COVID-19 Committee of the Lincei Academy

Drugs for the Prevention and Treatment of COVID-19 and its Complications: A 2023 update

What we learned in the past years

Carlo Patrono, Stefano Schiaffino, M. Gabriella Santoro, Garret A. FitzGerald, Gennaro Melino and Giuseppe Remuzzi, on behalf of the COVID-19 Committee of the Lincei Academy

Executive Summary

The COVID-19 Committee of the Lincei Academy has updated the evidence for the efficacy and safety of repurposed and new drugs for the prevention and treatment of COVID-19 and its complications, as well as the safety of some concomitant medications.

A number of pharmacological strategies could theoretically prevent the entry of SARS-CoV-2 into target cells and are currently being evaluated for efficacy and safety. These include neutralizing antibodies against the SARS-CoV-2 spike protein, a soluble recombinant form of the SARS-CoV-2 receptor angiotensin-converting enzyme (ACE)2, and drugs inhibiting the activity or expression of the transmembrane protease serine 2 (TMPRSS2) required for the spike protein proteolytic cleavage. A problem with the use of neutralizing antibodies has been the rapid emergence and spread of mutations of the spike protein, which are not recognized by available antibodies. Regarding the use of convalescent plasma for treatment of COVID-19 in hospitalized patients, it is not recommended in those without impaired humoral immunity.

Remdesivir was identified early as a promising therapeutic candidate for COVID-19 because of its ability to inhibit SARS-CoV-2 *in vitro*. Based on the results of a double-blind, placebo-controlled randomized clinical trial (RCT) of intravenous remdesivir in 1,063 adults hospitalized with COVID-19 with evidence of lower respiratory tract involvement, which demonstrated that remdesivir was superior to placebo in shortening the time to recovery in this setting, in 2020 the US Food and Drug Administration (FDA) has made remdesivir available under an emergency-use authorization (EUA) for the treatment of adults and children with severe COVID-19 disease (May 1st), followed by approval for use in adults and pediatric patients requiring hospitalization (October 22nd). On January 21 2022, based on new evidence, the FDA authorized remdesivir also for outpatient treatment of people at high risk of COVID-19 disease progression, and expanded the pediatric EUA to include treatment of non-hospitalized patients at high risk. It should be also noted that different studies now show that SARS-CoV-2 can develop resistance to remdesivir *in vitro* as well as in COVID-19 patients. In addition to remdesivir, two new antiviral drugs, molnupiravir (Lagevrio), and ritonavir-boosted nirmatrelvir (Paxlovid), received an EUA in different countries. Both drugs are available for oral use in non-hospitalized patients, with mild to moderate COVID-19 at high risk of developing severe disease. However, several factors may limit their use: molnupiravir might be able

to cause mutations in human DNA, leading the health authorities in some countries to advise against its use during pregnancy, while other countries have chosen not to authorize it. Regarding paxlovid, its possible interaction with a wide range of commonly used drugs may limit its use.

Early in the course of the SARS-CoV-2 pandemics, it was claimed that nonsteroidal anti-inflammatory drugs (NSAIDs), like ibuprofen, could aggravate the infection by masking its symptoms. However, after review of the evidence, the WHO and EMA advisories have been withdrawn. Therefore, until we have robust evidence, patients in chronic pain should continue to take their NSAIDs rather than turn to opiates. Given that the elderly comprises an at-risk group for severe COVID-19, an association between NSAIDs and the disease may merely reflect reverse causality. Low-dose aspirin appears minimally effective in patients hospitalized with COVID-19. Its utility in patients with milder disease remains to be assessed.

The benefit of corticosteroids in the treatment of COVID-19 has been established in large clinical trials in hospitalized critically ill patients, showing a significant reduction of mortality as compared to those allocated to usual care. The usefulness of dexamethasone in patients with severe pulmonary complications of COVID-19 infection has been confirmed by a recent WHO meta-analysis. Based on this evidence, the use of dexamethasone in hospitalized patients requiring respiratory support is widely recommended.

Several observational studies and RCTs have been performed with immunomodulatory drugs, particularly those targeting IL-6 and its downstream signaling, such as the Janus kinase (JAK) and signal transducer and activator of transcription (STAT) pathway. Based on available evidence, the FDA issued an EUA for the use of tocilizumab in combination with corticosteroids in hospitalized adult and pediatric patients (two years of age or older) with COVID-19 who require supplemental oxygen, non-invasive or invasive mechanical ventilation, or extracorporeal membrane oxygenation. A meta-analysis of nine completed JAK inhibitor trials showed that treatment with these drugs was associated with significant reduction in 28-day mortality in patients hospitalized with COVID-19, a finding supporting targeting the JAK/STAT axis in the setting of severe COVID-19.

The complement system is one of the host immune system's first line defense against invading pathogens. Collectively, early clinical findings and emerging clinical trial evidence suggests the potential therapeutic benefits of some complement inhibitors in severe COVID-19. Further studies, however, are required to clarify the best target(s) within the complement cascade and the optimal time of treatment initiation, and to characterize which patients may benefit the most.

Dysregulation of the coagulation cascade and fibrinolytic system is emerging as an important pathophysiologic component of COVID-19. Largely based on observational studies, the International Society on Thrombosis and Haemostasis (ISTH) suggested measuring D-dimer, prothrombin time and platelet count in all COVID-19 patients. ISTH also recommends that all COVID-19 patients admitted to hospital be treated with prophylactic doses of low-molecular-weight (LMW) heparin, unless contraindicated. Moreover, recent available evidence from RCTs supports the concept that therapeutic dose anticoagulation with LMW or unfractionated heparin is associated with improved outcomes in hospitalized patients with COVID-19 who are not critically ill or in the ICU setting, particularly in those with elevated D-dimer levels, but at the expense of increased risk of moderate or severe bleeding. There is not yet consensus on the role of extended thromboprophylaxis beyond the hospital stay. Routine administration of thromboprophylaxis is not recommended for

ambulatory patients with COVID-19. Additional RCTs of several antithrombotic agents are currently ongoing.

Earlier, the FDA had also issued an EUA allowing the temporary use of hydroxychloroquine (HCQ) and chloroquine (CQ) during the COVID-19 pandemic for treatment of the virus in hospitalized patients when clinical trials are not available, or participation is not feasible. This decision was largely based on mechanistic considerations and political pressure. Subsequent observational studies and a limited number of RCTs have not substantiated the clinical efficacy of these antimalarial drugs, while confirming their dose-dependent cardiac toxicity. At present, the US National Institutes of Health (NIH) COVID-19 Treatment Guidelines recommend against the use of CQ or HCQ for the treatment of COVID-19. In addition, the recent NIH guidelines have also indicated a list of drugs that are not recommended for the COVID-19 treatment because of proven lack of efficacy, including the anti-parasitic ivermectin, colchicine, interferons, fluvoxamine, and metformin.

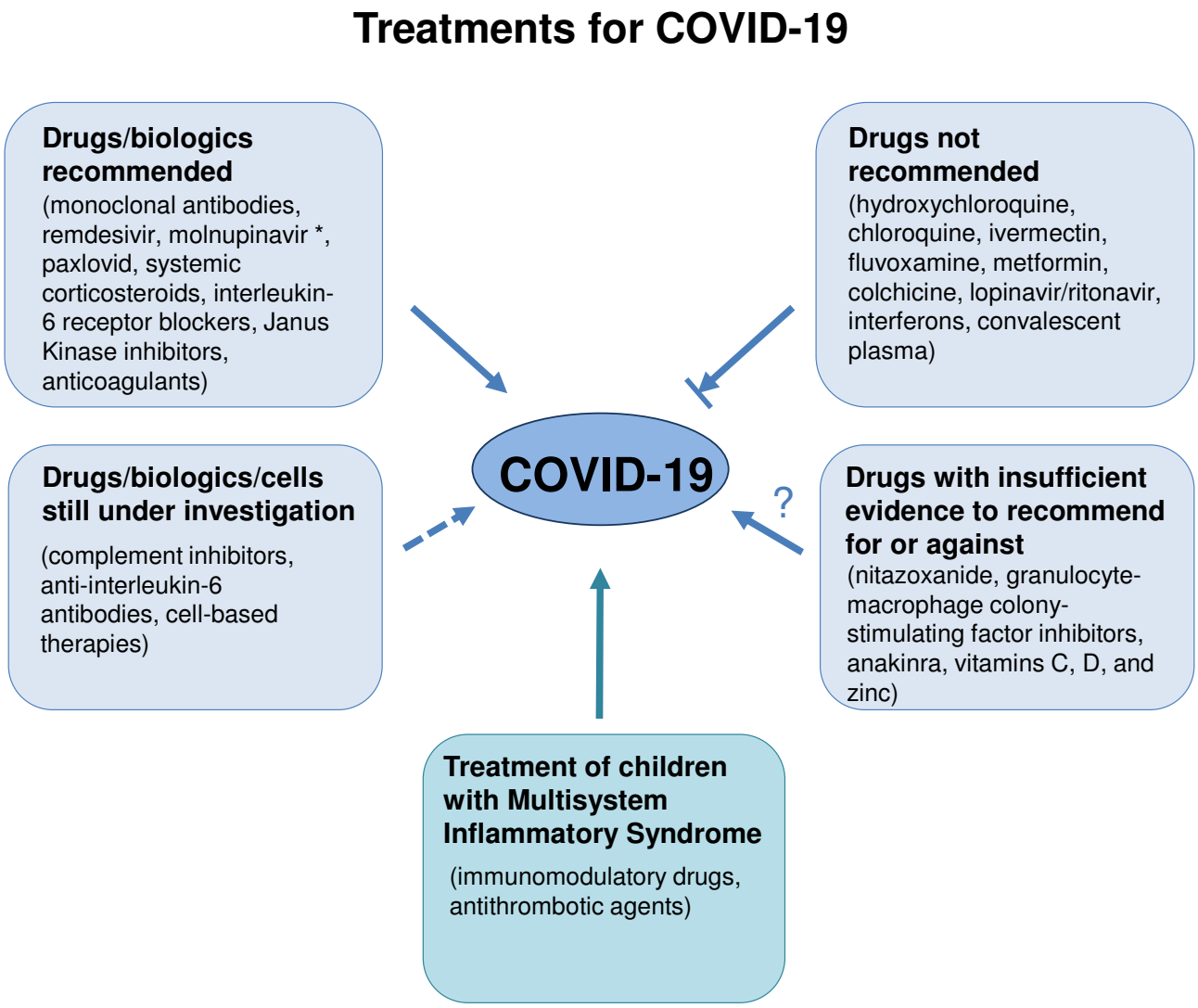
Several other drugs are still in clinical trials, and, considering the preliminary results, there is insufficient evidence to recommend for or against their use in COVID-19 patients. Among these, nitazoxanide, a broad spectrum, thiazolide antiparasitic agent, and anakinra, an interleukin-1 receptor antagonist. While the SAVE-MORE trial in hospitalized patient with moderate or severe COVID-19 pneumonia, reported a lower risk of clinical progression of the illness in patients given anakinra than in those receiving placebo, the REMAP-CAP, an open-label, adaptive platform, randomized controlled trial, showed no efficacy of the drug in reducing the combined endpoint of in-hospital mortality and days of organ support.

In view of the current understanding of the pathophysiologic mechanisms of COVID-19 and the unique biological characteristics of mesenchymal stromal cells (MSC), the development of cell-therapy has been seen as a promising approach for patients with SARS-CoV-2 infection, especially for those with severe illness. However, data supporting cell-based therapy with MSC in COVID-19 patients are limited to small open-label studies and few RCTs. Nonetheless, so far, no MSC products have been approved by the FDA for the treatment of COVID-19.

Individuals with underlying chronic conditions, such as cardiovascular disease (CVD), pulmonary disease, diabetes, and malignancy are at high risk for severe illness with COVID-19. These patients are usually prescribed medications to treat these disorders. Although ACE inhibitors and angiotensin-receptor blockers (ARBs) may enhance ACE2 gene expression, an effect that would increase the availability of receptor molecules for SARS-CoV-2 entry, there is no evidence that these commonly used drugs might be harmful in patients with COVID-19. Therefore, persons with COVID-19 who are prescribed ACE inhibitors or ARBs for CVD (or other indications) should continue these medications. A similar approach should be adopted for the use of statins. Thus, according to the NIH COVID-19 Treatment Guidelines Panel, patients with COVID-19 who are treated with concomitant medications for an underlying medical condition should not discontinue these medications during the acute management of COVID-19, unless discontinuation is otherwise warranted by their clinical condition. In addition, there is a recommendation against using medications off-label to treat COVID-19 if they have not been shown to be safe and effective for this indication in a RCT.

Finally, during the first peak of COVID-19 pandemic in Europe, multisystem inflammatory syndrome in children (MIS-C) was first described in reports of children presenting with a severe multisystem

hyperinflammatory illness temporally associated with preceding SARS-CoV-2 infection or exposure. The pathogenesis of MIS-C is still being elucidated. However, given the reported clinical similarity between MIS-C and Kawasaki's disease, the approach to treatment of MIS-C has been similar to that of Kawasaki's disease, the most commonly used therapy being intravenous immunoglobulin (IVIG) and glucocorticoids. There is also agreement that children with MIS should also be given low-dose aspirin, if they are not at risk for bleeding.



*In some countries health authorities advise against the use of molnupinavir during pregnancy; other countries have chosen not to authorize it at all.

1. Introduction

Coronaviruses (CoV), a group of enveloped positive-strand RNA viruses, were discovered in the 1960s and were originally thought to cause only mild disease in humans, with several strains being responsible for the common cold (1). This view changed in 2003 with the SARS (severe acute respiratory syndrome) pandemic and in 2012 with the MERS (Middle East respiratory syndrome) outbreak, two zoonotic infections that resulted in mortality rates greater than 10% and 35%, respectively (2).

The newly discovered (2019) SARS-CoV-2 coronavirus spread rapidly. On 30 January 2020, the WHO labelled it a public health emergency and on 25 May 2020 (the date our first Report was issued) the total number of laboratory-confirmed COVID-19 cases stood at over 5,470,900, having spread to at least 177 countries and caused over 346,000 deaths. On March 10, 2023, we dismayingly count 676,609,955 cases and 6,881,955 deaths (3). Given the unprecedented proportions of the pandemic in many countries, and the rise in the associated global death toll, over the past three years we have witnessed a race to find drugs/biologic treatments to save the lives of hospitalised, severely ill patients, as well as to develop vaccines. To this end, randomised clinical trials have been performed or are underway to test experimental drug candidates or repurposed medicines. Therapeutic approaches to the early, mild phase of COVID-19 are also being debated and here, too, there is an emphasis on the need for randomised clinical trials. However, in times like the present, Regulatory Authorities occasionally issue emergency use authorisations (EUAs) for drugs, as the US Food and Drug Administration (FDA) initially did for chloroquine and hydroxy-chloroquine for COVID-19. However, on June 15, 2020 the FDA informed that it was revoking EUA of the two drugs, saying that they are “unlikely to be effective” and that current national treatment guidelines don’t recommend using them outside of clinical trials.

Indeed, it is necessary to conduct rigorous studies on COVID-19 drug candidates that provide sufficient scientific data that can be evaluated meticulously, which make it possible to differentiate between anecdotes and evidence. Otherwise, there is a high risk of sowing confusion among physicians caring for COVID-19 patients under these high-pressure circumstances.

Working Group 1a of the COVID-19 Committee of the Lincei Academy has previously prepared a brief report of the available scientific evidence about the efficacy and safety of existing and new drugs for the prevention and treatment of COVID-19 and its complications. This is the second update reporting what we learned in the past three years. The focus is on drugs and neutralizing monoclonal antibodies that prevent the entry of SARS-CoV-2 into target cells; evidence on the benefits of the new drugs that inhibit SARS-CoV-2 replication; evidence of the risks/benefits of using non-steroidal anti-inflammatory drugs (NSAIDs); the place of corticosteroids in the treatment of critically ill patients with severe pulmonary complications of SARS-CoV-2 infection; evidence of the efficacy of immunomodulatory drugs, including anti-IL-6 receptor antagonists and Janus Kinase inhibitors; whether complement inhibitors, as well as anticoagulants and other anti-thrombotic agents have a place in the prevention and/or treatment of inflammatory and vascular complications of the disease; and on drugs that are not recommended because of proven lack of efficacy, as well as on drugs with insufficient evidence to recommend for or against. Moreover, this updated report discusses the risks/benefits of using cell therapies, in particular mesenchymal stromal cells; presents

evidence of the safety of concomitant medications prescribed for underlying conditions in COVID-19 patients; and evaluates the treatment management of children with Multi-system Inflammatory Syndrome (MSI-C), an illness temporally associated with preceding SARS-CoV-2 exposure (4).
This document does not intend to recommend any experimental drug, but to review the evidence supporting the efficacy and safety of these pharmacological treatments, highlights the official position of health authorities and panels of experts with regard to each drug or class of drugs considered, and briefly mentions the ongoing trials registered with clinicaltrials.gov or the WHO register.

References

1. Cui J, Li F, Shi Z. Origin and evolution of pathogenic coronaviruses. *Nature Rev. Microbiol.* 2019; 17:181-192.

2. T.S. Fung TS, Liu DX. Human coronavirus: host-pathogen interaction. *Annu. Rev. Microbiol.* 2019; 73:529-557.

3. Johns Hopkins CSSE. COVID-19 Map – Johns Hopkins Coronavirus Resource Center. Accessed March 10, 2023. Available at: https://www.coronavirus.jhu.edu

4. Li G, Hilgenfeld R, Whitley R , et al. Therapeutic strategies for COVID-19: progress and lessons learned. *Nat Rev Drug Discov.* 2023; Apr 19. doi: 10.1038/s41573-023-00672-y.

2. Drugs and neutralizing monoclonal antibodies preventing SARS-CoV-2 entry into target cells

A comprehensive review on therapeutic strategies for COVID-19, including drugs and monoclonal antibodies preventing SARS-CoV-2 entry into target cells, has been recently published (1). SARS-CoV-2 spike protein binds to its receptor, angiotensin- converting enzyme 2 (ACE2), and is proteolytically activated by the transmembrane protease serine 2 (TMPRSS2), thus enabling the fusion of the virus with the cell membrane (2,3) (Figure 1). Bioinformatics analyses based on protein structures predict that transmembrane dipeptidyl peptidase-4 (DPP4), which is the receptor for MERS-CoV, could also interact with SARS-CoV-2 (4). However, DPP4 was unable to mediate virus entry into cells lacking ACE2 (2). Another tissue protease, the proprotein convertase furin, is involved in the cleavage of the spike protein, possibly promoting the subsequent cleavage by TMPRSS2 (5). However, furin inhibitors, unlike TMPRSS2 inhibitors, can interfere with important cell functions, so furin is not an attractive drug target. Current approaches that aim to block SARS-CoV-2 cell entry are based on 1) treatments that inhibit the SARS-CoV-2 spike- ACE2 interaction or 2) TMPRSS2 inhibitors. At the moment (April 2023) only monoclonal antibodies that block the spike-ACE2 interaction have been approved.

Anti-spike antibodies and soluble ACE2 can block the interaction between the virus spike protein and ACE2. Monoclonal antibodies that block the spike-ACE2 interaction and are approved or granted emergency use authorization (EUA) in many countries are listed in Table 1. Passive immunization with convalescent plasma has been used in several countries to treat COVID-19, based on the rationale that neutralizing antibodies could both inhibit the virus from binding to the cell and promote immune cells clearance of the virus. Neutralizing antibodies are thus promising candidates for prophylactic and therapeutic treatment of COVID-19. Experience with other viral diseases indicates that donors with high serum titers of neutralizing antibody should be identified (a proportion of those who recover from COVID-19 have low titers) and the risk of antibody-dependent enhancement of infection (ADE) considered (6). The NIH guidelines (https://www.covid19treatmentguidelines.nih.gov/) recommend against using convalescent plasma to treat COVID-19 in hospitalized patients without impaired humoral immunity. For non-hospitalized, immunocompetent COVID-19 patients, data from well-designed clinical trials are conflicting. Some demonstrate efficacy (7,8), while others have found no benefits (9,10) regarding the incidence of disease progression and/or hospitalization. Monoclonal antibodies against SARS-CoV-2, many of which were derived from COVID-19 patients' B-cells, were found to neutralize the virus in cultured cells (11,12) and a study using a transgenic mouse model bearing human ACE2 confirmed that specific monoclonal antibodies can reduce virus titers in infected lungs (13). The latter report was complemented by a detailed structural analysis of the interaction between antibodies, the receptor binding domain (RBD) of the spike protein, and ACE2, providing important information on the development of vaccines and small molecule or peptide inhibitors. In outpatients with COVID-19, neutralizing anti-SARS-CoV-2 monoclonal antibodies have been shown to reduce the incidence of disease progression and hospitalization when given within 5–7 days of the onset of the illness (14-16). One serious problem with using neutralizing antibodies to treat COVID-19 has been the rapid emergence and spread of mutations of the spike protein, which are not recognized by the available antibodies (17). The NIH Guidelines (https://www.covid19treatmentguidelines.nih.gov/) indicate that almost all available antibodies have reduced activities against the Omicron variants of concern (VOC).

An alternative approach to blocking the interaction between the SARS-CoV-2 and the ACE2 receptor is to use picomolar miniprotein inhibitors that have a high affinity for binding to the SARS-CoV-2 spike protein and compete with ACE2 binding. Two of these recently designed inhibitors were found to prevent infection in cultured cells more efficiently than the most potent monoclonal antibodies described to date (18).

The administration of a large amount of soluble ACE2 may neutralize the virus and slow viral entry into cells. Interestingly, a recombinant human soluble ACE2 (rhsACE2), corresponding to the extracellular domain of ACE2, was developed several years ago and found to be safe in healthy volunteers and in a small cohort of patients with acute respiratory distress syndrome (ARDS) in completed Phase I and Phase II clinical trials (ClinicalTrials.gov identifier: NCT00886353) (19,20). This rhsACE2 was found to inhibit SARS-CoV-2 infection in cultured cells and in human blood vessel and kidney organoids (21) and a clinical trial has been launched to use rhsACE2 to treat patients with COVID-19 (ClinicalTrials.gov identifier: NCT04335136). RhsACE2 has been successfully applied

to COVID-19 patients (22) and a number of variations of this approach have been described. For example, engineering of ACE2 N-glycosylation through site-directed mutagenesis or glycosidase treatment resulted in enhanced binding affinities and improved virus neutralization (23). Treatment with recombinant human ACE2-Fc fusion protein (hACE2-Fc) effectively protected mice against SARS-CoV-2 infection: an advantage of this approach is that hACE2-Fc has a relative long half-life in vivo compared to soluble ACE2 (24).

