REVIEW

pharmadvances

Volume 4, issue 3, 2022: 197-226

PHARMACOLOGICAL APPROACHES TO SARS-CoV-2 INFECTION: FROM DRUG REPOSITIONING FOR COVID-19 TREATMENT TO DISEASE ARREST/ PREVENTION WITH MoAbs AND NOVEL ANTIVIRALS

A. Bergamo[1], A. Bitto[2], A. Grolla[3], G. Nocentini[4], S. Pierno[5], M. Pistis[6,7], G. Sava[8], G. Racagni[9]

[1] Department of Life Sciences, University of Trieste, Trieste, Italy
[2] Department of Clinical and Experimental Medicine, Pharmacology Unit, University of Messina, Messina, Italy
[3] Department of Pharmaceutical Sciences, Pharmacology Unit, University of Eastern Piedmont, Novara, Italy
[4] Department of Medicine and Surgery, Severi Square 1, University of Perugia, Perugia, Italy
[5] Department of Pharmacy and Drug Sciences, Pharmacology Unit, University of Bari, Bari, Italy
[6] Division of Neuroscience and Clinical Pharmacology, Department of Biomedical Sciences, Clinical Pharmacology Unit, University of Cagliari, Monserrato, Cagliari, Italy
[7] Neuroscience Institute, National Research Council of Italy (CNR), Section of Cagliari, Monserrato, Cagliari, Italy
[8] Director of SIF Magazine, Italian Society of Pharmacology, Milan, Italy
[9] President of SIF - Italian Society of Pharmacology, Milan, Italy

E-mail: g.sava@icloud.com. **ORCID:** 0000-0002-5138-6041

Doi: 10.36118/pharmadvances.2022.37

SUMMARY

COVID-19 disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is one of the major emergencies that have affected health care systems and society in recent decades. At the end of winter 2021-2022, the number of patients infected with SARS-CoV-2 and especially those suffering from severe COVID-19 is decreasing in Europe. This is due to the protective effect of anti-SARS-CoV-2 vaccines and the increasing number of people who had COVID-19, thus developing a certain immunity. However, vaccines to prevent the disease did not appear until more than one year after the emergence of SARS-CoV-2, so the initial medical approaches to control the disease focused on the existing drugs that were considered suitable for controlling the pathological events caused by the virus as far as was known at the time. Unfortunately, due in part to the limited initial knowledge of the molecular details of the pathology of COVID-19, many of the proposed drugs fell short of expectations and were abandoned. Over time, the challenge of understanding the mechanisms behind COVID-19 has generated a large body of knowledge about how this beta-coronavirus gains control of the host during infection, a knowledge that has been used to redefine treatment strategies by repurposing existing drugs and to explore new drugs. Here, we draw a picture of the major strategies and groups of drugs studied and provide a critical overview of their efficacy and safety based on the available literature data. The main topics covered are repurposed drugs, anticoagulants, anti-cytokine agents, monoclonal antibodies against SARS-CoV-2, and small antiviral molecules.

Key words
COVID-19; drug repositioning; anti-inflammatory and anticoagulants; antiviral MoAbs; antiviral small molecules.

Impact statement
The impact of the review is to collect together successes and failures in the use of drugs to treat COVID-19, the reasons for the repositioned drugs and the corresponding responses of the relevant clinical trials as well as the responses to new monoclonal antibodies and antiviral drugs.

INTRODUCTION

Severe Acute Respiratory Syndrome due to CoronaVirus-2 (SARS-CoV-2) appeared as a novel, highly dangerous, virus that caused the coronavirus disease-2019 (COVID-19) in humans at the end of 2019. First identified in Wuhan, China, SARS-CoV-2 rapidly spread throughout the world, leading to a public health emergency. SARS-CoV-2 infection caused patients to develop severe disease with an acute respiratory distress syndrome (ARDS), associated with coagulation disorders, an exuberant cytokine storm leading to multiple organ failure, and resulting in fatal events in about 3% of the infected people (1). The risk for the severity of COVID-19 disease depends on several comorbidities (diabetes, hypertension, lung-related diseases, cardiovascular diseases and obesity), older age, ethnicity, genetic factors, vaccination status and other conditions (2). The morbidity and mortality associated with the COVID-19 have pushed the development of SARS-CoV-2 vaccines as a priority for human health. As a result of that emergency, several effective vaccines, targeting the SARS-CoV-2 spike protein, rapidly emerged and gained conditioned approvals by the regulatory agencies (3).

However, vaccines dedicated to the prevention of the disease appeared after more than one year after SARS-CoV-2 appearance. Therefore, the initial medical approaches to this virus and the COVID-19 were focused on the existing drugs suitable for the control of the pathological events caused by the virus. Unfortunately, also because of the limited initial knowledge of the molecular pathology details of COVID-19, many of the proposed drugs have often missed expectations and were abandoned. Indeed, it was crucial to understand how this beta coronavirus gained control of the host during infection, a knowledge that was applied to the development of treatment strategies by the repurposing of existing drugs but also to the study of new ones. The emergency of the pandemic made the marketed drug repurposing the best approach to identify therapeutic options for COVID-19 in a limited time (4). In the absence of clear clinical evidence, many treatment regimens have been explored in the treatment of COVID-19. Some of these treatments could refer to the experience gained with the Middle East Respiratory Syndrome (MERS) and with the Severe Acute Respiratory Syndrome (SARS); some of them showed effects on COVID-19 patients (5). However, the published data often suffered from limited rigorousness of the clinical trials, particularly regarding randomization, genetic causes and differences in study design and treatment regimens, leading to contrasting results (6). In other cases, side effects precluded the use of the drug itself (4).

Although vaccines have made a difference in significantly reducing SARS-CoV-2 diffusion and COVID-19 frequency, with the acquired and increased knowledge on the modalities of virus infection, it became possible for researchers and pharmaceutical companies worldwide to work and develop new drug candidates. There is still a need for effective therapies for COVID-19 for many reasons: 1) some people do not properly respond to vaccines, 2) the appearance of virus variants that escape or reduce the vaccine effectiveness and 3) some patients develop severe forms of the pathology (7). Also, drugs can be useful in patients on chemotherapy, patients with hematologic malignancies, immunocompromised people or in other pathologic conditions.

Drug development is mainly focused on different strategies: i) to avoid the virus entry into the cells, ii) to inhibit viral replication and vitality, and iii) to regulate the human immune system. These drug categories include anticoagulants, immunosuppressors, anti-inflammatory, corticosteroids, janus kinase inhibitors, immunoglobulins, monoclonal antibodies, antivirals and cell therapy (8).

The aim of this review is to examine all the strategies adopted for the control and treatment of COVID-19, with particular emphasis of the role and effectiveness of the different categories of drugs on the stages of the disease.

REPURPOSED DRUGS FOR THE CONTROL OF COVID-19

In the search for an effective treatment for SARS-CoV-2 infection, many attempts have been made using existing drugs which, on the basis of their mechanisms of action, or given some preliminary clinical evidence, seemed to be effective in managing the disease (9). If, before vaccination and up to the spread of the omicron variant, the medical need was urgent, nowadays clinicians are more cautious in prescribing unapproved drugs for COVID-19. A database has also been developed (10) containing all the available in in vitro anti-SARS-CoV-2 activity and in vivo pharmacokinetic data to facilitate the extrapolation from in vitro antiviral activity to potential in vivo antiviral activity for choosing drugs that could be useful in saving lives.

The main problem concerning repurposed, or any other drug treatment for COVID-19 is the need for mechanical ventilation or high flux, as the availability of resources and the real severity of the patient respiratory function greatly influence the efficacy of the therapy. As an example, tocilizumab, an interleukin 6 antagonist, in randomized clinical trials has shown mixed results compared with control or usual care in hospitalized patients with COVID-19 (11). However, the real benefit was evident only for those patients who did not require invasive mechanical ventilation (IMV) at randomization and no further details were provided regarding the respiratory status. Despite the absence of these data, guidelines have suggested the use of tocilizumab in patients with either severe or critical COVID-19 independent of their respiratory condition. From a re-analysis of the published evidence, it appears that the real benefit of using tocilizumab might have been overestimated also in subjects without IMV, as it was used in association with high doses of corticosteroids that by themselves blunt the inflammatory reaction (12).

Another drug that has been used for treating COVID-19 was Ivermectin, an inexpensive, easy-to-administer, and widely available antiparasitic drug, because an in vitro study showed inhibitory effects against SARS-CoV-2. Despite the initial reports on its supposed efficacy in reducing viral load in 45 patients (13), a 5-day course of ivermectin, compared with placebo, did not significantly improve the time to resolution of symptoms in 400 patients (14). Further evidence on 490 high-risk hospitalized patients with mild to moderate COVID-19, demonstrated no benefit from this treatment regarding the need for mechanical ventilation, intensive care unit admission, or death (15).

Among the drugs used to treat COVID-19, the anti-malarial drugs chloroquine (CQ) and hydroxychloroquine (HCQ) deserve special attention. These have been suggested as promising agents, from early trials in China, for shortening the duration of the viral disease, reducing the fever duration, and improving lung health (16) also in combination with azithromycin, a commonly prescribed antibiotic for lung infections (17). The antiviral effects of CQ and HCQ have been demonstrated in vitro due to their ability to block viruses like coronavirus SARS in cell culture (18, 19). Given this preliminary evidence and considering the low cost of HCQ, most pharmacies, as a detrimental consequence of the rapid dissemination of over-interpreted data, in Europe and Italy have been struck by people asking for this drug, hoping for a miraculous cure for the deadly virus (20). However, a few months later, emerging clinical evidence demonstrated the ineffectiveness of HCQ, together with the severe adverse events, including death, when used at high dose. Currently, no direct supporting data on the effective role of CQ and HCQ in the treatment for COVID-19 exist, and the international RCTs for COVID-19 treatments launched by WHO (21) concluded that HCQ had little or no effect on overall mortality, initiation of ventilation, and duration of hospital stay in hospitalized patients (22, 23).

Finally, also azithromycin, a macrolide antibiotic with alleged antiviral efficacy against COVID-19, was widely prescribed up to the second quarter of 2021 because several

guidelines in 2020 recommended the use of empirical antimicrobial treatment (24). However, its alleged efficacy was eventually unsupported by the results of the Recovery trial that enrolled over 7000 patients that did not benefit from azithromycin treatment, in terms of the need for IMV or death (25). The same trial indeed revealed that corticosteroids are indeed useful in the treatment of COVID-19. In fact, before Recovery trial results were available, there was a wide debate regarding the role of corticosteroids in mitigating inflammatory organ injury (26, 27) and these were generally not included in most guidelines. However, the first results obtained in 2104 hospitalized patients showed that dexamethasone lowered mortality among those who were receiving either invasive mechanical ventilation or oxygen alone at randomization (28).

Considering the great medical need, a special effort has been made in these past 2 years to assess the safety and efficacy of drugs proposed or used to treat COVID-19, but little evidence exists to date on the prescribing patterns for repurposed and adjuvant drugs in routine clinical practice.

ANTICOAGULATION TREATMENTS IN COVID-19

Since the beginning of the COVID-19 pandemic, altered coagulation has been reported in hospitalized patients, with both thrombotic as well as hemorrhagic events. In some patients, a pro-thrombotic status was the alleged cause, but for many others, the problems seem related to the cytokine storm, leading to hyperinflammation, endothelial disruption, platelet activation, and thrombotic complications (29). Arterial and venous thrombotic complications are common in hospitalized patients with COVID-19 and are an independent predictor of poor outcome. Microvascular thrombi also determine multi-organ dysfunction, starting with acute respiratory distress and then involving other tissues (30). Early studies also indicated that standard prophylactic doses of anticoagulant therapy appeared to be inadequate for preventing thrombotic events in hospitalized patients (31).

More recently, larger trials have been published, providing more insight into treatment strategies for hospitalized patients with COVID-19. The ACTION trial (32) showed that clinically stable hospitalized patients with COVID-19 receiving rivaroxaban, compared to unstable patients receiving enoxaparin, did not improve the primary efficacy outcome on the death rate, duration of hospitalization, or duration of supplemental oxygen. Therapeutic anticoagulation was associated with increased bleeding in both clinically stable and clinically unstable patients.

The ATTACC, ACTIV-4a, and REMAP-CAP Investigators in 2 trials (33, 34) using therapeutic-doses anticoagulation compared with "usual-care" thromboprophylaxis in noncritically ill patients, defined as not needing respiratory or cardiovascular support, showed that therapeutic dosing improved survival and reduced the use of cardiovascular or respiratory organ support as compared with usual-care thromboprophylaxis.

In the HEP-COVID study (35), adult patients with evidence of coagulopathy (by laboratory means) affected by COVID-19 and randomized to receive standard prophylactic or intermediate-dose low-molecular-weight heparin (LMWH) or unfractionated heparin (UFH) or therapeutic-dose LMWH throughout hospitalization, demonstrated interesting results. The primary efficacy outcome of thromboembolic occurrence, or all-cause mortality, was reached only in non-severe patients with therapeutic-dose anticoagulation, but ICU patients did not improve with this therapeutic regimen.

Anticoagulation with LMWH or UFH at a therapeutic dose in COVID-19 hospitalized patients with an elevated D-dimer level did not significantly reduce the rate of death or severe consequences such as ICU admission, noninvasive or invasive mechanical ventilation, as demonstrated in the RAPID Trial (36). Also, considering the results of the INSPIRATION trial in ICU

patients, no benefit was obtained from using an intermediate dose of LMWH over standard prophylactic-dose anticoagulation in preventing thromboembolic events or death (37).
Despite the methodological differences in defining the criteria for considering critically or non-critically ill COVID-19 patients in these studies, the take-home message regarding the efficacy and safety of anticoagulant therapy in hospitalized patients can be summarized in 3 crucial points. First, patients that are non-critical and have elevated D-dimer levels benefit of therapeutic anticoagulation with LMWH or UFH; second, critically ill and/or ICU patients do not benefit from therapeutic anticoagulation and have a higher risk of hemorrhage; finally, a dose between prophylactic and therapeutic is not recommended in either ICU or non-ICU patients.
The use of LMWH in the prophylaxis of thromboembolic events or in patients with an acute respiratory infection is recommended by the main guidelines in the absence of contraindications. LMWH or UFH are necessary in case of thromboembolic manifestations; it is indeed reasonable to recommend enoxaparin prophylaxis or an intermediate dose when pneumonia is present and hypomobility occurs in the bed rest patient (38).
Although many limitations and a small number of high-quality, well-designed studies, heparin treatment should be preferred to anticoagulants in the treatment of COVID-19 patients at high risk or with thromboembolism.

