Act Farma Terap. 2021; 19(2):109-124

Immunotherapy: the role of monoclonal antibodies in COVID-19

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Resumen

COVID-19 es una gran amenaza sanitaria para la población mundial, aunque las vacunas ya están disponibles en forma de uso de emergencia. El riesgo para la vida está relacionado con la demora en alcanzar una amplia cobertura de vacunación y con la patogenicidad agresiva en los casos más graves de infección por SARS-CoV-2 que aún se están extendiendo por todo el mundo. Se ha demostrado que los anticuerpos monoclonales (mAb) son un enfoque farmacológico extremadamente atractivo para el tratamiento de pacientes que corren el riesgo de desarrollar consecuencias graves de COVID-19. Los resultados de los ensayos clínicos han mostrado una reducción del daño pulmonar, una disminución de la viremia y del riesgo de hospitalización, lo que llevó a agencias reguladoras como FDA, EMA y ANVISA a aprobar su uso de emergencia en casos específicos.

Palabras clave
COVID-19, anticuerpos monoclonales, SARS-COV-2.

Conflicto de intereses
Este artículo no presenta conflicto de interés.

Summary

COVID-19 is a great threat to the world health population, despite vaccines are already available in emergency use way. The risk to lives is connected to the delay in reaching an extensive vaccination coverage and to the aggressive pathogenicity for the most serious cases of the SARS-CoV-2 infection that are still growing overwide. Monoclonal antibodies (mAb) have been proved to be an extremely attractive pharmacological approach to the treatment for patients that are in the risk to develop severe consequences of COVID-19. The results of clinical trials have shown a reduction in pulmonary damage, a decrease in viremia and the risk of hospitalization, which led regulatory agencies such as FDA, EMA and ANVISA to approve its emergency use in specific cases.

Key words
COVID-19, monoclonal antibodies, SARS-COV-2.

Conflict of interests
This article does not present a conflict of interest.

PRESENTACIÓN

The high mortality linked to COVID-19 is associated with the immunological event called cytokine storm. The excess of cytokines and chemokines produced in the framework of lethal SARS-Cov-2 infection mainly involves antigen-presenting cells (APCs), especially macrophages, and T cells. Early in the pandemic, the possibility of successful use of mAbs in the treatment of COVID-19 was observed.

INTRODUCTION

In December 2019, the World Health Organization (WHO) was notified of an outbreak of pneumonia that arose in the city of Wuhan, in Hubei province, China, with an aetiology that was not identified. Bioinformatic studies showed that the aetiologic agent had typical characteristics of coronaviruses and researchers obtained the complete genome sequences of five patients infected with the virus (Zhou et al., 2020). It was found that the gene sequences shared 79.5% identity with the complete SARS-CoV sequence, which was, thus, the advent of the SARS-CoV-2 name for the new Wuhan beta-coronavirus (Zhou et al., 2020). Subsequently, the authors compared the complete genomic sequence of SARS-CoV-2 to that of other beta-coronaviruses from available genomes, and the results indicated a 96% proximity of SARS-CoV-2 with the bat SARS coronavirus strain of Bat-CoV RaTG13, suggesting that it evolved naturally from the bat beta-coronavirus. In addition, the natural emergence of SARS-CoV-2 variants in South Africa, United Kingdom and elsewhere that have improved capability, viral interaction with the cell receptor, and transmissibility have supported the hypothesis that the receptor-binding domain (RBD) of the virus at the beginning of the outbreak was suboptimal for interaction with ACE2 (angiotensin-converting enzyme 2), and therefore, the virus has been rapidly adapting to humans while increasing its virulence (Plante et al., 2021; Galloway et al., 2021).

Since the beginning, we faced a devastating scenario: high death rate, the inefficiency of known therapies, and reinfections generated by the incapacity of the affected individuals to produce memory antibodies. The difficulties of the moment led the topic of drug repositioning to gain notoriety. In the first months of the outbreak, the website ClinicalTrials.gov had approximately 24 studies involving more than 20 drugs, such as human immunoglobulin, interferons, arbidol, remdesivir, favipiravir, lopinavir, ritonavir, oseltamivir, methylprednisolone, and bevacizumab, among others (Rosa and Santos 2020). As for other lethal diseases, such as SARS-CoV-1, MERS-CoV, and Ebola, passive immunotherapy showed suitable results, and prior high titer convalescent plasma was used for the treatment of COVID-19, even before being recommended by the Food and Drug Administration (FDA). Nevertheless, the results from the randomised study by Libster et al. (2021) demonstrated the benefits of administering convalescent plasma to elderly patients, although they were limited to the early stages of the disease and non-severe patients. Hence, passive immunotherapy employing monoclonal antibodies (mAbs) might replace convalescent plasma, besides being more specific, precise, and safe.

Early in the pandemic, the possibility of successful use of mAbs in the treatment of COVID-19 was observed. Some were isolated from the blood of individuals infected with SARS-CoV-2 (Ju et al. 2020) and other anti-SARS-CoV-1 due to the structural proximity of the spike proteins of both (77.50%) (Wang et al. 2020). Cytokine storm, an immunological event observed in complicated COVID-19, also proves to be an extremely attractive target for mAbs (Fajgenbaum and June 2020).

To better comprehend the targets of mAbs, we will conduct a discussion about the virus and its mechanism of infection and pathogenesis.

SARS-CoV-2: EMERGENCE AND ELUCIDATION OF THE VIRAL STRUCTURE

SARS-CoV-2 has four main structural proteins: protein S (spike), protein M (membrane), protein N (nucleocapsid) and protein E (envelope). Proteins S, M and E are incorporated into the viral envelope and only protein N interacts with the viral RNA, forming the nucleocapsid inside the viral particle (Figure 1). The spike protein, when glycosylated, forms homotrimeric structures on the viral surface and mediates the interaction of the particle with the receptor present on the host cell membrane through the RBD S1. Proteins M

FIGURE 1

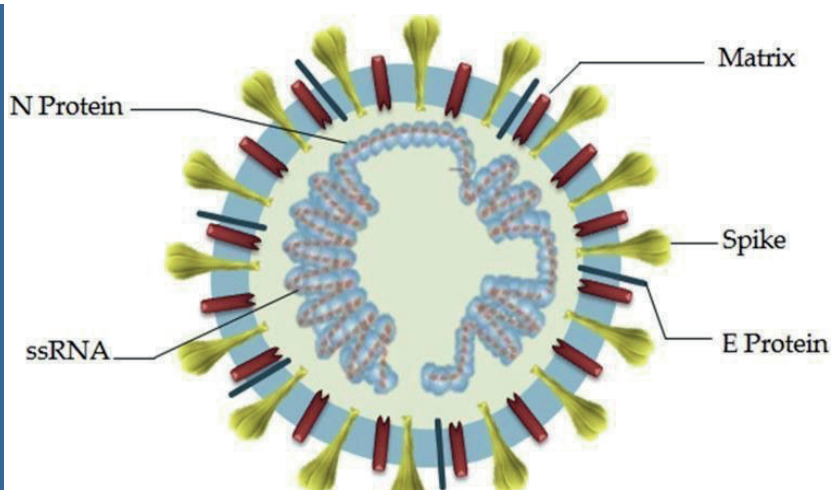

Figure 1. Schematic of the structure of the SARS-CoV-2 virus(10). Extracted from Astuti and Ysrafil (2020)[10]

and E are critically important in coordinating the assembly of the virus and mature envelopes. Protein N binds to viral RNA and is involved in the transcription and replication of genetic material and the packaging of the genome that makes up the virion.