A completely new approach to blocking virus entry into the cells is based on humoral innate immunity pattern recognition molecules. In particular, mannose-binding lectin (MBL), which binds the viral spike proteins, inhibited cell infection by SARS- CoV-2 variants of concern (VOCs), including Omicron (25). A potential problem with the therapeutical application of MBL is the possibility that in advanced disease MBL may contribute to complement activation and result in uncontrolled inflammation.

TMPRSS2 protease inhibitors could be used to block a crucial step in the fusion of the virus with the cell membrane. The TMPRSS2 protease inhibitor, camostat mesylate, was reported to inhibit SARS-CoV-2 entry into lung cell lines (3). This drug has been approved in Japan and Korea to treat chronic pancreatitis and was repurposed to treat COVID-19 in a clinical trial (ClinicalTrials.gov number NCT04353284). Nafamostat mesylate, another drug that has been used for many years in Japan to treat acute pancreatitis and disseminated intravascular coagulation (DIC), was recently reported to inhibit SARS-CoV-2 infection in Calu3 human lung cells in the nanomolar range, with 10–15 fold higher efficiency than camostat mesylate (26-28). The efficacy of nafamostat in COVID-19 patients is currently being evaluated in clinical trials (ClinicalTrials.gov identifier: NCT04352400; Japan Registry of Clinical Trials: jRCTs031200026; Korea CRIS: KCT0005003). It has been suggested that another TMPRSS2 inhibitor, bromhexine, presently used as a mucolytic cough suppressant, could be used to treat COVID- 19 (29,30). Novel TMPRSS2 inhibitors have recently been identified using high- throughput screening (31), and a highly potent inhibitor has recently been well characterized (32). This compound, N-0385, is a small peptidomimetic that is active at low doses (nanomolar) and inhibits SARS-CoV- 2 infection in human lung cells and in donor-derived colon organoids. N-0385 blocks SARS-CoV-2 in different variants of concern (alpha, beta, gamma and delta) and is protective against infection and mortality in mice that express the human ACE2 receptor, when delivered as a nasal spray 12h after infection.

Finally, since TMPRSS2 expression is controlled by androgens, which could explain the greater frequency of severe COVID-19 in males, it is possible that androgen receptor antagonists might reduce susceptibility to developing a serious COVID-19 infection (33). This possibility is supported by epidemiological studies that show that prostate cancer patients treated with anti-androgens are much less frequently affected by COVID-19 compared to patients who did not receive this treatment (34). This study is supported by new results from different laboratories and the effect of testosterone suppression in COVID-19 patients is being investigated in clinical trials, including a trial using Degarelix, an FDA-approved drug used to treat prostate cancer (ClinicalTrials.gov identifier: NCT04397718) (35). However, a recent randomized phase 2 clinical trial did not find evidence that anti-androgen therapy had a therapeutic effect on COVID-19 patients (36).

References

1. Li G, Hilgenfeld R, Whitley R , et al. Therapeutic strategies for COVID-19: progress and lessons learned. *Nat Rev Drug Discov.* 2023; Apr 19. doi: 10.1038/s41573-023-00672-y.

2. Zhou P, et al. A pneumonia outbreak associated with a new coronavirus of probable bat origin. *Nature* 2020; 579: 270–273.

3. Hoffmann M, et al. SARS-CoV-2 Cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. *Cell* 2020; 181:271-280.e8.

4. Li Y, et al. The MERS-CoV receptor DPP4 as a candidate binding target of the SARS-CoV-2 spike. *iScience* 2020; May 13:101160.

5. Hoffmann M, et al. A Multibasic cleavage site in the spike protein of SARS-CoV-2 is essential for infection of human lung cells. *Mol Cell* 2020; 78:779-784.

6. Casadevall A, Pirofski L-A. The convalescent sera option for containing COVID-19. *J Clin Invest.* 2020; 130:1545-1548.

7. Libster R, Perez Marc G, Wappner D, et al. Early high-titer plasma therapy to prevent severe COVID-19 in older adults. *N Engl J Med*. 2021; 384:610-618.

8. Sullivan DJ, Gebo KA, Shoham S, et al. Early outpatient treatment for COVID-19 with convalescent plasma. *N Engl J Med*. 2022; 386:1700-1711.

9. Korley FK, Durkalski-Mauldin V, Yeatts SD, et al. Early convalescent plasma for high-risk outpatients with COVID-19. *N Engl J Med*. 2021; 385:1951-1960.

10. Alemany A, Millat-Martinez P, Corbacho-Monne M, et al. High-titre methylene blue-treated convalescent plasma as an early treatment for outpatients with COVID-19: a randomised, placebo-controlled trial. *Lancet Respir Med*. 2022; 10(3):278-288.

11. Wang C, et al. A human monoclonal antibody blocking SARS-CoV-2 infection. *Nat Commun*. 2020; 11:2251.

12. Andreano E, et al. Extremely potent human monoclonal antibodies from COVID-19 convalescent patients. *Cells* 2021; 184: 1821-1835.

13. Wu Y, et al. A noncompeting pair of human neutralizing antibodies block COVID-19 virus binding to its receptor ACE2. *Science* 2020; 368: 1274-1278.

14. Weinreich DM, Sivapalasingam S, Norton T, *et al.* REGN-COV2, a Neutralizing Antibody Cocktail, in Outpatients with Covid-19. *N Engl J Med.* 2021; 384:238–51.

15. Dougan M, Nirula A, Azizad M, *et al.* Bamlanivimab plus Etesevimab in Mild or Moderate Covid-19. *N Engl J Med.* 2021; 385:1382–92.

16. 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; 385:1941–50.

17. Callaway E. COVID antibody drugs work best when given as early as possible. *Nature* 2022; published online March 30. DOI:10.1038/d41586-022-00893-y.

18. Cao L, et al. De novo design of picomolar SARS-CoV-2 miniprotein inhibitors. *Science* 2020; 370: 426-431.

19. Haschke M, et al. Pharmacokinetics and pharmacodynamics of recombinant human angiotensin-converting enzyme 2 in healthy human subjects. *Clin Pharmacokinet.* 2013; 52:783-792.

20. Khan A, et al. A pilot clinical trial of recombinant human angiotensin-converting enzyme 2 in acute respiratory distress syndrome. *Crit Care.* 2017; 21:234. doi: 10.1186/s13054-017-1823-x.

21. Monteil V, et al. Inhibition of SARS-CoV-2 infections in engineered human tissues using clinical-grade soluble human ACE2. *Cell.* 2020; 181:905-913.

22. Zoufaly A, et al. Human recombinant soluble ACE2 in severe COVID-19. *Lancet Respiratory Med.* 2020; 8: 1154-1158.

23. Capraz T, et al. Structure-guided glyco-engineering of ACE2 for improved potency as soluble SARS-CoV-2 decoy receptor. *eLife.* 2021; 10:e73641. doi: 10.7554/eLife.73641.

24. Zhang Z, et al. Potent prophylactic and therapeutic efficacy of recombinant human ACE2-Fc against SARS-CoV-2 infection in vivo. *Cell Discov.* 2021 Aug 12;7(1):65. doi: 10.1038/s41421-021-00302-0.

25. Stravalaci M, et al. Recognition and inhibition of SARS-CoV-2 by humoral innate immunity pattern recognition molecules. *Nat Immunol.* 2022; 23: 275-286. doi: 10.1038/s41590-021-01114-w.

26. Yamamoto M, et al. The anticoagulant nafamostat potently inhibits SARS-CoV-2 S protein-mediated fusion in a cell fusion assay system and viral infection in vitro in a cell-type-dependent manner. *Viruses.* 2020; 12 (6): 629. doi: 10.3390/v12060629.

27. Hoffmann M, et al. Nafamostat mesylate blocks activation of SARS-CoV-2: New treatment option for COVID-19. *Antimicrob. Agents Chemother.* 2020; 64 (6): e007544-20. doi:10.1128/AAC.00754-20.

28. Ko M, et al. Comparative analysis of antiviral efficacy of FDA-approved drugs against SARS-CoV-2 in human lung cells: Nafamostat is the most potent antiviral drug candidate. *J. Med. Virol.* 2021; 93: 1403-1408.

29. Maggio R, Corsini GU. Repurposing the mucolytic cough suppressant and TMPRSS2 protease inhibitor bromhexine for the prevention and management of SARS-CoV-2 infection. *Pharmacol Res*. 2020; 157:104837. doi: 10.1016/j.phrs.2020.104837.

30. Habtemariam S, et al. Possible use of the mucolytic drug, bromhexine hydrochloride, as a prophylactic agent against SARS-CoV-2 infection based on its action on the Transmembrane Serine Protease 2. *Pharmacol Res.* 2020; 157:104853. doi: 10.1016/j.phrs.2020.104853.

31. Mantzourani C, et al. The discovery and development of transmembrane serine protease 2 (TMPRSS2) inhibitors as candidate drugs for the treatment of COVID-19. *Expert Opin Drug Discov.* 2022;17(3):231-246.

32. Shapira T, et al. A TMPRSS2 inhibitor acts as a pan-SARS-CoV-2 prophylactic and therapeutic. *Nature* 2022; 605: 340-348.

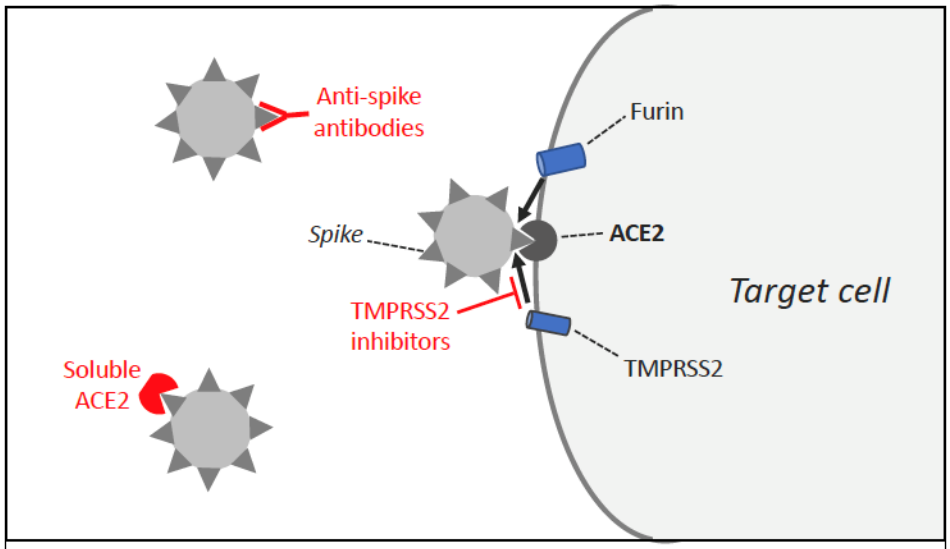
33. Stopsack KH, et al. TMPRSS2 and COVID-19: Serendipity or opportunity for intervention? *Cancer Discov.* 2020; 10: 779-782.

34. Montopoli M, et al. Androgen-deprivation therapies for prostate cancer and risk of infection by SARS-CoV-2: a population-based study (n=4532). *Ann Oncol.* 2020; 31: 1040-1045.

35. Wadman M. Why coronavirus hits men harder: sex hormones offer clues. *Science* 2020; 368: 1038-1039.

36. Welén K, et al. A Phase 2 trial of the effect of antiandrogen therapy on COVID-19 outcome: No evidence of benefit, supported by epidemiology and in vitro data. *Eur Urol.* 2022; 81(3):285-293.

Figure 1.



The entry of Sars-Cov-2 into target cells requires the binding of the spike protein present at the surface of the virus with cell membrane bound angiotensin converting enzyme 2 (ACE2), which acts as a receptor of the virus. Spike protein cleavage by the host cell proteases, transmembrane protease serine 2 (TMPRSS2) and furin, is also required for the fusion of the viral membrane with the plasma membrane of the cell. Ongoing clinical trials aim to determine whether cell entry of the virus can be prevented by 1) anti-spike antibodies, 2) soluble ACE2 extra-cellular domain or 3) inhibitors of TMPRSS2.

Table 1. Monoclonal antibodies that block the spike-ACE2 interaction (modified from Ref 1, Li et al, 2023)

Drug name	Type (delivery route)	Use	Eligible patients	Resistance likelihood[a]	Status
Bebtelovimab	mAb (i.v.)	Tx	Outpatients[b] ≤7 days of symptom onset	High (e.g., BQ.1, BQ.1.1)	EUA by the FDA; paused owing to resistance
Regdanvimab (Regkirona)	mAb (i.v.)	Tx	Outpatients[b] ≤7 days of symptom onset	High (e.g., Omicron, Gamma, Beta)	EUA in many countries; paused owing to resistance
Sotrovimab	mAb (i.v.)	Tx	Outpatients[b] ≤7 days of symptom onset	High (e.g., Omicron)	Approved or EUA in many countries; paused owing to resistance
Amubarvimab and romlusevimab	mAbs (i.v.)	Tx	Outpatients[b] ≤10 days of symptom onset	High (e.g., Omicron)	Approved in China; discontinued
Bamlanivimab and etesevimab	mAbs (i.v.)	Tx	Outpatients[b] ≤10 days of symptom onset	High (e.g., Omicron, beta)	EUA in many countries; paused owing to resistance
		PEP	Certain individuals at high risk of COVID-19		
Casirivimab and imdevimab (REGEN-COV)	mAbs (i.v. or s.c.)	Tx	Outpatients[b] ≤10 days of symptom onset	High (e.g., Omicron)	EUA in many countries, paused owing to resistance
		PEP	Certain individuals at high risk of COVID-19		
Cilgavimab and tixagevimab (Evusheld)	mAbs (i.m.)	PrEP	Certain individuals at high risk of COVID-19	High (e.g., Omicron)	Approved or EUA in many countries, paused owing to resistance

[a] High: >10-fold reduction in susceptibility of any SARS-CoV-2 variant. Low: <5-fold reduction in susceptibility. The data were obtained from the drug label.
[b] Nonhospitalized patients with mild-to-moderate COVID-19 and at high risk of progression to severe COVID-19, including hospitalization or death (see drug labels).
EUA, emergency use authorization; PEP, post-exposure prophylaxis; PrEP: pre-exposure prophylaxis; i.m., i.v, s.c., intramuscular, intravenous or subcutaneous injection; Tx, treatment.

3. Drugs inhibiting SARS-CoV-2 replication

Before the emergence of SARS-CoV-2 there was no specific antiviral treatment for coronavirus infections. Over the past three years considerable efforts have been directed towards identifying antivirals that are effective against SARS-CoV-2 and, in particular, toward repurposing of FDA-approved drugs that could become available much more quickly. So far, of the large number of antiviral drugs investigated in preclinical and clinical studies, three have been approved or have received emergency use authorization (EUA) from health authorities in different countries [https://www.bio.org/policy/human-health/vaccines-biodefense/coronavirus/pipeline-tracker]. As other antivirals used to treat RNA virus infections, these drugs target two key components of the virus replication machinery, the viral polymerase (remdesivir and molnupiravir) and the main viral protease (paxlovid), both of which are essential for viral replication. A different 3CL protease inhibitor (ensitrelvir) has recently received an EUA in Japan.

Remdesivir

Remdesivir, an adenosine analog prodrug originally developed to treat the Ebola virus (EBOV), was found to inhibit the replication of human and animal coronaviruses *in vitro* and in preclinical studies (1). Upon diffusion into the cell, remdesivir is metabolized into the nucleoside monophosphate form and ultimately into the active nucleoside triphosphate derivative, which is integrated into viral RNA by the viral RNA-dependent RNA polymerase (RdRp), resulting in chain termination (1). Remdesivir was identified early as a promising candidate for treating COVID-19 because of its ability to inhibit SARS-CoV-2 *in vitro*, as well as in animal studies when treatment was initiated early during the course of infection (reviewed in Eastman et al.) (1). These findings, along with the safety profile of remdesivir, as established in the clinical trial regarding Ebola virus (1), supported the decision to evaluate remdesivir as a potential therapeutic drug to be repurposed for treating SARS-CoV-2 infections.

Initial observations of the clinical improvement in a limited number of patients who were seriously ill with COVID-19 and treated with remdesivir under compassionate use, were sufficiently encouraging to prompt the initiation of a large number of studies to investigate the effectiveness of remdesivir, alone or in combination with other drugs, against COVID-19 (see *ClinicalTrials.gov*). However, many of these studies were small and have produced conflicting results (reviewed in Vegivinti et al.) (2).

No significant benefit was found in a randomized placebo-controlled trial of intravenous remdesivir conducted in China starting with 236 patients with COVID-19 (3). On the other hand, on April 2020 the US National Institute of Allergy and Infectious Diseases (NIAID) announced preliminary results from the Adaptive COVID-19 Treatment Trial (ACTT-1, NCT04280705), a double-blind, randomized, placebo-controlled phase 3 trial to evaluate the safety and efficacy of remdesivir in 1,062 adults hospitalized with COVID-19. The final report of the study concluded that remdesivir was superior to placebo in shortening patients' time to recovery: a median of 10 days in hospital as compared to 15 days for those assigned to the placebo group (4).

Based on these findings, on 1st May 2020 the FDA made remdesivir (VEKLURY®) available in the US under an EUA to treat adults and children with severe COVID-19 disease (https://www.fda.gov/news-events/press-announcements/coronavirus-covid-19-update-fda-issues-emergency-use-authorization-potential-covid-19-treatment). The drug was then also authorized in the EU (*https://www.ema.europa.eu/en/medicines/human/EPAR/veklury#authorisation-details-section*). On 22nd October 2020, the FDA approved VEKLURY for use in adults and pediatric patients (12-years old and above) who required hospitalization (https://www.fda.gov/news-events/press-announcements/fda-approves-first-treatment-covid-19).

However, the mortality rate recorded in the ACTT-1 study though lower in the patients treated with remdesivir, remained high: 11.4% as compared to 15.2% in the placebo group on day 29 after enrollment (4). Encouraging results regarding remdesivir-treated patients' survival, including a comparative analysis of the Phase 3 SIMPLE-Severe trial and a real-world retrospective cohort of patients with severe COVID-19 (NCT04292899 and EUPAS34303) were reported in 2021 (5). In this analysis, by day 14 remdesivir treatment was associated with both an improvement in clinical recovery, and a 62% reduction in the risk of mortality compared with standard-of-care treatment.

On the other hand, in an open-label multinational study (NCT04292730) (6) remdesivir was reported to be less effective in hospitalized patients with moderate COVID-19 pneumonia. In addition, the interim results of the WHO SOLIDARITY trial, a global, open-label, multicentric randomized four-arm trial comparing remdesivir, lopinavir/ritonavir, lopinavir/ritonavir with interferon beta-1a, and chloroquine or hydroxychloroquine (ISRCTN83971151/NCT04315948), concluded that not one of the four drugs produced any measurable benefits in terms of mortality or disease course (7). In the case of remdesivir, the study concluded that intravenous remdesivir had little or no effect on duration of hospital stay (the proportion still hospitalized on day 7, remdesivir *vs* control was: 69%v59%), or on mortality (301/2743 remdesivir *vs* 303/2708 control).

In March 2022, however, the Canadian Treatments for COVID-19 (CATCO) trial (NCT04330690), a substudy of the global WHO Solidarity trial, concluded that with regard to the 1282 patients admitted due to COVID-19, in-hospital mortality for patients treated with remdesivir was lower than for control patients: the 60-day mortality was 24.8% in the remdesivir arm, compared with 28.2% in the standard-of-care arm (RR 0.88, 95% CI 0.72 to 1.07) (8). In this study remdesivir treatment turned out to be especially beneficial in terms of preventing the need for mechanical ventilation [8.0% remdesivir *vs* 15.0% standard of care (RR 0.53, 95% CI 0.38 to 0.75)], again suggesting a better outcome for patients with less severe disease (8).

The differing results from the clinical trials that used remdesivir on hospitalized patients, described above, likely contributed to growing uncertainty in the medical community regarding the effectiveness of the drug, especially in terms of which patient subgroup would benefit the most from remdesivir treatment. In the attempt to answer this question, a new study (PROSPERO, CRD42021257134) was recently undertaken to reanalyze individual patient data from eight randomized clinical trials (RCT), covering 10,480 adult patients with COVID-19 (99% of COVID-19 patients who participated in an RCT investigating remdesivir between Feb 6, 2020 and April 1, 2021)

from over 40 countries. The individual patient data meta-analysis showed that remdesivir treatment resulted in a significant survival benefit and less progression to mechanical ventilation or death in patients who did not receive oxygen therapy or only received conventional oxygen support (9). In patients who were receiving high-flow oxygen or more intensified respiratory support before receiving remdesivir the evidence for the effect of the drug was instead inconclusive (9). Although remdesivir was reported to have specific side-effects, in particular severe bradycardia (10), the meta-analysis showed that remdesivir did not increase severe or serious adverse events compared with usual care (9).
The fact that most of the studies indicated that treatment with remdesivir is most successful when started in the early stages of infection, has created new perspectives. It should be noted that because remdesivir is administered as an infusion, treatment was until recently reserved only for hospitalized COVID-19 patients.

A recent placebo-controlled, randomized, double-blind trial that involved non-hospitalized COVID-19 patients who experienced symptom onset within the previous 7 days and presented at least one risk factor for disease progression, including age ≥60 years and obesity (PINETREE, NCT04501952) showed that a 3-day course of remdesivir had an acceptable safety profile and resulted in an 87% lower risk of hospitalization or death than placebo (11). The results of this study were published during a surge in COVID-19 cases and the reduced susceptibility to several anti-SARS-CoV-2 monoclonal antibodies due to the Omicron variant. Thus, on 21st January 2022 the FDA authorized remdesivir for outpatient treatment for people at high risk of COVID-19 disease progression, and expanded the pediatric EUA to include treatment for non-hospitalized pediatric patients who are at high risk (https://www.fda.gov/news-events/press-announcements/fda-takes-actions-expand-use-treatment-outpatients-mild-moderate-covid-19).

In the United States, remdesivir is actually indicated for treating COVID-19 in adults and pediatric patients (12 years of age and older and weighing at least 40 kg) who are either hospitalized or not hospitalized and are at high risk of progression to severe COVID-19. Remdesivir is also authorized for these uses in pediatric patients below the age of 12, provided they weigh at least 3.5 kg. Detailed information can be found in the NIH COVID-19 Treatment Guidelines (https://www.covid19treatmentguidelines.nih.gov/therapies/antivirals-including-antibody-products/remdesivir/). Based on the safety data from the REDPINE (12) and CATCO (13) studies, and following the recent FDA indication, the NIH COVID-19 Panel also indicates that remdesivir can be used without dose adjustment in COVID-19 patients with estimated GFR of <30 ml/min, including those on renal replacement therapy with dialysis.