ANTI-CYTOKINE AGENTS FOR COVID-19 TREATMENT

SARS-CoV-2 virus infection triggers an inflammatory response and subsequent production of immune mediators such as cytokines, chemokines, and complement, initially locally and in moderate amounts: this response is essential to fight the infection. However, in severe COVID-19 infection, cytokines and chemokines are released in increased amounts, leading to massive recruitment of immune cells and consequent hyperinflammation, which eventually causes the cytokine storm (CS) (39). This increased inflammatory response leads to severe complications such as acute respiratory distress syndrome (ARDS) in the lungs, intravascular coagulation, multiorgan failure, and ultimately death. Higher concentrations of cytokines in the plasma of patients have been associated with disease severity (40, 41). These pro-inflammatory cytokines and chemokines include tumour necrosis factor alpha (TNF-α), interleukin 1beta (IL-1β), IL-6, IL-10, IL-17, Granulocyte/macrophage colony-stimulating factor (GM-CSF), interferon gamma (IFN-γ), monocyte chemoattractant protein-1 (MCP-1), and macrophage inflammatory protein-1-alpha (MIP-1α) (42-45). The IL-1/IL-6 axis is probably one of the most biologically relevant signalling pathways in the SARS-CoV-2-induced hyperinflammatory response (44, 46, 47). Consequently, monoclonal antibodies or drugs targeting specific cytokines among the host defence immune mediators triggered by the virus were considered early on as a potential class of adjunctive therapies for COVID-19 (48).

IL-1 blockers

IL-1 induces local effects such as macrophage activation, endothelial leakage, and fluid extravasation, as well as systemic effects such as fever, drowsiness, and synthesis of acute-phase proteins. Blocking IL-1 signals reduces inflammation, which in turn may reduce the need for respiratory support and deaths from COVID-19. Three IL-1 blockers are available: anakinra, canakinumab, and rilonacept.
Anakinra is a recombinant soluble IL-1 receptor antagonist (IL -1Ra) that competitively inhibits the binding of both IL-1α and IL-1β to their receptor (IL-1 type I) (49-51) and is currently approved for rheumatoid arthritis and other autoinflammatory diseases. Randomized trials with anakinra, compared to placebo, in patients with mild to moderate COVID-19 pneumonia reported no significant effect on the proportion of patients who died or re-

quired non-invasive or mechanical ventilation, or on survival without the need for mechanical or non-invasive ventilation, or on discharge from organ support in the intensive care unit (ICU) (52, 53). These findings are consistent with a Cochrane systematic review that examined the effects of IL-1 blockers compared with standard of care (SoC) alone or placebo on efficacy and safety in patients with moderate to severe COVID-19 (54). Overall, there was no evidence of a significant beneficial effect of IL-1 blockers or of adverse effects. Similarly, a study on canakinumab, a monoclonal antibody that blocks only IL -1β, did not reach significance for its primary outcome, survival without invasive mechanical ventilation at day 29 (55). Again, the results are supported by the findings of the Cochrane systematic review, which states that canakinumab is likely to result in little or no improvement in COVID -19 symptoms, defined as improvement on a clinical scale or discharge from hospital at day 28 after treatment. No studies of rilonacept in COVID-19 were found in either the EU Clinical Trials Register or on ClinicalTrials.gov (accessed February 17, 2022).

In contrast to these disappointing results, a different approach based on stratifying patients by immunologic profiles identified patients who would likely benefit from IL-1 blockade. In the SAVE-MORE trial, treatment with anakinra was guided by plasma levels of soluble urokinase plasminogen receptor (suPAR) as a biomarker of risk of progression to severe respiratory failure (56-58). Treatment with anakinra resulted in significant clinical improvement on the 11-point WHO clinical outcome scale, both toward complete resolution and toward critical illness or death at 28 days (59). In this study, anakinra also improved outcomes in patients treated concomitantly with dexamethasone, suggesting that suPAR-based treatment with anakinra is a therapeutic strategy before critical illness occurs. Other useful information on the use of anakinra comes from a retrospective observational study suggesting that a shorter time between hospitalization and treatment with anakinra in patients with moderate/severe COVID-19 is associated with a significantly lower number of intensive care admissions and lower mortality (60).

IL-6 blockers

In severe COVID -19 patients, a significant increase in the levels of IL-6 is observed (47, 61). IL-6 is a strong predictive marker of acute severe systemic inflammatory response requiring support by mechanical ventilation. Moreover, elevated levels of IL-6 activate the coagulation cascade and increase the risk of death (62-64). Accordingly, blockade of IL-6 has emerged as a potentially promising approach to control SARS-CoV-2-associated cytokine release syndrome (CRS). IL-6 promotes monocyte differentiation into macrophages, recruits immune cells to the site of injury, and increases cytokine production. Interaction of IL-6 with its transmembrane IL-6 receptor (IL-6R) leads to dimerization of glycoprotein 130 and the "classical" signalling process via JAK /STAT, MAPK and RAS /RAF. However, cells that do not express IL-6R also respond to IL-6 through circulating soluble IL-6Rα (sIL-R), known as "trans-signaling". Recently, three drugs have been used to treat COVID-19 infections and are in clinical trials: tocilizumab, sarilumab, siltuximab.

Tocilizumab is a humanized IgG1-type mAb that targets both the membrane-bound and soluble forms of IL-6R (63), inhibiting both classical and trans-signalling. It is used to treat rheumatoid arthritis (RA) and CRS concomitant with CAR-T therapy in cancer, a syndrome similar to the hyperinflammatory phase of COVID-19 (64, 65). A prospective meta-analysis of clinical trials of patients hospitalized for COVID -19 showed an association with lower 28-day all-cause mortality in patients treated with IL-6 antagonists compared with patients receiving usual care or placebo (65). Tocilizumab resolved respiratory symptoms and improved overall health (11). In addition, patients with hypoxemia requiring oxygen therapy have benefited from anti-IL-6 strategies,

as shown by the results of two large-scale randomized clinical trials (65, 66). In the open-label trial RECOVERY, which enrolled predominantly non-critically ill patients, a significant reduction in mortality was observed in the tocilizumab arm compared with the usual care arm (66). In the REMAP-CAP trial, both tocilizumab and sarilumab were effective compared with the control group and likely equivalent in improving survival and discharge from organ support (67). In the same study, treatment with anakinra was not effective, as previously reported. Overall, these data support the use of blockade of IL-6 in patients with COVID-19 who are hospitalized and require oxygenation. Unlike tocilizumab and sarilumab, which target the IL-6 receptor, siltuximab modulates IL-6 signalling by directly binding the cytokine (68). The COV-AID study examined the effects of tocilizumab and siltuximab within the anti-IL-6 therapy group and found no significant difference between the two different anti-IL-6 strategies (69).

Inhibitors of JAK /STAT

Several studies suggest that activation of host NF-kB and IL-6/JAK/STAT signalling pathways by SARS-CoV-2 viral proteins is likely a critical factor in virulence, promoting overexpression of proinflammatory cytokines, viral replication, and pathogenicity. The JAK/STAT pathway transmits extracellular signals conveyed by a large number of cytokines, lymphokines, and growth factors, with IL-6 being one of the most important activators (70). Binding of IL-6 to its receptor activates STAT3, which contributes to the cytokine storm, then the ability of STAT3 to promote IL-6 gene expression leads to an autocrine loop that enhances cytokine expression (71). JAK/STAT signalling pathway in COVID-19 has also been implicated in the inflammatory response of IFN-γ, the signalling of which involves JAK1 and JAK2 as well as STAT1 (72). Last but not least, detachment of ACE2 from the cell surface after endocytosis increases angiotensin II levels (Ang II), whose effects are also mediated by the JAK/STAT pathway and contribute to the development of ARDS (73). Therefore, it is not surprising that one of the therapeutic strategies being investigated for COVID-19 is targeting the JAK/STAT pathway, whose inhibition may have pleiotropic effects on the actions of multiple cytokines, including IL-6 and GM-CSF, while overcoming the limitations of mAbs that normally target only one cytokine. There are several JAK/STAT inhibitors that differ in their selectivity toward members of the family, namely JAK1, JAK2, JAK3, and Tyk2 (74).

The efficacy and safety of the pan-JAK inhibitor tofacitinib were evaluated in a clinical trial of 289 patients hospitalized with COVID-19 pneumonia (75). Tofacitinib resulted in a lower risk of death or respiratory failure than placebo by day 28, with serious adverse events occurring in 14.1% in the tofacitinib group and 12.0% in the placebo group. Further promising results were also obtained in combination with hydroxychloroquine (76). Further evidence is available on the use of ruxolitinib and baricitinib, both inhibitors of JAK1 and JAK2. Ruxolitinib is a potent JAK1/2 inhibitor and significantly suppresses the increase in IL-6 and TNF-α levels in COVID-19 patients. Compared with placebo, treatment with ruxolitinib resulted in significantly improved chest computed tomography and faster recovery from lymphopenia (77). In addition, ruxolitinib in combination with steroids reduced mortality and resulted in a 75% recovery rate in COVID-19 patients enrolled in the MAP program (78). Nevertheless, ruxolitinib failed to significantly reduce inflammation in patients who experienced respiratory failure or ICU admission. Baricitinb is not only a JAK inhibitor but also impedes the entry of SARS-CoV-2 into target cells (73). Although the virus enters the host cell mainly through ACE2 receptors, JAK and AP-2 (Adaptor Protein Complex 2) associated protein kinase-1 (AAK1) are also involved in viral attack and endocytosis (79, 80). Baricitinb inhibits viral endocytosis and assembly by inhibiting AAK1 and cyclin G-associated kinase (GAK). Baricitinib treatment attenuates the cy-

tokine storm by decreasing expression levels of IL-6, IL-1β, and TNF-α, resulting in improvement in lymphocyte counts in patients with COVID-19 (81). A double-blind, randomized, placebo-controlled trial of 1,033 adults hospitalized with COVID-19 who were randomized to receive either baricitinib or placebo showed that patients receiving this JAK inhibitor had a shorter time to recovery than patients in the placebo group (82). Importantly, the effect was more pronounced in the subgroup that required high-flow oxygen or non-invasive ventilation compared with placebo. Encouraging results came from a double-blind phase 3 trial of 1,525 participants randomized to baricitinib or placebo (83). The relative reduction in mortality was 38.2% for baricitinib versus placebo when considering 28-day all-cause mortality; this effect is in addition to standard treatment, including corticosteroids. Positive feedback comes from the use of baricitinib in combination with remdesivir, better than baricitinib alone, in accelerating recovery time and improving the clinical condition of COVID-19 patients dependent on high-flow oxygen or non-invasive ventilation, with fewer adverse events (82). The FDA recently approved baricitinib for the emergency treatment of COVID-19 (July 2021). On the other hand, it should also be considered that baricitinib, as a potent immunosuppressant, may lead to an additional risk of infection in critically ill patients.

One concern with the use of pan-JAK inhibitors for COVID-19 is that such inhibitors may interfere with host responses mediated by type I and type II interferons, which have important antiviral effects through their ability to inhibit viral replication in infected cells (84,85). Because JAK2 is not involved in cell signalling that regulates type I interferons and is not essential for type II and III interferons in host immunity, selective JAK2 inhibitors might be preferred over other JAK inhibitors to block signalling by cytokines such as IL-6 and GM-CFS, leading to suppression of COVID-19-associated CRS. The hypothesized benefits of JAK2 inhibition in the treatment of COVID-19-associated CRS are currently being investigated with FDA-approved inhibitors. Fedratinib is an FDA-approved JAK2 inhibitor that has nanomolar activity in the treatment of myelofibrosis (MF) (86); it has also been reported to prevent the worsening outcomes that follow Th17 cell differentiation and the associated cytokine storm, helping to control pulmonary oedema in COVID-19 (87). Several other JAK2 inhibitors are currently under investigation for the treatment of various human diseases, including acute myeloid leukemia, MF, psoriasis, GvHD (graft versus host disease) (88, 89). Given that JAK2 inhibitors likely do not interfere with the type I interferon response in immunity but inhibit cytokines including IL-6 and GM-CSF in COVID-19 associated CRS, JAK2 inhibition should be an attractive therapeutic option for blocking the cytokine storm in COVID-19.

The need to find effective therapies against COVID-19 in the shortest possible time has forced the entire scientific community to make great efforts. The experience accumulated so far suggests that host-specific therapy is a rather complex approach and that the heterogeneity of the immunological milieu of COVID-19 patients must be taken into account. It is now clear that not all patients benefit from the same immunomodulatory treatment and that the same patient may respond differently depending on the stage and severity of the disease. In particular, the experience with the IL-1 antagonist anakinra points to the need to evaluate and use biomarkers to guide patient-specific immunotherapy.