SARS-CoV-2: PATHOGENIC MECHANISM

Wrapp et al., (2020) performed a study in which they compared the binding affinity of SARS-CoV-2 and SARS-CoV-1 to ACE2 and found that the RBD of the spike protein of SARS-CoV-2 is approximately 10–20 times more avid to interact with the host receptor than the spike protein of SARS-CoV-1, which may be relat-

FIGURE 1

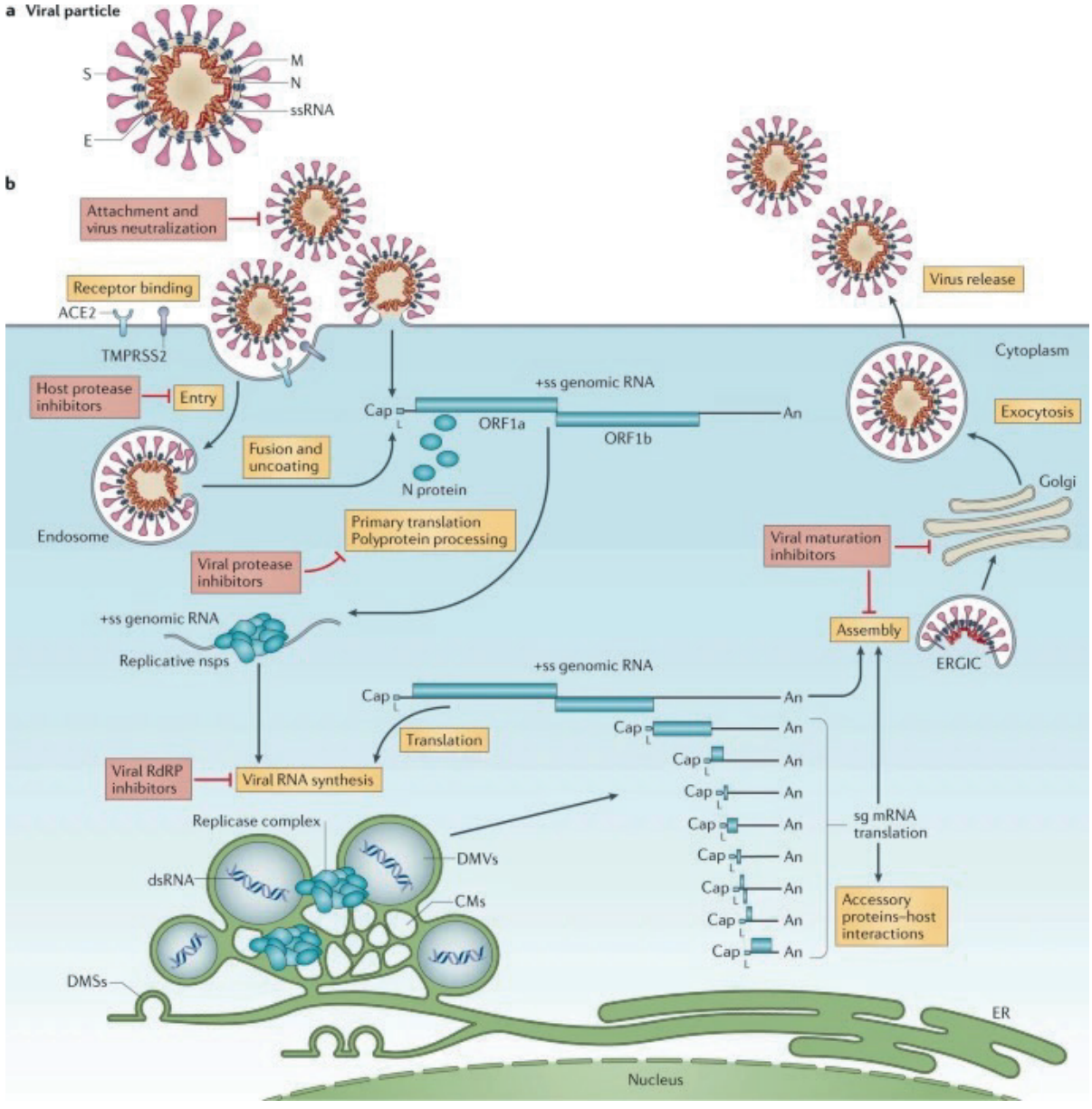

Figure 2. SARS-CoV-2 Cycle[13]

a. SARS-CoV-2 consists of structural proteins, namely spike (S), envelope (E), membrane (M), nucleocapsid (N). The positive-sense single-stranded RNA genome (+ssRNA) is encapsidated by N, while M and E ensure its incorporation into the viral particle during the assembly process. S trimers project from the host-derived viral envelope and provide specificity for host cell receptors.

b. Coronavirus particles bind to cell attachment factors and specific S interactions with the cell receptor (such as angiotensin-2 converting enzyme (ACE2)), together with host factors (such as cell surface serine protease TMPRSS2), promote viral uptake and fusion with the cell or endosomal membrane. After entry, release and uncoating of viral RNA, it is subjected to immediate translation of two large open reading frames, ORF1a and ORF1b. The resulting pp1a and pp1ab polyproteins are co-translated and post-translated into individual non-structural proteins (nsps) that form the viral replication and transcription complex. According to nsps (non-structural proteins) expression, the biogenesis of viral replication organelles consisting of characteristic perinuclear double-membrane vesicles (DMVs), convoluted membranes (CMs) and small open double-membrane beads (DMSs) create a protective microenvironment for the replication of viral genomic RNA and transcription of subgenomic mRNAs (sg mRNAs) comprising the characteristic nested set of coronavirus mRNAs. The translated structural proteins translocate to the membranes of the endoplasmic reticulum (ER) and transit through the ER-Golgi intermediate compartment (ERGIC), where interaction with the newly produced N-encapsulated genomic RNA results in budding in the lumen of the vesicular secretory compartments. Finally, virions are secreted from the infected cell by exocytosis. Key steps inhibited by compounds being validated that represent attractive antiviral targets are highlighted in red.

Extracted from V'Kovski et al. (2021)[13]

ed to greater infectivity and dissemination of SARS-CoV-2. After binding to ACE2, viral RNA is released into the cytoplasm and begins to use the cellular machinery to its own benefit. Once sufficient amounts of structural and genome proteins are reached, virions are formed that will be stored in vesicles and subsequently released by membrane fusion. Both viral assembly and its subsequent release are caused by a series of interactions that occur in the intermediate compartment of the endoplasmic reticulum and Golgi complex (ERGIC) (Sevajol et al., 2014) (Figure 2).