It should also be mentioned that remdesivir in combination with the JAK inhibitor baricitinib was found to be superior to remdesivir alone in reducing recovery time and accelerating improvement in clinical status among COVID-19 patients (NIAID ACTT-2 trial NCT04401579) (14). In a different study (ACTT-3 trial NCT04492475), on the other hand, remdesivir plus interferon beta-1a was, instead, not found to be superior to remdesivir alone in hospitalized patients with COVID-19 pneumonia; moreover, patients who required high-flow oxygen at baseline had worse outcomes after treatment with interferon beta-1a compared with those given placebo (15).

Finally, it should be noted that different studies now show that SARS-CoV-2 can develop resistance to remdesivir *in vitro* after prolonged exposure to the drug (16), as well as in COVID-19 patients (17).

Molnupiravir

As discussed above, most studies indicate that antivirals such as remdesivir work best when given early in the course of infection, before severe disease occurs; since one major drawback of remdesivir is that the drug is administered as an infusion, the focus began to shift to oral drugs that could be used outside of hospital settings to treat mild illness, in order to prevent progression to severe disease. In late 2021, a different polymerase inhibitor, molnupiravir (Lagevrio), jointly developed by Merck and Ridgeback Biotherapeutics, became available as a pill.

Molnupiravir (MK-4482/EIDD-2801), β-D-N4-hydroxycytidine-5'-isopropyl ester, is a bioactive prodrug of β-D-N4-hydroxycytidine (NHC, EIDD-1931), an orally bioavailable ribonucleoside analogue originally described in 2003 and characterized by a broad-spectrum activity against RNA viruses, including influenza, the Ebola virus and several zoonotic coronaviruses (reviewed in Tian et al.) (18). In the case of SARS-CoV-2, molnupiravir inhibits virus replication in human lung tissue (19), and blocks SARS-CoV-2 transmission in ferrets (20).

Molnupiravir, like remdesivir, is a nucleoside analogue, but the two drugs work in entirely different ways. Whereas remdesivir interferes with RNA chain elongation acting as a 'chain terminator' (1), molnupiravir acts as a mutagenizing agent that causes an 'error catastrophe' during viral replication, thus hindering the formation of infectious viral particles (21).

A series of preclinical and clinical studies have indicated that molnupiravir is effective in the treatment of SARS-CoV-2 infection (18). After oral administration, molnupiravir is rapidly transformed into the active NHC metabolite in plasma, distributed to various organs, and converted into the NHC 5'-triphosphate by host kinases (18). Molnupiravir has been tested in several clinical trials, some of which are completed (https://clinicaltrials.gov/ct2/results?cond=COVID-19&term=molnupiravir).

The most informative evidence of the efficacy of molnupiravir in COVID-19 patients comes from the MOVe-OUT trial (NCT04575597), an international phase 2/3, double-blind, randomized, placebo-controlled trial, involving 1433 patients with mild or moderate COVID-19, which started in October 2020. The trial evaluated the efficacy and safety of treatment with molnupiravir (800 mg twice daily for 5 days) started within 5 days of the onset of symptoms in non-hospitalized, unvaccinated adults with mild-to-moderate, laboratory-confirmed COVID-19 and at least one risk factor for severe COVID-19 illness. The interim results of the trial, announced by Merck in October 2021, found that the number of patients in the molnupiravir arm who died or needed to be hospitalized was approximately half the number of patients with such outcomes in the placebo arm. However, the recently published final results of the study, including all 1433 participants, showed that hospitalization and deaths were approximately 30% lower in the molnupiravir group: for participants who received the drug the risk of death or hospitalization through day 29 was 6.8% (48 of 709 participants), as compared to 9.7% (68 of 699 participants) in the placebo arm (difference,

−3.0 percentage points; 95% CI, −5.9 to −0.1) (22). The proportion of patients who experienced adverse events was similar in the two groups.

The final results of the study, which were suggested to be linked to the emergence of the SARS-CoV-2 Delta variant - which had not yet become globally dominant during the first half of the trial - lowered expectations and limited the initial enthusiasm for the drug (23). Furthermore, even before the final trial results were released, concerns about molnupiravir's mutagenic potential had been raised. Although animal tests indicated that the risk is low, laboratory tests suggested that there might be a risk of molnupiravir generating mutations in human DNA, especially in quickly reproducing cells such as blood cells or spermatozoa (23,24).

In November 2021, the MHRA (Medicines and Healthcare products Regulatory Agency) in the UK approved the use of molnupiravir for at-risk patients with mild to moderate COVID-19, as the world's first approved oral medication for SARS-CoV-2 (https://www.gov.uk/government/news/first-oral-antiviral-for-covid-19-lagevrio-molnupiravir-approved-by-mhra). On 23rd December 2021 in the US the FDA also granted molnupiravir an EUA for the treatment of mild to moderate COVID-19 in at-risk adults for whom alternative COVID-19 treatment options are not accessible or clinically appropriate (https://www.fda.gov/news-events/press-announcements/coronavirus-covid-19-update-fda-authorizes-additional-oral-antiviral-treatment-covid-19-certain). Following the FDA's decision, the use of molnupiravir was also authorized in other countries, while the Indian Council of Medical Research excluded molnupiravir from its COVID-19 treatment guidelines over toxicity concerns (24) on 13th January 2022, and In March 2022 WHO recommended that children and pregnant or breastfeeding patients should not be given molnupiravir, and that those who take it should have a contraceptive plan (https://www.who.int/news/item/03-03-2022-molnupiravir*).* Finally, on 23 February 2023 the European Medicines Agency (EMA) has recommended the refusal of the marketing authorization for Lagevrio for the treatment of COVID-19 in adults (https://www.ema.europa.eu/en/medicines/human/summaries-opinion/lagevrio#opinion-section).

Several trials have recently been launched to establish the efficacy and safety of molnupiravir, including the PANORAMIC study in the UK (https://www.panoramictrial.org/), the results of which were reported on January 2023 (25). Between Dec 8, 2021, and April 27, 2022, 26,411 participants (vaccinated adults at increased risk of an adverse outcome with confirmed SARS-CoV-2 infection) were randomly assigned to molnupiravir plus usual care (12,821) or to usual care alone (12,962). Data for the primary outcome (all-cause hospitalization or death) available for 25,054 (>90%) participants, indicated that early treatment with molnupiravir did not reduce the frequency of COVID-19-associated hospitalizations or death among high-risk vaccinated adults; molnupiravir treatment was, however, associated with reduced time to recovery and reduced viral load (25).

Also, in March 2022 a pharmacovigilance program was launched by WHO in low- and middle-income countries to provide further evidence of molnupiravir's safety in the general population (26). WHO recently recommended the use of molnupiravir only when alternative treatment options are not

accessible or clinically appropriate in patients with confirmed non-severe COVID-19, excluding pregnant or breastfeeding women and children (≤ 18 years), at highest risk for hospitalization; and with symptoms less than 5 days (https://www.who.int/publications/i/item/WHO-2019-nCoV-Therapeutics-Molnupiravir-Poster_A-2022.1).

Finally a new study, posted on the medRxiv preprint server in January 2023, has received considerable attention, as the analysis of global SARS-CoV-2 sequencing databases uncovered sequences that bear molnupiravir fingerprints, suggesting that the drug has triggered the evolution of viral lineages carrying several mutations that, in some cases, may be able to spread to other individuals (27).

A day after molnupiravir was approved in the UK, Pfizer announced that its antiviral drug Paxlovid cut hospitalizations by 89%.

Paxlovid

Distinct from remdesivir and molnupiravir, which target the viral polymerase, paxlovid targets the highly conserved SARS-CoV-2 main protease (Mpro, also called 3CLpro), a three-domain chymotrypsin–like cysteine protease (28). Paxlovid is a co-packaged combination of nirmatrelvir (PF-07321332) and ritonavir tablets, developed for COVID-19 treatment and post-exposure prophylaxis. Nirmatrelvir is a peptidomimetic irreversible inhibitor of the SARS-CoV-2 Mpro, while ritonavir is a HIV-1 protease inhibitor and CYP3A inhibitor. As Nirmatrelvir is metabolized mainly by CYP3A4, coadministration of nirmatrelvir with a low dose (100 mg) of ritonavir, enhances nirmatrelvir pharmacokinetics which increases therapeutic benefit (29).

The SARS-CoV-2 genome encodes two polyproteins, pp1a and pp1ab, and four structural proteins (28). The polyproteins are cleaved by Mpro at multiple sites to generate a set of shorter, nonstructural proteins that are critical for viral RNA transcription and replication, including the RdRp complex (29). In addition to the key role that Mpro plays in viral replication, the lack of closely related homologs in humans, identifies Mpro as an attractive antiviral drug target (29). Based on early studies on the small molecule protease inhibitor PF-00835231, which was investigated to ascertain whether it could be used intravenously to treat SARS-CoV-1 (30), nirmatrelvir/PF-07321332 was recently discovered and characterized as an orally bioavailable SARS-CoV-2 Mpro inhibitor with *in vitro* pan-human coronavirus antiviral activity (28). Nirmatrelvir was also found to have good selectivity and safety profiles, as well as oral activity in a mouse-adapted SARS-CoV-2 model (28).

On 14th December 2021, Pfizer announced that paxlovid significantly reduced hospitalization and death, based on an interim analysis of the Phase 2/3 EPIC-HR trial (Evaluation of Protease Inhibition for COVID-19 in High-Risk Patients, NCT04960202), a randomized, double-blind study of non-hospitalized adult patients with COVID-19, who are at high risk of progressing to severe illness (https://www.pfizer.com/news/press-release/press-release-detail/pfizer-announces-additional-phase-23-study-results).

The interim analysis showed an 89% reduction in the risk of COVID-19-related hospitalization or death from any cause compared to placebo in patients treated within three days of symptom onset. The final results of the study were published on 14th April, 2022, and confirmed the interim results (31). A total of 2246 patients were assigned in a 1:1 ratio to receive either 300 mg of nirmatrelvir plus 100 mg of ritonavir or placebo twice daily for 5 days. Efficacy was maintained in the final analysis with a difference of −5.81 percentage points (95% CI, −7.78 to −3.84; P<0.001; relative risk reduction, 88.9%). The viral load was lower with nirmatrelvir plus ritonavir than with placebo on day 5 of treatment, and all deaths reported occurred in the placebo group (29). The incidence of adverse events that emerged during the treatment period was similar in the two groups; however, dysgeusia (5.6% vs. 0.3%) and diarrhea (3.1% vs. 1.6%) occurred more frequently with nirmatrelvir plus ritonavir than with placebo (31).

One important consideration on the use of paxlovid is that the concomitant use of nirmatrelvir plus ritonavir and certain other drugs may result in potentially serious drug interactions; therefore paxlovid is contraindicated for patients already receiving certain drugs because of the risk of serious adverse events (32, 33) (Table 1). Since paxlovid is a highly effective antiviral for the treatment of COVID-19, its use should not be precluded in patients receiving concomitant medications, if drug interactions can be safely managed by adjusting the medication dose and monitoring for adverse events (Table 1). Unfortunately, for drugs listed in Table 1, information on how to adjust the dosage, also based on the pharmacokinetics interaction with ritonavir in the combined formulation of paxlovid, is not easily available. A list of common medications without clinically relevant interactions with ritonavir in the paxlovid formulation is given in Table 2 (33).

It should also be noted that the EPIC-HR trial was restricted to unvaccinated persons; a separate phase 2/3 trial of nirmatrelvir plus ritonavir, EPIC-SR (EPIC-Standard Risk, NCT05011513) included vaccinated persons and a third phase 2/3 EPIC-PEP trial (NCT05047601) evaluated the efficacy and safety of paxlovid in preventing symptomatic SARS-CoV-2 infection in the adult household contacts of individuals with SARS-CoV-2 infection. Interim analyses of the EPIC-SR showed a 57% reduction in hospitalization and death in PAXLOVID-treated vaccinated patients with at least one risk factor for severe COVID-19 as compared to placebo; however, the primary endpoint of sustained alleviation of all symptoms for four consecutive days was not met (https://www.pfizer.com/news/press-release/press-release-detail/pfizer-reports-additional-data-paxlovidtm-supporting).

Results of several clinical studies were recently reviewed (34), and a retrospective cohort study in a highly vaccinated US outpatient population, showed that paxlovid effectively reduced the incidence of hospitalization or death within 30 days of a positive test for SARS-CoV-2 compared to standard of care; early treatment (within 5 days of symptom onset) was associated with the greatest clinical benefit (34). In addition, a recent study showed that, also during the omicron surge, the rates of hospitalization and death due to COVID-19 were significantly lower among patients 65 years of age or older who received paxlovid than among those who did not; however, no evidence of benefit was found in younger adults (35). Notably, a very recent Italian study compared for the first time data

on mortality in COVID-19 patients treated with paxlovid (11,576) or molnupiravir (17,977) using data collected in the nationwide, population-based, cohort of patients registered in the database of the Italian Medicines Agency (AIFA) between February and April 2022. During this timeframe, Italy pandemic was mainly characterized by the BA.1, BA.1.1 and BA.2 Omicron variants/subvariants (36). Early initiation (within 5 days after the test date and symptom onset) of paxlovid was associated with a significant reduced risk of all-cause mortality by day 28 compared to molnupiravir, both in the overall population and in patients subgroups, including those fully vaccinated with the booster dose. These findings strongly support paxlovid rather than molnupiravir as a preferred option for early treatment of SARS-CoV-2 infected patients at risk of clinical progression in the Omicron era. Further studies are required to extent and validate these findings across different viral strains.

Paxlovid received its first EUA on the 22nd December 2021 in the USA for the treatment of mild-to-moderate COVID-19 in adults and pediatric patients (≥ 12 years of age and weighing ≥ 40 kg) who are at increased risk of progression to severe COVID-19. Paxlovid also received conditional authorization for the treatment of COVID-19 in the UK on the 31st December 2021, and more recently in the EU (January 2022) (32).
Treatment with paxlovid is currently recommended by NIH as the first choice for antiviral therapy for nonhospitalized adults who are at high risk for disease progression, regardless of vaccination status (NIH COVID-19 Treatment Guidelines: https://www.covid19treatmentguidelines.nih.gov/therapies/antivirals-including-antibody-products/ritonavir-boosted-nirmatrelvir--paxlovid-/); on the other hand, apparently the drug has not been deployed to the extent that health officials had expected. Worries about cases of recurrence of COVID-19 clinical symptoms after completion of paxlovid treatment (post-Paxlovid 'rebound') have been reported; however, recent studies have found that viral rebound and the recurrence of COVID-19 symptoms can also occur in the absence of treatment (37).

Finally, it should be mentioned that a recent study demonstrates that high-level resistance to nirmatrelvir can readily be achieved by SARS-CoV-2 via multiple pathways in vitro (38). The mechanism of resistance is being presently studied in detail to inform the design of next-generation protease inhibitors. Among these, a promising new 3CL protease inhibitor, ensitrelvir (also known as S-217622), has demonstrated clinical efficacy (39); in November 2022 ensitrelvir, a once-daily antiviral made by the Japanese pharmaceutical company Shionogi, received an EUA for COVID-19 treatment in Japan.

In conclusion, in the last months, in addition to the first antiviral approved for COVID-19 treatment – remdesivir - two new antiviral drugs, molnupiravir and paxlovid, have received an EUA in different countries. In the case of paxlovid, recently, on May 25, 2023, the FDA has approved the use of the drug for the treatment of mild to moderate COVID-19 in adults at high risk of progressing to severe COVID-19 (https://www.fda.gov/news-events/press-announcements/fda-approves-first-oral-antiviral-treatment-covid-19-adults).

Both molnupiravir and paxlovid are available for oral use in non-hospitalized patients, but neither drug is a panacea: molnupiravir may cause mutations in human DNA, leading the health authorities

in some countries to advise against its use during pregnancy, while other countries have chosen not to authorize it at all. And paxlovid's possible interaction with a wide range of commonly used drugs limits its use.

In addition to ensitrelvir, a large number of antivirals that target the SARS-CoV-2 main protease or the polymerase are currently being developed. It is also expected that, as in the case of other viral diseases, such as AIDS and Hepatitis C, combinations of antivirals that target different viral or host proteins will be able to boost their effectiveness and reduce the risk of developing drug resistance (40).

Table 1. Main drug-drug interaction of nirmatrelvir/ritonavir (Paxlovid)

Paxlovid co-administration contraindicated/not recommended

Anticonvulsants	**Cardiovascular**	**Pulmonary hypertension**
Carbamazepine	Amiodarone	Sildenafil
Phenobarbital	Clopidogrel	Tadalafil
Phenytoin	Disopyramide	Vardenafil
Primidone	Dofetilide	
	Dronedarone	**Miscellaneous**
Anti-Infectives	Eplerenone	Bosentan
Glecaprevir/pibrentasvir	Flecainide	Certain chemotherapeutic agents
Rifampin	Ivabradine	Ergot derivatives
Rifapendine	Propafenone	Lumacaftor/ivacaftor
	Quinidine	St John's wort
Neuropsychiatric		Tolvaptan
Clozapine	**Immunosuppressants**	
Lurasidone	Voclosporin	
Midazolam (oral)		
Pimozide		

Paxlovid administration needs temporary withhold of concomitant drugs (if clinically appropriate)

Anticoagulants	**Immunosuppressants**	**Neuropsychiatric**
Rivaroxaban	Everolimus	Suvorexant
	Sirolimus	Triazolam
Anti-Infectives	Tacrolimus	
Erythromycin		**Erectile dysfunction**
	Lipid-modifiers	Avanafil
BPH	Atorvastatin	
Alfuzosin	Lomitapide	**Respiratory**
Silodosin	Lovastatin	Salmeterol
	Rosuvastatin	
Cardiovascular	Simvastatin	**Miscellaneous**
Aliskiren		Certain chemotherapeutic agents
Ranolazine	**Migraine**	Colchicine
Ticagrelor	Eletriptan	Finerenone
Vorapaxar	Rimegepant	Flibanserin
	Ubrogepant	Naloxegol

Adjustment of concomitant medication dose and monitoring for adverse effects

Anticoagulants	**Anti-Infectives**	**Cardiovascular**
Apixaban	Clarithromycin	Amlodipine
Dabigatran	Itraconazole	Cilostazol
Edoxaban	Ketoconazole	Digoxin
	Maraviroc	Diltiazem
BPH	Rifabutin	Felodipine
Tamsulosin		Nifedipine
	Neuropsychiatric	Verapamil
Immunosuppressants	Alprazolam	
Cyclosporine	Aripiprazole	**Pain**
Dexamethasone	Brexpiprazole	Fentanyl
Fedratinib	Buspirone	Hydrocodone
Ruxolitinib	Cariprazine	Oxycodone
Tofacitinib	Chlordiazepoxide	
Upadacitinib	Clobazam	**Pulmonary Hypertension**
	Clonazepam	Riociguat
Erectile Dysfunction	Clorazepate	
Sildenafil	Diazepam	**Miscellaneous**
Tadalafil	Estazolam	Certain chemotherapeutic agents
Vardenafil	Flurazepam	Darifenacin
	Iloperidone	Elexacaftor/tezacaftor/ivacaftor
Migraine	Lumateperone	Eluxadoline
Almotriptan	Pimavanserin	Ivacaftor
	Quetiapine	Solifenacin
Diabetes	Trazodone	Tezacaftor/ivacaftor
Saxagliptin		

Continue concomitant medication and monitor for adverse effects

Anticoagulants	**Cardiovascular**	**Neuropsychiatric**
Warfarin	Mexiletine	Haloperidol
	Sacubitril	Hydroxyzine
Anti-Infectives	Valsartan	Mirtazapine
Brincidofovir		Risperidone
Cobicistat- or ritonavir-boosted	**Pain**	Ziprasidone
antiretrovirals	Buprenorphine	Zolpidem
Isavuconazole	Hydromorphone	
Posaconazole	Methadone	**Migraine**
Voriconazole	Morphine	Zolmitriptan
	Tramadol	
BPH		**Miscellaneous**
Doxazosin		Certain chemotherapeutic agents
Terazosin		Certain conjugated monoclonal antibodies
Diabetes		Oxybutynin
Glyburide		

From COVID-19 Treatment Guidelines Panel. Coronavirus Disease 2019 (COVID-19) Treatment Guidelines. National Institutes of Health. https://www.covid19treatmentguidelines.nih.gov/ (21 July 2023) (31).

Table 2. A list of common medications without clinically relevant interactions with ritonavir in the paxlovid formulation

Medications without clinically relevant interactions

Acid Reducers Famotidine Omeprazole Pantoprazole **Allergy** Cetirizine Diphenhydramine Fexofenadine Loratadine **Anti-Infectives** Azithromycin Cidofovir Hydroxychloroquine Ticovirimat Valacyclovir **Diabetes** Empagliflozin Insulin Metformin Pioglitazone **Migraine** Frovatriptan Naratriptan Riztriptan Sumatriptan	**Cardiovascular** Aspirin Atenolol Carvedilol Furosemide Hydrochlorothiazide Irbesartan Isosorbide dinitrate Lisinopril Losartan Metoprolol Prasugrel **Immunosuppressants** Abrocitinib Baricitinib Methotrexate Mycophenolate Prednisone **Neuropsychiatric** Amitriptyline Bupropion Citalopram Duloxetine Escitalopram Fluoxetine Gabapentin Lorazepam Nortriptyline Olanzapine Paroxetine Sertraline Venlafaxine	**Pain** Acetaminophen Aspirin Codeine Ibuprofen Meloxicam Naproxen **Respiratory** Corticosteroids (inhaled/nasal) Formoterol Montelukast **Miscellaneous** Allopurinol Contraceptives (oral) Cyclobenzaprine Donepezil Enoxaparin Finasteride Levothyroxine Most mAb products Ondansetron

From COVID-19 Treatment Guidelines Panel. Coronavirus Disease 2019 (COVID-19) Treatment Guidelines. National Institutes of Health. https://www.covid19treatmentguidelines.nih.gov/ (21 July 2023) (31).

References

1. Eastman RT, et al. Remdesivir: a review of its discovery and development leading to emergency use authorization for treatment of COVID-19. *ACS Cent Sci.* 2020; 6:672–683.

2. Vegivinti CT, et al. Efficacy of antiviral therapies for COVID-19: a systematic review of randomized controlled trials. *BMC Infect Dis.* 2022; 22: 107.

3. Wang Y, et al. Remdesivir in adults with severe COVID-19: a randomised, double-blind, placebo-controlled, multicentre trial. *Lancet* 2020; 395: 1569-1578.