ANTI-SARS-CoV-2 MONOCLONAL ANTIBODIES

Soon after the discovery that SARS-CoV-2 enters the cells, after binding the human angiotensin-converting enzyme 2 (ACE2) receptor through the spike protein (90) the idea of preparing monoclonal antibodies (MoAb) capable of binding the Spike protein in the receptor-binding domain (RBD) and inhibiting the

Spike/ACE2 binding was pursued. Neutralizing MoAbs (NMoAbs) would inhibit viral replication and cure patients. However, in the following months, it became progressively clear that what might seem a simple and successful idea presented some critical issues: 1) the role of antibodies in the fight against SARS-CoV-2 infection, 2) the decreasing efficacy of the MoAbs during the development of the infection so that the treatment has become half a way between cure and prevention, 3) the cost of antibodies considering the relatively low mortality rate also in patients at high risk of death, 4) the need to use the parental route, making more difficult the administration, 5) relevant changes in the Spike protein over time.

Point 1. The role of antibodies in COVID-19 remains to be fully defined. After SARS-CoV-2 appearance, it was clear within months that the more severe the COVID-19 disease, the higher the anti-Spike antibody titers (91), possibly suggesting that naturally arising antibodies were not protective. The idea seemed to be confirmed when several studies demonstrated that administration of polyclonal antibody-containing sera of patients who recovered from COVID-19 did not cure patients (92-94). In addition, some data suggest that certain patient-produced antibodies may lead to antibody-dependent potentiation (ADE) of the disease, favoring the entry of the virus into cells, as observed for other viruses, including coronavirus (95-98). On the contrary, some studies suggested a protective role of naturally arising antibodies. For example, hospitalized patients with no anti-Spike antibodies showed a mortality rate almost twice that of patients with anti-Spike antibodies (99).

Thus, it seemed reasonable to conclude that antibodies inhibiting ACE2/Spike binding and the entry of virus in the cells are protective if devoided of ADE effect. Indeed, the first clinical studies using one monoclonal antibody (MoAb) or two MoAbs in association demonstrated a relevant protective activity (100, 101).

A second crucial issue (point 2) was the timing of antibody administration relative to the evolution of the infection. Some studies demonstrated that antibodies were effective when administered early (*e.g.*, in the patient positive for SARS-CoV-2 but with few symptoms) and inactive when the patient is hospitalized and/or in intensive care (102,103). Therefore, all antibodies entered in the clinical use must be given as soon as possible, even if the patient does not have a serious disease. The need for early administration made it necessary to establish the type of patients that need to be treated. Indeed, it was and is still impossible to treat all the COVID-19 patients with antiviral MoAb, due to the shortage of the drugs (particularly soon after their approval) and their cost. Moreover, considering the very low mortality rate of COVID-19 in a large portion of the young-adult population, the administration may be non-ethical due to the very low benefit versus the potential risk of adverse events. Therefore, each Health Organization established the patient categories that should be treated, including old patients and those with co-morbidities known to increase the mortality rate (see below). Nonetheless, when treating paucisymptomatic patients, the NNT of antiviral monoclonal antibodies is quite high, ranging between 25 and 29 in the hypothesis of 5% risk of hospitalization (104). Therefore, the cost of the treatments is rather high (point 3).

The need for MoAb administration as soon as possible means that they are given to patients still at home and in a relatively good condition (see below for details). Considering that they must be given through the endovenous route, home administration of the drug to a patient positive for SARS-CoV-2 was a critical issue (point 4), considering the susceptibility of specialized personnel to CoViD-19 (especially before vaccination) and the lack of available medical and paramedical personnel, especially during the pandemic peaks.

The emerging SARS-CoV-2 variant: the most relevant issue

A crucial issue concerning antibodies efficacy is the appearance of variants of concern of

SARS-CoV-2 (point 5). Errors (point mutation) in RNA viruses such as SARS-CoV-2 are a rule. Despite SARS-CoV-2 codes for a polymerase with proofreading activity (105, 106), SARS-CoV-2 variants are quite frequent.

The issue was well known at the beginning of the pandemic. Now we know that the frequency of mutation in the viral RNA coding the Spike is much higher than the frequency of mutation in the RNA coding the other viral proteins (107). In particular, comparing 303,250 human SARS-CoV-2 spike protein sequences with the reference sequence of Wuhan-Hu, authors found mutations of each of the 195 amino acid residues forming the RBD, including the amino acid residues crucial for ACE2 binding (8 residues), which is somewhat surprising. We can conclude that: 1) no amino acid residues are indispensable to bind ACE2, 2) more importantly, we cannot bet on the efficacy over time of neutralizing MoAbs binding the RBD. Reasonably, the high frequency of mutation is due to a selective advantage for the virus having a Spike with a higher affinity for the ACE2 receptor, more able to favor virus entry or not recognized by anti-SARS-CoV-2 Abs produced by the host following infection with another variant of SARS-CoV-2 or the vaccination with a vaccine expressing the Spike of Wuhan-Hu virus.

In theory, the same use of monoclonal antibodies favors the appearance of variants, but we believe that their use in the population had been so infrequent that it did not exert sufficient selective pressure. Moreover, most MoAbs are administered as an association of two antibodies, making unlike the appearance in one virus particle of mutations conferring resistance to both antibodies (108). The consequence of the appearance of specific variants on the efficacy of the antibodies in clinical use will be discussed later.

Interestingly, forty-four invariant residues are present in the Spike protein outside the RBD and correspond to ten domains/regions in the SARS-CoV-2 Spike protein (107), possibly suggesting that MoAbs binding these amino acid residues may be effective not only against the present but also future SARS-CoV-2 variants.

Patients for which MoAbs treatment is indicated

As reported above, not all patients affected by COVID-19 are treated with MoAbs. Treatment is indicated soon after the occurrence of COVID-19 symptoms in non-hospitalized patients with laboratory-confirmed SARS-CoV-2 infection who are at high risk for progressing to severe disease and/or hospitalization. The patients must be aged > 64 years or aged 12-64 years with relevant comorbidities or conditions, such as obesity (BMI > 25), diabetes, cardiovascular and chronic lung diseases, including hypertension. Other patients poorly represented in the study leading to MoAb authorization but considered to be at high risk when infected with SARS-CoV-2 are patients under immunosuppressive treatment or immunocompromised, with chronic kidney disease, pregnant, with neurodevelopmental disorders, conditions that confer medical complexity and dependant on medical-related technological devices. Even infants with less than 1 year are considered at high risk. For sure, the anti-SARS-CoV-2 MoAbs are not authorized for use in the patients hospitalized for COVID-19 and/or who require oxygen therapy due to COVID-19, because MoAbs do not improve any parameter, including survival.

More recently, some anti-SARS-CoV-2 MoAbs have been found to be effective in reducing the risk of infection when used as pre-exposure prophylaxis (109) and as post-exposure prophylaxis in a household and other high-risk settings (110, 111).

The list of patients who are to be treated with anti-SARS-CoV-2 monoclonal antibodies overlaps with that of patients that should be treated with anti-SARS-CoV-2 small molecules (see paragraph Antiviral small molecules). Future studies will indicate which drug class has to be preferred in a specific category of patients also regarding the safety, the cost, and availability of the drugs.

Anti-SARS-CoV-2 MoAbs with emergency use authorization/full authorizations from EMA/FDA

Eight anti-SARS-CoV-2 MoAb products have received emergency use authorizations from EMA and/or FDA. They are bamlanivimab plus etesevimab given in association (previously called LY-CoV555 and LY-CoV016, respectively), casirivimab plus imdevimab given in association (previously called REGN10933 and REGN10987, respectively), regdanvimab (previously called CT-P59), tixagevimab and cilgavimab (previously called COV2-2196 and COV2-2130, respectively), and sotrovimab (previously called VIR-7831, the parent MoAb of S309).

Bamlanivimab, etesevimab, casirivimab, imdevimab, regdanvimab, tixagevimab, and cilgavimab are neutralizing mAbs binding to the RBD of SARS-CoV-2 Spike protein. Bamlanivimab and etesevimab bind to different but overlapping epitopes, whereas casirivimab/imdevimab and tixagevimab/cilgavimab bind to non-overlapping epitopes. Regdanvimab is not given in association.

Phase 3 BLAZE-1 trial had demonstrated that bamlanivimab plus etesevimab, compared to placebo, was associated with 4.8% absolute reduction and 70% relative reduction in COVID-19-related hospitalizations or all-cause deaths (112). Casirivimab plus imdevimab, compared to placebo, was associated with 7.5% absolute reduction and 70% relative risk reduction in COVID-19-related hospitalizations or all-cause deaths (113). Regdanvimab, compared to placebo, was associated with 2.2% absolute reduction and 78% relative risk reduction in progression to severe COVID-19 disease (114).

In March 2021 EMA's Committee for Medicinal Products for Human undertook the review of data on bamlanivimab plus etesevimab as part of a rolling review and supported the use at the National level before market authorization. On November 2, 2021, the manufacturer informed the EMA of the decision to withdraw from the approval process. The broad distribution of bamlanivimab plus etesevimab has been paused in the United States because the Omicron variant has markedly reduced in vitro susceptibility to this mAb regimen (see below) (115). In Italy, the authorization for the temporary use of bamlanivimab as monotherapy was revoked in May 2021, while, on March 1, 2022, the authorization for the use of the association has not yet been revoked.

On November 11, 2021, EMA's CHMP has recommended authorizing regdanvimab and the association of casirivimab with imdevimab for treating patients with COVID-19. The recommended dosage of regdanvimab in adults is a single IV infusion of 40 mg/kg within 7 days of developing symptoms of COVID-19. Casirivimab and imdevimab are administered at the dose of 600 mg each by IV infusion or by SC injection within 7 days of developing symptoms of COVID-19. Moreover, Casirivimab and imdevimab can be used to prevent COVID-19 after contact with an infected person or even when no contact has occurred. Moreover, a recent study demonstrated that hospitalized patients receiving high doses of casirivimab plus imdevimab (4,000 mg each) showed a significant reduction in 28-day all-cause mortality when seronegative for the anti-spike protein antibody (24% mortality in the mAb-treated group *vs.* 30% mortality in the standard care group) (99). However, the treatment of hospitalized patients is authorized by neither EMA nor FDA.

Tixagevimab and cilgavimab are in rolling review at EMA and have received emergency use authorization by FDA for the pre-exposure prophylaxis of COVID-19. In Italy, its temporary distribution was authorized for the prophylaxis of COVID-19 on 28 January 2022. Tixagevimab and cilgavimab were optimised using a proprietary half-life extension technology, which could afford up to 12 months of protection. An interim analysis of the PROVENT phase III trial having as the primary efficacy endpoint the first case of any SARS-CoV-2 RT-PCR positive symptomatic illness occurring post-dose prior to 6 months, demonstrated a reduced risk of

developing symptomatic COVID-19 (HR 0.23 with a median follow-up 83 days and HR 0.17 with a median follow-up 6.5 months) (116). Moreover, there were no severe or critical COVID-19 events in the antibody group compared to 5 in the placebo group. Tixagevimab and cilgavimab should be given as separate, sequential IM injections at different injection sites, preferably one in each of the gluteal muscles. The recommended dosage is 150 mg of each mAb every 6 months. The incidence of serious cardiac adverse events (*e.g.*, myocardial infarction, cardiac failure, arrhythmia) was higher in the antibody group than in the placebo group (0.6% *vs.* 0.2%) (116).

Sotrovimab is a neutralizing mAb binding to SARS-CoV-2 Spike protein outside the RBD. In particular, it recognizes an epitope that is highly conserved within the Sarbecovirus subgenus and prevents the virus from entering the cell by inhibiting the mechanisms downstream of the spike/ACE2 bond (117). Interestingly, it was derived from a parent antibody (S309) isolated for the first time in 2003 from an individual who recovered from SARS (118). Sotrovimab was designed to possess an Fc LS mutation (M428L/N434S) which confers greater binding to the neonatal Fc receptor resulting in prolonged half-life. Sotrovimab also demonstrated antiviral activity through antibody-dependent cellular cytotoxicity (ADCC) and antibody-dependent cell phagocytosis (ADCP) of virus-infected cells. In the first studies demonstrating the efficacy of Sotrovimab, three patients (1%) in the sotrovimab group, as compared with 21 patients (7%) in the placebo group had disease progression leading to hospitalization or death with a relative risk reduction of 85%. Moreover, only in the placebo group, five patients were admitted to the intensive care unit, including one who died (119). On December 17, 2021, EMA's CHMP has recommended sotrovimab for treating patients with COVID-19. The recommended dosage of sotrovimab in adults is a single IV infusion of 500 mg within 5 days from the developing symptoms.

Safety

The safety of anti-SARS-CoV-2 antibodies is quite high. Anaphylaxis and infusion-related reactions have been reported in a few patients who received anti-SARS-CoV-2 mAbs. More frequently, it is observed nausea, vomiting, diarrhea, dizziness, hyperglycemia, rash, and pruritis (120-124).

Neutralizing activity of MoAbs on SARS-CoV-2 variants

The above-mentioned MoAbs have been tested in clinical studies when the SARS-CoV-2 variant of concern Omicron was not present and most of them were effective in the treatment of patients infected with variants other than the Omicron variant. The Omicron variant encodes 37 amino acid substitutions in the Spike protein, 15 of which are in the RBD, and represents a major antigenic shift in SARS-CoV-2. Indeed it determines a marked reduction in neutralizing activity in plasma from convalescent patients and individuals who had been vaccinated against SARS-CoV-2. Due to the high infectivity of Omicron, currently, most patients are infected by this variant.

Some studies evaluated whether the above-described MoAbs retain neutralizing activity against Omicron variant (125, 126). For all the MoAbs binding the RBD of the Spike protein, a significant drop in the neutralizing activity was described (in practice, loss of activity), with the only exception of cilgavimab, which showed a slight drop only (about 12 fold decrease). Interestingly, the neutralizing activity of sotrovimab, binding to the Spike protein outside the RBD, was minimally affected. Consequently, FDA assessed that "the broad distribution of bamlanivimab plus etesevimab and casirivimab plus imdevimab has been paused because the products have reduced activities against Omicron variant of concern" (115, 127).