It is important to note that patients infected with SARS-CoV-2 have higher plasma levels of cytokines, such as interleukin 2 (IL-2), interleukin-7 (IL-7), and tumour necrosis factor alpha (TNF-α). In addition, in individuals who are in critical conditions, the reduction of T lymphocytes and the number of CD4+ and CD8+ T cells in the peripheral blood, which remain hyperactivated, is marked characteristic. All these observations lead to a belief that SARS-CoV-2 infection overactivates the host's immune system by interacting with toll-like receptors (TLR) present on the surface of dendritic cells and macrophages (antigen-presenting), thus resulting in the event of a storm of cytokines, which tends to potentiate the infection of host cells and aggravate the clinical picture of those affected by the virus (Zhu et al., 2020). Inhibition of the mTOR (mammalian target of rapamycin) pathway by the immunosuppressant rapamycin may be useful in controlling the overproduction of macrophages at the lung level and of circulating inflammatory cytokines (Teixeira and Santos 2020).

SPIKE PROTEIN: MAIN VIRAL TARGET OF MONOCLONAL ANTIBODIES

As previously mentioned, the spike protein is responsible for mediating the interaction of the virus with the cell receptor. The spike protein is classified as a transmembrane glycoprotein capable of forming homotrimers that project from the viral surface to interact with ACE2. Because it is critical for cellular infection, glycoprotein S is an extremely attractive target for potential drugs. Structurally, protein S is composed of two functional subunits: S1 and S2. The S1 subunit consists of the N-terminal domain (NTD) and RBD; its specific function is to bind to the receptor present in the host cell. The S2 subunit has a series of components responsible for the fusion of viral and host cell membranes, namely: fusion peptide (FP), heptad 1 repeat region (HR1), central helix (CH), connection domain (CD), heptad 2 repeat region (HR2),

FIGURE 3

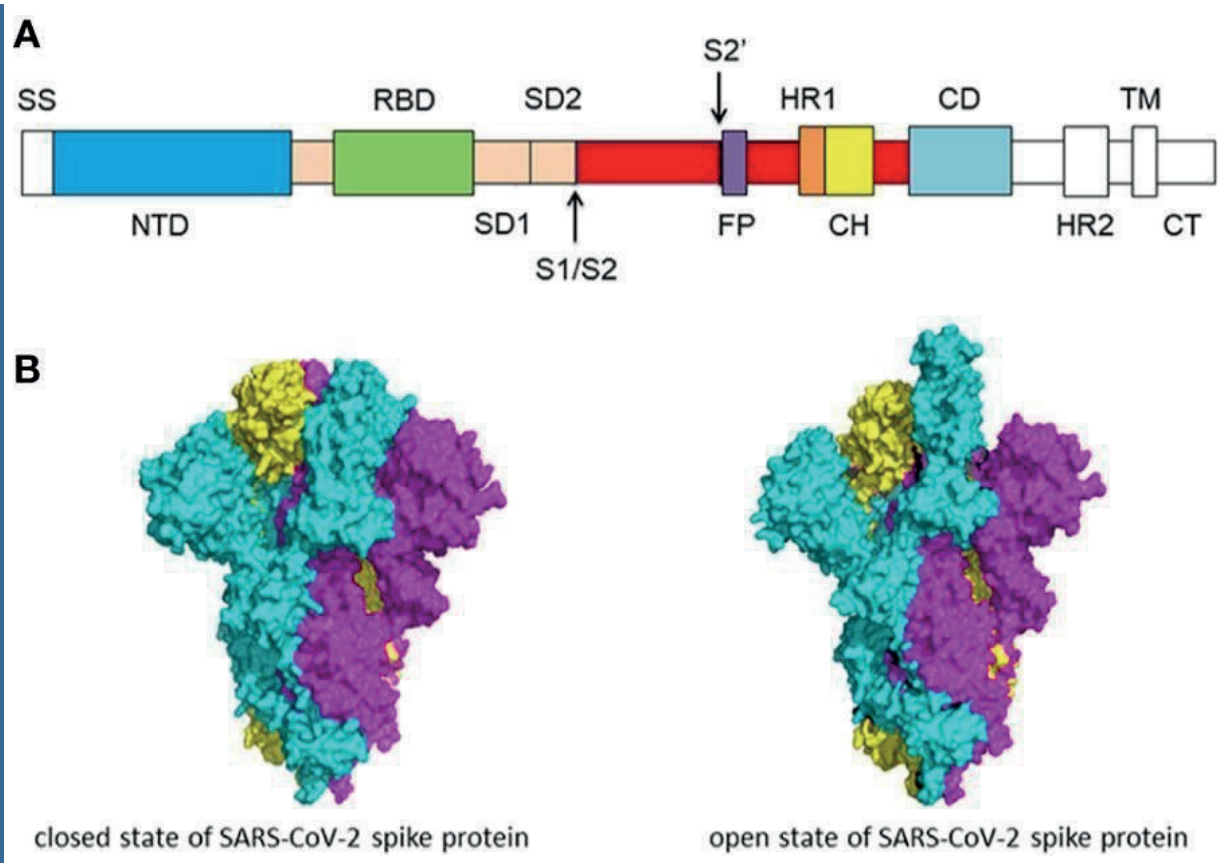

Figure 3. Spike protein[16]

a. Schematic of the primary structure of the SARS-CoV-2 peak protein. Different domains are shown in different colours. SS, single string; NTD, N-terminal domain; RBD, receptor-binding domain; SD1, subdomain 1; SD2, subdomain 2; S1/S2, S1/S2 protease cleavage site; S2', S2' protease cleavage site; FP, fusion peptide; HR1, heptad 1 repeat region; CH, central helix; CD, connector domain; HR2, heptad 2 repeat region; TM, transmembrane domain; CT, cytoplasmic tail. The protease cleavage site is indicated by arrows.

b. Cryo-EM structure of the SARS-CoV-2 Spike protein. The closed state (PDB: 6VXX) of the SARS-CoV-2 S glycoprotein on the left and the open state (PDB: 6VYB) of the SARS-CoV-2 S glycoprotein on the right.

Extracted from Wang et al.(2020)[16]

transmembrane domain (TM) and cytoplasmic tail (CT). The point at which the cleavage between the S1 and S2 subunits occurs is called the S1/S2 cleavage point. It is common to all coronaviruses that host proteases cleave the spike protein to activate domains that are critical for viral membrane fusion with the cell membrane through irreversible conformational changes. Protein S occurs in two forms, in the open and closed states. When present in the closed state, the three recognition motifs do not project to the protein interface. In the open state, the RBD presents itself in "upward" conformation, which is necessary for the fusion of the virus with the host cell, thus allowing the entry of viral material into the intracellular environment (Walls et al., 2020).

CYTOKINE STORM

The high mortality linked to COVID-19 is associated with the immunological event called cytokine storm, defined by Cron and Behrens (2019) as a self-amplifying cascade of activation of cytokine production due to the host's deregulated immune response to different antigens. Tisoncik et al. (2012) described the cytokine storm as a systemic inflammatory response associated with a wide variety of infectious and non-infectious diseases that have been the consequence of ineffective therapeutic intervention attempts. Indeed, they have pointed out the main inflammatory cytokines involved in the cytokine storm process (Table 1):

Interferons

Interferons (IFNs) represent a family of cytokines that play a central role in the innate immunity to viral infections and microbial pathogens. They are classified into three main types (I, II and III) according to their specificity, regarding their receptors. The interaction with the receptor initiates signalling cascades that result in the activation of transcription factors and the induction of genes stimulated by IFNs, that is, genes that encode proteins with antiviral, antiproliferative or immunomodulatory activities. For all these properties, often in combination with other drugs, IFNs are used in the treatment of viral diseases (such as hepatitis C and B), in certain types of leukaemia and lymphomas, and also in the treatment of multiple sclerosis (Borden et al., 2007).