4. Beigel JH, et al. Remdesivir for the treatment of Covid-19 - Final Report. *N Engl J Med.* 2020; 383: 1813-1826.

5. Olender SA, et al. Remdesivir for severe COVID-19 versus a cohort receiving standard of care. *Clin Infect Dis.* 2021; 73: e4166–e4174.

6. Spinner CD, 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: 1048-1057.

7. WHO Solidarity Trial Consortium. Repurposed Antiviral Drugs for Covid-19 -- Interim WHO Solidarity Trial Results. *N Engl J Med.* 2021; 384: 497-511.

8. Ali K, et al. Remdesivir for the treatment of patients in hospital with COVID-19 in Canada: a randomized controlled trial. *CMAJ* 2022; 194: E242-251.

9. Amstutz A, et al. Effects of remdesivir in patients hospitalised with COVID-19: a systematic review and individual patient data meta-analysis of randomised controlled trials*. Lancet Respir Med.* 2023; doi: 10.1016/S2213-2600(22)00528-8. Epub ahead of print.

10. Devgun JM, et al. Identification of bradycardia following remdesivir administration through the US Food and Drug Administration American College of Medical Toxicology COVID-19 toxic pharmacovigilance project. *JAMA Netw Open.* 2023; 6(2):e2255815. doi: 10.1001/jamanetworkopen.2022.55815.

11. Gottlieb RL, et al. Early remdesivir to prevent progression to severe Covid-19 in outpatients. *N Engl J Med.* 2022; 386: 305-315.

12. Santos JR, Goldman JD, Tuttle KR, et al. The REDPINE study: efficacy and safety of remdesivir in people with moderately and severely reduced kidney function hospitalized for COVID-19 pneumonia: Presented at: 33rd European Congress of Clinical Microbiology and Infectious Diseases; April 15-18, 2023; Copenhagen, Denmark. Available at: https://www.askgileadmedical.com/docs/comference/JoseRamon_ECCMID2023_Redpine_P2635.pdf

13. Cheng M, Fowler R, Murthy S, et al. Remdesivir in patients with severe kidney dysfunction: a secondary analysis of the CATCO randomized trial. JAMA Netw Open. 2022; 5:e2229236.

14. Kalil AC, et al. Baricitinib plus remdesivir for hospitalized adults with Covid-19. *N Engl J Med.* 2021; 384: 795-807.

15. Kalil AC, et al. Efficacy of interferon beta-1a plus remdesivir compared with remdesivir alone in hospitalised adults with COVID-19: a double-bind, randomised, placebo-controlled, phase 3 trial. *Lancet Respir Med.* 2021; 9: 1365-1376.

16. Stevens LJ, et al. Mutations in the SARS-CoV-2 RNA dependent RNA polymerase confer resistance to remdesivir by distinct mechanisms. *Sci. Transl. Med.* 2022; 14:eabo0718.

17. Gandhi S, et al. De novo emergence of a remdesivir resistance mutation during treatment of persistent SARS-CoV-2 infection in an immunocompromised patient: a case report. *Nat. Commun.* 2022; 13: 1547. doi: 10.1038/s41467-022-29104-y.

18. Tian L, et al. Molnupiravir and its antiviral activity against COVID-19. *Front Immunol.* 04 April 2022; https://doi.org/10.3389/fimmu.2022.855496.

19. Wahl A, et al. SARS-CoV-2 infection is effectively treated and prevented by EIDD-2801. *Nature* 2021; 59: 451-457.

20. Cox RM, et al. Therapeutically administered ribonucleoside analogue MK-4482/EIDD-2801 blocks SARS-CoV-2 transmission in ferrets. *Nat Microbiol.* 2021; 6: 11-18.

21. Kabinger F, et al. Mechanism of molnupiravir-induced SARS-CoV-2 mutagenesis. *Nat Struct Mol Biol.* 2021; 28: 740–746.

22. Bernal AJ, et al. Molnupiravir for oral treatment of Covid-19 in nonhospitalized patients. *N Engl J Med.* 2022; 386: 509-520.

23. Extance A. Covid-19: what is the evidence for the antiviral molnupiravir? *BMJ* 2022; 377:o926http://dx.doi.org/10.1136/bmj.o926.

24. Zhou S, et al. β-d-N4-hydroxycytidine inhibits SARS-CoV-2 through lethal mutagenesis but is also mutagenic to mammalian cells. *J Infect Dis.* 2021; 224: 415–419.

25. Butler CC, et al. Molnupiravir plus usual care versus usual care alone as early treatment for adults with COVID-19 at increased risk of adverse outcomes (PANORAMIC): an open-label, platform-adaptive randomised controlled trial. *Lancet* 2023; 401: 281-293.

26. World Health Organization. Safety monitoring of molnupiravir for treatment of mild to moderate covid-19 infection in low and middle-income countries using cohort event monitoring: a WHO study. World Health Organization. https://apps.who.int/iris/rest/bitstreams/1413629/retrieve.

27. Sanderson T, et al. Identification of a molnupiravir-associated mutational signature in SARS-CoV-2 sequencing database. *Preprint at medRxiv*, 2023: https://doi.org/10.1101/2023.01.26.23284998.

28. Owen DR, et al. An oral SARS-CoV-2 Mpro inhibitor clinical candidate for the treatment of COVID-19. *Science* 2021; 374: 1586-1593.

29. Wen W, et al. Efficacy and safety of three new oral antiviral treatment (molnupiravir, fluvoxamine and Paxlovid) for COVID-19: a meta-analysis. *Annals Med.* 2022; 54: 516-523.

30. Boras B, et al. Preclinical characterization of an intravenous coronavirus 3CL protease inhibitor for the potential treatment of COVID-19. *Nat Commun.* 2021; 12: 6055.

31. Hammond J, et al. Oral Nirmatrelvir for high-risk, nonhospitalized adults with Covid-19. *N Engl J Med.* 2022; 386: 1397-1408.

32. Lamb YN. Nirmatrelvir Plus Ritonavir: First Approval. *Drugs* 2022, 19: 1-7.

33. COVID-19 Treatment Guidelines Panel. Coronavirus Disease 2019 (COVID-19) Treatment Guidelines. National Institutes of Health. Available at https://www.covid19treatmentguidelines.nih.gov/. Accessed 21 July 2023.

34. Lewnard JA, et al. Effectiveness of nirmatrelvir–ritonavir in preventing hospital admissions and deaths in people with COVID-19: a cohort study in a large US health-care system. *Lancet Infect Dis.* 2023 DOI:https://doi.org/10.1016/S1473-3099(23)00118-4.

35. Arbel R, et al. Nirmatrelvir use and severe Covid-19 outcomes during the Omicron surge. *N Engl J Med.* 2022; 387: 790-798.

36. Torti C, Olimpieri P.P., Bonfanti P et al. Real-life comparison of mortality on patients with SARS-CoV-2 infection at risk for clinical progression treated with molnupiravir or nirmatrelvir plus ritonavir during the Omicron era in Italy: a nationwide, cohort study. *Lancet Regional Health – Europe.* 2023; published online doi.org/10.1016/j.lanepe.2023.100684.

37. Anderson AS, et al. Nirmatrelvir–Ritonavir and Viral Load Rebound in Covid-19. *N Engl J Med.* 2022; 387: 1047-1049.

38. Iketani S, et al. Multiple pathways for SARS-CoV-2 resistance to nirmatrelvir. *Nature* 2023; 613: 558-564.

39. Mukae, H. et al. A randomized phase 2/3 study of ensitrelvir, a novel oral SARS-CoV-2 3C-like protease inhibitor, in Japanese patients with mild-to-moderate COVID-19 or asymptomatic SARS-CoV-2 infection: results of the phase 2a part. *Antimicrob. Agents Chemother*. 2022; 66**:** e0069722.

40. Schultz DC, et al. Pyrimidine inhibitors synergize with nucleoside analogues to block SARS-CoV-2. *Nature* 2022; 604: 134–140.

4. Non-steroidal anti-inflammatory drugs and COVID-19

As the incidence of COVID-19 began to accelerate in Europe, the French Health Minister, Olivier Véran, claimed that nonsteroidal anti-inflammatory drugs (NSAIDs), like ibuprofen could aggravate the infection (1). This led to an advisory on the WHO and EMA websites. However, evidence has not emerged to substantiate this claim. The advisories have been withdrawn.

NSAIDs work by suppressing prostaglandin synthases 1 and 2, colloquially known as cyclooxygenase (COX)-1 and COX-2. These enzymes produce prostaglandins (PGs), lipids that can trigger pain and fever. COX-2 produces most of the PGs relevant to pain and inflammation. NSAIDs selective for inhibiting COX-2 include celecoxib, etoricoxib and diclofenac; ibuprofen is an NSAID that blocks both COXs.

The French Health Minister advised people to take paracetamol (acetaminophen) for fever instead of NSAIDs (1). However, acetaminophen is an NSAID (2). The most common oral daily dose – 1000mg - inhibits prostaglandin PG formation by both COX-1 and COX-2 enzymes by about 50% (3). Common daily doses of drugs like ibuprofen hit ~100% at time of peak action (3).

Acetaminophen and other NSAIDs reduce body temperature the same way – by inhibiting the central PGE_2 dependent activation of EPr3 (4). They are also analgesic through the same mechanism, by reducing PGE_2-dependent central and peripheral activation of EPrs (5). One must move up the dose response curve with NSAIDs to achieve maximal PG inhibition (as is achieved on common daily doses of other NSAIDs that inhibit both COXs like ibuprofen) to gain anti-inflammatory efficacy. Thus, at acetaminophen 3-4000mg/day, there is a similar GI (6) and hypertensive (7) adverse effect profile as with other NSAIDs.

However, acetaminophen has a particular risk of hepatotoxicity at higher doses which are avoided for that reason. The makers of acetaminophen made a virtue of necessity and marketed acetaminophen as an anti-pyretic, analgesic. They claimed that it was not an NSAID because it did not cause GI toxicity. At that time (before the discovery of COX-2), all NSAIDs competed in direct-to-consumer advertising in the US by claiming a safer GI profile. So, the myth that acetaminophen was not an NSAID was marketed and widely believed. Like other NSAIDs, acetaminophen has PG independent effects of unestablished relevance to their clinical profile. Most commonly, we also use aspirin, another NSAID, at doses that are not anti-inflammatory and take advantage of its particular action on the platelets at low doses, thereby minimizing its GI toxicity.

Given the thrombotic complications of COVID-19, it was suggested that aspirin might be beneficial in treating COVID-19. Thrombotic events appear to be no more common in patients with Acute Respiratory Distress Syndrome caused by COVID-19 than by other viral or bacterial causes. The RECOVERY Collaborative Group has shown that in patients hospitalised with COVID-19, aspirin was not associated with reductions in 28 day mortality or in the risk of progressing to invasive mechanical ventilation or death, but was associated with a small increase in the rate of being discharged alive within 28 days (8). Ongoing trials are assessing the potential utility of low dose aspirin in delaying or postponing hospitalization in patients with milder disease.

Membrane sphingolipids (9) and membrane cholesterol (10) modulate viral entry into cells. Furthermore, activation of phospholipases by viral attachment to its cellular receptors releases many bioactive lipids, including PGs, such as PGE_2, PGD_2, and prostacyclin (PGI_2), which can both promote and restrain inflammation (11).

For example, the infection of certain immune cells (microglia) with a related coronavirus (not the one that causes COVID-19) activates a proinflammatory response (the inflammasome) to combat the pathogen; however, PGD_2 increases the expression of PYDC3, a putative inflammasome inhibitor, in certain immune cells in mice (12). The SARS coronavirus responsible for the 2003 outbreak directly binds to the COX-2 promotor and increases its expression (13), boosting PG

production capacity. There is also evidence that PGE2 inhibits SARS coronavirus replication (14). Indomethacin, an NSAID, blocks coronavirus RNA synthesis, but independently of COX inhibition (15). In contrast, COX-2–dependent PGE2 attenuates the chronic antiviral lymphocyte response of unresolved viral infection (16). Based on these findings, multiple contrasting possibilities are plausible, but evidence has yet to emerge of the relevance of these observations to the course or treatment of COVID-19.
Perhaps the most provocative finding relates to PGD2, the predominant COX-2 product of mast cells. It acts through its two receptors, DPr1 and DPr2. DPr1 signalling delays the migration of dendritic cells (DCs) to lung and lymph nodes by down-regulating the expression of C-C chemokine receptor type 7 (CCR7) on respiratory DCs in response to infection. DPr1 inhibition enhances DC migration and, in turn, T cell proliferation, which increased survival in older, but not younger mice after SARS-CoV infection (17). More recently, DPr1 deletion or blockade with an antagonist, asapiprant, or the deletion of an upstream biosynthetic enzyme, the phospholipase, PLA2G2D, protected middle-aged mice against lethal infection with SARS CoV-2 (18). While asapiprant is being investigated in ongoing clinical trials, other studies are exploring the spectrum of the lipidomic response to infection in search of predictive signatures and therapeutic opportunity (19).

Patterns of individual PG formation may turn out to reflect the intensity of disease and forecast its course but also signal the opportunity to intervene with potentially preventative therapies before patients progress to severe disease. For example, microangiopathy and hemostatic activation are features of severe COVID-19 and roughly 30% of our patients have elevated d-dimers at hospitalization. To give just one example, thromboxane (Tx) biosynthesis is markedly elevated in the acute respiratory distress syndrome (ARDS) and preclinical studies have shown that Tx receptor (TPr) antagonism prevents the evolution of a lipopolysaccharide (LPS)-induced syndrome of ARDS in sheep (20). Unlike NSAIDs, which suppress the vasodilator PGs that maintain renal blood flow (RBF) in syndromes such as ARDS, TPr antagonism would be expected to sustain RBF even in renoprival syndromes, such as ARDS, where NSAIDs cannot be used (21). Thus, serial analysis of PGs in patients with COVID-19 may suggest that the modulation of individual PGs be considered for therapeutic intervention or as biomarkers that are predictive of disease progression. Interestingly, *ex-vivo* studies of monocyte-derived macrophages, which are themselves strongly implicated in ARDS pathogenesis, suggest that even mild infections with COVID-19 inflict a lasting pro-inflammatory eicosanoid signature, which remains evident 1 month after infection (22).

A large study of patients with sepsis of bacterial and viral origin linked clinical outcomes to lipidomic, proteomic and peripheral immune cellular phenotypes (23). This revealed a broad based lipidomic storm driven predominantly by secretory phospholipase A_2 dependent eicosanoid production. Elevations in the cyclooxygenase products of arachidonic acid, PGD_2 and PGI_2, and the AA lipoxygenase product, 12-HETE, and a reduction in the high abundance lipids, ChoE 18:3, LPC - O-16:0 and PC-O-30:0 exhibit relative specificity for COVID-19 amongst such patients, correlate with the inflammatory response and link to disease severity. Linoleic acid binds directly to SARS-CoV-2 (24) and both linoleic acid and its di-HOME products reflected disease severity in COVID-19 in this study.

While this study revealed potential therapeutic targets, little has yet been published targeting specific eicosanoids. A randomized controlled trial of inhaled prostacyclin in patients with acute respiratory distress syndrome failed to establish benefit (25). A comparison of intravenous n-3 fatty acid infusion with control in 20 patients hospitalized with COVID-19 depressed proinflammatory eicosanoids and isoprostane generation while augmenting prostacyclin but was not designed to assess clinical outcome (26).

Conflicting public health messaging has surrounded the question of whether concomitant NSAIDs might blunt the immune response to vaccination against SARS-CoV-2. A recent pilot longitudinal study of repeated vaccination in 5 deeply phenotyped volunteers (27) revealed that they all mounted a vaccine specific immune response, irrespective of NSAID consumption. The quantified response for SARS CoV-2-specific RBD IgG, measured by ELISA, showed a transient dampening in the NSAID group compared to controls. However, this study merely provided a basis for power calculations necessary for a definitive study, which would require 90 patients per group to address this response.

Summary

If there is no clear evidence of risk from NSAIDs, should patients with clinically complicated SARS-CoV-2 infections receive them? No. There is no evidence of benefit, either. If such a patient also had poor kidney function, maintenance of renal blood flow would become critically dependent on vasodilator PGs, such as PGE_2 and PGI_2. This situation might also predispose the patient to the gastrointestinal and cardiovascular complications of NSAIDs. However, until we have robust evidence, patients who are in chronic pain should continue to take their NSAIDs, rather than turning to opiates. Given that the elderly are an at-risk group for severe COVID-19, an association between NSAIDs and the disease may merely reflect reverse causality. Low-dose aspirin appears minimally effective in patients hospitalized with COVID- 19. Its utility in patients with milder disease remains to be reported.

References

1. Willsher K. Anti-inflammatories may aggravate Covid-19, France advises, *The Guardian* (2020).

2. Smyth E, Grosser T and FitzGerald GA. Chapter 38: Anti-inflammatory, antipyretic, and analgesic agents; Pharmacotherapy of Gout. In Goodman & Gilman's: The Pharmacological Basis of Therapeutics, 13e Laurence L. Brunton, Bruce A. Chabner, Björn C. Knollmann Editors. 2018.

3. Catella-Lawson F, Reilly MP, Kapoor SC, Cucchiara AJ, DeMarco S, Tournier B, Vyas SN, FitzGerald GA. Cyclooxygenase inhibitors and the antiplatelet effects of aspirin. *N Engl J Med.* 2001; 345(25):1809-17.

4. Ushikubi F, Segi E, Sugimoto Y, Murata T, Matsuoka T, Kobayashi T, Hizaki H, Tuboi K, Katsuyama M, Ichikawa A, Tanaka T, Yoshida N, Narumiya S. Impaired febrile response in mice lacking the prostaglandin E receptor subtype EP3. *Nature.* 1998; 395(6699):281-4.

5. Grosser T, Theken KN, FitzGerald GA. Cyclooxygenase inhibition: pain, inflammation, and the cardiovascular system. *Clin Pharmacol Ther.* 2017 Oct;102(4):611-622.

6. García Rodríguez LA, Hernández-Díaz S. Relative risk of upper gastrointestinal complications among users of acetaminophen and nonsteroidal anti-inflammatory drugs. *Epidemiology.* 2001 Sep;12(5):570-6.

7. Sudano I, Flammer AJ, Périat D, Enseleit F, Hermann M, Wolfrum M, Hirt A, Kaiser P, Hurlimann D, Neidhart M, Gay S, Holzmeister J, Nussberger J, Mocharla P, Landmesser U, Haile SR, Corti R, Vanhoutte PM, Lüscher TF, Noll G, Ruschitzka F. Acetaminophen increases blood pressure in patients with coronary artery disease. *Circulation.* 2010; 122(18):1789-96. doi: 10.1161/CIRCULATIONAHA.110.956490. Epub 2010 Oct 18.

8. RECOVERY Collaborative Group. Aspirin in patients admitted to hospital with COVID-19 (RECOVERY): a randomised, controlled, open-label, platform trial. *Lancet.* 2022 Jan 8;399(10320):143-151.

9. van der Meer-Janssen YPM, van Galen J, Batenburg JJ, Bernd Helms J. Lipids in host–pathogen interactions: Pathogens exploit the complexity of the host cell lipidome. *Prog Lipid Res.* 2010; 49(1): 1–26.

10. Hu B., Höfer C., Thiele C., Veit M. Cholesterol binding to the transmembrane region of a group 2 hemagglutinin (HA) of influenza virus is essential for virus replication, affecting both virus assembly and HA fusion activity. *J Virol.* 2019; 93(15): e00555-19. PMID: 31118253

11. Theken K, et al. The roles of lipids in SARS-CoV-2 viral replication and the host immune response. *J Lipid Res.* 2021;62: 100129.

12. Vijay R., et al. Virus-induced inflammasome activation is suppressed by prostaglandin D 2/DP1 signalling. *Proc. Natl. Acad. Sci. U.S.A.* 2017; 114, E5444.

13. Yan W., et al. Nucleocapsid protein of SARS-CoV activates the expression of cyclooxygenase-2 by binding directly to regulatory elements for nuclear factor-kappa B and CCAAT/enhancer binding protein. *Int J Biochem Cell Biol.* 2006; 38, 1417.

14. Sander W.J., et al. Prostaglandin E 2 as a modulator of viral infections. *Front. Physiol.* 2017; 8: 89.

15. Amici C., et al. Indomethacin has a potent antiviral activity against SARS coronavirus. Antivir. Ther. 2006; 11: 1021.

16. Schaeuble K., et al. Attenuation of chronic antiviral T-cell responses through constitutive COX2-dependent prostanoid synthesis by lymph node fibroblasts. *PLOS Biol.* 2019; 17: e3000072.

17. Theken K. and FitzGerald G.A. Bioactive lipids in antiviral immunity. *Science* 2021; 371(6526):237-238. doi: 10.1126/science.abf3192.

18. Zhao J., et al. Age-related increases in PGD(2) expression impair respiratory DC migration, resulting in diminished T cell responses upon respiratory virus infection in mice. *J. Clin. Invest.* 2011; 121: 4921.

19. Roy Wong, L.-Y. et al. Eicosanoid signaling blockade protects middle-aged mice from severe COVID-1. *Nature*. 2022 Mar 21. doi: 10.1038/s41586-022-04630-3. Online ahead of print.

20. Kühl PG, Bolds JM, Loyd JE, Snapper JR, FitzGerald GA. Thromboxane receptor-mediated bronchial and hemodynamic responses in ovine endotoxemia. *Am J Physiol* 1988; 254(2 Pt 2):R310-9.

21. Qi Z, Hao CM, Langenbach RI, Breyer RM, Redha R, Morrow JD, Breyer MD. Opposite effects of cyclooxygenase-1 and -2 activity on the pressor response to angiotensin II. *J Clin Invest.* 2002; 110(1):61-9.

22. Bohnacker S., Hartung F., Henkel F., et al. Mild COVID-19 imprints a long-term inflammatory eicosanoid- and chemokine memory in monocyte-derived macrophages. *Mucosal Immunol.* 2022. https://doi.org/10.1038/s41385-021-00482-8.

23. Meng H et al. Deep Phenotyping of the Lipidomic Response in COVID and non-COVID Sepsis. https://www.biorxiv.org/content/10.1101/2023.06.02.543298v1

24. Toelzer C et al. Cryo-EM reveals binding of linoleic acid to SARS-CoV-2 spike glycoprotein, suggesting an antiviral treatment strategy. *Acta Crystallogr D Struct Biol.* 2023; 79:111–121.

25. Haeberle H et al. Inhaled prostacyclin therapy in the acute respiratory distress syndrome: a randomized controlled multicenter trial. *Respir Res.* 2023; 24:58.