Anti-SARS-CoV-2 MoAbs in the clinical study

Several MoAbs are still in the clinical study. Two approaches appear very interesting: 1) MoAbs

binding to the Spike protein outside the RBD and active against Omicron variants (128); 2) The MoAbs MAD0004J08 showing an extremely high affinity for the RBD of the S protein and being one of the most potent antibodies selected by screening 453 neutralizing antibodies produced by B lymphocytes from 14 COVID-19 survivors (129). Its potency allows administration by i.m. injection and lower production cost.

ANTIVIRAL SMALL MOLECULES

At the outbreak of the pandemic, the available antiviral drugs seemed the obvious choice to fight the SARS-CoV-2 virus responsible for COVID-19. The virus was new, but it was an RNA virus of which much was known about biological and pathological characteristics. The pathology caused by SARS-CoV-2 infection, that is COVID-19, indeed showed entirely new and unexpected characteristics. We were therefore faced with a new virus and a new pathology. Obviously, neither against the first nor against the second there were already specific drugs available. The biological characteristics of the virus, in particular being an RNA virus, have however suggested the possibility of contrasting it with anti-retroviral drugs developed for similar viruses, such as those against HIV. This is why the WHO immediately suggested carrying out a multicenter study using the Lopinavir-Ritonavir combination, a drug capable of inhibiting viral- RNA-dependent RNA-polymerase. Also, the fact that SARS-CoV-2 was a member of the beta coronavirus family suggested that it could be contrasted with other drugs such as those developed for the treatment of the less-lethal but very widespread influenza viruses. Thus, antiviral drugs such as Favipiravir, Oseltamivir, Umifenovir and Ribavirin have been studied in different combinations. Obviously, antiviral drugs with activity on liver RNA viruses, such as those for hepatitis B (Remdesivir) and for hepatitis C (Sofosbuvir), have not been ignored.

The results obtained using these antiviral drugs have often been very disappointing. These drugs were expected to reduce the spread of the virus in the body and, consequently, the severity of COVID-19. Indeed, in the various clinical studies of which the outcomes have been reported, there have been no significant advantages both in reducing the severity of the disease and even less in mortality. In some studies, the lack of therapeutic success was attributed to the viral load, while in others to the advanced state of the disease. Ultimately, regardless of the drug combinations used, the state of the temporal course of the infection or the state of the pathology, with the exception of Remdesivir, which, in some cases, has reduced the risk of aggravation of the disease and consequent hospitalization of the patient, all other approaches have reported negative or unsuitable results for planning the use of these drugs in an appropriate and more extensive manner.

Of these antiviral drugs, their pharmacological and therapeutic characteristics and their effects in patients with COVID-19 have been revised in an exhaustive review that summarizes their value in controlling COVID-19 and in the progression of this disease to more severe stages leading to hospitalization and/or death (130). Indeed, another review (131) written at the end of 2020, already anticipated the often discordant and almost always negative results of the use of these drugs in patients with different statuses of COVID-19 severity. In this work, the reader can find the tables that summarize the results of clinical studies, often well-controlled, which highlight Remdesivir, among all the antiviral drugs examined, for which a certain response, expressed as a reduction in hospitalization and the risk of disease progression, was found in 3 of the four studies examined.

In the present review we will focus on the three antivirals currently authorized by regulatory agencies, remdesivir, molnupiravir and paxlovid; the second with a mechanism similar to that of remdesivir, and the third totally new and with a new and different molecular target.

Remdesivir

Remdesivir is the first antiviral medicine to be authorised by the European Medicines Agen-

cy (EMA) with specific indication for the "treatment of coronavirus disease 2019 (COVID-19) in adults and adolescents (aged 12 years and over and weighing at least 40 kg) with pneumonia requiring supplemental oxygen therapy". In December 2021, the EMA authorized an extension of indication relating to the treatment of coronavirus disease 2019 (COVID-19) in "adults who do not require supplemental oxygen therapy and have an increased risk of progression to severe COVID-19".

Remdesivir is a monophosphoramidate nucleoside analogue prodrug that was originally developed for Ebola virus and utilized in response to the 2014–2016 outbreak in West Africa (132, 133). It displayed broad-spectrum activity against different coronaviruses in preclinical models and has been suggested for COVID-19 clinical trials (132,134,135). It competes with endogenous nucleotides for incorporation into replicating viral RNA through the RNA-dependent RNA polymerase (RdRp) and inhibits viral replication (132). The RdRp is an attractive target for antiviral drugs, as it is highly conserved across coronaviruses. As a prodrug, remdesivir undergoes intracellular conversion by kinases to its active nucleoside triphosphate metabolite. Remdesivir and its metabolites display higher selectivity for RdRp compared to human polymerases (132).

Coronaviruses express a unique exoribonuclease (ExoN) which functions as a proofreading enzyme correcting errors in the growing RNA chain (136). The development of effective nucleoside analogues is, therefore, particularly challenging. Remdesivir is able to partly evade proofreading and maintain potent antiviral activity in the presence of ExoN. The reason remdesivir's activity is only modestly decreased by ExoN relates to two unique properties: i) it is incorporated into replicating RNA more efficiently than natural nucleotides (136-138); ii) it functions as a non-obligate or delayed RNA chain terminator (136-138). The incorporation of the delayed chain terminators perturbs the RNA structure, and synthesis is halted at some point downstream (138). In SARS-CoV-1, SARS-CoV-2, and MERS-CoV, remdesivir consistently induces chain termination after the addition of three nucleotides (136, 137), thus escaping ExoN excision.

Remdesivir is administered intravenously and is a substrate of several cytochrome P450 enzymes in vitro, however clinical implications are unclear since the prodrug is rapidly metabolized by plasma hydrolases (139). Consequently, hepatic impairment has little effect on remdesivir plasma levels, although specific studies have not been conducted in patients with hepatic impairment, and the drug is contraindicated in patients with severe hepatic impairment (139). Remdesivir exhibits low renal excretion (< 10%) (140). To date, there are no recommendations for dose adjustments in patients with mild to moderate renal impairment. There are no PK data available for children or women who are pregnant or breastfeeding.

The main randomized studies that evaluated the clinical efficacy of remdesivir in the treatment of hospitalized subjects, albeit open and with different primary endpoints, consistently did not show clinical benefit of remdesivir regarding mortality (141-145), with the exception of a clinical trial carried out among non-hospitalized patients who were at high risk for COVID-19 progression (146). In this study, a 3-day course of remdesivir had an acceptable safety profile and resulted in an 87% lower risk of hospitalization or death than placebo (146). Beneficial effects on time to recovery are confirmed in a single study, especially in the lower-risk population (subjects receiving low-flow oxygen therapy and starting treatment within 10 days of the onset of symptoms) (147).

Remdesivir is generally well tolerated and adverse effects are rare. However, since early reports, transient asymptomatic alanine amino-transferase (ALT) elevations were observed in most subjects in PK studies (148, 149). Transaminase increases have also been reported in COVID-19 patients treated with compassionate use remdesivir (150-152). Although transaminase elevation has been reported as a feature of COVID-19, there is a concern for possible

hepatotoxicity associated with remdesivir (152, 153). Based on the data regarding the adverse effects of remdesivir on hepatic function, caution must be taken by evaluating baseline liver function, avoiding the use of potentially hepatotoxic drugs, and monitoring liver function when using remdesivir in patients hospitalized with COVID-19 (153).

Molnupiravir

On 19/11/2021, the EMA's Committee for Medicinal Products for Human Use issued an opinion on the use of Lagevrio (the trade name of molnupiravir) for the treatment of COVID-19. The medicine can be used to treat adults with COVID-19 at high risk of developing severe forms of the disease.

Molnupiravir is an oral antiviral also known by the names EIDD-2801 and MK4482. The drug was originally developed by Drug Innovation Ventures at Emory University and subsequently acquired by Ridgeback Therapeutics in partnership with Merck & Co, USA. It belongs to the class of ribonucleoside analogues with broad-spectrum antiviral activity against a series of RNA viruses, including coronaviruses. MK-4482 was first developed as a flu shot and later "repositioned" as an oral treatment for adults with COVID-19 in a mild to moderate form. MK-4482 is a prodrug that is rapidly absorbed in the intestine and hydrolyzed into the ribonucleoside analogue N-hydroxycytidine (NHC) (154), which is widely distributed to tissues (including lungs and brain) and, similarly with remdesivir, converted to the pharmacologically active triphosphate form (NHC-TP).

The mechanism of the antiviral activity of MK-4482 is a two-step process that inhibits the RdRp through an accumulation of viral mutations beyond a biologically tolerable threshold, with consequent impairment of the normal fitness of the virus, leading to its death (154, 155). In fact, coronaviruses use the RdRp for the replication and transcription of their RNA genomes and it is therefore clear that this enzyme represents an important target for hitting the virus (156). This mechanism is distinct from that of remdesivir in which its incorporation into nascent RNA causes premature termination of RNA synthesis, stopping the growth of the RNA strand after the addition of some nucleotides. Because of this, MK-4482 has demonstrated *in vitro* activity against remdesivir-resistant SARS-CoV-2. Given its unique mechanism of action, NHC is expected to be active against viruses resistant to other antiviral agents.

At the start of the pandemic, MK-4482 was in the preclinical phase as an anti-flu drug, but a number of factors helped to move the molecule quickly into phase 1. These include: i) the favorable characteristics of the molecule to meet public health needs, 2) the in-depth non-clinical program that included model testing of various viral diseases and 3) collaboration between sponsors, multinational CROs and regulatory agencies in the US and UK (157,158). Based on the results of the planned interim analysis of the Phase 3 MOVe-OUT study (NCT04575597), Merck has discontinued patient enrollment and sought approval from the FDA. The planned interim analysis evaluated data from 775 patients enrolled in the Phase 3 MOVe-OUT study through August 5, 2021. Specifically, molnupiravir significantly reduced the risk of hospitalization or death in non-hospitalized at-risk adult patients with COVID-19 mild to moderate. In the interim analysis, molnupiravir reduced the risk of hospitalization or death by approximately 50%; 7.3% of patients receiving molnupiravir were hospitalized or died until day 29 after randomization (28/385), versus 14.1% of patients treated with placebo (53/377); p = 0.0012. Up to day 29, no deaths were reported in patients who received molnupiravir, compared with 8 deaths in patients who received placebo. The incidence of any adverse events was comparable in the molnupiravir and placebo groups (35% and 40%, respectively). Similarly, the incidence of drug-related adverse events was also comparable (12% and 11%, respectively). Fewer subjects discontinued study therapy due to an adverse event in the molnupiravir group

(1.3%) compared to the placebo group (3.4%). On the recommendation of an independent data monitoring committee and in consultation with US FDA, recruitment into the study was terminated early based on these positive results. In England, on November 4, 2021 molnupiravir was approved by the UK drug regulatory agency (Mhra) under the trade name of Lagevrio.
Molnupiravir displays in vitro activity against SARS-CoV-2 variants of concern such as B1.1.529 (omicron) (159-164), and B.1.1.7 (alpha), B.1351 (beta), P.1 (gamma) and B.1.617.2 (delta) (Merck Sharp & Dohme (UK) Limited. Lagevrio 200 mg hard capsules: UK prescribing information 2021) (165).

Paxlovid

Paxlovid, is the combination of Pfizer's investigational antiviral PF-07321332 (nirmatrelvir) and a low dose of ritonavir, an antiretroviral drug traditionally used to treat HIV. On April 6, 2021, Pfizer released the structure of an inhibitor of the 3-CL^{PRO} enzyme of the SARS-CoV-2 virus, named PF-07321332, which has been shown to be able to suppress the replication of the virus in human cells at submicromolar concentrations (166-168). PF-07321332 is the first molecule to target the SARS-CoV-2 main protease (3-CL^{PRO}). 3-CL^{PRO} is responsible for the cleavage of SARS-CoV-2 polyproteins 1a and 1b. Without the activity of SARS-CoV-2 3-CL^{PRO}, non-structural proteins 1a and 1b (including proteases) cannot perform their functions and, consequently, viral replication is inhibited (169-170). In particular, PF-07321332 is an inhibitor of a cysteine residue of 3-CL^{PRO} responsible for the enzymatic activity of the protease. The co-administration of a low dose of ritonavir (a drug used to treat HIV) helps slow down the metabolism, in which cytochrome p450 enzymes are involved, and breakdown of PF-07321332 and, consequently, to maintain higher concentrations for longer times resulting in a prolongation of its activity. A Phase 1 study (NCT04756531), conducted in double-blind and in which both single and multiple doses were tested, evaluated the safety, tolerability and pharmacokinetics of PF-07321332 in healthy individuals (171).
On November 5, 2021, Pfizer announced the first results of the NCT04960202 EPIC-HR (Evaluation of Protease Inhibition for COVID-19 in High-Risk Patients) trial. The EPIC-HR trial is a quadruple-blind study (NB: double-blind is specified in the title of the trial, but under MASK it is reported that the study was conducted so that the "Participant, Care Provider, Investigator, Outcomes Assessor" the type of treatment proposed was masked (171)) on non-hospitalized adult patients with COVID-19, who are at high risk of developing severe disease. The interim analysis assessed data from 1219 adults enrolled by September 29, 2021. By the time of the decision to stop patient recruitment, enrollment had reached 70% of the expected 3,000 patients from clinical trial centers throughout North and South America, Europe, Africa and Asia, with 45% of patients in the United States. Enrolled individuals had a laboratory-confirmed diagnosis of SARS-CoV-2 infection within a five-day period, with mild to moderate symptoms, and must have had at least one medical condition associated with an increased risk of developing COVID-19 severe. Each patient was randomized (1:1) to receive orally Paxlovid or placebo every 12 hours for five days. The scheduled interim analysis showed an 89% reduction in the risk of hospitalization or death from any cause related to COVID-19 compared to placebo in patients treated within three days of symptom onset (primary endpoint). On day 28, 0.8% of patients treated with Paxlovid went into hospitalization (3/389 hospitalized and 0 deaths), compared with 7.0% of patients who received placebo and were hospitalized or died (27/385 hospitalized with 7 subsequent deaths) ($p < 0.0001$). Similar rates of COVID-19-related hospitalization or death have been observed in patients treated within five days of symptom onset. Specifically, 1.0% of patients treated with Paxlovid were hospitalized (6/607 hospitalized, 0 deaths), compared to 6.7% of pa-

tients who received placebo (41/612 hospitalized with 10 subsequent deaths). ($p < 0.0001$). Overall, in the global population, no deaths were reported in patients who received Paxlovid compared with 17 (1.6%) deaths in patients who received placebo.

