that harbor certain mutations have markedly reduced susceptibility to almost of the authorized anti-SARS-CoV-2 mAbs. The clinical relevance of the reduced in vitro susceptibility of select variants to anti-SARS-CoV-2 mAbs remains under investigation. This leads researchers to transition from monotherapy towards combination therapies with mAbs, in the means of lowering potential viral escape, ensuring longevity of the therapies and reducing the potential rate of treatment failure.

In recent times EMA and FDA issued statements that currently authorized mAbs are no longer effective against current SARS-CoV-2 VOC. Consequently, the use of mAbs for the prevention or treatment of COVID-19 in patients at increased risk for progressing to severe COVID-19 will likely not provide a clinical benefit in regions in which BQ.1.1, BQ.1, BA.4.6, BA.2.75.2, XBB and BJ.1 variants are spreading. Furthermore, it remains unknown to what extent the approved mAbs that have decreased neutralization activity against the Omicron sublineages BA.1, BA.2, BA.4 and BA.5 will be clinically effective. The healthcare professionals are advised to check first the current epidemiological situation in their region and to consider alternative antiviral treatment options. In the future development of efficient mAbs against circulating strains should include dynamics of mAb resistance by SARS-CoV-2 variants, epitope mutations, epistatic effects and evolutionary dynamics. mAb development must be supported by epitope identification, in vitro studies and the monitoring of resistance mutations. Also, genomic surveillance of circulating strains and longitudinal clinical studies that track genetic changes during treatment should be included.

In summary, the present studies demonstrated the benefit of current therapeutic mAbs against COVID-19 despite decreasing their efficacy from the viral mutations. Research and development in this therapeutic area should continue by means of developing therapies that are more efficient and will resist the viral mutations. This can be a potential area for research of treatments for other infectious diseases.

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ABBREVIATIONS:

ACE2 – angiotensin converting enzyme 2

ADA – anti drug antibody

ADC – antibody-drug conjugate

ADCC – antibody dependent cellular cytotoxicity

ADCP – antibody dependent cellular phagocytosis

ARDS – acute respiratory distress syndrome

CDC – Complement-Dependent Cytotoxicity

cDNA – complementary DNA

CDR – complementarity determining region

CH – constant region heavy chain

CL – constant region light chain

CNS – Central nervous system

COVID-19 – Corona virus disease 2019

CP – cytoplasm domain

CRP – C-reactive protein

CRS – cytokine release syndrom

ER – endoplasmic reticulum

ERGIC – endoplasmic reticulum-Golgi intermediate compartment

Fab – fragment antigen binding

Fc – Fragment crystallizable region

GM-CSF – granulocyte-macrophage colony-stimulating factor

HGPRT – hypoxanthine-guanine phosphoribosyltransferase

HE – hemagglutinin-esterase

HP – heptad repeat

IP-10 – interferon gamma-induced protein 10

IL – interleukin

Ig – immunoglobulin

LDH – lactate dehydrogenase

mAB – monoclonal antibody

MAC – membrane attack complex

MCP1 –monocyte chemoattractant protein 1

MIP – macrophage inflammatory protein

MOA – mechanism of action

NK cell – natural killer cell

nsp – non structural proteins

RBD – receptor - binding domain

SARS - severe acute respiratory syndrome

scFv – single chain fragment variable

SP – signal peptide

RTC – replication – transcription complex

TNF-α – tumour necrosis factor

VL – variable region light chain

VH – variable region heavy chain

VOC – variant of concern

7. CURRICULUM VITAE

Personal Information

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Mobile phone:	+389 70 804 995
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Nationality:	Macedonian
Date of birth:	05.09.1984
Marital status:	Married with two children

Education

Dates	**March 2021 - Current**
Title of qualification awarded:	University Master of Drug Development (Univ. Mag. Pharm.)
Name of organisation providing education:	University of Zagreb, Faculty of Pharmacy and Biochemistry
Dates	**October 2003 to June 2008**
Title of qualification awarded:	5 year M.Sc. degree in Pharmaceutical sciences
Name of organisation providing education:	Faculty of Pharmacy, at the University "Ss Cyril and Methodius" Skopje, Macedonia