Interleukins

Interleukins (ILs) are a family of cytokines that regulate the immune system that acts mainly in the differentiation and activation of immune cells. They can be classified as pro- or anti-inflammatory and induce a wide variety of responses. The acute phase of the response to infections is the result of a wide range of local effects and systemic changes that contribute to generally pro-inflammatory changes, such as the increased production of specific cytokines. For instance, they might be linked to viral clearance or increased system activation complement. IL-1α and IL-1β are pro-inflammatory cytokines that mediate the host's response to infection through direct and indirect mechanisms. Among their biological functions, these cytokines increase acute phase signalling, immune cell trafficking to the primary infection site, epithelial cell activation, and secondary cytokine production (Brocker et al., 2010).

Chemokines

Representing the largest family of cytokines, chemokines are small proteins that are classified into four types (CXC, CC, C and CX3C) according to the positioning of their first and second cysteine residues. Chemokines are chemoattractant compounds that control the migration of cells, especially those belonging to the immune system. They contribute to several processes, such as embryogenesis, development and functioning of the innate and adaptive immune system and cancer metastasis. Most chemokines are considered pro-inflammatory and are released by cell varieties responsive to viral infections, their release resulting in the highly selective recruitment of immune system cells - neutrophils, monocytes/macrophages and lymphocytes - to the site of infection (Comerford and McColl, 2011).

Colony Stimulating Factors

Colony stimulating factors (CSFs) are divided into three types: granulocyte-macrophage colony stimulating factor (GM-CSF), macrophage colony stimulating factor (M-CSF) and granulocyte colony stimulating factor (G-CSF). CSFs are responsible for stimulating the proliferation and differentiation of haematopoietic progenitor cells. They are also associated with inflammation due to evidence that these factors may increase the number of cytokine-producing macrophages at the site of inflammation, possibly amplifying the signalling cascades that perpetuate inflammatory reactions (Hamilton, 2008).

Tumour Necrosis Factor

Considered by many to be the best known and most studied among pro-inflammatory cytokines, TNF is considered a central molecule in acute viral diseases, such as those caused by the dengue, Ebola and influenza viruses (Aggarwal 2003). It also plays a prominent role in cytokine storms (Aggarwal 2003). TNF is expressed by a variety of immune system cells and its primary receptor, TNFR1, seems to be expressed by

Table 1. Main types and actions of cytokines[18]

Type	Actions
Interferons	Regulation of innate immunity, activation of antiviral properties, antiproliferative effects
Interleukins	Leukocyte growth and differentiation; many are pro-inflammatory
Chemokines	Chemotaxis control, leukocyte recruitment; many are pro-inflammatory
Colony Stimulating Factors	Stimulation of proliferation and differentiation of hematopoietic progenitor cells
Tumour Necrosis Factor	Pro-inflammatory activates cytotoxic T lymphocytes

Extracted from Tisoncik et al.(2012)[18]

all types of cells, ensuring widespread effects on its actions. Excessive TNF synthesis is associated with many chronic inflammatory and autoimmune diseases (Aggarwal 2003); therefore, TNF inhibitors have been approved for the treatment of diseases such as psoriasis and rheumatoid arthritis (Kopf et al., 2010). However, the use of TNF inhibitors does not affect the treatment of sepsis, possibly due to the early release and short half-life of the cytokine (Clark 2007).

While the general concept of excessive and/or uncontrolled release of pro-inflammatory cytokines is well known, a real definition of what constitutes a cytokine storm is missing. In addition, there is no complete understanding of the molecular events that trigger the cytokine storm, or the actual contribution to the magnification of the pathogenesis of COVID-19 or other diseases, nor has a possible therapeutic strategy that might be used to prevent or overcome it once it has already started been established.

COVID-19 patients admitted to the intensive care unit (ICU), when compared to patients in less severe condition (who do not require ICU), have high counts of leukocytes and neutrophils, high levels of procalcitonin, C-reactive protein and other inflammatory indices (Tang et al., 2020). Huang et al. (2020) demonstrated that critically ill patients had higher concentrations of pro-inflammatory interleukins, especially interleukin 6 (IL-6), than patients with moderate COVID-19. Bronchoalveolar lavage fluid (BALF) tested by transcriptome sequencing revealed an excessive release of chemokines due to SARS-CoV-2 infection. Molecules, such as CXCL10 and CCL2, can activate apoptosis and the P53 signalling pathway in lymphocytes (Xiong et al., 2020). Huang et al. (2020) examined several types of cytokines and found that the most severe patients had higher levels of G-CSF, GM-CSF, IP-10, MCP-1, MIP-1a, MIP-1b, RANTES and IL-8. Pathological analysis of the post-mortem examination of lungs of fatal victims of the disease demonstrated the existence of the acute respiratory distress syndrome (ARDS) and T cell hyperactivation, a phenomenon that is due to the increase of the number of helper T cells (Th) and high toxicity of CD8+ T cells (Xu et al., 2020). The innate and adaptive responses activated by the infection caused by SARS-CoV-2 can lead to uncontrolled immune responses that can culminate in the cytokine storm. This can lead to apoptosis of the epithelial and endothelial cells, in addition to vascular leakage, thus resulting in ARDS and other equally serious syndromes that can lead to the death of the affected patients. Therefore, a high level of inflammatory cytokines indicates a poor prognosis associated with COVID-19 disease (Cao 2020).

The excess of cytokines and chemokines produced in the framework of lethal coronavirus infection mainly involves antigen-presenting cells (APCs), especially macrophages, and T cells. SARS-CoV-2, especially in critically ill patients, causes decreased lymphocyte count and hyperactivation and increased C-reactive protein (CRP) and TNF-α. This leads us to a dichotomy regarding the formation of critical thinking: cytokines secreted by the immune system aim to eliminate the viral infection and their deficiency can lead to physiological damage; however, their excessive secretion can lead to severe conditions that can progress to death. Therefore, identifying the threshold at which the lack of control occurs and modulating the action of these substances would represent a pronounced advance. The physiopathological characteristics of COVID-19 include lungs infiltrated with excess CCR6+ Th17 cells and high cytotoxicity of CD8+ T cells, which does not mean that these cells are exercising their normal function. The distinctiveness of the cytokine storm caused by COVID-19 is the increased secretion of cytokines related to T helper 2 cells (Th2), namely IL-4 and IL-10, which are known to suppress inflammation (by inhibiting the synthesis of other cytokines and Th1-mediated response). The virus can also lead to exhaustion of cytotoxic lymphocytes, especially those concerning NK

and CD8+ T cells, which manifests as positively regulated exhaustion markers (e.g., NKG2) that return to normal rates in recovered or convalescent patients (Tang et al., 2020). The aforementioned results corroborate the development of lymphopenia (absolute lymphocyte count [ALC] < 1.0 x 10^9/L, which can be explained by an inadequate immune response to the virus) in critically ill patients. A study carried out of the peripheral blood of affected individuals revealed that non-structural proteins (nsp 9 and nsp10) of SARS-CoV-2 direct the NF-κB (NKRF) factor to the production of IL-6 and IL-8, which leads to an increase in neutrophil recruitment and induction of an uncontrollable inflammatory response as the disease worsens (Li et al. 2021). Figure 4 illustrates a summary diagram addressing the mechanism of pathogenesis and immune response to SARS-CoV-2.