The review of the safety data included a larger cohort of 1881 patients in EPIC-HR, whose data were available at the time of the analysis. Treatment-associated adverse events were comparable between Paxlovid (19%) and placebo (21%), most of which were mild in intensity. Among patients evaluable for av events, fewer serious adverse events (1.7% *vs.* 6.6%) and fewer study drug discontinuation (2.1% *vs.* 4.1%) were observed in patients treated with Paxlovid versus those receiving placebo, respectively.

Paxlovid will be administered twice daily for five days at a dose of 300 mg (two 150 mg tablets) of PF-07321332 with one 100 mg tablet of ritonavir.

CONCLUSIONS AND PERSPECTIVES

The pharmacologic approach to control the SARS-CoV-2 diffusion in humans and the consequent COVID-19 pathology has been challenging the scientific community in the last couple of years. Here we focus on the two main aspects governing the pharmacological approach to this pandemic: i) the possibility of using drugs already available and ii) the need for new and appropriate drugs for this specific virus. After two years of a considerably high number of experiences (clinical trials of different kinds with a number of drug candidates, mainly based on the concept of "try-and-error" research) we can conclude that we have selected and adapted old drugs (treatment of COVID-19) and we have developed new drugs for the SARS-CoV-2 (prevention of COVID-19). It is reasonable to think that the results obtained are the best we could get in this short time-lapse.

The main medical aspects of the SARS-CoV-2 infection are a strong inflammation, variably distributed in different organs but with a particular propensity for the respiratory system, associated with the risk of blood coagulation. We were prepared for treating such diseases since anti-inflammatory drugs were available either from the panel of anti-cytokine medicines (small molecules or MoAbs) or with corticosteroids. By generalizing the observed results, we can admit that corticosteroids helped COVID-19 patients much more than the anti-cytokine drugs. The anticoagulants were the other family of drugs that made the difference between life and death in COVID-19 patients. All major international Societies on thrombosis rapidly produced and diffused the guidelines for the best use of anticoagulation in high-risk patients.

These approaches can be considered the best treatment options for patients with COVID-19. It must be said that all the other drugs tested on COVID-19, all of them selected on the basis of their mechanism of pharmacological action, almost failed or showed minimal effectiveness, often because of the low degree of the trial with which they were examined.

Better results, considering the appearance of new drugs, were observed with the prevention of the COVID-19, namely the control of SARS-CoV-2 infectivity. In this case, we have two separate approaches being developed: MoAbs directed to control the virus's ability to bind to the target cells and small molecules (conventional antiviral drugs) hampering the viral replication inside the infected cells. The knowledge of the virus's chemical structure and the molecular biology of its replication has considerably helped the research for optimal treatment options.

Similar to what was shown with anti-COVID-19 drugs, also these approaches suffered from successes and failures. At the beginning of the pandemic, when the only available data simply indicated that SARS-CoV-2 was a RNA coronavirus, the idea of the control of the infection lead to the use of antiviral agents already in our hands and known to be active against viruses with similar replicative steps. Unfortunately, among all the antiviral drugs tested, only

remdesivir showed some activity, sufficient to convince the regulatory agencies to suggest its use to contain the viral diffusion inside the body of the infected patients. Similar success has been documented with another repositioned anti-viral small molecule, molnupiravir. The target is the same as remdesivir, *e.g.* the RdRp, but the consequences on the viral replication, at least from the molecular aspect of this interaction, are dramatically greater, leading to the accumulation of mutations ending with a sort of replicative catastrophe. However, the most real advantage in the control of viral diffusion in the body is given by the new anti-viral small molecule nirmatrelvir that, when used in combination with ritonavir (namely Paxlovid), warrants a greater than 90% protection against the development of a severe COVID-19. The target of nirmatrelvir (the viral protease) is a specific locus of the protein that is relevant for SARS-CoV-2, and this makes the difference from the other re-positioned drugs that were tested and found inactive.

On the other hand, the control of patient's infection towards a severe COVID-19 with MoAbs showed the most intense activity by the pharmaceutical companies. A number of MoAbs became rapidly available, mostly targeting the viral proteins responsible for the viral attack on the target cells. The chapter on these drugs is exhaustive and here we simply remark the advantages and limitations of these therapies. The most important advantage is the high specificity of the MoAbs therapy and the rapid washout of the viruses from the body. At the same time, the high specificity of these drugs is also their weakness, given the high rate of mutations of their target operated by the SARS-CoV-2. In fact, the experience with these drugs showed how the virus mutated the target proteins without losing its ability to infect the target cells. This viral behaviour made the MoAbs to rapidly reduce their effectiveness with the appearance of the new variants of SARS-CoV-2, a process that forced the use of combinations of these MoAbs to prevent the viral escape.

In conclusion, the take-home messages of the pharmacological experience for the control of the SARS-CoV-2 pandemic, a very important message also for the pharmacological discipline as a whole, can be summarized as follows. Drug repositioning cannot be successful simply based on the knowledge of their molecular mode of action and the new drugs, even though based on a specific and selective target, may need a continuous arrangments in order to fulfill a complete therapeutic success.

Nonetheless, the experiences gained during these two years in the pharmacological treatment of the virus responsible for the pandemic and COVID-19 has demonstrated the possibility of significantly accelerating the development of new drugs with measurable innovation.

The MoAbs have highlighted the rapid versatility of their curvature on the targets of the virus in constant evolution and, in perspective, solve the pharmacokinetic problems of the earlier preparations with measures that significantly extend the therapeutic range. It is hoped that the new MoAbs under development will meet the needs of prescribers and patients and, together with the development of new vaccines, will prevent the spread of the virus in the body, hospitalizations and deaths of patients. In this context, an important role is attributed to the new antiviral drug nirmatrelvir, whose most intriguing advantage over existing antivirals is that it has a peculiar mechanism of action on a specific target of SARS-CoV-2 and can be easily taken orally compared to MoAbs. Considering that the SARS-CoV-2 pandemic may stabilize with an annual frequency very similar to winter flu, it is desirable that this molecule provides the inpetus for the development of other specific agents against SARS-CoV-2. Thus, this virus can be expected to offer a range of therapeutic options for its control, regardless of the type of mutations that will occur in the future. Indeed, in addition to nirmaltrevir, other products could be developed that target conserved viral pathways or whose mutations are

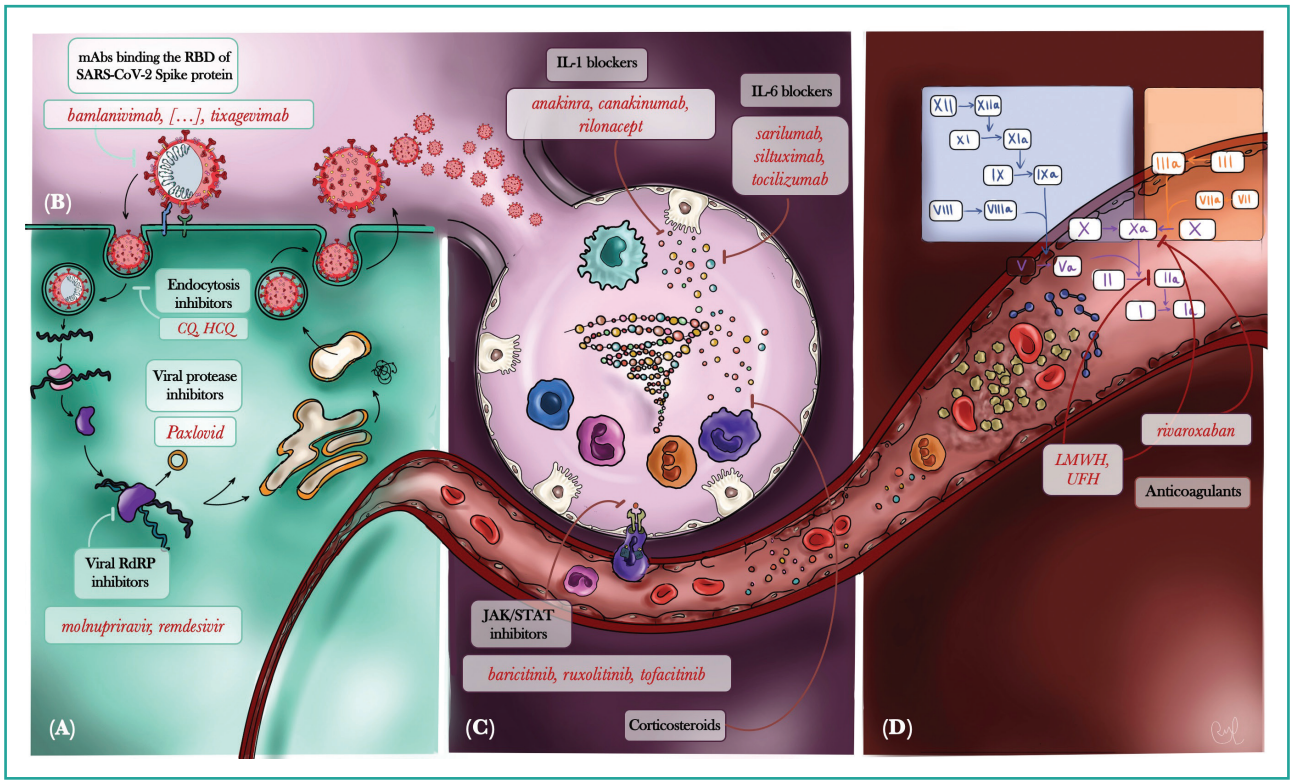

Figure 1. The main drugs studied and/or recommended for the treatment of CoViD-19 and their therapeutic targets. This figure summarizes the available or proposed medications for COVID-19 prevention or treatment and their sites of action.
(A) Drugs that inhibit viral replication. Remdesivir, paxlovid (nirmatrelvir/ritonavir) and molnupiravir are small antiviral molecules recommended by AIFA for the treatment of adults with CoViD-19 at high risk to develop severe disease. These drugs inhibit viral replication. Chloroquine (CQ) or hydroxychloroquine (HCQ) are not recommended. **(B) Monoclonal antibodies (mAbs) to prevent virus attachment to cellular proteins**. Monoclonal antibodies directed towards the RBD of the SARS-CoV-2 Spike proteins are approved as an early treatment or as pre-exposure or post-exposure prophylaxis in high-risk patients (see text for further details). **(C) Drugs that modulate the host inflammatory response**. A number of IL-1 blockers, IL-6 blockers, Jak-Stat inhibitors have been tested as repurposed drugs to dampen the host inflammatory response and the "cytokine storm" that might lead to severe complications such as acute respiratory distress syndrome (ARDS) in the lungs, intravascular coagulation, multiorgan failure, and ultimately death. Corticosteroids are also standard therapy for hospitalised patients requiring supplemental oxygen therapy (with or without mechanical ventilation) and are also recommended for home management of patients with severe CoViD-19 disease requiring supplemental oxygen. **(D) Anticoagulants**. Unfractionated heparins (UFH) or low molecular weight heparins (LMWH) are approved for the prophylaxis of thromboembolic events in patients with an acute respiratory infection and limited mobility. Oral anticoagulants such as rivaroxaban have also been tested in clinical trials but showed no evidence of efficacy.
CQ: chloroquine; HCQ: hydroxychloroquine; LMWH: low molecular weight heparins; UFH: unfractionated heparins.

extremely rare, ensuring the stability of the product in therapy.

ACKNOWLEDGEMENTS

This work was done in the frame of FIS-R2020IP_03103 granted to ABe, GN, and S.P. by Ministero dell'Università e della Ricerca (MUR). The authors thank dr. Rita Lauro for her unvaluabe support and the representation of the mechanism of drug's action summarized in **figure 1**.

ETHICS

Fundings

There were no institutional or private fundings for this article.

Conflict of interests
The authors declare that they have no conflict of interests.

Authors' contributions
AB contributed to Anticytokine agents for COVID-19 treatment, ABi contributed to Repurposed drugs for the control fo COVID-19, AG contributed to Anticoagulation treatment for COVID-19, GN contributed to Anti-SARS-CoV-2 monoclonal antibodies, SP contributed to the Indroduction, MP contribute to Antiviral small molecules, GS contributed to Introduction, Antiviral small molecules, Conclusions and to the complete revision of the review, and GR contributed to the revision of the review.

Availability of data and materials
The data underlying this manuscript are available in the article.

Ethical approval
N/A.