26. Pawelzik S.C. et al. Decreased oxidative stress and altered urinary oxylipidome by intravenous omega-3 fatty acid emulsion in a randomized controlled trial of older subjects hospitalized for COVID-19. Free *Radic Biol Med.* 2023; 194:308–315.

27 Skarke C et al. Modulation of the immune response to SARS-CoV-2 vaccination by NSAIDs. *J Pharmacol Exp Ther.* 2023; DOI: 10.1124/jpet.122.001415.

5. Corticosteroids

Several randomized clinical trials indicate that systemic corticosteroids improve clinical outcomes and reduce mortality in hospitalized patients with COVID-19 who require supplemental oxygen (1-5). This benefit is likely to be achieved by virtue of corticosteroid therapy suppressing the COVID-19-induced systemic inflammatory response that can lead to lung injury and multisystem organ dysfunction (6).

The place of corticosteroids in the treatment of COVID-19 has been first established by the outcome of the RECOVERY trial (1). This was a randomized, controlled, open-label, adaptive, platform trial

comparing a range of possible treatments with usual care in patients hospitalized with COVID-19. Around 10% of all UK hospitalized patients with COVID-19 were enrolled in the trial, and the control arm fatality rate is consistent with the overall case fatality rate of hospitalized patients with COVID-19 in the UK (1).

Prior to this trial there was considerable confusion about the place of steroids in the treatment of severe viral infections. On the one hand, slower clearance of viral RNA had been observed in patients with SARS, MERS and influenza treated with systemic corticosteroids. On the other, steroids offered a theoretical benefit after the phase of viral replication when immunopathology is dominant.

However, prior to the RECOVERY trial, clinical trials of sufficient size and rigor had not been performed in such settings. In this trial, the comparison of dexamethasone 6 mg given once daily for up to ten days vs. usual care alone was assessed (1). The primary outcome was 28-day mortality. In contrast to SARS and MERS, the phase of viral replication in COVID-19 is early after infection, declining thereafter.

As reported (1), 2104 patients randomly allocated to receive dexamethasone were compared with 4321 patients concurrently allocated to usual care. Overall, 482 (22.9%) patients allocated dexamethasone and 1110 (25.7%) patients allocated usual care died within 28 days (age adjusted rate ratio [RR], 0.83; 95% confidence interval [CI], 0.75 to 0.93; P<0.001). Based on sub-group analysis, the proportional and absolute mortality rate reductions varied significantly depending on the level of respiratory support at randomization: dexamethasone reduced deaths by one-third in patients receiving invasive mechanical ventilation (29.3% vs. 41.4%; RR, 0.64; 95% CI, 0.51 to 0.81), by one-fifth in patients receiving oxygen without invasive mechanical ventilation (23.3% vs. 26.2%; RR, 0.82; 95% CI, 0.72 to 0.94), but had no significant protective effect in those who were not receiving any respiratory support (1).

Most deaths were due to Covid-19, and such deaths were less frequent in the dexamethasone group than in the usual care group. The very small number of reported serious adverse reactions consisted of recognized adverse effects of glucocorticoids (1).

Despite some methodologic caveats about the platform design of RECOVERY (2), this trial provides clear evidence that treatment with dexamethasone 6 mg once daily for up to 10 days reduces 28-day mortality in patients with COVID-19 who are receiving respiratory support. Based on these results, one death would be prevented by treatment of around 8 patients requiring invasive mechanical ventilation or around 34 patients requiring oxygen without invasive mechanical ventilation.

In contrast, in hospitalized patients with COVID-19 who do not require supplemental oxygen, the use of systemic corticosteroids has not shown any benefits and may cause harm (1,3). There are no data to support the use of systemic corticosteroids in non-hospitalized patients with COVID-19.

The usefulness of dexamethasone in patients with severe pulmonary complications of COVID-19 infection has been supported by further investigations (4,5). In particular, a subsequent meta-analysis of seven trials of glucocorticoids (dexamethasone, hydrocortisone, or methylprednisolone) for critically ill patients with COVID-19, including RECOVERY (but limited to patients who received invasive mechanical ventilation), has confirmed the findings of this trial (5).

Based on this evidence, the use of dexamethasone in hospitalized patients requiring respiratory support is widely recommended. Details on specific recommendations for or against corticosteroid therapy are provided by several treatment guidelines (6,7).

Inhaled corticosteroids have been proposed as an early COVID-19 treatment on the basis of their targeted anti-inflammatory effects in the lung and their antiviral properties (8-10). Few randomized controlled trials have explored treatment with inhaled corticosteroids (budesonide, ciclesonide, fluticasone) in nonhospitalized patients with COVDI-19 within 7 to 14 days of the onset of mild to moderate symptoms (11-15). Overall, evidence from these trials suggest that the use of inhaled corticosteroids in outpatients COVID-19 does not adversely affect clinical outcomes nor does it increase the risk of side-effects compared with usual care or placebo. Nonetheless, the effectiveness of these medications in early COVID-19 management remains ill-defined. Therefore the NIH COVID-19 Treatment Guidelines Panel concludes that there is insufficient evidence to recommend either for or against the use of inhaled corticosteroids for the treatment of COVID-19 in nonhospitalized patients (6). Nonetheless, the Panel indicates that patients with COVID-19 who are receiving an inhaled corticosteroid for an underlying condition should continue this treatment according to their family physician advice.

References

1. Horby P, Lim WS, Emberson JR, et al. RECOVERY Collaborative Group. Dexamethasone in hospitalized patients with COVID-19. *N Engl J Med.* 2021; 384: 693-704. doi: 10.1056/NEJMoa2021436.

2. Normand S-L T. The RECOVERY Platform. *N Engl J Med.* 2021; 384: 757-758. doi: 10.1056/NEJMe2025674.

3. Crothers K, DeFaccio R, Tate J, et al. Dexamethasone in hospitalised coronavirus-19 patients not on intensive respiratory support. *Eur Respir J.* 2022;60:2102532. doi: 10.1183/13993003.02532-2021.

4. Tomazini BM, Maia IS, Cavalcanti AB, et al. Effect of dexamethasone on days alive and ventilator-free in patients with moderate or severe acute respiratory distress syndrome and COVID-19: The CoDEX randomized clinical trial. *JAMA* 2020; 324:1307-1316. doi: 10.1001/jama.2020.17021.

5. WHO REACT Working Group. Association between administration of systemic corticosteroids and mortality among critically ill patients with COVID-19: A meta-analysis. *JAMA* 2020; 324:1330-1341. doi: 10.1001/jama.2020.17023.

6. COVID-19 Treatment Guidelines Panel. Coronavirus Disease 2019 (COVID-19) Treatment Guidelines. National Institutes of Health. Available at https://www.covid19treatmentguidelines.nih.gov/. Last updated July 21, 2023.

7. Infectious Diseases Society of America Guidelines on the Treatment and Management of Patients with COVID-19. Last updated February 8, 2023 and posted online at www.idsociety.org/COVID19guidelines

8. Nicolau DV, Bafadhel M. Inhaled corticosteroids in virus pandemics: a treatment for COVID-19? *Lancet Respir Med* 2020; 8**:** 846–47.

9. Halpin DMG, Faner R, Sibila O, Badia JR, Agusti A. Do chronic respiratory diseases or their treatment affect the risk of SARS-CoV-2 infection? *Lancet Respir Med* 2020; 8**:** 436–38.

10. Pinna SM, Scabini S, Corcione S, Lupia T, De Rosa FG. COVID-19 pneumonia: do not leave the corticosteroids behind! *Future Microbiol* 2021; 16**:** 317–22.

11. Ramakrishnan S, Nicolau DV Jr, Langford B, et al. Inhaled budesonide in the treatment of early COVID-19 (STOIC): a phase 2, open-label, randomised controlled trial. *Lancet Respir Med* 2021; 9**:** 763–72.

12. Yu L-M, Bafadhel M, Dorward J, et al. Inhaled budesonide for COVID-19 in people at high risk of complications in the community in the UK (PRINCIPLE): a randomised, controlled, open-label, adaptive platform trial. *Lancet* 2021; 398**:** 843–55.

13. Ezer N, Belga S, Daneman N, et al. Inhaled and intranasal ciclesonide for the treatment of COVID-19 in adult outpatients: CONTAIN phase II randomised controlled trial. *BMJ* 2021; 375**:** e068060.

14. Clemency BM, Varughese R, Gonzalez-Rojas Y, et al. Efficacy of inhaled ciclesonide for outpatient treatment of adolescents and adults with symptomatic COVID-19: a randomized clinical trial. *JAMA Intern Med* 2022; 182**:** 42–49.

15. Duvignaud A, Lhomme E, Onaisi R, et al. Inhaled ciclesonide for outpatient treatment of COVID-19 in adults at risk of adverse outcomes: a randomised controlled trial (COVERAGE). *Clin Microbiol Infect* 2022; 28**:** 1010–16.

6. Immunomodulatory drugs

Severe COVID-19 is characterized by interstitial pneumonia/acute respiratory distress syndrome and systemic inflammation, with elevated levels of proinflammatory cytokines, such as interleukin-1 (IL-1), IL-6 and tumor necrosis factor alpha 1. Increased serum levels of IL-6 were found to predict

adverse outcomes, especially the need for mechanical ventilation, and mortality (1,2). Several observational studies and randomized controlled trials (RCTs) have therefore targeted IL-6 and its downstream signaling, such as the JAK and signal transducer and activator of transcription (STAT) pathway.

Most therapeutic strategies have to date focused on the inhibition of the IL-6 receptor using the monoclonal antibody tocilizumab, which had already been approved for treating rheumatologic diseases and cytokine release syndrome induced by chimeric antigen receptor therapy. In observational studies tocilizumab appeared to improve clinical outcomes for hospitalized patients with COVID-19 pneumonia (3-5). However, the initial RCTs that examined this monoclonal antibody led to conflicting results (6–9). Many of these clinical trials seem, though, to be constrained by their small size, heterogeneous patient populations, and/or low frequency of concomitant administration of corticosteroids, which are now used as the standard of care for patients with severe COVID-19. The two largest RCTs to evaluate tocilizumab - REMAP-CAP and RECOVERY - both reported a survival benefit for tocilizumab in certain COVID-19 patients when used on background corticosteroid therapy. In particular, the multiplatform, adaptive REMAP-CAP trial showed that in critically ill COVID-19 patients who received organ support in the intensive care unit (ICU), treatment with tocilizumab markedly improved outcomes, in terms of the number of days free from organ support and in-hospital mortality, compared to standard care, which included glucocorticoids in the majority of patients (>80%) (10). The open-label, platform RECOVERY trial found that among 4,116 hospitalized COVID-19 patients with hypoxia and systemic inflammation, adding tocilizumab to standard care significantly reduced the primary outcome of 28-days mortality, compared to standard care alone. The finding that consistent results were obtained in patients who received systemic glucocorticoid treatment at randomization (82%), suggests that the benefits of tocilizumab were additional to those of glucocorticoids (11).

Based on available evidence from these RCTs, tocilizumab received an FDA EUA for COVID-19 on 24th June 2021. In December 2022 the FDA finally approved tocilizumab for treatment of COVID-19 in hospitalized adults who are receiving systemic corticosteroids and require supplemental oxygen, non-invasive or invasive mechanical ventilation, or extracorporeal membrane oxygenation.

Sarilumab is the second most commonly studied monoclonal antibody blocking IL-6 receptor in COVID-19 patients. Parallel with encouraging data on tocilizumab, early uncontrolled studies that tested sarilumab also produced promising results (12,13), leading to the initiation of several RCTs. An adaptive design trial involving 420 patients with severe or critical COVID-19 found that sarilumab did not meet its primary endpoint of time to improvement by two or more points on an ordinal seven-point clinical status scale compared to placebo (14). Likewise, there was no significant difference between groups regarding the key secondary endpoint, the proportion of patients alive on day 29 (14). A similar adaptive trial showed that in 298 critically ill COVID-19 patients who required mechanical ventilation, sarilumab failed to exhibit any benefits over placebo with regards to the primary endpoint - the proportion of patients with a ≥1 point improvement in clinical status on day 22 (15). Among critical patients who received mechanical ventilation and corticosteroids at baseline, there was a numerical but not significant reduction in the risk of mortality with sarilumab

compared to placebo (15). In the REMAP-CAP trial, the efficacy results for sarilumab were similar to those for tocilizumab. Compared to patients randomized to standard of care, those allocated to sarilumab had more organ support-free days and a greater likelihood of survival during hospitalization (10). In summary, sarilumab had a favorable effect on survival in patients with severe COVID-19 pneumonia in one RCT, while it was neutral in two other trials.

A meta-analysis of 27 RCTs on IL-6 antagonists (9 of whom were published in peer-reviewed journals) showed that administration of these monoclonal antibodies (tocilizumab, n=19; sarilumab, n=9; siltruximab, n=1, with two trials having randomized patients to more than one IL-6 antagonist) was associated with a lower risk of 28 day mortality compared to usual care or placebo in 10,930 patients hospitalized with COVID-19 (16). Importantly, the mortality benefit was only found when IL-6 antagonists were co-administered with corticosteroids (16).

JAK inhibitors exert immunomodulatory effects by inhibiting the STAT-mediated signaling pathways of several cytokines. Baricitinib, specifically, is an orally administered inhibitor of JAK1 and JAK2 that blocks the intracellular signaling pathways of several cytokines which are known to be elevated in severe COVID-19, especially IL-6. Moreover, unlike other JAK inhibitors, baricitinib was also thought to inhibit AP2-associated protein kinase 1, a pivotal regulator of clathrin-dependent endocytosis, thereby preventing viral entry into target cells (17). This evidence, along with promising results from early observational studies that tested baricitinib treatment for hospitalized patients with COVID-19 (18,19), prompted the initiation of RCTs. The ACTT-2 trial found that for 1,033 patients with COVID-19 pneumonia who received supplemental oxygen, high-flow oxygen or noninvasive ventilation, the combination of baricitinib and remdesivir was superior to remdesivir alone in terms of the primary outcome of time to recovery, as measured on an 8-category ordinary scale (20). The combination treatment group also had 30% higher odds of improvement in terms of clinical status on day 15 than the control group (20). The COV-BARRIER trial showed that for 1,525 COVID-19 patients who did not require mechanical ventilation, and who had at least one elevated inflammatory marker, treatment with baricitinib in addition to standard of care (which predominantly included corticosteroids) did not reduce the incidence of primary composite endpoint of progression to high-flow oxygen, noninvasive ventilation, invasive mechanical ventilation or mortality compared to standard of care alone (21). However, treatment with baricitinib reduced the key secondary outcome of mortality by 38.2% within 28 days (21). Similarly, the large RECOVERY trial found that adding baricitinib to usual care (which included corticosteroids in 95% of cases) reduced the primary endpoint of 28 day mortality by 13% compared to usual care alone in 8,156 patients hospitalized with COVID-19 (22). Taken together, the results of these trials suggest that baricitinib may have additive or even synergistic effects with standards of care, including remdesivir and corticosteroids. Based on evidence from RCTs, baricitinib received EUA from the FDA for the treatment of severe COVID-19, in combination with remdesivir, in November 2020, and then as monotherapy in July 2021. In May 2022 the FDA finally approved baricitinib for the treatment of adult patients hospitalized with COVID-19 who required supplemental oxygen, non-invasive or invasive mechanical ventilation, or extracorporeal membrane oxygenation (23).

A meta-analysis of all nine completed JAK inhibitor trials (baricitinib, n=4; ruxolitinib, n=3; tofacitinib, n=2) showed that treatment with baricitinib or another JAK inhibitor was associated with a significant reduction in 28 day mortality by 20% in 11,888 patients hospitalized with COVID-19 (22) These findings support targeting the JAK/STAT axis in the setting of severe COVID-19.

References

1. Del Valle DM, Kim-Schulze S, Huang H-H, Beckmann ND, Nirenberg S, Wang B, et al.: An inflammatory cytokine signature predicts COVID-19 severity and survival. *Nat Med.* 2020; 26: 1636–1643.

2. Herold T, Jurinovic V, Arnreich C, Lipworth BJ, Hellmuth JC, von Bergwelt-Baildon M, et al.: Elevated levels of IL-6 and CRP predict the need for mechanical ventilation in COVID-19. *J Allergy Clin Immunol.* 2020; 146: 128-136.e4.

3. Xu X, Han M, Li T, Sun W, Wang D, Fu B, et al.: Effective treatment of severe COVID-19 patients with tocilizumab. *Proc Natl Acad Sci U S A* 2020; 117: 10970–10975.

4. Gupta S, Wang W, Hayek SS, Chan L, Mathews KS, Melamed ML, et al.: Association Between Early Treatment With Tocilizumab and Mortality Among Critically Ill Patients With COVID-19. *JAMA Intern Med.* 2021; 181: 41–51.

5. Morrison AR, Johnson JM, Griebe KM, Jones MC, Stine JJ, Hencken LN, et al.: Clinical characteristics and predictors of survival in adults with coronavirus disease 2019 receiving tocilizumab. *J Autoimmun.* 2020; 114: 102512.

6. Stone JH, Frigault MJ, Serling-Boyd NJ, Fernandes AD, Harvey L, Foulkes AS, et al.: Efficacy of Tocilizumab in Patients Hospitalized with Covid-19. *N Engl J Med.* 2020; 383: 2333–2344.

7. Hermine O, Mariette X, Tharaux P-L, Resche-Rigon M, Porcher R, Ravaud P, et al.: Effect of Tocilizumab vs Usual Care in Adults Hospitalized With COVID-19 and Moderate or Severe Pneumonia: A Randomized Clinical Trial. *JAMA Intern Med.* 2021; 181: 32–40.

8. Salvarani C, Dolci G, Massari M, Merlo DF, Cavuto S, Savoldi L, et al.: Effect of Tocilizumab vs Standard Care on Clinical Worsening in Patients Hospitalized With COVID-19 Pneumonia: A Randomized Clinical Trial. *JAMA Intern Med.* 2021; 181: 24–31.

9. Rosas IO, Bräu N, Waters M, Go RC, Hunter BD, Bhagani S, et al.: Tocilizumab in Hospitalized Patients with Severe Covid-19 Pneumonia. *N Engl J Med.* 2021; 384: 1503–1516.

10. REMAP-CAP Investigators, Gordon AC, Mouncey PR, Al-Beidh F, Rowan KM, Nichol AD, et al.: Interleukin-6 Receptor Antagonists in Critically Ill Patients with Covid-19. *N Engl J Med.* 2021; 384: 1491–1502.

11. RECOVERY Collaborative Group: Tocilizumab in patients admitted to hospital with COVID-19 (RECOVERY): a randomised, controlled, open-label, platform trial. *Lancet* 2021; 397: 1637–1645.

12. Benucci M, Giannasi G, Cecchini P, Gobbi FL, Damiani A, Grossi V, et al.: COVID-19 pneumonia treated with Sarilumab: A clinical series of eight patients. *J Med Virol.* 2020; 92: 2368–2370.

13. Gremese E, Cingolani A, Bosello SL, Alivernini S, Tolusso B, Perniola S, et al.: Sarilumab use in severe SARS-CoV-2 pneumonia. *EClinicalMedicine* 2020; 27: 100553.

14. Lescure F-X, Honda H, Fowler RA, Lazar JS, Shi G, Wung P, et al.: Sarilumab in patients admitted to hospital with severe or critical COVID-19: a randomised, double-blind, placebo-controlled, phase 3 trial. *Lancet Respir Med.* 2021; 9: 522–532.

15. Sivapalasingam S, Lederer DJ, Bhore R, Hajizadeh N, Criner G, Hosain R, et al.: Efficacy and Safety of Sarilumab in Hospitalized Patients With COVID-19: A Randomized Clinical Trial. *Clin Infect Dis.* 2022; ciac153.

16. WHO Rapid Evidence Appraisal for COVID-19 Therapies (REACT) Working Group, Shankar-Hari M, Vale CL, Godolphin PJ, Fisher D, Higgins JPT, et al.: Association Between Administration of IL-6 Antagonists and Mortality Among Patients Hospitalized for COVID-19: A Meta-analysis. *JAMA* 2021; 326: 499–518.

17. Stebbing J, Krishnan V, de Bono S, Ottaviani S, Casalini G, Richardson PJ, et al.: Mechanism of baricitinib supports artificial intelligence-predicted testing in COVID-19 patients. *EMBO Mol Med*. 2020; 12: e12697.

18. Cantini F, Niccoli L, Matarrese D, Nicastri E, Stobbione P, Goletti D: Baricitinib therapy in COVID-19: A pilot study on safety and clinical impact. *J Infect.* 2020; 81: 318–356.

19. Titanji BK, Farley MM, Mehta A, Connor-Schuler R, Moanna A, Cribbs SK, et al.: Use of Baricitinib in Patients With Moderate to Severe Coronavirus Disease 2019. *Clin Infect Dis.* 2021; 72: 1247–1250.

20. Kalil AC, Patterson TF, Mehta AK, Tomashek KM, Wolfe CR, Ghazaryan V, et al.: Baricitinib plus Remdesivir for Hospitalized Adults with Covid-19. *N Engl J Med.* 2021; 384: 795–807.

21. Marconi VC, Ramanan AV, de Bono S, Kartman CE, Krishnan V, Liao R, 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; 9: 1407–1418.

22. RECOVERY Collaborative Group: Baricitinib in patients admitted to hospital with COVID-19 (RECOVERY): a randomised, controlled, open-label, platform trial and updated meta-analysis. *Lancet* 2022; 400: 359–368.

23. Rubin R: Baricitinib Is First Approved COVID-19 Immunomodulatory Treatment. *JAMA* 2022; 327: 2281.

7. Complement inhibitors

The complement system is one of the host immune system's first lines of defense against invading pathogens (1). However, its potentially beneficial role in providing immunity to SARS-CoV-2 has

been called into question by multiple lines of evidence that implicate uncontrolled complement activation in the pathogenesis of severe COVID-19 (2,3).