Cytokine storms in severe pulmonary infections are characterised by local inflammation that spreads to the systemic circulation, potentially producing systemic sepsis, characterised by persistent hypotension, hyper- or hypothermia, leukocytosis or leukopenia, and often, thrombocytopenia. Another abnormality observed in SARS-CoV-2 infections is related to blood clotting in some patients, which led to the emergence of the term COVID-19-associated coagulopathy (CAC), in which elevated circulating levels of prothrombin, fibrinogen and D-dimer, in addition to elevated pro-inflammatory markers such as CRP and IL-6, are used as CAC markers. This might explain, for instance, the high morbidity rate in patients with diabetes for the high risk of emergence of inflammatory events (Johnson et al., 2021).

FIGURE 4

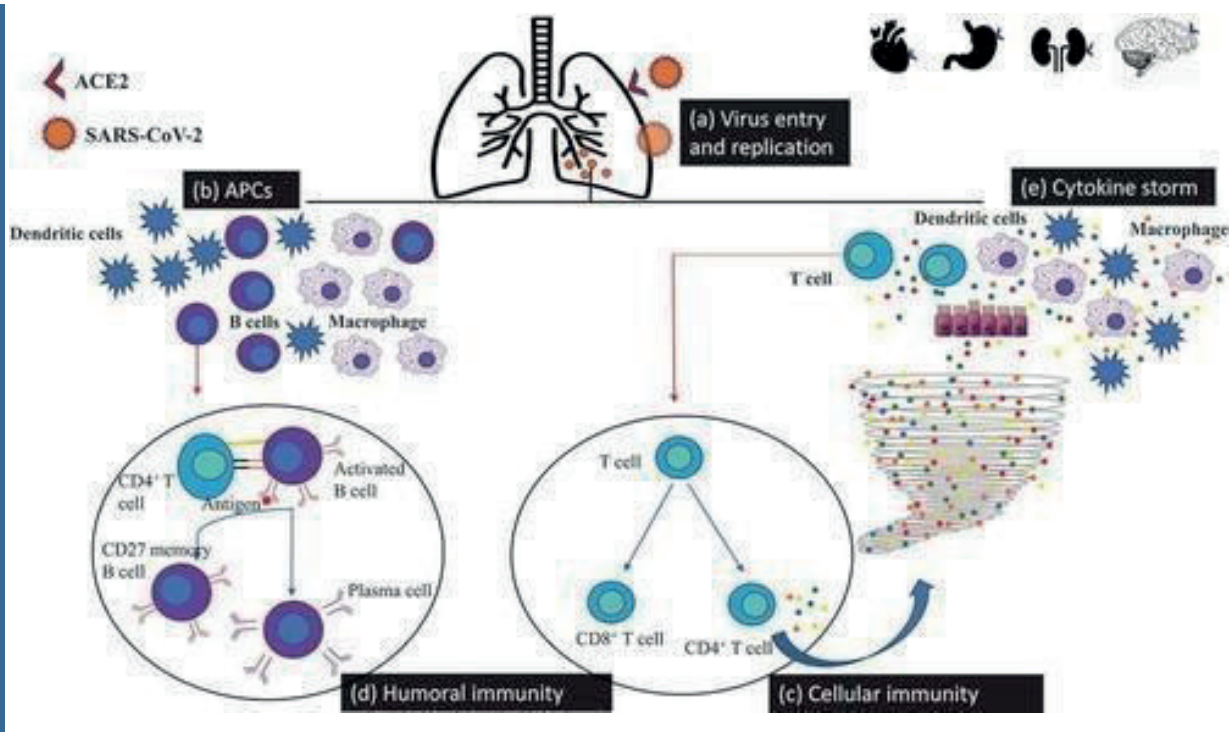

Figure 4. Simplified representation of the pathogenic phases of COVID-19 [31]

a. SARS-CoV-2 can cross the mucosa of the nasal epithelium and lungs, binding to the ACE2 receptor and replicating soon after entry.
b. c. Virus entry and replication attract antigen-presenting cells, such as macrophages, B lymphocytes, and dendritic cells, which process and present viral antigens to T cells to trigger cellular immunity. Phagocytes, antigen-specific cytotoxic T cells (CD8 +) and helper T cells (CD4 +) interact to produce a flow of cytokines (cytokine storm).
d. Helper T cells (CD4+) and naive B cells interact and process specific antigens of SARS-CoV-2 to produce specific content for the specific (adaptive) response.
e. Simultaneously, large-scale replication of SARS-CoV-2 in the lungs leads to infiltration of immune cells, causing an increase in the level of cytokines in the area of infection. This pathologically manifests itself through vasodilation and increased capillary permeability, causing a phenomenon called cytokine storm. Vital organs, such as the heart, kidney and brain, also express ACE2 receptors at appropriate levels, therefore, they may change with the manifestation of the disease in patients infected with SARS-CoV-2.
Extracted from Johnson et al. (2021)[31]

MONOCLONAL ANTIBODIES: CURRENT PERSPECTIVES

After a simple search on the website of the National Institutes of Health (NIH)[32] with the filters "COVID-19" and "monoclonal antibody", we obtained a result of 100 clinical studies involving mAbs for the treatment of COVID-19 in progress in the world, with some of these mAbs with approval for emergency use by some regulatory agencies. Although there are some vaccines approved for emergency use, it is known that efforts to define effective and affordable antiviral drugs, as well as support therapies for complications of COVID-19, should not be ruled out, given the delay in production, availability and application of vaccines on the world stage. In addition, we must consider that vaccination is a collective measure that, if it does not reach the entire population, it loses its effectiveness. Another important aspect is that cases of infections may arise even in immunised people and we must have effective therapies for these cases.

The previously driven topics had as objectives the habituation with potential therapeutic targets for mAbs, from the viral structure aiming at the spike protein, passing through the infection mechanism to also address the cytokine storm, which is responsible for worsening the condition of many affected.

Figure 5 shows potential therapeutic targets for possible new mAbs aimed at the treatment of COVID-19, showing the so-called antiviral targets (related to viral aspects of the infection) and anti-inflammatory targets (related to the aspects of the host's response to the infection). Humanised mAbs are highly specific to the proposed epitope. They have clinically favourable pharmacokinetic properties and are, therefore, promising therapeutic tools to deal with pathological and clinical effects, mainly associated with cytokine storms (Johnson et al., 2021). Other important aspects, as addressed by Johnson et al. (2021), is that most patients were able to obtain marked improvements in respiratory function, rapid decrease in fever and successful discharge from a single dose; however, cytokine-oriented mAbs are associated with adverse events that can affect the results of clinical trials mainly in COVID-19 patients in critical condition, especially those with comorbidities (Figure 5).