REFERENCES

1. Huang C, Wang Y, Li X et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. Lancet. 2020; 395(10223):497-506. Erratum in: Lancet 2020; 395(10223):496. doi: 10.1016/S0140-6736(20)30183-5.
2. Niknam Z, Jafari A, Golchin A, et al. Potential therapeutic options for COVID-19: an update on current evidence. Eur J Med Res 2022; 27(1):6. doi: 10.1186/s40001-021-00626-3.
3. Parker EPK, Shrotri M, Kampmann B. Keeping track of the SARS-CoV-2 vaccine pipeline. Nat Rev Immunol. 2020;20(11):650. doi: 10.1038/s41577-020-00455-1.
4. Gordon DE, Jang GM, Bouhaddou M, et al. A SARS-CoV-2 protein interaction map reveals targets for drug repurposing. Nature. 2020;583(7816):459-68. doi: 10.1038/s41586-020-2286-9.
5. Shanmugaraj B, Siriwattananon K, Wangkanont K, Phoolcharoen W. Perspectives on monoclonal antibody therapy as potential therapeutic intervention for Coronavirus disease-19 (COVID-19). Asian Pac J Allergy Immunol. 2020;38(1):10-8. doi: 10.12932/AP-200220-0773.
6. Al-Horani RA, Kar S. Potential Anti-SARS-CoV-2 Therapeutics That Target the Post-Entry Stages of the Viral Life Cycle: A Comprehensive Review. Viruses. 2020;12(10):1092. doi: 10.3390/v12101092.
7. Cari L, Alhosseini MN, Fiore P, et al. Cardiovascular, neurological, and pulmonary events following vaccination with the BNT162b2, ChAdOx1 nCoV-19, and Ad26.COV2.S vaccines: An analysis of European data. J Autoimmun. 2021;125:102742. doi: 10.1016/j.jaut.2021.102742.
8. Diamond MS, Kanneganti TD. Innate immunity: the first line of defense against SARS-CoV-2. Nat Immunol. 2022; 23(2):165-76. doi: 10.1038/s41590-021-01091-0.
9. Prats-Uribe A, Sena AG, Lai LYH, et al. Use of repurposed and adjuvant drugs in hospital patients with covid-19: multinational network cohort study. BMJ. 2021;373:n1038. doi: 10.1136/bmj.n1038.
10. Zhang X, Yang Y, Grimstein M, et al. Anti-SARS-CoV-2 Repurposing Drug Database: Clinical Pharmacology Considerations. CPT Pharmacometrics Syst Pharmacol. 2021;10(9):973-82. doi: 10.1002/psp4.12681.
11. Shankar-Hari M, Vale CL, Godolphin PJ, et al. WHO Rapid Evidence Appraisal for COVID-19 Therapies (REACT) Working Group. Association between administration of IL-6 antagonists and mortality among patients hospitalized for COVID-19: a meta-analysis. JAMA. 2021;326(6):499-518. doi: 10.1001/jama.2021.11330.
12. Albuquerque AM, Tramujas L, Sewanan LR, et al. Mortality Rates Among Hospi-

talized Patients With COVID-19 Infection Treated With Tocilizumab and Corticosteroids: A Bayesian Reanalysis of a Previous Meta-analysis. JAMA Netw Open. 2022;5(2):e220548. doi: 10.1001/jamanetworkopen.2022.0548.

13. Krolewiecki A, Lifschitz A, Moragas M, et al. Antiviral effect of high-dose ivermectin in adults with COVID-19: a proof-of-concept randomized trial. EClinicalMedicine. 2021;37:100959. doi: 10.1016/j.eclinm.2021.100959.
14. López-Medina E, López P, Hurtado IC, et al. Effect of ivermectin on time to resolution of symptoms among adults with mild COVID-19: a randomized clinical trial. JAMA. 2021;325(14):1426-35. doi: 10.1001/jama.2021.3071.
15. Lim SCL, Hor CP, Tay KH, et al. Efficacy of Ivermectin Treatment on Disease Progression Among Adults With Mild to Moderate COVID-19 and Comorbidities: The I-TECH Randomized Clinical Trial. JAMA. Intern Med. doi:10.1001/jamainternmed.2022.0189.
16. Huang M, Li M, Xiao F, et al. Preliminary evidence from a multicenter prospective observational study of the safety and efficacy of chloroquine for the treatment of COVID-19. Natl Sci Rev. 2020;7(9):1428-36. doi: 10.1093/nsr/nwaa113.
17. Gautret P, Lagier J-C, Parola P, et al. Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open-label non-randomized clinical trial. Int J Antimicrob Agents. 2020;56(1):105949. doi: 10.1016/j.ijantimicag.2020.105949.
18. Arshad S, Kilgore P, Chaudhry ZS, et al. Henry Ford COVID-19 Task Force . Treatment with hydroxychloroquine, azithromycin, and combination in patients hospitalized with COVID-19. Int J Infect Dis. 2020;97:396-403. doi: 10.1016/j.ijid.2020.06.099.
19. Wang M, Cao R, Zhang L, et al. Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. Cell Res. 2020;30(3):269-71. doi: 10.1038/s41422-020-0282-0.
20. Kim AHJ, Sparks JA, Liew JW, et al. A rush to judgment? Rapid reporting and dissemination of results and its consequences regarding the use of hydroxychloroquine for COVID-19. Ann Intern Med. 2020;172(12):819-21. doi: 10.7326/M20-1223.
21. World Health Organization. "Solidarity" clinical trial for COVID-19 treatments. 2020. Available from: www.who.int/emergencies/diseases/novel-coronavirus-2019/global-research-on-novel-coronavirus-2019-ncov/solidarity-clinical-trial-for-covid-19-treatments. Accessed: June 1, 2022.
22. Das RR, Jaiswal N, Dev N, et al. Efficacy and safety of anti-malarial drugs (chloroquine and hydroxy-chloroquine) in treatment of COVID-19 infection: a systematic review and meta-analysis. Front Med. 2020;7:482. doi: 10.3389/fmed.2020.00482. eCollection 2020.
23. Gasmi A, Peana M, Noor S, et al. Chloroquine and hydroxychloroquine in the treatment of COVID-19: the never-ending story. Appl Microbiol Biotechnol. 2021;105(4):1333-43. doi: 10.1007/s00253-021-11094-4.
24. Dagens A, Sigfrid L, Cai E, et al. Scope, quality, and inclusivity of clinical guidelines produced early in the covid-19 pandemic: rapid review. BMJ. 2020;369:m1936. doi: 10.1136/bmj.m1936.
25. RECOVERY Collaborative Group. Azithromycin in patients admitted to hospital with COVID-19 (RECOVERY): a randomised, controlled, open-label, platform trial. Lancet. 2021;397:605-12. doi: 10.1016/S0140-6736(21)00149-5.
26. Shang L, Zhao J, Hu Y, et al. On the use of corticosteroids for 2019-nCoV pneumonia. Lancet. 2020;395:683-4. doi: 10.1016/S0140-6736(20)30361-5.

27. Russell CD, Millar JE, Baillie JK. Clinical evidence does not support corticosteroid treatment for 2019-nCoV lung injury. Lancet. 2020;395:473-5. doi: 10.1016/S0140-6736(20)30317-2.
28. RECOVERY Collaborative Group, Horby P, Lim WS, et al. Dexamethasone in Hospitalized Patients with Covid-19. N Engl J Med. 2021;384(8):693-704. doi: 10.1056/NEJMoa2021436.
29. McGonagle D, O'Donnell JS, Sharif K, et al. Immune mechanisms of pulmonary intravascular coagulopathy in COVID-19 pneumonia. Lancet Rheumatol. 2020;2(7):e437-e445. doi: 10.1016/S2665-9913(20)30121-1.
30. Shaw RJ, Bradbury C, Abrams ST, et al. COVID-19 and immunothrombosis: emerging understanding and clinical management. Br J Haematol. 2021;194:518-29. doi: 10.1111/bjh.17664.
31. Middeldorp S, Coppens M, van Haaps TF, et al. Incidence of venous thromboembolism in hospitalized patients with COVID-19. J Thromb Haemost. 2020;18(8):1995-2002. doi: 10.1111/jth.14888.
32. Lopes RD, de Barros E Silva PGM, Furtado RHM, et al. Therapeutic versus prophylactic anticoagulation for patients admitted to hospital with COVID-19 and elevated D-dimer concentration (ACTION): an open-label, multicentre, randomised, controlled trial. Lancet. 2021;397(10291):2253-63.
33. Lawler PR, Goligher EC, Berger JS, et al. Therapeutic anticoagulation with heparin in noncritically ill patients with COVID-19. N Engl J Med. 2021;385(9):790-802.
34. Goligher EC, Bradbury CA, McVerry BJ, et al. Therapeutic anticoagulation with heparin in critically ill patients with COVID-19. N Engl J Med. 2021;385(9):777-89.
35. Spyropoulos AC, Goldin M, Giannis D, et al. Efficacy and safety of therapeutic-dose heparin vs standard prophylactic or intermediate-dose heparins for thromboprophylaxis in high-risk hospitalized patients with COVID-19: the HEP-COVID randomized clinical trial. JAMA Intern Med. 2021;181(12):1612-1620.
36. Sholzberg M, Tang GH, Rahhal H, et al. Heparin for moderately ill patients with COVID-19. medRxiv. 2021; 2021.07.08.21259351. doi:10.1101/2021.07.08.21259351.
37. Sadeghipour P, Talasaz AH, Rashidi F, et al. Effect of intermediate-dose vs standard-dose prophylactic anticoagulation on thrombotic events, extracorporeal membrane oxygenation treatment, or mortality among patients with COVID-19 admitted to the intensive care unit: the INSPIRATION randomized clinical trial. JAMA. 2021; 325(16):1620-1630.
38. Lazaridis D, Leung S, Kohler L, et al. The Impact of Anticoagulation on COVID-19 (SARS CoV-2) Patient Outcomes: A Systematic Review. J Pharm Pract. 2021; 8971900211015055. doi: 10.1177/08971900211015055.
39. Satarker S, Tom AA, Shaji RA, et al. JAK-STAT pathway inhibition and their implications in COVID-19 therapy. Postgrad Med. 2020;1-19. doi: 10.1080/00325481.2020.1855921.
40. Mulchandani R, Lyngdoh T, Kakkar AK. Deciphering the COVID-19 cytokine storm: systematic review and meta-analysis. Eur J Clin Invest. 2020;51:e13429. doi: 10.1111/eci.13429.
41. Zhang JJ, Dong X, Liu GH, et al. Risk and protective factors for COVID-19 morbidity, severity, and mortality. Clin Rev Allergy Immunol. 2022;1-18. doi: 10.1007/s12016-022-08921-5.
42. Guo YR, Cao QD, Hong ZS et al. The origin, transmission and clinical therapies on coronavirus disease 2019 (COVID-19) outbreak – an update on the status. Mil Med Res. 2020;7:11. doi: 10.1186/s40779-020-00240-0.
43. Mehta P, McAuley DF, Brown M, et al. COVID-19: consider cytokine storm syn-

dromes and immunosuppression. Lancet. 2020; 395:1033-4. doi: 10.1016/S0140-6736(20)30628-0.
44. Giamarellos-Bourboulis EJ, Netea MG, Rovina N et al. Complex immune dysregulation in COVID-19 patients with severe respiratory failure. Cell Host Microbe. 2020;27:992-1000. doi: 10.1016/j.chom.2020.04.009.
45. Melenotte C, Silvin A, Goubet AG, et al. Immune responses during COVID-19 infection. Oncoimmunology. 2020;9:1807836. doi: 10.1080/2162402X.2020.1807836.
46. Chen G, Wu D, Guo W, et al. Clinical and immunological features of severe and moderate coronavirus disease 2019. J Clin Invest. 2020;130(5): 2620-9. doi: 10.1172/JCI137244.
47. Chen X, Zhao B, Qu Y, et al. Detectable serum severe acute respiratory syndrome coronavirus 2 viral load (RNAemia) is closely correlated with drastically elevated interleukin 6 level in critically ill patiens with coronavirus disease 2019. Clin Infect Dis. 2020;71(8):1937-42. doi: 10.1093/cid/ciaa449.
48. Sanders JM, Monogue ML, Jodlowski TZ, et al. Pharmacological treatments for coronavirus disease 2019 (COVID-19) a review. JAMA. 2020;323:1824-36. doi: 10.1001/jama.2020.6019.
49. Langer-Gould A, Smith JB, Gonzales EG, et al. Early identification of COVID-19 cytokine storm and treatment with anakinra or tocilizumab. Int J Infect Dis. 2020;99:291-7. doi: 10.1016/j.ijid.2020.07.081.
50. Gritti G, Raimondi F, Ripamonti D, et al. IL-6 signalling pathway inactivation with siltuximab in patients with COVID-19 respiratory failure: an observational cohort study. 2020. Available from: https://www.medrxiv.org/content/10.1101/2020.04.01.20048561v4. Accessed: June 9, 2022.
51. Della Torre E, Campochiaro C, Cavalli G, et al. Interleukin-6 blockade with sarilumab in severe COVID-19 pneumonia with systemic hyperinflammation: an open-label cohort study. Ann Rheum Dis. 2020;79:1277-85. doi: 10.1136/annrheumdis-2020-218122.
52. The CORIMUNO-19 Collaborative group. Effect of anakinra versus usual care in adults in hospital with COVID-19 and mild-to-moderate pneumonia (CORIMUNO-ANA-1); a randomized controlled trial. Lancet Respir Med. 2021;9:295-304. doi: 10.1016/S2213-2600(20)30556-7.
53. The REMAP-CAP Investigators, Derde LPG. Effectiveness of tocilizumab, sarilumab, and anakinra for critically ill patients with COVID-19 The REMAP-CAP COVID-19 Immune Modulation Therapy Domain Randomized Clinical Trial. medRxiv 2021. doi: /10.1101/2021.06.18.21259133.
54. Davidson M, Menon S, Chaimani A, et al. Interleukin-1 blocking agents for treating COVID-19. Cochrane Database of Systematic Reviews. 2022;1(1):CD015308. doi: 10.1002/14651858.
55. Caricchio R, Abbate A, Gordeev I, et al. Effect of canakinumab vs placebo on survival without invasive mechanical ventilation in patients hospitalized with severe COVID-19. JAMA. 2021;326:230-9. doi: 10.1001/jama.2021.9508.
56. Rovina N, Akinosoglou K, Eugen-Olsen J, et al. soluble urokinase plasminogen activator receptor (suPAR) as an early predictor of severe respiratory failure in patients with COVID-19 pneumonia. Crit Care. 2020;24:187. doi: 10.1186/s13054-020-02897-4.
57. Azam TU, Shadid HR, Blakely P, et al. Soluble urokinase receptor (SuPAR) in COVID-19-related AKI. J Am Soc Nephrol. 2020;31:2725-35. doi: 10.1681/ASN.2020060829.
58. Kyriazopoulou E, Panagopoulos P, Metallidis S, et al. An open label trial of anakinra to prevent respiratory failure in COVID-19. eLife. 2021;10: e66125. doi: 10.7554/eLife.66125.
59. Kyriazopoulou E, Poulakou G, Milionis H, et al. Early treatment of COVID-19 with