Several uncontrolled studies and controlled clinical trials using different complement inhibitors have been performed in patients with severe COVID-19, while others are underway. To date, most of the strategies employed to target complement activation in COVID-19 have focused on C5 inhibition, in particular using the monoclonal antibody eculizumab, which has already been approved for the treatment of paroxysmal nocturnal hemoglobinuria, atypical hemolytic uremic syndrome, myasthenia gravis and neuromyelitis optica spectrum disorder. Initial case series and explorative studies found that patients with severe COVID-19 who were treated with eculizumab, including in combination with the JAK1/2 inhibitor ruxolitinib (4–8), experienced more positive clinical outcomes. In a non-randomized controlled study involving 80 patients with severe COVID-19 who were admitted to an ICU, treatment with eculizumab with more frequent and higher dosing compared to what is indicated for atypical hemolytic uremic syndrome appeared to improve 15-day survival compared to standard therapy alone. However, serious infectious complications and ventilator-associated pneumonia were two-fold more common in eculizumab-treated patients than in controls (9). Another non-randomized controlled study showed that adding two 900 mg doses of eculizumab to standard therapy for ten patients with severe COVID-19, who were receiving continuous positive airway pressure support for ≤ 24 h, safely improved respiratory dysfunction and reduced the risk of reaching the combined endpoint of mortality, or discharge with chronic complications, compared to 65 contemporary similar controls who were given standard therapy alone (Ruggenenti et al., 2021) (10). Similarly, in a proof-of-concept phase II RCT involving 81 COVID-19 patients with signs of hypoxia not yet requiring mechanical ventilation, adding the C5 peptide inhibitor zilucoplan to standard of care resulted in numerically relevant improvements in respiratory function (primary endpoint) and clinical outcomes (mortality and 6-minute walk test) compared to standard of care alone, even though statistical significance was not reached (11).

On the other hand, a phase III randomized controlled trial (RCT) with ravulizumab, another anti-C5 monoclonal antibody with a prolonged half-life compared to eculizumab, (NCT04369469), that involved patients with severe COVID-19 who required mechanical ventilation has been stopped after an interim analysis revealed a lack of efficacy (12).

The impact of blocking the C5a-C5aR1 axis has also been investigated. An initial phase II open-label RCT tested the blockade of C5a with the monoclonal antibody vilobelimab in 30 patients with severe COVID-19. Although there was no significant difference between patients randomized to vilobelimab and those allocated to standard of care alone in terms of the primary endpoint of change in the ratio of arterial oxygen tension over fraction of inspired oxygen (PaO2/FiO2) on day five, there was a trend toward improved survival in the anti-C5a treatment group (13). These findings supported the investigation of vilobelimab in a phase III trial, whose results showed that addition of the C5a inhibitor compared with placebo to standard of care resulted in a reduction of 28 day mortality (primary outcome) in 369 critically ill COVID-19 patients who received invasive mechanical ventilation within 48 hours of intubation at time of the first infusion (14). Based on these findings, on April 4, 2023 vilobelimab received an Emergency Use Authorization from the FDA which

allows the drug to be employed to treat hospitalized patients with COVID-19 within 48 hours of starting invasive mechanical ventilation or extracorporeal membrane oxygenation. Despite the promising results from C5aR1 blockade with the monoclonal antibody avdoralimab in a mouse model of acute lung injury (Casadevall and Pirofski, 2020) (15), where it decreased pulmonary neutrophil and macrophage infiltration, treatment with avdoralimab in a double-blind RCT in patients with severe COVID-19 pneumonia, did not meet its primary endpoint of improving clinical status over placebo on days 14 or 28 (16).

The inhibition of C3, which is upstream of C5 in the complement cascade, does not appear to be a safe approach for patients with COVID-19, since it could reduce the antiviral response and prevent immunity to other infectious diseases. Nonetheless, the C3 inhibitor AMY-101 was initially used in two case series to treat four COVID-19 patients with acute respiratory distress syndrome, all of whom eventually recovered (6,17). A larger phase II RCT involving 144 patients with acute respiratory distress syndrome due to COVID-19 has been planned (NCT04395456) but is not yet recruiting.

Other strategies that aim to target the early stages of complement activation involve the inhibition of C1 esterase or the mannose-binding lectin-associated serine proteases (MASPs) of the lectin pathway. In a preliminary case series in five patients with severe COVID-19 pneumonia, the administration of the human recombinant C1 esterase inhibitor conestat alpha was found to be safe and associated with clinical improvements (18). A larger phase II RCT, where conestat alpha was administered to hospitalized COVID-19 patients with respiratory involvement, symptom onset within the previous 10 days, and at least one risk factor for progression to mechanical ventilation, was terminated early for several reasons, including the changes in standard of care (19). By contrast, the first interim analysis of a phase II RCT with a similar protocol showed that adding conestat alpha to standard of care improved clinical status in hospitalized patients with early-stage COVID-19 (20). Another clinical trial found that neither an inhibitor of C1 esterase/kallikrein nor icatibant, a bradykinin B2 receptor antagonist, reduced time to clinical improvement compared to standard of care in 30 patients with severe COVID-19. However, both drugs were safe and ameliorated lung computed tomography scores (21).

The use of the anti-MASP2 antibody narsoplimab was initially reported in six patients with severe COVID-19 and acute respiratory distress syndrome, all of whom subsequently recovered and were discharged from hospital (22). More recently, the Omeros Corporation reported the results from the narsoplimab arm of the I-SPY COVID, an adaptive platform trial designed to evaluate promising investigational agents in reducing the time to recovery or mortality risk in critically ill COVID-19 patients. Analysis of the randomized patient population showed that addition of narsoplimab to standard of care reduced the mortality risk without shortening the time to recovery (23).

Collectively, early clinical findings and emerging clinical trial evidence suggest that some complement inhibitors may have therapeutic benefits in severe COVID-19. Further studies are required to clarify the best target(s) within the complement cascade and the optimal time of treatment initiation.

References

1. Noris M, Remuzzi G: Overview of complement activation and regulation. *Semin Nephrol*. 2013; 33: 479–492.

2. Noris M: The case of complement inhibitors. *Adv Biol Regul*. 2021; 81: 100822.

3. Afzali B, Noris M, Lambrecht BN, Kemper C: The state of complement in COVID-19. *Nat Rev Immunol*. 2022; 22: 77–84.

4. Diurno F, Numis FG, Porta G, Cirillo F, Maddaluno S, Ragozzino A, et al.: Eculizumab treatment in patients with COVID-19: preliminary results from real life ASL Napoli 2 Nord experience. *Eur Rev Med Pharmacol Sci*. 2020; 24: 4040–4047.

5. Giudice V, Pagliano P, Vatrella A, Masullo A, Poto S, Polverino BM, et al.: Combination of Ruxolitinib and Eculizumab for Treatment of Severe SARS-CoV-2-Related Acute Respiratory Distress Syndrome: A Controlled Study. *Front Pharmacol.* 2020; 11: 857.

6. Mastellos DC, Pires da Silva BGP, Fonseca BAL, Fonseca NP, Auxiliadora-Martins M, Mastaglio S, et al.: Complement C3 vs C5 inhibition in severe COVID-19: Early clinical findings reveal differential biological efficacy. *Clin Immunol*. 2020; 220: 108598.

7. Laurence J, Mulvey JJ, Seshadri M, Racanelli A, Harp J, Schenck EJ, et al.: Anti-complement C5 therapy with eculizumab in three cases of critical COVID-19. *Clin Immunol.* 2020; 219: 108555.

8. Pitts TC: Soliris to Stop Immune-Mediated Death in COVID-19 (SOLID-C19)-A Compassionate-Use Study of Terminal Complement Blockade in Critically Ill Patients with COVID-19-Related Adult Respiratory Distress Syndrome. *Viruses* 2021; 13: 2429.

9. Annane D, Heming N, Grimaldi-Bensouda L, Frémeaux-Bacchi V, Vigan M, Roux A-L, et al.: Eculizumab as an emergency treatment for adult patients with severe COVID-19 in the intensive care unit: A proof-of-concept study. *EClinicalMedicine* 2020; 28: 100590.

10. Ruggenenti P, Di Marco F, Cortinovis M, Lorini L, Sala S, Novelli L, et al.: Eculizumab in patients with severe coronavirus disease 2019 (COVID-19) requiring continuous positive airway pressure ventilator support: Retrospective cohort study. *PLoS One* 2021; 16: e0261113.

11. De Leeuw E, Van Damme KFA, Declercq J, Bosteels C, Maes B, Tavernier SJ, et al.: Efficacy and safety of the investigational complement C5 inhibitor zilucoplan in patients hospitalized with COVID-19: an open-label randomized controlled trial. *Respir Res*. 2022; 23: 202.

12. Covid-19 roundup: Alexion's C5 inhibitor Ultomiris misses the PhIII bar; Lonza in waiting game to use ingredients in Moderna vaccine, Endpoints News. https://endpts.com/covid-19-roundup-alexions-c5-inhibitor-ultomiris-misses-the-phiii-bar/.

13. Vlaar APJ, de Bruin S, Busch M, Timmermans SAMEG, van Zeggeren IE, Koning R, et al.: Anti-C5a antibody IFX-1 (vilobelimab) treatment versus best supportive care for patients with severe COVID-19 (PANAMO): an exploratory, open-label, phase 2 randomised controlled trial. *Lancet Rheumatol.* 2020; 2: e764–e773.

14. Vlaar APJ, Witzenrath M, van Paassen P, Heunks LMA, Mourvillier B, de Bruin S, et al.: Anti-C5a antibody (vilobelimab) therapy for critically ill, invasively mechanically ventilated patients with COVID-19 (PANAMO): a multicentre, double-blind, randomised, placebo-controlled, phase 3 trial. *Lancet Respir Med.* 2022; 10: 1137–1146.

15. Carvelli J, Demaria O, Vély F, Batista L, Chouaki Benmansour N, Fares J, et al.: Association of COVID-19 inflammation with activation of the C5a-C5aR1 axis. *Nature* 2020; 588: 146–150.

16. Carvelli J, Meziani F, Dellamonica J, Cordier P-Y, Allardet-Servent J, Fraisse M, et al.: Avdoralimab (Anti-C5aR1 mAb) Versus Placebo in Patients With Severe COVID-19: Results From a Randomized Controlled Trial (FOR COVID Elimination [FORCE]). *Crit Care Med.* 2022; 50: 1788–1798.

17. Mastaglio S, Ruggeri A, Risitano AM, Angelillo P, Yancopoulou D, Mastellos DC, et al.: The first case of COVID-19 treated with the complement C3 inhibitor AMY-101. *Clin Immunol*. 2020; 215: 108450.

18. Urwyler P, Moser S, Charitos P, Heijnen IAFM, Rudin M, Sommer G, et al.: Treatment of COVID-19 With Conestat Alfa, a Regulator of the Complement, Contact Activation and Kallikrein-Kinin System. *Front Immunol.* 2020; 11: 2072.

19. Conestat Alfa in the Prevention of Severe SARS-CoV-2 Infection in Hospitalized Patients With COVID-19.https://clinicaltrials.gov/ct2/show/NCT04414631

20. Giannetti, B. et al. Recombinant Human C1 Esterase Inhibitor Treatment in Addition to Standard of Care for the Prevention of Cytokine Storm in Hospitalized Patients with COVID-19: A Randomized, Open-Label Trial. Available at SSRN: https://ssrn.com/abstract=4078179.

21. Mansour E, Palma AC, Ulaf RG, Ribeiro LC, Bernardes AF, Nunes TA, et al.: Safety and Outcomes Associated with the Pharmacological Inhibition of the Kinin-Kallikrein System in Severe COVID-19. *Viruses* 2021; 13: 309.

22. Rambaldi A, Gritti G, Micò MC, Frigeni M, Borleri G, Salvi A, et al.: Endothelial injury and thrombotic microangiopathy in COVID-19: Treatment with the lectin-pathway inhibitor narsoplimab. *Immunobiology* 2020; 225: 152001.

23. OMEROS CORPORATION REPORTS NARSOPLIMAB TREATMENT RESULTS IN CRITICALLY ILL COVID-19 PATIENTS IN I-SPY COVID TRIAL. Press Release. Available at: https://investor.omeros.com/news-releases/news-release-details/omeros-corporation-reports-narsoplimab-treatment-results

8. Anticoagulants and other antithrombotic agents

Thrombotic complications, including arterial but especially venous thromboembolism (VTE), are common in patients admitted to hospital with COVID-19 (1,2). A systematic review estimated the VTE event rate to be about 17% among COVID-19 inpatients, increasing to around 28% for those admitted to the ICUs (3). Microvascular thrombi also contribute to organ dysfunction, including

acute respiratory distress syndrome. The pathogenesis of thrombosis in COVID-19 is closely linked with the inflammatory response to the virus, endothelial dysfunction and hypercoagulability (4).

Several organizations have released guidelines regarding the prevention and management of VTE in patients with COVID-19. All agree that hospitalized, non-pregnant patients with COVID-19 should receive, at a minimum, a prophylactic dose of anticoagulants to prevent VTE (5–9). Nevertheless, the optimal antithrombotic strategy across the spectrum of COVID-19 severity remains ill defined. Many RCTs have been performed and others are ongoing to evaluate the efficacy and safety of a variety of antithrombotic regimens in COVID-19 patients during all phases of the illness, from the community to hospital admission, when critically ill, and after hospital discharge.

The ACTIV-4B RCT showed that in 657 outpatients with COVID-19, treatment with aspirin, prophylactic or therapeutic doses of apixaban (a selective inhibitor of factor Xa) compared to placebo did not reduce the rate of the primary composite endpoint of mortality, symptomatic venous or arterial thromboembolism, myocardial infarction, stroke, or hospitalization for cardiovascular or pulmonary causes (10). However, the study was terminated when only 9% of the planned total number of participants could be enrolled due to the lower than anticipated primary event rates. Two RCTs evaluated the impact of low-molecular-weight heparin on hospital admission and death in COVID-19 outpatients, with remarkably similar results. In particular, the ETHIC trial showed that prophylaxis with enoxaparin 40 mg once or twice daily based on bodyweight did not reduce the primary composite endpoint on any hospitalization or mortality at 21 days compared to standard of care among 219 symptomatic unvaccinated COVID-19 outpatients (11). The trial was terminated early due to a low event rate and slow enrolment (11). Likewise, the OVID trial was stopped for futility, since prophylaxis with enoxaparin 40 mg daily did not reduce the primary composite endpoint of any hospitalization or mortality at 30 days compared to standard of care among 472 symptomatic COVID-19 outpatients (12). Thus, routine administration of thromboprophylaxis is not recommended for ambulatory patients with COVID-19.

Several RCTs have been conducted to evaluate the role of therapeutic doses of heparin in reducing VTE events, disease progression or mortality in hospitalized patients with COVID-19 who do not require ICU-level care. An international, multiplatform RCT that combined data from the ATTACC, REMAP-CAP and ACTIV-4A studies showed that among 2219 moderately ill COVID-19 patients, a therapeutic dose of anticoagulation with unfractionated or low-molecular-weight heparin was more effective than usual care thromboprophylaxis with regards to the primary outcome of survival free from organ support (13). Major bleeding occurred in 1.9% of patients who received therapeutic dose anticoagulation and in 0.9% of those given thromboprophylaxis (13). In the FREEDOM trial there was no difference in the therapeutic (with either enoxaparin or apixaban) and prophylactic anticoagulation (with enoxaparin) in the occurrence of the 30 day primary composite outcome of all-cause mortality, need for ICU-level care, systemic thrombosis or ischemic stroke among 3398 noncritically ill patients hospitalized with COVID-19 (14). However, fewer patients who were treated with therapeutic-dose anticoagulation died. The RAPID trial found that among 465 moderately ill COVID-19 patients with increased D-dimer levels, therapeutic compared to prophylactic anticoagulation treatment with unfractionated or low-molecular-weight heparin did not

significantly reduce the primary composite outcome of non-invasive or invasive mechanical ventilation, ICU admission, or mortality up to 28 days (5). However, therapeutic dose anticoagulation was associated with a decrease in the secondary outcome of all-cause mortality, and there was no increase in major bleeding compared to prophylactic anticoagulation (16). The HEP-COVID trial showed that among 253 hospitalized COVID-19 patients with elevated D-dimer levels or a high sepsis-induced coagulopathy score, most of whom (67%) did not require an ICU-level of care, therapeutic dose low-molecular-weight heparin significantly reduced the primary composite outcome of thromboembolism or death compared to standard prophylactic or intermediate dose heparins (16). There was no meaningful difference in terms of major bleeding between groups, even though confidence intervals were wide (16).

Together, the available evidence from RCTs supports the hypothesis that therapeutic anticoagulation with low-molecular-weight heparin or unfractionated heparin is associated with improved outcomes for hospitalized patients with COVID-19 who are not critically ill or in the ICU setting, particularly for those with elevated D-dimer levels. The beneficial effect of therapeutic anticoagulation in moderately ill COVID-19 patients using heparin does not seem to extend to other classes of anticoagulant agents. The ACTION trial showed that in 615 hospitalized patients with COVID-19 and elevated D-dimer levels, therapeutic anticoagulation with the factor Xa inhibitor rivaroxiban (and with enoxaparin in the small number of clinically unstable patients) did not reduce the primary composite endpoint of death, duration of hospitalization, or duration of oxygen use compared to prophylactic anticoagulation with heparin but did increase the risk of bleeding (17). Therefore, the use of therapeutic doses of rivaroxiban or other direct oral anticoagulants is not recommended for hospitalized COVID-19 patients.

Both rivaroxiban and heparin exert their anticoagulant effect against activated factor X, but the former does so directly and the latter indirectly via the natural inhibitor antithrombin III. Heparin also inhibits thrombin (Factor II) and other coagulation factor serine proteases (18). Moreover, it elicits anti-inflammatory functions through mechanisms that are independent of its anticoagulant activity, which include binding to inflammatory cytokines, inhibition of neutrophil chemotaxis and leukocyte migration, neutralization of the positively charged peptide complement factor C5a, and sequestration of acute phase proteins (19). Experimental evidence also suggests that heparin could have antiviral potential. Indeed, the structure of heparin highly resembles heparan sulfate, a linear polyanionic polysaccharide used by a large number of human viruses, including coronaviruses, to attach to target cells (20). One study has used spectroscopic techniques along with molecular modeling to show that heparin binds to and induces a conformational change in the Spike (S1) protein receptor binding domain of SARS-CoV-2 (21). This observation raises the intriguing possibility, which remains to be addressed, that heparin could compete with heparan sulfate to bind to SARS-CoV-2, thereby preventing virus entry into cells. Overall, the proposed extended anticoagulant and nonanticoagulant properties of heparin may explain why heparin, unlike rivaroxiban, showed benefits in RCTs.

The role of therapeutic doses of heparin in reducing VTE events, disease progression and mortality has also been investigated in hospitalized patients who require ICU-level care. The ATTACC, REMAP-

CAP and ACTIV-4A multiplatform trial showed that in critically ill patients, therapeutic dose anticoagulation with unfractionated or low-molecular-weight heparin did not improve the primary outcome of survival free from organ support compared to usual care thromboprophylaxis, and was associated with more major bleeding events (22). The INSPIRATION trial found that in 562 COVID-19 patients admitted to the ICU, intermediate dose (1 mg/kg enoxaparin daily) compared to standard dose (40 mg enoxaparin daily) thromboprophylaxis did not reduce the primary composite outcome of venous or arterial thrombosis, treatment with extracorporeal membrane oxygenation, or mortality within 30 days (23). Major bleeding occurred in 2.5% of patients in the intermediate dose group and in 1.4% of those in the standard dose group (23). More recently, the COVID-PACT trial showed that among 382 critically ill patients with COVID-19, therapeutic versus prophylactic dose of anticoagulation (mainly with low-molecular-weight heparin) reduced the primary composite endpoint of VTE or arterial thrombotic events, but at the expense of an increased incidence of moderate or severe bleeding (24).

Thus, current evidence from RCTs supports the use of standard dose thromboprophylaxis in critically ill patients with COVID-19. In this patient population, therapeutic dose heparin is only indicated for documented thromboembolic complications (25,26). It is not known why therapeutic doses of heparin appear to improve clinical outcomes in moderately ill, but not in critically ill, COVID-19 patients. It is conceivable that the disease may be too advanced in patients requiring ICU-level care or organ support for them to benefit from therapeutic heparin, with organized thrombi that are quite resistant to the action of antithrombin III, the endogenous anticoagulant potentiated by heparin (27).

Two RCTs evaluated the role that extended thromboprophylaxis plays beyond a patient's hospital stay. Recently, the MICHELLE trial has showed that for 320 patients at high risk who were discharged after COVID-19 hospitalization, thromboprophylaxis with rivaroxiban for 35 days –compared to no extended anticoagulation – significantly reduced the primary composite outcome of venous thromboembolic events (these included events that were either symptomatic or detected using routine imaging tests, and arterial thrombotic events and cardiovascular death) without increasing the risk of major bleeding (28). More recently, the ACTIV-4c trial randomized 1217 patients who were hospitalized for symptomatic COVID-19 for 48 hours or more to receive apixaban 2.5 mg orally twice daily or placebo at hospital discharge (29). The 30 day composite endpoint of all-cause mortality, venous thrombosis, or arterial thrombosis was comparable between groups. However, because of the early trial termination due to a lower-than-expected primary event rate, the results were imprecise and the study was inconclusive. Based on these findings, routinely continuing venous thromboembolism prophylaxis in patients with COVID-19 after hospital discharge is not recommended unless they have another indication for anticoagulation.

Since platelets play a central role in the pathogenesis of COVID-19, the use of antiplatelet agents has been investigated in the framework of RCTs. In particular, the large RECOVERY trial showed that aspirin (150 mg daily) plus usual care (which included anticoagulation therapy in 94% of cases) did not reduce the primary endpoint of 28 day mortality, but increased the risk of major bleeding, compared to usual care alone in 14,892 hospitalized patients with COVID-19 (30). The ACTIV-4a trial

found that in 562 non-critically ill patients hospitalized with COVID-19, adding a PAY12 inhibitor (63% ticagrelor, 37% clopidogrel) to a therapeutic dose of heparin did not improve the primary endpoint of organ support-free days compared to a therapeutic dose of heparin only (31). The REMAP-CAP trial showed that in 1,557 critically ill patients hospitalized with COVID-19 who were already receiving anticoagulation thromboprophylaxis, administration of aspirin or a PAY12 inhibitor (ticagrelor, clopidogrel or prasugrel) did not ameliorate the primary endpoint of organ support-free days compared to no antiplatelet therapy (32). Finally, in the COVID-PACT trial, the addition of clopidogrel versus no antiplatelet agents to anticoagulation therapy did not reduce VTE or arterial thrombotic events in critically ill COVID-19 patients (24).

Thus, despite the compelling rationale for adding antiplatelet therapy to anticoagulation in order to prevent the thrombotic complications of COVID-19, available evidence from RCTs does not support this strategy.

References

1. Piazza G, Campia U, Hurwitz S, Snyder JE, Rizzo SM, Pfeferman MB, et al.: Registry of Arterial and Venous Thromboembolic Complications in Patients With COVID-19. *J Am Coll Cardiol*. 2020; 76: 2060–2072.