Below are listed some mAbs that have clinical trials in progress and those that have already been approved for emergency use or are under analysis by the North American (Food and Drug Administration [FDA]), European (European Medicines Agency [EMA]), and Brazilian (Agência Nacional de Vigilância Sanitária [ANVISA]) regulatory agencies.

FIGURE 5

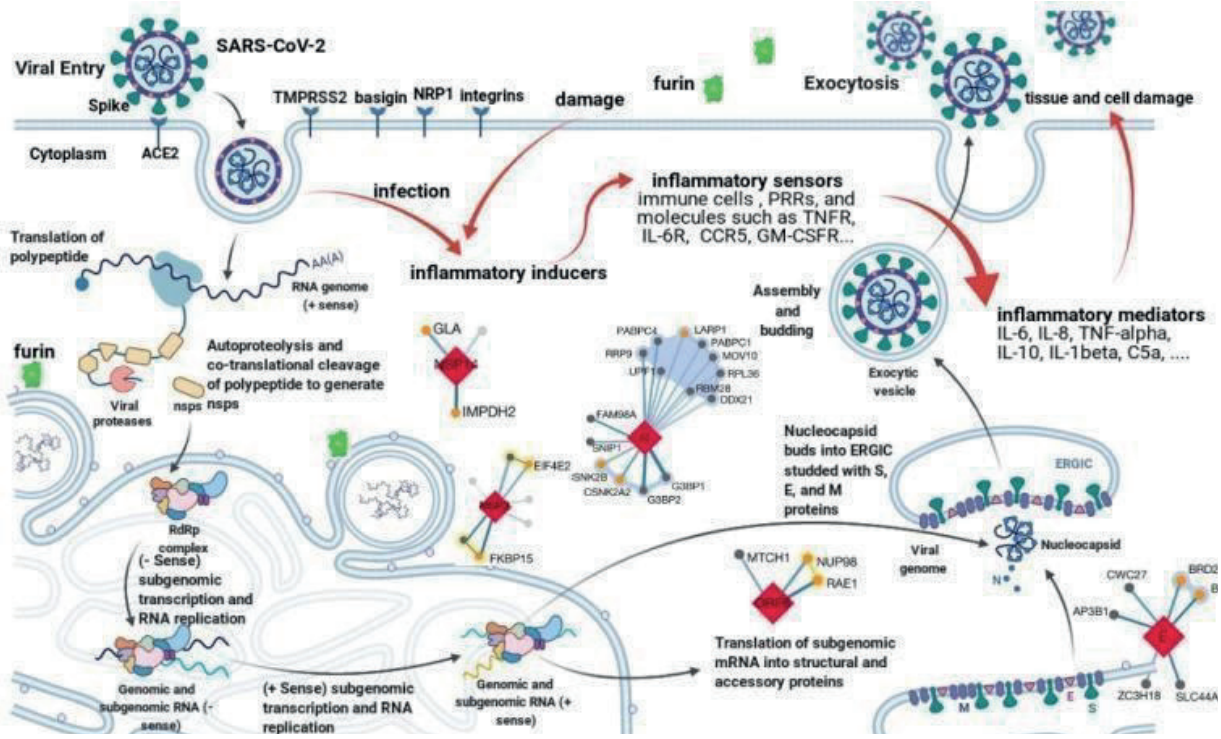

Figure 5. Potential targets for monoclonal antibodies against COVID-19[33]
Extracted from Ning et al.[33]

Tocilizumab

Tocilizumab (Actemra®) is a recombinant, humanised monoclonal antibody of the IgG1 subtype, which targets the interleukin 6 receptor (IL-6R) and, therefore, has been proposed as a therapeutic possibility in severe cases of COVID-19. This mAb specifically binds to the IL-6R and acts as a competitive inhibitor of IL-6-mediated signalling. Tocilizumab is licensed in the US and UK with some limitations for the treatment of rheumatoid arthritis, active systemic juvenile idiopathic arthritis, among other chronic inflammatory diseases. This monoclonal antibody presents as the cause of the most common adverse events: infections of the upper respiratory tract and hypercholesterolemia (Ruiz et al. 2021).

From a search of the NIH database using the terms "COVID-19" and "Tocilizumab", 85 clinical trials, ranging from tocilizumab alone evaluated against placebo to the combined use of the mAb with other therapeutic classes or other mAbs, were obtained in response. "A Study to Evaluate the Efficacy and Safety of Tocilizumab in Hospitalized Participants With COVID-19 Pneumonia (Evaluating Minority Patients with Actemra®) [EMPACTA])" is one of the most visible clinical trials on the use of tocilizumab in the treatment of COVID-19, featuring the identifier code NCT04372186 in ClinicalTrials.gov website and funded by Genentech, Inc. EMPACTA was registered on the NIH platform on 01 May 2020, and has an estimated completion date of 01 December 2021. This trial, currently in phase 3[35], is defined as a randomised, double-blind, placebo-controlled and multicenter study to assess the efficacy and safety of tocilizumab in hospitalised patients with pneumonia caused by COVID-19. Salama et al. (2021) published an update of the EMPACTA trial that drew attention to the information that in patients hospitalised with COVID-19 pneumonia who were not receiving mechanical ventilation, tocilizumab reduced the likelihood of progression to mechanical ventilation or death outcomes but did not improve survival.

Although its use has been explored in several clinical trials and it targets one of the main cytokines involved in aggravation of the disease (IL-6), tocilizumab has not yet shown satisfactory results that would justify authorisation for use by any of the regulatory agencies considered in this study (Johnson et al. al., 2021).

Infliximab

Infliximab (Remicade®) is a chimeric monoclonal antibody that targets TNF-α, with at least seven known biosimilars[37]. This mAb binds with high affinity to both the soluble and transmembrane forms of TNF-α, but not to lymphotoxin (TNF-β) (Melsheimer et al., 2019). Infliximab has a clear and accurate indication for autoimmune diseases, such as Crohn's disease, rheumatoid arthritis, ulcerative colitis and psoriatic arthritis. Its use is associated with serious adverse events in patients who have recovered from tuberculosis or with the latent form of the disease, those who live in endemic regions of histoplasmosis and coccidioidomycosis, among other fungal diseases, and those with diabetes mellitus or diseases of the immune system (Johnson et al. al., 2021).

The NIH has five ongoing clinical trials that include infliximab, in regard to the treatment of COVID-19 and its complications (Johnson et al. 2021). Although infliximab has an extremely attractive target, especially in light of the immunological complications reported in severe COVID-19, adverse events ranging from recurrent infections to heart failure, hepatitis B infection and nervous system disorders (e.g., multiple sclerosis) have been reported after treatment. These events may be associated with potentially fatal adverse effects; thus, infliximab is contraindicated in combination with other TNF-α-based immunotherapies, such as anakira, abatacept and tocilizumab (Johnson et al., 2021). All of these limitations affect the use of infliximab as a therapeutic option for critically ill patients with pneumonia, thus reducing the widespread adoption of this mAb as standard care in individuals affected by COVID-19. It is noteworthy that infliximab has not received any authorisation from the FDA, EMA or ANVISA for use in any stage of COVID-19 disease.