anakinra guided by soluble urokinase plasminogen receptor plasma levels: a double-blind, randomized controlled phase 3 trial. Nat Med. 2021;27:1752-60. doi: 10.1038/s41591-021-01499-z.
60. Rich C, Eriksson D, Dolfi F, et al. Patients diagnosed with COVID-19 and treated with anakinra: a real-world study in the USA. Clin Exp Immunol. 2022;1-9. doi: 10.1093/cei/uxab024.
61. Atal S, Fatima Z. IL-6 inhibitors in the treatment of serious COVID-19: a promising therapy? Pharmaceut Med. 2020;34:223-31. doi: 10.1007/s40290-020-00342-z.
62. Tanaka T, Narazaki M, Kishimoto T. Immunotherapeutic implications of IL-6 blockade for cytokine storm. Immunotherapy. 2016;8:959-70. doi: 10.2217/imt-2016-0020.
63. Zhang C, Wu Z, Li JW, et al. Cytokine release syndrome in severe COVID-19: interleukin-6 receptor antagonist tocilizumab may be the key to reduce mortality. Int J Antimicrob Agents. 2020;55:105954. doi: 10.1016/j.ijantimicag.2020.105954.
64. Sheppard M, Laskou F, Stapleton PP, et al. Tocilizumab (Actemra). Human Vaccines Immunother. 2017;13:1972-88. doi: 10.1080/21645515.2017.1316909.
65. Xu X, Han M, Li T et al. Effective treatment of severe COVID-19 patients with tocilizumab. PNAS. 2020;117:10970-5. doi: 10.1073/pnas.2005615117.
66. Recovery Collaborative Group. Tocilizumab in patients admitted to hospital with COVID-19 (RECOVERY): a randomised, controlled, open-label, platform trial. Lancet. 2021;397:1637-45. doi: 10.1016/S0140-6736(21)00676-0.
67. The REMAP-CAP Investigators. Interleukin-6 receptor antagonists in critically ill patients with COVID-19. NEJM. 2021;384:1491-502. doi: 10.1056/NEJMoa2100433.
68. Jones SA, Hunter CA. Is IL-6 a key cytokine target for therapy in COVID-19? Nat Rev Immunol. 2021;21:337-9. doi: 10.1038/s41577-021-00553-8.
69. Declercq J, Van Damme KFA, De Leeuw E, et al. Effect of anti-interleukin drugs in patients with COVID-19 and signs of cytokine release syndrome (COV-AID): a factorial, randomises, controlled trial. Lancet Respir Med. 2021;9:1427-38. doi: 10.1016/S2213-2600(21)00377-5.
70. Potere N, Batticciotto A, Vecchiè A, et al. The role of IL-6 and IL-6 blockade in COVID-19. Exp Rev Clin Immunol. 2021;17:601-18. doi: 10.1080/1744666X.2021.1919086.
71. Grivennikov S, Karin M. Autocrine IL-6 signaling: a key event in tumorigenesis? Cancer Cell. 2008;13:7-9. doi: 10.1016/j.ccr.2007.12.020.
72. Zhang X, Zhang Y, Qiao W, et al. Baricitinib, a drug with potential effect to prevent SARS-CoV-2 from entering target cells and control cytokine storm induced by COVID-19. Int Immunopharmacol. 2020;86:106749. doi: 10.1016/j.intimp.2020.106749.
73. Seif F, Aazami H, Khoshmirsafa M, et al. JAK inhibition as a new treatment strategy for patients with COVID-19. Int Arch Allergy Imunol. 2020;181:467-75. doi: 10.1159/000508247.
74. Choy EH. Clinical significance of Janus Kinase inhibitor selectivity. Rheumathology. 2019;58:953-62. doi: 10.1093/rheumatology/key339.
75. Guimaraes PO, Quirk D, Furtado RH, et al. Tofacitinib in patients hospitalized with Covid-19 pneumonia. NEJM. 2021;385:406-15. doi: 10.1056/NEJMoa2101643
76. Ip A, Berry DA, Hansen E, et al. Hydroxychloroquine and tocilizumab therapy in COVID-19 patients – an observational study. PLoS ONE. 2020;15: e0237693. doi: 10.1371/journal.pone.0237693.
77. Cao Y, Wei J, Zou L, et al. Ruxolitinib in treatment of severe coronavirus disease 2019 (COVID-19): a multicentre, single-blind, randomized controlled trial.

J Allergy Clin Immunol. 2020;146:137-46.e.3. doi: 10.1016/j.jaci.2020.05.019.
78. D'Alessio A, Del Poggio P, Bracchi F, et al. Low-dose ruxolitinib plus steroid in severe SARS-CoV-2 pneumonia. Leukemia. 2021;35:635-8. doi: 10.1038/s41375-020-01087-z.
79. Goker BB, Biray AC. The potential of JAK/STAT pathway inhibition by ruxolitinib in the treatment of COVID-19. Cytokine Growth Factor Rev. 2020;51-61. doi: 10.1016/j.cytogfr.2020.06.013.
80. Luo W, Li YX, Jiang LJ, et al. Targeting JAK-STAT signalling to control cytokine release syndrome in COVID-19. Trends Pharmacol Sci. 2020;41:531-43. doi: 10.1016/j.tips.2020.06.007.
81. Bronte V, Ugel S, Tinazzi E, et al. Baricitinib restrains the immune dysregulation in patients with severe COVID-19. J Clin Invest. 2020;130:6409-16. doi: 10.1172/JCI141772.
82. Kalil AC, Patterson TF, Mehta AK, et al. Baricitinib plus remdesivir for hospitalized adults with COVID-19. NEJM. 2021;384:975-807. doi: 10.1056/NEJMoa2031994.
83. Marconi VC, Ramanan AV, de Bono S, et al. Efficacy and safety of baricitinib for the treatment of hospitalised adults with COVID-19 (COV-BARRIER): a randomised, double-blind, parallel-group, placebo-controlled phase 3 trial. Lancet Respir Med. 2021;19:1-12. doi: 10.1016/S2213-2600(21)00331-3.
84. Chow KT, Gale M Jr. Snapshot: interferon signaling. Cell. 2015;163:1808. doi: 10.1016/j.cell.2015.12.008.
85. Platanias LC. Mechanisms of type I- and type II-interferon-mediated signalling. Nat Rev Immunol. 2005;5:375-86. doi: 10.1038/nri1604.
86. Blair HA. Fedratinib: first approval. Drugs. 2019;79:1719-25. doi: 10.1007/s40265-019-01205-x.
87. Wu D, Yang XO. Th17 responses in cytokine storm of COVID-19: an emerging target of JAK2 inhibitor fedratinib. J Microbil Immunol Infect. 2020;53:368-70. doi: 10.1016/j.jmii.2020.03.005. doi: 10.1016/j.jmii.2020.03.005.
88. Gadina M, Le MT, Schwartz DM, et al. Janus kinases to jakinibs: from basic insights to clinical practice. Rheumatology. 2019;50:i4-i16. doi: 10.1093/rheumatology/key432.
89. Spinelli FR, Conti F, Gadina M. HiJAKing SARS-CoV-2? The potential role of JAK inhibitors in the management of COVID-19. Sci Immunol. 2020;5:eabc5367. doi: 10.1126/sciimmunol.abc5367.
90. Hoffmann M, Kleine-Weber H, Schroeder S, et al. SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. Cell. 2020;181(2):271-80. e8. doi: 10.1016/j.cell.2020.02.052.
91. Legros V, Denolly S, Vogrig M, et al. A longitudinal study of SARS-CoV-2-infected patients reveals a high correlation between neutralizing antibodies and COVID-19 severity. Cell Mol Immunol. 2021;18(2):318-27. doi: 10.1038/s41423-020-00588-2.
92. Ameratunga R, Woon ST, Lea E, et al. The (apparent) antibody paradox in COVID-19. Expert Rev Clin Immunol. 2022;18(4):335-45. doi: 10.1080/1744666X.2022.2044797.
93. Ortigoza MB, Yoon H, Goldfeld KS, et al. Efficacy and Safety of COVID-19 Convalescent Plasma in Hospitalized Patients: A Randomized Clinical Trial. JAMA Intern Med. 2022;182(2):115-26. doi: 10.1001/jamainternmed.2021.6850.
94. van den Berg K, Glatt TN, Vermeulen M, et al. Convalescent plasma in the treatment of moderate to severe COVID-19 pneumonia: a randomized controlled trial (PROTECT-Patient Trial). Sci Rep. 2022;12(1):2552. doi: 10.1038/s41598-022-06221-8.
95. Fierz W, Walz B. Antibody Dependent Enhancement Due to Original Antigenic Sin and the Development of SARS. Front

Immunol. 2020;11:1120. doi: 10.3389/fimmu.2020.01120.
96. Lee WS, Wheatley AK, Kent SJ, et al. Antibody-dependent enhancement and SARS-CoV-2 vaccines and therapies. Nat Microbiol. 2020; 5(10):1185-91. doi: 10.1038/s41564-020-00789-5.
97. Wan Y, Shang J, Sun S, et al. Molecular Mechanism for Antibody-Dependent Enhancement of Coronavirus Entry. J Virol. 2020;94(5):e02015-19. doi: 10.1128/JVI.02015-19.
98. Taylor PC, Adams AC, Hufford MM, de la Torre I, Winthrop K, Gottlieb RL. Neutralizing monoclonal antibodies for treatment of COVID-19. Nat Rev Immunol. 2021;21(6):382-93. doi: 10.1038/s41577-021-00542-x.
99. RECOVERY Collaborative Group, Horby PW, Mafham M, et al. Casirivimab and imdevimab in patients admitted to hospital with COVID-19 (RECOVERY): a randomised, controlled, open-label, platform trial. medRxiv. 2021;399(10325):665-76. doi: 10.1101/2021.06.15.21258542
100. Chen P, Nirula A, Heller B, et al. SARS-CoV-2 Neutralizing Antibody LY-CoV555 in Outpatients with Covid-19. N Engl J Med. 2021;384(3):229-37.
101. Gottlieb RL, Nirula A, Chen P Effect of Bamlanivimab as Monotherapy or in Combination With Etesevimab on Viral Load in Patients With Mild to Moderate COVID-19: A Randomized Clinical Trial. JAMA. 2021; 325(7):632-44
102. ACTIV-3/TICO LY-CoV555 Study Group, Lundgren JD, Grund B, et al. A Neutralizing Monoclonal Antibody for Hospitalized Patients with Covid-19. N Engl J Med. 2021; 384(10):905-14.
103. ACTIV-3/Therapeutics for Inpatients with COVID-19 (TICO) Study Group. Efficacy and safety of two neutralising monoclonal antibody therapies, sotrovimab and BRII-196 plus BRII-198, for adults hospitalised with COVID-19 (TICO): a randomised controlled trial. Lancet Infect Dis. 2021;S1473-3099(21)00751-9. doi: 10.1016/S1473-3099(21)00751-9.
104. Lee TC, Morris AM, Grover SA, Murthy S, McDonald EG. Outpatient Therapies for COVID-19: How Do We Choose? Open Forum Infect Dis. 2022;9(3):ofac008. doi: 10.1093/ofid/ofac008.
105. Wu F, Zhao S, Yu B, et al. A new coronavirus associated with human respiratory disease in China. Nature 579(7798):265-269. 2020. doi: 10.1038/s41586-020-2008-3.
106. Zhao J, Guo S, Yi D, et al. A cell-based assay to discover inhibitors of SARS-CoV-2 RNA dependent RNA polymerase. Antiviral Res. 2021;190:105078. doi: 10.1016/j.antiviral.2021.105078.
107. Guruprasad K. Mutations in human SARS-CoV-2 spike proteins, potential drug binding and epitope sites for COVID-19 therapeutics development. Curr Res Struct Biol. 2022;4:41-50. doi: 10.1016/j.crstbi.2022.01.002.
108. Miguez-Rey E, Choi D, Kim S, et al. Monoclonal antibody therapies in the management of SARS-CoV-2 infection. Expert Opin Investig Drugs. 2022;31(1):41-58. doi: 10.1080/13543784.2022.2030310.
109. Mahase E. Covid-19: AstraZeneca says its antibody drug AZD7442 is effective for preventing and reducing severe illness. BMJ. 2021;375:n2860. doi: 10.1136/bmj.n2860.
110. O' Brien MP, Forleo-Neto E, Musser BJ, et al. Subcutaneous REGEN-COV Antibody Combination to Prevent Covid-19. N Engl J Med. 2021;385(13):1184-95. doi: 10.1056/NEJMoa2109682.
111. Cohen MS, Nirula A, Mulligan MJ, et al. Effect of Bamlanivimab vs Placebo on Incidence of COVID-19 Among Residents and Staff of Skilled Nursing and Assisted Living Facilities: A Randomized Clinical Trial. JAMA. 2021;326(1):46-55. doi: 10.1001/jama.2021.8828.
112. Dougan M, Nirula A, Azizad M, et al. Bamlanivimab plus Etesevimab in Mild or Moderate Covid-19. N Engl J Med.