2. Nadkarni GN, Lala A, Bagiella E, Chang HL, Moreno PR, Pujadas E, et al.: Anticoagulation, Bleeding, Mortality, and Pathology in Hospitalized Patients With COVID-19. *J Am Coll Cardiol.* 2020; 76: 1815–1826.

3. Jiménez D, García-Sanchez A, Rali P, Muriel A, Bikdeli B, Ruiz-Artacho P, et al.: Incidence of VTE and Bleeding Among Hospitalized Patients With Coronavirus Disease 2019: A Systematic Review and Meta-analysis. *Chest* 2021; 159: 1182–1196.

4. Shaw RJ, Bradbury C, Abrams ST, Wang G, Toh C-H: COVID-19 and immunothrombosis: emerging understanding and clinical management. *Br J Haematol.* 2021; 194: 518–529.

5. Barnes GD, Burnett A, Allen A, Blumenstein M, Clark NP, Cuker A, et al.: Thromboembolism and anticoagulant therapy during the COVID-19 pandemic: interim clinical guidance from the anticoagulation forum. *J Thromb Thrombolysis* 2020; 50: 72–81.

6. Marietta M, Ageno W, Artoni A, De Candia E, Gresele P, Marchetti M, et al.: COVID-19 and haemostasis: a position paper from Italian Society on Thrombosis and Haemostasis (SISET). *Blood Transfus.* 2020; 18: 167–169.

7. Thachil J, Tang N, Gando S, Falanga A, Cattaneo M, Levi M, et al.: ISTH interim guidance on recognition and management of coagulopathy in COVID-19. *J Thromb Haemost.* 2020; 18: 1023–1026.

8. National Institute for Health Care Excellence. COVID-19 rapid guideline: managing COVID-19. Available at: https://www.nice.org.uk/guidance/ng191.

9. American Society of Hematology. ASH Guidelines on Use of Anticoagulation in Patients with COVID-19. Available at: https://www.hematology.org/education/clinicians/guidelines-and-quality-care/clinical-practice-guidelines/venous-thromboembolism-guidelines/ash-guidelines-on-use-of-anticoagulation-in-patients-with-covid-19.

10. Connors JM, Brooks MM, Sciurba FC, Krishnan JA, Bledsoe JR, Kindzelski A, et al.: Effect of Antithrombotic Therapy on Clinical Outcomes in Outpatients With Clinically Stable Symptomatic COVID-19: The ACTIV-4B Randomized Clinical Trial. *JAMA* 2021; 326: 1703–1712.

11. Cools F, Virdone S, Sawhney J, Lopes RD, Jacobson B, Arcelus JI, et al.: Thromboprophylactic low-molecular-weight heparin versus standard of care in unvaccinated, at-risk outpatients with COVID-19 (ETHIC): an open-label, multicentre, randomised, controlled, phase 3b trial. *Lancet Haematol.* 2022; 9: e594–e604.

12. Barco S, Voci D, Held U, Sebastian T, Bingisser R, Colucci G, et al.: Enoxaparin for primary thromboprophylaxis in symptomatic outpatients with COVID-19 (OVID): a randomised, open-label, parallel-group, multicentre, phase 3 trial. *Lancet Haematol.* 2022; 9:e585–e593.

13. ATTACC Investigators, ACTIV-4a Investigators, REMAP-CAP Investigators, 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: 790–802.

14. Stone GW, Farkouh ME, Lala A, Tinuoye E, Dressler O, Moreno PR, et al.. Randomized Trial of Anticoagulation Strategies for Noncritically Ill Patients Hospitalized With COVID-19. *J Am Coll Cardiol* 2023;81: 1747-1762.

15. Sholzberg M, Tang GH, Rahhal H, AlHamzah M, Kreuziger LB, Áinle FN, et al.: Effectiveness of therapeutic heparin versus prophylactic heparin on death, mechanical ventilation, or intensive care unit admission in moderately ill patients with covid-19 admitted to hospital: RAPID randomised clinical trial. *BMJ* 2021; 375: n2400.

16. Spyropoulos AC, Goldin M, Giannis D, Diab W, Wang J, Khanijo S, 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: 1612–1620.

17. Lopes RD, de Barros E Silva PGM, Furtado RHM, Macedo AVS, Bronhara B, Damiani LP, 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: 2253–2263.

18. Hirsh J, Anand SS, Halperin JL, Fuster V: Mechanism of action and pharmacology of unfractionated heparin. *Arterioscler Thromb Vasc Biol.* 2001; 21: 1094–1096.

19. Poterucha TJ, Libby P, Goldhaber SZ: More than an anticoagulant: Do heparins have direct anti-inflammatory effects? *Thromb Haemost.* 2017; 117: 437–444.

20. Milewska A, Zarebski M, Nowak P, Stozek K, Potempa J, Pyrc K: Human coronavirus NL63 utilizes heparan sulfate proteoglycans for attachment to target cells. *J Virol.* 2014; 88: 13221–13230.

21. Mycroft-West CJ, Su D, Pagani I, Rudd TR, Elli S, Gandhi NS, et al.: Heparin Inhibits Cellular Invasion by SARS-CoV-2: Structural Dependence of the Interaction of the Spike S1 Receptor-Binding Domain with Heparin. *Thromb Haemost.* 2020; 120: 1700–1715.

22. REMAP-CAP Investigators, ACTIV-4a Investigators, ATTACC Investigators, 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: 777–789.

23. INSPIRATION Investigators, Sadeghipour P, Talasaz AH, Rashidi F, Sharif-Kashani B, Beigmohammadi MT, 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: 1620–1630.

24. Bohula EA, Berg DD, Lopes MS, Connors JM, Babar I, Barnett CF, et al.: Anticoagulation and Antiplatelet Therapy for Prevention of Venous and Arterial Thrombotic Events in Critically Ill Patients With COVID-19: COVID-PACT. *Circulation* 2022; 146: 1344–1356.

25. Flaczyk A, Rosovsky RP, Reed CT, Bankhead-Kendall BK, Bittner EA, Chang MG: Comparison of published guidelines for management of coagulopathy and thrombosis in critically ill patients with COVID 19: implications for clinical practice and future investigations. *Crit Care* 2020; 24: 559.

26. Cuker A, Tseng EK, Nieuwlaat R, Angchaisuksiri P, Blair C, Dane K, et al.: American Society of Hematology 2021 guidelines on the use of anticoagulation for thromboprophylaxis in patients with COVID-19. *Blood Adv.* 2021; 5: 872–888.

27. Ten Cate H: Surviving Covid-19 with Heparin? *N Engl J Med*. 2021; 385: 845–846.

28. Ramacciotti E, Barile Agati L, Calderaro D, Aguiar VCR, Spyropoulos AC, de Oliveira CCC, et al.: Rivaroxaban versus no anticoagulation for post-discharge thromboprophylaxis after hospitalisation for COVID-19 (MICHELLE): an open-label, multicentre, randomised, controlled trial. *Lancet* 2022; 399: 50–59.

29. Wang TY, Wahed AS, Morris A, Baumann Kreuziger L, Quigley JG, Lamas GA, et al.. Effect of Thromboprophylaxis on Clinical Outcomes After COVID-19 Hospitalization. *Ann Intern Med* 2023;176: 515-523.

30. RECOVERY Collaborative Group: Aspirin in patients admitted to hospital with COVID-19 (RECOVERY): a randomised, controlled, open-label, platform trial. *Lancet* 2022; 399: 143–151.

31. Berger JS, Kornblith LZ, Gong MN, Reynolds HR, Cushman M, Cheng Y, et al.: Effect of P2Y12 Inhibitors on Survival Free of Organ Support Among Non-Critically Ill Hospitalized Patients With COVID-19: A Randomized Clinical Trial. *JAMA* 2022; 327: 227–236.

32. REMAP-CAP Writing Committee for the REMAP-CAP Investigators, Bradbury CA, Lawler PR, Stanworth SJ, McVerry BJ, McQuilten Z, et al.: Effect of Antiplatelet Therapy on Survival and Organ Support-Free Days in Critically Ill Patients With COVID-19: A Randomized Clinical Trial. *JAMA* 2022; 327: 1247–1259.

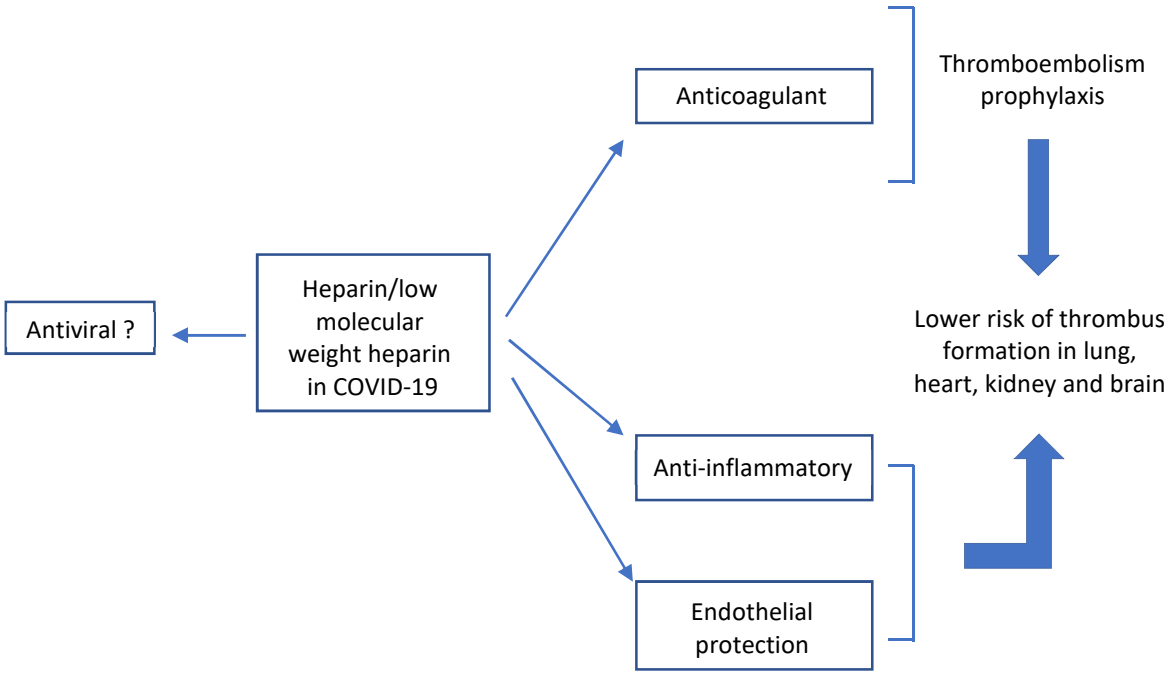


Figure. Possible effects of heparin and low molecular weight heparin in COVID-19

9. Drugs that are not recommended because of proven lack of efficacy

Hydroxychloroquine/chloroquine

Introduced in the early 1960 as an antimalarial drug, it is also used to treat autoimmune diseases such as lupus and rheumatoid arthritis. Despite the in vitro evidence (1,2) and the results of some preliminary anecdotal reports, there is no evidence of efficacy for the use of chloroquine or hydroxychloroquine, either alone or with azithromycin, for the treatment of COVID-19 patients according to NIH COVID-19 Treatment Guidelines (3-8). The NIH guidelines, also, recommend against the use of hydroxychloroquine/chloroquine for the prophylaxis of SARS-CoV-2 infection (3). Therefore the NIH Panel recommends against the use of hydroxychloroquine/chloroquine prophylaxis of COVID-19.

Ivermectin

This FDA-approved antiparasitic drug, has demonstrated a degree of efficacy in vitro against SARS-CoV-2 infection (9,10), and uncontrolled, interventional studies have reported a degree of efficacy or no benefits (11-13). In the CORVETTE-01 trial, 248 patients diagnosed with COVID-19 were treated with a single oral dose of ivermectin (200 μg/kg) showing ineffective results (14). The NIH

Panel indicates that there is insufficient evidence for the COVID-19 treatment, and recommended against its use (3).

Fluvoxamine

Fluvoxamine is a selective serotonin reuptake inhibitor, cytochrome P450 inhibitor and regulator of autophagy, that is approved by the FDA for the treatment of obsessive-compulsive disorder as well as depression. Based on the anti-inflammatory effects of fluvoxamine documented in pre-clinical studies (15,16), trials involving humans are exploring the possibility of using this drug to treat COVID-19. Several clinical studies (NCT04668950; NCT04718480; NCT04342663; NCT04510194) are still in progress, whilst the one completed failed to show any significant benefits. The TOGETHER trial, an adaptive platform, supported a double blind randomized placebo-controlled trial involving non-hospitalized adults with COVID-19 and a known risk factor for severe illness, which showed there was a lower risk of the primary composite outcome of retention in the emergency department for >6 hours or admission to a tertiary hospital in fluvoxamine-treated patients than in the placebo group (17). However, there was no significant difference in mortality rates for the two study groups. This finding is consistent with the recent results of a phase 3, double-blind, randomized, placebo-controlled trial that tested the efficacy of fluvoxamine (and also metformin and ivermectin) in preventing serious SARS-CoV-2 infection in 1,431 nonhospitalized adult patients, enrolled early after the onset of COVID-19 symptoms (18). Fluvoxamine, as well as metformin and ivermectin failed to prevent the occurrence of hypoxemia, an emergency department visit, hospitalization, or death associated with COVID-19 (the primary composite endpoint).

Lopinavir/ritonavir

The replication of SARS-CoV-2 requires the enzymatic proteolysis into an RNA-dependent RNA polymerase and a helicase.1 Two distinct proteases are involved in this cat: 3-chymotrypsin-like protease (3CLpro) and papain-like protease (PLpro). The major peptidase of the virus (Mpro) is a 3-chymotrypsin-like cysteine protease (3CLpro). Subsequent the translation of the SARS-CoV-2 mRNA, Mpro results in the synthesis of non-structural proteins by a selective cleavage in eleven distinct sites on the two viral proteins (polyproteins 1a and 1ab). Like other HIV protease inhibitors (darunavir/cobicistat), lopinavir/ritonavir have been used in clinical trials (6,19). Despite these efforts, the NIH Panel in its guidelines recommends against their use for the treatment of COVID-19 in hospitalized, as well as non-hospitalized, patients for insufficient evidence of efficacy (3).

Colchicine

Used as an anti-inflammatory drug for a variety of conditions (20), the NIH Panel guidelines recommend against its use to treat hospitalized and non-hospitalized COVID-19 patients (3). Indeed, two early large randomized RECOVERY (21) and COLCORONA (22) studie have been conducted with limited output. In the RECOVERY trial involving hospitalized COVID-19 patients, colchicine did not show benefits in term of 28-day mortality or other secondary outcomes (21). Similarly, in non-hospitalized patients with COVID-19 of the large randomized, placebo-controlled COLCORONA trial, colchicine failed to reach the primary end-point of reducing hospitalization and death (22). Therefore, the drug is not recommend.

Interferons

Approved to treat hepatitis B and hepatitis C virus infections, interferons have been in used clinical trials for hospitalized adults with COVID-19 in early 2020. The drug has also been administered with lopinavir/ritonavir and hydroxychloroquine. Interferon beta-1a plus remdesivir showed no clinical benefit when compared to remdesivir alone (6, 23). The same trial tested also its combination to corticosteroids, with very limited results (23). Pegylated interferon lambda was studied in a randomized, double-blind adaptive clinical trial, with 1,941 patients, that enrolled nonhospitalized patients with COVID-19 in Brazil and Canada (24), with a 51% decrease in the occurrence of the primary outcome. Accordingly, the NIH Panel recommends against the use of systemic interferon beta for the treatment of hospitalized patients with COVID-19, against the use of interferon alfa or lambda for the treatment of hospitalized patients with COVID-19, except in a randomized clinical trial, as well as against the use of interferons for the treatment of non-hospitalized patients with mild or moderate COVID-19, except in a randomized clinical trial (3).

COVID-19 convalescent plasma (CCP)

Plasma from donors who have recovered from COVID-19 (regardless of vaccination status) may contain antibodies to SARS-CoV-2 that could help suppress viral replication; therefore its use was approved by FDA and EUA, limited to the use of products that contain high antibody titre. However, currently plasma from patients who recovered from COVID-19 is not recommended from the NIH Panel (3). In details, there is insufficient evidence for the NIH Panel to recommend either for or against the use of high-titer CCP for the treatment of COVID-19 in hospitalized or nonhospitalized patients who are immunocompromised. The Panel recommends against the use of CCP for the treatment of COVID-19 in hospitalized patients who are immunocompetent. Whilst this is based on insufficient evidence of its efficacy, the treatment seems superseded by the use of monoclonal antibodies.

Lactoferrin

Lactoferrin, or lactotransferrin, is an iron-binding glycoprotein used in clinical trials (NCT04526821; NCT04412395; NCT04475120; NCT04847791) for its immune-regulatory effects in distinct infections, including SARS-CoV-2 (25). There is a very limited (26) or no evidence (27) of proven efficacy against COVID-19 both in prophylaxis and treatment of patients. Hence, its use is not recommended.

Metformin

Outpatient treatment with metformin reduced long COVID incidence by about 41%, with an absolute reduction of 4·1%, compared with placebo (28). Metformin has clinical benefits when used as outpatient treatment for COVID-19 and is globally available, low-cost, and safe. However, the NIH Panel recommends against the use of metformin for the treatment of COVID-19 in nonhospitalized and hospitalized patients, except in a clinical trial (3); patients with COVID-19 who are receiving metformin for an underlying condition should continue this therapy as directed by their health care provider (3).

References

1. Liu, J, Cao R, Xu M et al. Hydroxychloroquine, a less toxic derivative of chloroquine, is effective in inhibiting SARS-CoV-2 infection in vitro. *Cell Discov.* 2020; 6:16.

2. Fantini J, Chahinian H, Yahi N. Synergistic antiviral effect of hydroxychloroquine and azithromycin in combination against SARS-CoV-2: what molecular dynamics studies of virus-host interactions reveal. *Int J Antimicrob Agents* 2020; 56: 106020.

3. COVID-19 Treatment Guidelines Panel. Coronavirus Disease 2019 (COVID-19) Treatment Guidelines. National Institutes of Health. Available at https://www.covid19treatmentguidelines.nih.gov/

4. Cavalcanti AB, Zampieri FG, Rosa RG, et al. Hydroxychloroquine with or without azithromycin in mild-to-moderate COVID-19. *N Engl J Med.* 2020; 383: 2041-2052.

5. Recovery Collaborative Group, Horby P, Mafham M, et al. Effect of hydroxychloroquine in hospitalized patients with COVID-19. *N Engl J Med.* 2020; 382:2030-2040.

6. WHO Solidarity Trial Consortium, Pan H, Pert R, et al. Repurposed antiviral drugs for COVID-19 – interim WHO Solidarity Trial results. *N Engl J Med.* 2021; 384: 497-511.

7. Self WH, Semler MW, Leither LM, et al. Effect of hydroxychloroquine on clinical status at 14 days in hospitalized patients with COVID-19: a randomized clinical trial. *JAMA* 2020; 324: 2165-2176.

8. Geleris J, Sun Y, Platt J, et al. Observational study of hydroxychloroquine in hospitalized patients with COVID-19. *N Engl J Med.* 2020; 382: 2411-2428.

9. Yang SNY, Atkinson SC, Wang C, et al. The broad spectrum antiviral ivermectin targets the host nuclear transport importin alpha/beta 1 heterodimer. *Antiviral Res.* 2020; 177: 104760.

10. Caly L, Druce JD, Catton MG, et al. The FDA-approved drug ivermectin inhibits the replication of SARS-CoV-2 in vitro. *Antiviral Res.* 2020; 178: 104787.

11. Khan MSI, Debnath CR, et al. Ivermectin treatment may improve the prognosis of patients with COVID-19. *Arch Broncopneumol. 2020*; 56: 828-830.

12. Amhed S, Karim MM, Ross AG, et al. A five-day course of ivermectin for the treatment of COVID-19 may reduce the duration of illness. *Int J Infect Dis*. 2020; 103: 214-216.

13. Chachar AZK, Khan KA, Asif M, et al. Effectiveness of ivermectin in SARS-CoV-2/COVID-19 patients. *Int J of Sci.* 2020; 9: 31-35.

14. Wada T, Hibino M, Aono H, et al. CORVETTE-01 Study Group. Efficacy and safety of single-dose ivermectin in mild-to-moderate COVID-19: the double-blind, randomized, placebo-controlled CORVETTE-01 trial. *Front Med (Lausanne).* 2023 May 22;10:1139046. doi: 10.3389/fmed.2023.1139046. PMID: 37283627; PMCID: PMC10240959.

15. Rosen DA, Seki SM, Fernandez-Castaneda A, et al. Modulation of the sigma-1 receptor-IRE1 pathway is beneficial in preclinical models of inflammation and sepsis. *Sci Transl Med.* 2019; 11(478): eaau5266.

16. Rafiee L, Hajhashemi V, Javanmard SH. Fluvoxamine inhibits some inflammatory genes expression in LPS/stimulated human endothelial cells, U937 macrophages, and carrageenan-induced paw edema in rat. *Iran J Basic Med Sci.* 2016; 19: 977-994.

17. Reis G, Dos Santos Moreira-Silva EA, Silva DCM, et al. Effect of early treatment with fluvoxamine on risk of emergency care and hospitalization among patients with COVID-19: the TOGETHER randomized, platform clinical trial. *Lancet Glob Health* 2022; 10: e42-e51.

18. Bramante CT, Huling JD, Tignanelli CJ, et al. Randomized trial of metformin, ivermectin, and fluvoxamine for COVID-19. *N Engl J Med.* 2022; 387: 599-610.

19. RECOVERY Collaborative Group. Lopinavir-ritonavir in patients admitted to hospital with COVID-19 (RECOVERY): a randomized, controlled, open-label, platform trail. *Lancet* 2020; 396: 1345-1352.

20. Van Echteld I, Wechalekar MD, Schlesinger N. Cochicine for acute gout. 2014 Oxford, United Kingdom: *Cochrane Database Sys Rev*, CD006190.

21. RECOVERY Collaborative Group. Colchicine in patients admitted to hospital with COVID-19 (RECOVERY): a randomized, controlled, open-label, platform trial. *Lancet Respir Med.* 2021; 9: 1419-1426.

22. Tardif JC, Bouabdallaoui N, L'Allier PL, et al. Colchicine for community-treated patients with COVID-19 (COLCORONA): a phase 3, randomized, double-blinded, adaptive, placebo-controlled, multicentre trial. *Lancet Respir Med.* 2021; 9: 924-932.