Adalimumab

Adalimumab (Humira®), originally developed for the treatment of rheumatoid arthritis and other inflammatory diseases, has at least six biosimilars[39]. Adalimumab is the first fully human recombinant IgG1 monoclonal antibody that binds and neutralises soluble and membrane-bound TNF-α to block its interaction with p55 and p75 receptors. It also induces apoptosis in mononuclear cells with TNF-α receptors (Mease 2007). Regarding adverse effects, it appears to be less critical than infliximab. However, there are still reservations about the benefits of adalimumab regarding the treatment of COVID-19 that are linked to its adverse effects, especially when infected patients have comorbidities, such as diabetes mellitus and cardiovascular and liver diseases. However, a quick review of the safety database of adalimumab suggests that dose improvement and other precautions can circumvent the potential adverse events associated with the drug. Some adverse events of adalimumab that have been reported include a rare type of lymphoma of the liver, spleen and bone marrow, as well as heart failure. In addition, this mAb is potentially associated with hypersensitivity reactions, reactivation of hepatitis B virus and adverse neurological and haematological reactions. Another important aspect linked to the metabolism of adalimumab is that it can act as an inducer of cytochrome P450, so it

is likely that the clinical use of this mAb can potentiate the metabolism and adverse effects of other co-administered drugs whose metabolism is dependent on cytochrome P450 (Johnson et al. al., 2021).

According to the NIH database, only one clinical trial is currently in progress evaluating the use of adalimumab in the treatment of COVID-19, "Study of Adalimumab or Placebo in Patients with Mild to Moderate COVID-19 (COMBAAT)"[41], which is sponsored by Ology Bioservices and identified on ClinicalTrials.gov by the code NCT04705844. It was posted on 12 January 2021, last updated on 24 March 2021, and it is estimated to be completed in January 2022. It is a multicentre, randomised, double-blind, placebo-controlled, interventional study in patients with low to moderate COVID-19, and this study is in phase 3 of testing. No results have been published on ClinicalTrials.gov database to date and adalimumab does not have FDA, EMA, and ANVISA approval for use in the treatment of COVID-19.

Bamlavinimab and etesevimab

Produced by the pharmaceutical company Eli Lilly, bamlanivimab and etesevimab are mAbs that bind to different, but overlapping, sites located in the RBD of the spike protein of SARS-CoV-2, blocking its binding to the human ACE2 receptor. Both are investigational drugs that are not currently approved for any indications. The effectiveness of this combination in reducing the rates of hospitalisation and deaths related to COVID-19 by up to 70% has led agencies such as the FDA, ANVISA and EMA to position themselves in favour of its use in specific cases of the disease (Bamlanivimab and Etesevimab EUA094 Letter of Authorization)[42].

On 9 February 2021, the FDA approved emergency use authorisation (EUA094) for intravenous (IV) bamlanivimab 700 mg and IV etesevimab 1400 mg given together for the treatment of mild to moderate COVID-19 in adult and paediatric patients (> 12 years of age, weighing at least 40 kg) with positive direct viral test results for SARS-CoV-2 and who are at high risk of progression to severe COVID-19 and/or hospitalisation. On 25 February 2021, after concluding that the EUA094 revision would be appropriate for the protection of public health and safety, the FDA began re-editing the approval letter in its entirety, incorporating instructional conditions and educational materials. New conditions were also incorporated in the establishment of a process to monitor the effectiveness of bamlanivimab and etesevimab in genomic databases, regarding the emergence of new variants of SARS-CoV-2 and their therapeutic employability (Bamlanivimab and Etesevimab EUA094 Letter of Authorization)[42]. The release for use of this combination was based on the data of the BLAZE-1 trial [44] in phase 2, also in progress; both are classified as randomised, double-blind, placebo-controlled clinical trials.

On 11 March 2021, the EMA began an ongoing review of the use of the antibodies bamlanivimab and etesevimab in combination for the treatment of COVID-19, as well as the use of bamlanivimab alone (EMEA/H/A-5 (3)/1502). The results considered for the evaluation belong to the ongoing study BLAZE-1[43], which evaluates the potential use of bamlanivimab (monotherapy) in a single administration of 700 mg IV and the combination of bamlanivimab and etesevimab in a single administration of 700 mg and 1400 mg IV, respectively, for the treatment of COVID-19. After considering the analysed data, the EMA established that monotherapy and the combination of mAbs can provide a therapeutic option for the treatment of confirmed COVID-19 in patients aged ≥ 12 years who weigh over 40 kg, do not use supplemental oxygen and are at high risk of progression to severe COVID-19. A continuous review is a regulatory tool that the EMA uses to accelerate the evaluation of promising drugs or vaccines during a public health emergency, in which all data on a drug's efficacy, safety and quality and all necessary documents must be presented at the start of the evaluation in a formal application for marketing authorisation. In the case of an ongoing review, EMA's human medicines committee (CHMP) analyses data as it becomes available from ongoing studies, and as soon as the CHMP decides that there is sufficient data available, the formal request must be submitted by the company, allowing the agency to reach a faster conclusion regarding the regulation of the drug (Salama et al. 2021).

On 13 May 2021, at the Eighth Extraordinary Public Meeting of the Collegiate Board, ANVISA approved the emergency use of the association of the antibodies bamlanivimab and etesevimab for the treatment of COVID-19. The Brazilian agency followed the same indication as the American agency (FDA), that the mAbs should be administered together; that is, bamlanivimab monotherapy was not approved[45].

Casirivimab and imdevimab (REGN-COV2)

Casirivimab (IgG1-kappa) and imdevimab (IgG1-lambda) are recombinant human mAbs that target the RBD of the spike protein of SARS-CoV-2. They bind to non-overlapping epitopes of the RBD; thus, blocking binding to the human ACE2 receptor. These mAbs were developed and produced by Regeneron Pharmaceuticals, Inc. and F. Hoffman-La Roche, Ltd (Roche), are under investigation, and are not currently approved for any indications. Clinical trials that are still in progress are determining the effectiveness of REGN-COV2 in the treatment of COVID-19, which led agencies such as the FDA, ANVISA and EMA to present favourable opinions on its use in specific cases of the disease[46].

On 21 November 2020, the FDA issued a statement (EUA091) authorising the emergency use of casirivimab and imdevimab, administered together for the treatment of low to moderate COVID-19 in adult and paediatric patients (minimum age of 12 years, weighing at least 40 kg) who tested positive on the direct viral test for SARS-CoV-2 and are at high risk of progressing to severe COVID-19 and/or hospitalisation. On 25 February 2021, after the completion of this EUA review, the FDA revised the letter of authorisation in its entirety, incorporating new conditions in the establishment of a gene bank monitoring process of the effectiveness of REGN-COV2 against new variants of SARS-CoV-2[46]. The decision of emergency authorisation was based on the results obtained in phases 1 and 2 of the study R10933-10987-COV-2067[47], a phase 1/2/3 randomised, double-blinded, placebo-controlled clinical trial that seeked to assess the safety and efficacy of casirivimab and imdevimab 2400 mg IV (1200 mg casirivimab + 1200 mg imdevimab) or 8000 mg IV (4000 mg casirivimab + 4000 mg imdevimab) versus placebo in non-hospitalised patients diagnosed with SARS-CoV-2 infection. Preliminary results showed that the antibody cocktail reduced viral load (by up to 50%) with greater effect in patients whose immune response had not yet been initiated or who had a high viral load at baseline (Weinreich et al., 2020).