2021;385(15):1382-92. doi: 10.1056/NEJMoa2102685.
113. Weinreich DM, Sivapalasingam S, Norton T, et al. REGEN-COV Antibody Combination and Outcomes in Outpatients with Covid-19. N Engl J Med. 2021;385(23):e81. doi: 10.1056/NEJMoa2108163.
114. Lee JY, Lee JY, Ko JH, et al. Effectiveness of Regdanvimab Treatment in High-Risk COVID-19 Patients to Prevent Progression to Severe Disease. Front Immunol. 2021;12:772320. doi: 10.3389/fimmu.2021.772320.
115. Available from: https://www.phe.gov/emergency/events/COVID19/investigation-MCM/Bamlanivimab-etesevimab/Pages/important-update-24Jan2022.aspx. Accessed: June 9, 2022.
116. Tixagevimab and Cilgavimab (Evusheld) for Pre-Exposure Prophylaxis of COVID-19. JAMA. 2022;327(4):384-85. doi:10.1001/jama.2021.24931
117. Cameroni E, Bowen JE, Rosen LE, et al. Broadly neutralizing antibodies overcome SARS-CoV-2 Omicron antigenic shift. Nature. 2022;602(7898):664-70. doi: 10.1038/s41586-021-04386-2.
118. Pinto D, Park YJ, Beltramello M, et al. Cross-neutralization of SARS-CoV-2 by a human monoclonal SARS-CoV antibody. Nature. 2020;583(7815):290-95. doi: 10.1038/s41586-020-2349-y.
119. Gupta A, Gonzalez-Rojas Y, Juarez E, et al. Early Treatment for Covid-19 with SARS-CoV-2 Neutralizing Antibody Sotrovimab. N Engl J Med. 2021;385(21):1941-50. doi: 10.1056/NEJMoa2107934.
120. Available from: https://www.fda.gov/media/149534/download. Accessed: June 9, 2022.
121. Available from: https://www.fda.gov/media/145611/download. Accessed: June 9, 2022.
122. Available from: https://www.fda.gov/media/149535/download. Accessed: June 9, 2022.
123. Available from: https://www.ema.europa.eu/documents/referral/regn-cov2-antibody-combination-casirivimab/imdevimab-covid19-article-53-procedure-conditions-use-conditions-distribution-patients-targeted_en.pdf. Accessed: June 9, 2022.
124. Available from: https://www.ema.europa.eu/documents/referral/sotrovimab-also-known-vir-7831-gsk4182136-covid19-article-53-procedure-conditions-use-conditions_en.pdf. Accessed: June 9, 2022.
125. Shah M, Woo HG. Omicron: A Heavily Mutated SARS-CoV-2 Variant Exhibits Stronger Binding to ACE2 and Potently Escapes Approved COVID-19 Therapeutic Antibodies. Front Immunol. 2022;12:830527. doi: 10.3389/fimmu.2021.830527.
126. VanBlargan LA, Errico JM, Halfmann PJ, et al. An infectious SARS-CoV-2 B.1.1.529 Omicron virus escapes neutralization by therapeutic monoclonal antibodies. Nat Med. 2022;28(3):490-95. doi: 10.1038/s41591-021-01678-y.
127. Available from: https://www.phe.gov/emergency/events/COVID19/investigation-MCM/cas_imd/Pages/default.aspx. Accessed: June 9, 2022.
128. Duan X, Shi R, Liu P, et al. A non-ACE2-blocking neutralizing antibody against Omicron-included SARS-CoV-2 variants. Signal Transduct Target Ther. 2022;7(1):23. doi: 10.1038/s41392-022-00879-2.
129. Andreano E, Nicastri E., Paciello I et al. Extremely potent human monoclonal antibodies from COVID-19 convalescent patients. Cell. 2021;184(7):1821-35.e16. doi: 10.1016/j.cell.2021.02.035.
130. Rommasi F, Nasiri MJ, Mirsaiedi M. Antiviral drugs proposed for COVID-19: action mechanism and pharmacological data. Eur Rev Med Pharmacol Sci. 2021;25:4163-73. doi: 10.26355/eurrev_202106_26060.
131. Zhao M, Zhang J, Li H, et al. Recent progress of antiviral therapy for coro-

navirus disease. J Eur J Pharmacol. 2021;5;890:173646. doi: 10.1016/j.ejphar.2020.173646.
132. Siegel D, Hui HC, Doerffler E, et al. Discovery and Synthesis of a Phosphoramidate Prodrug of a Pyrrolo[2,1-f][triazin-4-amino] Adenine C-Nucleoside (GS-5734) for the Treatment of Ebola and Emerging Viruses. J Med Chem. 2017;60(5):1648-61. doi: 10.1021/acs.jmedchem.6b01594
133. Mulangu S, Dodd LE, Davey RT, et al. A Randomized, Controlled Trial of Ebola Virus Disease Therapeutics. N Engl J Med. 2019;381:2293-303.
134. Sheahan TP, Sims AC, Graham RL, et al. Broad-spectrum antiviral GS-5734 inhibits both epidemic and zoonotic coronaviruses. Sci Transl Med. 2017;9(396):eaal3653. doi: 10.1126/scitranslmed.aal3653.
135. McCreary EK, Pogue JM. Coronavirus Disease 2019 Treatment: A Review of Early and Emerging Options. Open Forum Infect Dis. 2020;7(4):ofaa105. doi: 10.1093/ofid/ofaa105.
136. Gordon CJ, Tchesnokov EP, Feng JY, et al. The antiviral compound remdesivir potently inhibits RNA-dependent RNA polymerase from Middle East respiratory syndrome coronavirus. J Biol Chem. 2020;295:4773-79. doi: 10.1074/jbc.AC120.013056.
137. Gordon CJ, Tchesnokov EP, Woolner E, et al. Remdesivir is a direct-acting antiviral that inhibits RNA-dependent RNA polymerase from severe acute respiratory syndrome coronavirus 2 with high potency. J Biol Chem. 2020;295:6785-97. doi: 10.1074/jbc.RA120.013679.
138. Shannon A, Le NT-T, Selisko B, et al. Remdesivir and SARS-CoV-2: Structural requirements at both nsp12 RdRp and nsp14 Exonuclease active-sites. Antiviral Res. 2020;178:104793. doi: 10.1016/j.antiviral.2020.104793.
139. Available from: https://www.ema.europa.eu/en/documents/other/summary-compassionate-use-remdesivir-gilead_en.pdf. Accessed: June 9, 2022.
140. Williamson BN, Feldmann F, Schwarz B, et al. Clinical benefit of remdesivir in rhesus macaques infected with SARS-CoV-2. Nature. 2020;585(7824):273-76. doi: 10.1038/s41586-020-2423-5.
141. WHO Solidarity Trial Consortium, Pan H, Peto R, et al. Repurposed Antiviral Drugs for Covid-19 - Interim WHO Solidarity Trial Results. N Engl J Med. 2021;384(6):497-511. doi: 10.1056/NEJMoa2023184.
142. Goldman JD, Lye DCB, Hui DS, et al. Remdesivir for 5 or 10 Days in Patients with Severe Covid-19. N Engl J Med. 2020;383(19):1827-37. doi: 10.1056/NEJMoa2015301.
143. Spinner CD, Gottlieb RL, Criner GJ, et al. Effect of Remdesivir vs Standard Care on Clinical Status at 11 Days in Patients With Moderate COVID-19: A Randomized Clinical Trial. JAMA. 2020;324(11):1048-57. doi: 10.1001/jama.2020.16349.
144. Wang Y, Zhang D, Du G, et al. Remdesivir in adults with severe COVID-19: a randomised, double-blind, placebo-controlled, multicentre trial. Lancet. 2020;395(10236):1569-78. doi: 10.1016/S0140-6736(20)31022-9.
145. Ader F, Bouscambert-Duchamp M, Hites M, et al. Remdesivir plus standard of care versus standard of care alone for the treatment of patients admitted to hospital with COVID-19 (DisCoVeRy): a phase 3, randomised, controlled, open-label trial. Lancet Infect Dis. 2022;22(2):209-21. doi: 10.1016/S1473-3099(21)00485-0.
146. Gottlieb RL, Vaca CE, Paredes R, et al. Early Remdesivir to Prevent Progression to Severe Covid-19 in Outpatients. N Engl J Med. 2022;386(4):305-15. doi: 10.1056/NEJMoa2116846.
147. Beigel JH, Tomashek KM, Dodd LE, et al. Remdesivir for the Treatment of Covid-19 - Final Report. N Engl J Med. 2020;383(19):1813-26. doi: 10.1056/NEJMoa2007764.
148. Grein J, Ohmagari N, Shin D, et al. Compassionate Use of Remdesivir for Patients with Severe Covid-19. N Engl J Med.

2020;382(24):2327-36. doi: 10.1056/NEJMoa2007016.
149. Munster VJ, Feldmann F, Williamson BN, et al. Respiratory disease and virus shedding in rhesus macaques inoculated with SARS-CoV-2. bioRxiv [Preprint]. doi: 10.1101/2020.03.21.001628.
150. COVID-19 Investigation Team. Clinical and virologic characteristics of the first 12 patients with coronavirus disease 2019 (COVID-19) in the United States. Nat Med. 2020;26(6):861-8. doi: 10.1038/s41591-020-0877-5.
151. Lescure FX, Bouadma L, Nguyen D, et al. Clinical and virological data of the first cases of COVID-19 in Europe: a case series. Lancet Infect Dis. 2020;20(6):697-706. doi: 10.1016/S1473-3099(20)30200-0.
152. Jorgensen SCJ, Kebriaei R, Dresser LD. Remdesivir: Review of Pharmacology, Pre-clinical Data, and Emerging Clinical Experience for COVID-19. Pharmacotherapy. 2020;40(7):659-71. doi: 10.1002/phar.2429.
153. Aleem A, Mahadevaiah G, Shariff N, et al. Hepatic manifestations of COVID-19 and effect of remdesivir on liver function in patients with COVID-19 illness. Proc (Bayl Univ Med Cent). 2021;34(4):473-77. doi: 10.1080/08998280.2021.1885289.
154. Malone B, Campbell EA. Molnupiravir: coding for catastrophe. Nat Struct Mol Biol. 2021;(9):706-8. doi: 10.1038/s41594-021-00657-8.
155. Imran M, Kumar Arora M, Asdaq SMB, et al. Discovery, Development, and Patent Trends on Molnupiravir: A Prospective Oral Treatment for COVID-19. Molecules. 2021;26(19):5795. doi: 10.3390/molecules26195795.
156. Kabinger F, Stiller C, Schmitzová J, et al. Mechanism of molnupiravir-induced SARS-CoV-2 mutagenesis. Nat Struct Mol Biol. 2021;28(9):740-46. doi: 10.1038/s41594-021-00651-0.
157. Holman W, Holman W, McIntosh S, et al. Accelerated first-in-human clinical trial of EIDD-2801/MK-4482 (molnupiravir), a ribonucleoside analog with potent antiviral activity against SARS-CoV-2. Trials. 2021;22(1):561. doi: 10.1186/s13063-021-05538-5.
158. Painter WP, Holman W, Bush JA, et al. Human Safety, Tolerability, and Pharmacokinetics of Molnupiravir, a Novel Broad-Spectrum Oral Antiviral Agent with Activity Against SARS-CoV-2. Antimicrob Agents Chemother. 2021;65(5):e02428-20. doi: 10.1128/AAC.02428-20.
159. Dabrowska A, Szczepanski A, Botwina P, et al. Efficacy of antiviral drugs against the omicron variant of SARS-CoV-2. bioRxiv. 2021. doi: 10.1101/2021.12.21.47326.
160. Bojkova D, Widera M, Ciesek S, et al. Reduced interferon antagonism but similar drug sensitivity in Omicron variant compared to Delta variant of SARS-CoV-2 isolates. Cell Res. 2022;32(3):319-21.
161. Li P, Wang Y, Lavrijsen M, et al. SARS-CoV-2 Omicron variant is highly sensitive to molnupiravir, nirmatrelvir, and the combination. Cell Res. 2022;32(3):322-24. doi: 10.1038/s41422-022-00618-w.
162. Rosales R, McGovern BL, Rodriguez ML, et al. Nirmatrelvir, Molnupiravir, and Remdesivir maintain potent in vitro activity against the SARS-CoV-2 Omicron variant. bioRxiv. 2022. doi: 10.1101/2022.01.17.476685
163. Takashita E, Kinoshita N, Yamayoshi S, et al. Efficacy of Antibodies and Antiviral Drugs against Covid-19 Omicron Variant. N Engl J Med. 2022;386(10):995-98. doi: 10.1056/NEJMc2119407.
164. Vangeel L, Chiu W, De Jonghe S, et al. Remdesivir, Molnupiravir and Nirmatrelvir remain active against SARS-CoV-2 Omicron and other variants of concern. Antiviral Res. 2022;198:105252. doi: 10.1016/j.antiviral.2022.105252.
165. Available from: https://products.mhra.gov.uk/. Accessed: June 9, 2022.
166. Macchiagodena M, Pagliai M, Procacci P. Characterization of the non-covalent interaction between the PF-07321332 inhibitor and the SARS-CoV-2 main protease. J

Mol Graph Model. 2022;110:108042. doi: 10.1016/j.jmgm.2021.108042.
167. Halford B, Howes L, Widener A. How Covid-19 Has Changed the Culture of Science. Chem Eng News. 2021;99:31-7. Available from: https://cen.acs.org/biological-chemistry/infectious-disease/How-COVID-19-has-changed-the-culture-of-science/99/i3. Accessed: June 10, 2022.
168. Ionescu MI. An Overview of the Crystallized Structures of the SARS-CoV-2. Protein J. 2020;6:600-18. doi: 10.1007/s10930-020-09933-w.
169. Reina J, Iglesias C. Nirmatrelvir plus ritonavir (Paxlovid) a potent SARS-CoV-2 3CL-pro protease inhibitor combination. Rev Esp Quimioter. 2022;21:reina21feb2022. doi: 10.37201/req/002.2022.
170. Owen DR, Allerton CMN, Anderson AS, et al. An oral SARS-CoV-2 M^{pro} inhibitor clinical candidate for the treatment of COVID-19. Science. 2021;374(6575):1586-93. doi: 10.1126/science.abl4784.
171. Available from: https://clinicaltrials.gov/ct2/show/study/NCT04960202. Accessed: June 9, 2022.