Working experience

Dates:	**May 2022 - Current** (Alkaloid AD – Quality Control Department)
Position held:	**Stability project lead**
Activities and responsibilities:	▪ Manage stability studies ▪ Provide technical oversight, planning, coordination and support to projects related with stability studies, CMO product transfers ▪ Life-Cycle management support. ▪ Manage high-quality analytical registration documents for regulatory submission (CMC documentation). ▪ Works closely with internal cross-functional impacted areas QA, CMC/Regulatory affairs, R&D, Technical services to resolve open issues with in a project in a timely manner. ▪ Review, approval and disposition of deliverables (batch record, SOP) ▪ Providing GAP analysis for stability studies and transfer processes ▪ Bilateral aligning with CMO's according regulatory requirements and company policies ▪ Risk analysis of the processes ▪ Control projects ensuring overall success and effective closure on time. ▪ Lead initiatives to ensure proactive compliance and continuous improvement.

- Working with GMP QMS documentation including Change controls, Deviations, CAPA, OOS, OOE and OOT.
- Manage, follow up, and conduct root-cause analysis and CAPA determination.
- Manage and maintain a culture of enhancing safety and corporate ethics throughout the entire laboratory.

Dates: **January 2021 – April 2022** (Alkaloid AD – Quality Control Department)

Position held: **Stability lead at On-going stability of commercial batches**

Activities and responsibilities:

- Manage stability studies, responsible for planning and execution of stability programs for commercial drug products (on going stability).
- Manage stability studies data and documentation.
- Management of stability chambers and sample management for stability studies.
- Manage high-quality analytical registration documents for regulatory submission (CMC documentation).
- Works closely with internal cross-functional impacted areas QA, CMC/Regulatory affairs, R&D, Technical services to resolve open issues in a timely manner resulting from record reviews and deviation events.
- Control projects ensuring overall success and effective closure on time.
- Lead initiatives to ensure proactive compliance and continuous improvement.
- Working with GMP QMS documentation including Change controls, Deviations, CAPA, OOS, OOE and OOT.
- Manage, follow up, and conduct root-cause analysis and CAPA determination.
- Manage and maintain a culture of enhancing safety and corporate ethics throughout the entire laboratory.

Dates: **May 2009 – January 2021** (Alkaloid AD – Quality Control Department)

Position held: **Senior lead at Validation/Development /Transfer of analytical methods team**

Activities and responsibilities:

- Strong experience in Validation of Analytical methods and preparing Validation documentation
- Lead innovative developments, improvement and optimization of Analytical methods according to latest technology and pharmaceutical regulatory, quality and safety requirements (Pharmacopoeias, ICH guidelines).
- Life cycle management of analytical methods with trend analysis.
- Plan, prepare and execute Analytical Method Transfer for transfer of out-license and in-license products.
- Lead in troubleshooting analytical technical challenges during the development, validation, qualification and transfer of analytical methods.
- Experience with analytical techniques HPLC, GC, Spectroscopy, KF, ICP, NIR

- CDS documentation user and involved in establishing CDS, DMS and SAP user.
- Data integrity implementation
- Preparing answers for letters of deficiency from regulatory agencies (according to latest pharmaceutical regulatory, quality and safety requirements)
- Working 12 years in GMP, GLP and ISO standards environment and have a good understanding of regulatory requirements and how they relate to existing and new business systems and practices, primarily GxP globally and locally.
- Experience with international regulatory GMP inspections (MHRA, JZAMP, Russian regulatory agency) and familiar with current EU regulations (ICH, EMEA, EDQM).

Employer:	Alkaloid AD, Skopje, Macedonia
Dates:	**June 2008 – May 2009 (**Galafarm – Quality Control Department)
Position held:	**Analyst at practice**
Activities and responsibilities:	▪ Getting familiar with laboratory work, analytical methods, preparing documentation for regulatory submission
Dates:	**June 2008 – May 2009 (**Galafarm – Quality Control Department)
Position held:	**Analyst at practice**

Languages

Mother tongue Macedonian

Other languages

	English	**German**	**Russian**	**Greek**
Understanding	Advanced	Intermediate	Intermediate	Beginner
Speaking	Advanced	Intermediate	Intermediate	Beginner
Writing	Advanced	Intermediate	Intermediate	Beginner