On 1 February 2021, the EMA began the process of continuous review regarding the use of the casirivimab and imdevimab association in the treatment of COVID-19 in adult and paediatric patients (minimum age of 12 years, weighing at least 40 kg) who were not hospitalised who tested positive on the direct viral test for SARS-CoV-2, and who are at high risk of progressing to severe COVID-19 and/or hospitalisation. The conditions of use and its benefits are being attested in the clinical trial R10933-10987-COV-2067[47], still in progress. The recommendations for use follow the same rules presented by the FDA and were documented in a letter published on 25 February 2021, issuing a favourable scientific opinion aimed at the use of the antibody cocktail in the treatment of COVID-19 under specific conditions (the same clinical conditions presented by FDA)[48].

On 20 April 2021, the ANVISA approved the emergency use of the REGN-COV2 cocktail for the treatment of COVID-19 in adults and children (minimum age of 12 years, weighing at least 40 kg) who were not hospitalised that might progress to a serious condition and/or hospitalisation. The dose recommended by the agency is 1200 mg (600 mg of casirivimab + 600 mg of imdevimab)[49].

Sotrovimab

Sotrovimab is a recombinant human monoclonal antibody (IgG1κ) that binds to an epitope in the RBD ACE2 present on the spike protein of SARS-CoV-2 and does not compete for the receptor binding site. This mAb, produced by GlaxoSmithKline and Vir Biotechnology, Inc. (GSK), is an investigational drug that is not yet approved for any indications. After being proven to reduce the progression of COVID-19 (by up to 85%) in patients with mild to moderate disease, well-tolerated and proven safe, sotrovimab received a favourable opinion from the EMA and FDA agencies for the treatment of specific cases of COVID-19 (Gupta et al., 2021).

On 7 May 2021, the EMA began an ongoing review of the use of sotrovimab for the treatment of mild to moderate COVID-19 in adults and children (minimum age of 12 years, weighing at least 40 kg) not hospitalised under risk of progression to serious illness and/or hospitalisation. On 21 May 2021, CHMP issued a harmonised scientific opinion at the European Union level to support national decision-making on the use of the antibody[51]. The decision was based on a provisional analysis of the COMET-ICE study[52], classified as a phase 1/2/3, double-blind, placebo-controlled randomised clinical trial aimed at evaluating the safety and efficacy of sotrovimab (500 mg IV) in outpatients (non-hospitalised). Preliminary results have shown a reduction in the risk of hospitalisation for more than 24 hours or death in 85% of cases compared to placebo: hospitalisation for more than 24 hours or death occurred in 1% (3 of 291) of patients who received sotrovimab and 7% (21 of 292) of those who received placebo (Gupta et al., 2021).

On 26 May 2021, the FDA issued an emergency use authorisation for sotrovimab (EUA100) for the treatment of mild to moderate COVID-19 in adults and paediatric patients (≥ 12 years of age, weighing at least 40 kg) who test positive on the direct viral test for SARS-CoV-2, and who are at high risk of progression to severe COVID-19, including hospitalisation or death. The recommended dose is 500 mg of sotrovimab administered via the IV route[53].

Regdanvimab

Regdanvimab, a human recombinant monoclonal antibody (IgG1) developed and produced by Celltrion, acts in the treatment of SARS-CoV-2 infection by binding to the spike protein RBD, which inhibits the interaction between the spike protein (RBD) and cellular receptor (ACE2). Consequently, it acts in the cellular blocking of viral infection (Tuccori et al., 2020). This monoclonal antibody is an investigational drug and does not yet have a specific indication.

On 24 February 2021, the EMA started a continuous review aimed at the use of regdanvimab for the treatment of mild to moderate COVID-19 in adults not hospitalised, with the possibility of progression to serious condition and/or hospitalisation. This re-

view is based on the clinical trial CT-P59[55], classified as randomised in phases 2/3, double-blind, placebo-controlled, and aims to supplement the decision of EU countries to administer the standardised form of regdanvimab for the treatment of COVID-19. The dosage recommended by the EMA is 40 mg/kg by single IV infusion. It is important to emphasise that treatment must be started as soon as possible after diagnosis (use is not recommended after seven days of onset of symptoms;[56]).

CONCLUSION

This study aimed to demonstrate, contextualise and update data regarding the therapeutic applicability of mAbs in mild, moderate and complicated COVID-19-positive cases. Considering the panorama discussed in the present article, mAbs represent a very suitable and promising therapeutic class of medicines for the treatment of the clinical aspects of COVID-19, whether linked to the virus or its complications. It is notorious that the mAbs approved for emergency use target the spike protein, which is critical for cellular infection in the acute phase of the disease. Other mAbs, such as tocilizumab, which has a critical target for the cytokine storm (IL-6) have not yet achieved the same success due to their adverse effects and low impact on increasing the survival of those affected by COVID-19. We should emphasise the term "still" when referring to the therapeutic success of tocilizumab, as we have several studies (85 registered at the NIH) in progress, aiming to adjust its parameters to achieve therapeutic success.

The balance of advantages and disadvantages regarding the use of mAbs as a therapeutic strategy is illustrated at various moments in the present work. Advantages, such as specificity with regard to therapeutic target and long plasma half-life, balance very well the disadvantage of mAbs as an extremely expensive technology, as in almost all cases, a single dose can meet therapeutic expectations. Another advantage is linked to the legal field, as they usually have a shorter analysis time for approval. In general, mAbs act effectively in the control of fever and show marked improvements in SARS-CoV-2-positive individuals. However, adverse reactions represent the greatest disadvantage and possible hindrance to mAb application, since most of them might put individuals at risk. As mAb x therapeutic target interactions are extremely specific, monitoring therapeutic efficacy for new viral strains is of supreme importance. Johnson et al., (2021) summarised the advantages and disadvantages of therapeutic strategies based on mAbs, which are exhibited in Table 2.

Table 2. Advantages and disadvantages linked to the use of mAbs in the treatment of COVID-19[31]

Advantages	Disadvantages
Specificity in molecular interactions and involvement with therapeutic targets.	Possible resistant viral mutations can alter mAb affinities and efficacy.
Long plasma half-life, thus decreasing the need for repeated doses.	Adverse drug reactions are serious and often fatal.
Shorter timeframes for testing and approval compared to other small-molecule therapeutics.	Varying bioavailabilities can affect efficacy.
Marked improvements in respiratory function in COVID-19 patients.	Affordability and affordability across the global economic spectrum and in low-income countries are questionable.
Rapid defervescence.	

Extracted from Johnson et al. (2021)[31]

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