23. Kalil AC, Mehta AK, Patterson TF, et al. Efficacy of interferon beta-1a plus remdesivir compared with remdesivir alone in hospitalized adult with COVID-19: a double-blind, randomized, placebo-controlled, Phase 3 trial. *Lancet Respir Med.* 2021; 9: 1365-1376.

24. Reis G, Moreira Silvia EAS, Medeiros Silva DC, et al. Early treatment with pegylated interferon lambda for COVID-19. *N Engl J Med.* 2023; 388:518-528.

25. Cutone A, Rosa L, Bonaccorsi di Patti MC, et al. Lactoferrin binding to SARS-CoV-2 spike glycoprotein blocks pseudoviral entry and relieves iron protein dysregulation in several in vitro Models. *Pharmaceutics* 2022;14(10):2111. doi: 10.3390/pharmaceutics14102111.

26. Yazawa S, Yamazaki E, Saga Y, et al. Evaluation of SARS-CoV-2 isolation in cell culture from nasal/nasopharyngeal swabs or saliva specimens of patients with COVID-19. *Sci Rep*. 2023;13(1):8893. doi: 10.1038/s41598-023-35915-w.

27. Algahtani FD, Elabbasy MT, Samak MA, et al. The prospect of lactoferrin use as adjunctive agent in management of SARS-CoV-2 patients: A randomized pilot study. *Medicina* 2021; 57: 842.

28. Bramante CT, Buse JB, Liebovitz DM, et al. Outpatient treatment of COVID-19 and incidence of post-COVID-19 condition over 10 months (COVID-OUT): a multicentre, randomised, quadruple-blind, parallel-group, phase 3 trial. *Lancet Infect Dis.* 2023 Jun 8:S1473-3099(23)00299-2. doi: 10.1016/S1473-3099(23)00299-2.

10. Drugs with insufficient evidence to recommend for or against

Several drugs are still in clinical trials (see for latest update: www.ClinicalTrials.gov) and, considering the preliminary results there is insufficient evidence to recommend for or against (1). Here are some examples.

Nitazoxanide

This FDA-approved, broad-spectrum thiazolide antiparasitic agent, or its metabolite, tizoxanide, exibit in vitro activity against a number of viral infections, including Ebola virus, MERS-CoV, SARS-CoV, SARS-CoV-2 (2-4). Nitazoxanide inhibits host enzymes, which impairs the posttranslational processing of viral proteins. On the basis of the early clinical trials (4), however, the NIH Panel did not find the evidence sufficient to recommend nitazoxanide for the treatment of COVID-19 (1). On the other hand, a recent multicenter, randomized, double-blind, placebo controlled trial in 405 patients hospitalized with COVID-19 pneumonia found that nitazoxanide did not prevent ICU admission compared to placebo (5). However, treatment with nitazoxanide may accelerate symptom resolution, shorten duration of oxygen therapy, and reduce levels of inflammatory mediators (5).

Granulocyte-macrophage colony-stimulating factor (GM-CSF) inhibitors

Granulocyte-macrophage colony-stimulating factor (GM-CSF) is a myelopoietic growth factor and proinflammatory cytokine, FDA-approved, that plays a central role in a broad range of immune-mediated diseases (6). Anti-GM-CSF monoclonal antibodies may limit inflammation by minimizing the production of several pro-inflammatory mediators involved in COVID-19 (7). Several clinical trials have been completed (NCT04411680; NCT04326920; NCT04707664) or are still open (NCT04569877; NCT04341116), with inconclusive results. Preliminary data published of small randomized trials with anti-GM-CSF monoclonal antibodies provided conflicting results (8-10). Lenzilumab produced a significant improvement in the ventilator-free survival through day 28 for COVID-19 patients compared to patients treated with placebo (9). Other studies, however, did not report a survival benefit for otilimab (8) or mavrilimumab (10) compared to placebo. Thus, the NIH

COVID-19 Treatment Guidelines Panel states that there is insufficient evidence for the treatment of hospitalized COVID-19 patients (1).

Anakinra

Anakinra, interleukin-1 receptor antagonist, is used to treat the Multisystem Inflammatory Syndrome in Children (11-12). Among 235 patients results were mixed (12). There is insufficient evidence for the COVID-19 Treatment Guidelines Panel to recommend either for or against the use of anakinra for the treatment of COVID-19, except in clinical trials (1). Indeed, while the SAVE-MORE trial in hospitalized patients with moderate or severe COVID-19 pneumonia, reported a lower risk of clinical progression of the illness in patients given anakinra than in those receiving placebo (11), the REMAP-CAP, an open-label, adaptive platform, randomized controlled trial, showed that anakinra did not have any efficacy in reducing the combined endpoint of in-hospital mortality and days of organ support (12-13).

Vitamin C, D and zinc

Vitamin C, D, and zinc have been used for the therapy or the prophylaxis of COVID-19 rather than as supplements. There is insufficient evidence for the COVID-19 Treatment Guidelines Panel to recommend either for or against the use of vitamin C for the treatment of COVID-19 in non-critically and critically ill patients (1). There are no controlled trials that have definitely demonstrated a clinical benefit regarding the use of vitamin C in ambulatory or critically ill patients with COVID-19, and the available observational data are inconclusive (14-15). Similarly, the few randomized clinical trials of vitamin D in patients with moderate to severe COVID-19 preclude robust conclusions regarding the effectiveness of this treatment on major disease outcomes (16). In patients with COVID-19, studies regarding zinc, known to inhibit in vitro coronavirus and arterivirus RNA polymerase activity and virus replication (17) have significant limitations including small sample size, lack of randomization or blinding (18-20). Moreover, the results of currently available clinical trials do not provide consistent or compelling evidence of a clinical benefit of zinc for the treatment or prevention of COVID-19. Therefore the NIH Panel conclude that there is insufficient evidence to recommend either for or against the use of zinc for COVID-19.

References

1. COVID-19 Treatment Guidelines Panel. Coronavirus Disease 2019 (COVID-19) Treatment Guidelines. National Institutes of Health. Available at https://www.covid19treatmentguidelines.nih.gov/

2. Jasenosky LD, Cadena C, Mire CE, et al. The FDA-approved oral drug nitazoxanide amplifies host antiviral responses and inhibits Ebola virus. *iScience* 2019; 19: 1279-1290.

3. Rossignol JF. Nitazoxanide: a first-in-class broad-spectrum antiviral agent. *Antiviral Res.* 2014; 110; 94-103.

4. Rocco PRM, Silvis PL, Cruz FF, et al. Early use of nitazoxanide in mild COVID-19 disease: randomized, placebo-controlled trial. *Eur Respir J.* 2021; 58: 2003725.

5. Rocco PRM, Silvia PL, Cruz FF et al. Nitazoxanide in patients hospitalized with COVID-19 pneumonia: A multicenter, randomized, double-blind, placebo-controlled trial. Front Med 2022; 9: 844728.Hamilton JA. GM-CSF in inflammation and autoimmunity. *Trends Immunol*. 2002; 23: 403-408.

6. De Luca G, Cavalli G, Campochiaro C, et al. GM-CSF blockade with mavrilimumab in severe COVID-19 pneumonia and systemic hyperinflammation: A single-centre, prospective cohort study. *Lancet Rheumatol.* 2020; 2: e465-e473.

7. Patel J, Bass D, Beishuizen A, et al. A randomised trial of anti-GM-CSF otilimab in severe COVID-19 pneumonia (OSCAR). *Eur Respir J.* 2023; 61(2):2101870. doi: 10.1183/13993003.01870-2021.

8. Temesgen Z, Burger CD, Baker J, et al. Lenzilumab in hospitalized patients with COVID-19 pneumonia (LIVE-AIR): a phase 3, randomised, placebo-controlled trial. *Lancet Respir Med.* 2022; 10: 237-246.

9. Cremer PC, Abbate A, Hudock K, et al. Mavrilimumab in patients with severe COVID-19 pneumonia and systemic hyperinflammation (MASH-COVID): an investigator initiated, multicentre, double-blind, randomised, placebo-controlled trial. *Lancet Rheumatol.* 2021; 3: e410-e418.

10. Kyriazopoulou E, Poulaku 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: 1752-1760.

11. Arcani R, Correard F, Suchon P, et al. Tocilizumab versus anakinra in COVID-19: results from propensity score matching. *Front Immunol.* 2023; 14:1185716. doi: 10.3389/fimmu.2023.1185716.

12. Writing Committee for the REMAP-CAP Investigators, Higgins AM, Berry LR, Lorenzi E, et al. Long-term (180-Day) Outcomes in Critically Ill Patients With COVID-19 in the REMAP-CAP Randomized Clinical Trial. *JAMA* 2023; 329(1):39-51. doi: 10.1001/jama.2022.23257.

13. Thomas S, Patel D, Bittel B, et al. Effect of high-dose zinc and ascorbic acid supplementation vs usual care on symptoms length and reduction among ambulatory patients with SARS-CoV-2 infection: the COVID A to Z randomized clinical trial. *JAMA Netw Open* 2021; 4: e210369.

14. Zhang J, Rao X, Li Y, et al. Pilot trial of high-dose vitamin C in critically ill COVID-19 patients. *Ann Intensive Care* 2021; 11: 5.

15. Murai IH, Fernandes AL, Antonangelo L, et al. Effect of a single high-dose vitamin D3 on the length of hospital stay of severely 25-hydroxyvitamin D-deficient patients with COVID-19. *Clinics (Sao Paulo)* 2021; 76: e3549.

16. te Velthuis AJW, van den Worm SHE , Sims AC et al. Zn^{2+} inhibits coronavirus and arterivirus RNA polymerase activity in vitro and zinc ionophores block the replication of these viruses in cell culture. *PloS Pathog.* 2010; 6:e10001176.

17. Abdallah SB, Mhalla Y, Trabelsi I, et al. Twice-daily oral zinc in the treatment of patients with coronavirus disease 2019: a randomized double-blind controlled trial. *Clin Infect Dis.* 2023; 76:185-191.

18. Thomas S, Patel D, Bittel B, et al. Effect of high-dose zinc and ascorbic acid supplementation vs usual care on symptom length and reduction among ambulatory patients with SARS-Cov-2 infection: The COVID A to Z randomized clinical trial. *JAMA Netw Open* 2021; 4:e210369.

19. Abd-Elsalam S, Soliman S, Esmail ES, et al. Do zinc supplements enhance the clinical efficacy of hydroxychloroquine? : a randomized, multicenyte trial. *Biol Trace Elem Res.* 2021; 199: 3642-3646.

11. Cell-based therapy

Mesenchymal stromal cells, rational for use in COVID-19, and clinical evidence of efficacy

In recent years, stem cells have attracted much attention in the field of medicine, and it is generally believed that they have the potential to treat several diseases (1). Compared with embryonic stem cells, mesenchymal stromal cells (MSC) are easily available, more ethical to work with, and easy to freeze and thaw under standard conditions in vitro, making clinical application more convenient and safer[2]. MSC have low immunogenicity and possess homing properties, and have been shown to modulate overactive immune and hyperinflammatory processes, promote tissue repair, and secure antimicrobial molecules (1,3-6). MSC have been reported to limit inflammation and fibrosis in the lungs (7), and have generated variable yet promising results in acute respiratory distress syndrome (ARDS) of viral (8) and non-viral etiology (9,10).

Together, these observations present the rationale for hypothesizing that MSC could reduce the acute lung injury and inhibit the cell-mediated inflammatory response induced by SARS-CoV-2 (11,12). Notably, because MSC lack the ACE2 receptor for SARS-CoV-2 entry into the host cells, they are resistant to the infection (13).

Data supporting cell-based therapy with MSC in COVID-19 patients are limited to small open-label studies and few randomized control trials.

Initial pilot studies on intravenous infusion of bone marrow-derived- or umbilical cord-MSC (hUC-MSC) for hospitalized COVID-19 patients with severe illness were performed in China. They consistently reported that MSC treatment was safe, and accelerated pulmonary function recovery as compared to standard of care therapies (14-16). Some confirmatory evidence has been obtained

in small studies (17). Notably, in an uncontrolled observational cohort of 210 severe/critically ill COVID-19 patients, significantly higher survival rates were reported in those who received UC-MSC infusion before intubation (18).

More informative are the results of the few randomized controlled trials published so far. In a double-blind, phase 1/2a trial in COVID-19 patients with acute respiratory distress syndrome, subjects were randomized to either UC-MSC treatment (n=12, two cell infusions) or placebo (also two infusions of vehicle solution), both in addition to best standard of care (19). Inflammatory cytokines decreased significantly in UC-MSC treated subjects at day 6. Moreover, cell-treatment significantly improved patients' survival by day 31, compared to placebo group. Although the reported benefits of UC-MSC infusion in this study, the interpretation of the results is again limited by the small sample size and the change in an eligibility criterion from enrolling only individuals who were on invasive mechanical ventilation to including those who were receiving high-flow oxygen or non-invasive ventilation. Another randomized controlled trial in critically ill patients with COVID-19, albeit still with a small sample size (n=40), showed that the survival rate in those given a single intravenous infusion of UC-MSC was 2.5 times higher than that in the control group. However, the length of stay in the intensive care unit (ICU) and ventilator usage was comparable in the two groups (20). Less encouraging are recent results of the STROMA-CoV-2 study, a multicenter, double-blind, randomized, placebo-controlled trial involving adult patients with SARS-CoV-2-induced early mild-to-severe ARDS (21). Although the three UC-MSC infusions were not associated with any serious adverse event during treatment or thereafter (until day 28), changes in the partial pressure of oxygen to fractional inspired oxygen (PaO_2/FiO_2)-ratio between baseline and day 7 post-infusion did not differ significantly in UC-MSC versus placebo group.

The above are all short term studies. As part of a previous UC-MSC clinical trial, the long-term consequences of this cell treatment has been reported in a prospective, longitudinal, randomized, double-blind, placebo-controlled phase 2 trial, in which 100 COVID-19 patients were followed up at 3-month intervals for 1 year (22). Interestingly, 17.9% of patients in the UC-MSC group had normal lung CT images at month 12, but none in the placebo group, indicating that UC-MSC administration may confer long-term benefits in terms of recovery from lung lesions and symptoms in COVID-19 patients.

Thus, evidence regarding the extent that MSC may improve clinical outcomes in patients with COVID-19 remains in the preliminary stage and is limited by marked inter-study heterogeneity, inconsistent product characterization and appreciable risk of bias. This conclusion is supported by a very recent systematic review ad meta-analysis of eight randomized controlled trials, overall including 316 patients, 165 administered MSC and 151 controls (23). All studies reported mortality at study endpoint. The results showed that MSC decreased relative risk of death, with no significant difference in absolute risk of death. MSC improved secondary clinical outcomes, namely cell treatment decreased length of hospital stay and C-reactive protein levels compared with controls. Nonetheless, variable outcome reporting, inconsistent MSC characterization and variable control group treatments, remain barriers to higher-quality evidence and may constrain clinical usage.

References

1. Najar M, *et al.* Therapeutic Mesenchymal Stem/Stromal Cells: Value, Challenges and Optimization. *Front Cell Dev Biol*. 2022; doi: 10.3389/fcell.2021.716853.

2. Shivakumar SB *et al.* DMSO- and serum-free cryopreservation of Wharton's jelly tissue isolated from human umbilical cord. *J Cell Biochem.* 2016; 117: 2397-2412.

3. Caplan AI, Correa D. The MSC: an injury drugstore. *Cell Stem Cell* 2011; 9:11-15.

4. Thompson M, *et al.* Cell therapy with intravascular administration of mesenchymal stromal cells continues to appear safe: an update systematic review and meta-analysis. *EClinicalMedicine* 2020; 19:100249.

5. Alagesan S, *et al.* Enhancement strategies for mesenchymal stem cells and related therapies. *Stem Cell Research & Therapy* 2022; 13:75 doi.org/10.1186/s13287-022-02747-w.

6. Perico N, Casiraghi F, Remuzzi G. Clinical Translation of Mesenchymal Stromal Cell Therapies in Nephrology. *J Am Soc Nephrol.* 2018; 29:362-375. doi: 10.1681/ASN.2017070781.

7. Harrell CR, *et al.* Mesenchymal stem cell-based therapy of inflammatory lung diseases: current understanding and future perspectives. *Stem Cells Int.* 2019; 2019: 4236973.

8. Chen J, Hu C, Chen L, *et al.* Clinical study of mesenchymal stem cell treating acute respiratory distress syndrome induced by epidemic influenza A (H7N9) infection, a hint for COVID-19 treatment. *Engineering (Beijing)* 2020; 6: 1153-1161.

9. Yip HK, Fang WF, Li YC, *et al.* Human umbilical cord-derived mesenchymal stem cells for acute respiratory distress syndrome. *Crit Care Med*. 2020; 48: e391-e399.

10. Matthay MA, Calfee CS, Zhuo H, *et al.* Treatment with allogeneic mesenchymal stromal cells for moderate to severe acute respiratory distress syndrome (START study): a randomized phase 2a safety trial. *Lancet Respir Med.* 2019; 7: 154-162.

11. Sadeghi S, Soudi S, Shafiee A, et al. Mesenchymal stem cell therapies for COVID-19: current status and mechanism of action. *Life Sci.* 2020; 262: 118493.

12. Cai Q, Yin F, Hao L, *et al.* Research progress of mesenchymal stem cell therapy for severe COVID-19. *Stem Cells Development* 2021; 30: 459-472.

13. Lukomska B Stanaszek L, Zuba-Surma E, *et al.* Challenges and controversies in human mesenchymal stem cell therapy. *Stem Cells Int.* 2019; 2019: 9628536.

14. Leng Z, Zhu R, Hou W, *et al.* Transplantation of $ACE2^{-}$ mesenchymal stem cells improves the outcome of patients with COVID-19 pneumonia. *Aging and Dis.* 2020; 11: 216-228.

15. Shu L, Niu C, Li R, *et al.* Treatment of severe COVID-19 with human umbilical cord mesenchymal stem cells. *Stem Cell Res Ther.* 2020; 11: 361.

16. Feng G, Shi L, Huang T, *et al.* Human umbilical cord mesenchymal stromal cell treatment of severe COVID-19 patients: A 3-month follow-up study following hospital discharge. *Stem Cells Development* 2021; 30: 773-781.

17. Iglesias M, Butròn P, Torre-Villalvazo I, *et al.* Mesenchymal stem cells for the compassionate treatment of severe acute respiratory distress syndrome due to COVID 19. *Aging and Dis.* 2021; 12: 360-370.

18. Ercelen N, Pekkoc-Uyanik KC, Alpaydin N, *et al.* Clinical experience on umbilical cord mesenchymal stem cell treatment in 210 severe and critical COVID-19 cases in Turkey. *Stem Cell Reviews and Reports* 2021; 17: 1917-1925.

19. Lanzoni G, Linetsky E, Correa D, *et al.* Umbilical cord mesenchymal stem cells for COVID-19 acute respiratory distress syndrome: A double-blind, phase 1/2a, randomized controlled trial. *Stem Cells Transl Med.* 2021; 1-14.

20. Dilogo IH, Aditianingsih D, Sugiarto A, *et al.* Umbilical cord mesenchymal stromal cells as critical COVID-19 adjuvant therapy: A randomized controlled trial. *Stem Cells Transl Med.* 2021; 10: 1279-1287.

21. Monsel A, Hauw-Berlemont C, Mebarki M, *et al.* Treatment of COVID-19-associated ARDS with mesenchymal stromal cells: a multicenter randomized double-bind trial. *Crit Care* 2022; 26: 48.

22. Shi L, Yuan X, Yao W, *et al.* Human mesenchymal stem cells treatment for severe COVID-19: 1-year follow-up results of a randomized, double-blind, placebo-controlled trial. *eBioMedicine* 2022; 75: 103789.

23. Kirkham AM, Bailey AJM, Shorr R, Lalu MM, Fergusson DA, Allan DS. Systematic review and meta-analysis of randomized controlled trials of mesenchymal stromal cells to treat coronavirus disease 2019: is it too late? *Cytotherapy* 2023; 25:341-352.

12. Concomitant medications for underlying conditions in COVID-19 patients

Individuals with underlying chronic conditions, such as cardiovascular disease (CVD), pulmonary disease, diabetes, and malignancy are at higher risk of severe illness with COVID-19. These patients are usually prescribed medications to treat these disorders. Early in the pandemic, some of these agents, such as angiotensin-converting enzyme inhibitors (ACEi), angiotensin receptor blockers (ARBs) (1), HMG-CoA reductase inhibitors (statins) (2,3), and H-2 receptor antagonists (4), were reported to offer potential as COVID-19 therapeutic agents or, in the case of nonsteroidal anti-inflammatory drugs (NSAIDs), to present potential hazards (5).

A predictable aftermath of the discovery that membrane-bound ACE2 is the functional receptor through which SARS-CoV-2 enters human cells was the concern that ACEi and ARBs, through up-regulation of the expression of ACE2, may contribute to adverse outcomes related to COVID-19 (6). Currently, based on at least three randomized clinical trials, there is no evidence that discontinuing renin-angiotensin system inhibitors for underlying medical conditions offers a clinical benefit for patients with COVID-19 (6-8). The American Heart Association, the American College of Cardiology,

and the Heart Failure Society of America issued a joint statement that renin-angiotensin-aldosterone system antagonists, such as ACEi and ARBs, should be continued as prescribed in patients with COVID-19 (9).

Although simvastatin has been reported to downregulate the SARS-CoV-2-induced inflammatory response and to impair viral infection through disruption of lipid rafts (10), a large retrospective

cohort study of the US Veterans Health Administration found that statin use was associated with lower odds of 30-day mortality both among subjects with and without a positive respiratory swab for SARS-CoV-2, indicating that statins may not exert COVID-19 specific beneficial effects (11).

Other agents, such as NSAIDs, in particular ibuprofen, were postulated to have a negative impact without a clear mechanistic explanation (see section 4 of this document) (12). However, after review of the evidence, the US Food and Drug Administration (FDA) stated that there is no evidence linking the use of NSAIDs with worsening of COVID-19 and advised patients to use them as directed (13).

According to the US National Institutes of Health (NIH) COVID-19 Treatment Guidelines Panel, patients with COVID-19 who are treated with concomitant medications for an underlying medical condition should not discontinue these medications during the acute management of COVID-19, unless discontinuation is otherwise warranted by their clinical condition (14).

The same Panel recommends against using medications off-label to treat COVID-19 if they have not been shown to be safe and effective for this indication in a randomized clinical trial (14).

Finally, when prescribing medications to treat COVID-19, clinicians should always assess the patient's current medications for potential drug-drug interactions and/or additive adverse effects (14).

References

1. Bavishi C, Whelton PK, Mancia G, Corrao G, Messerli FH. Renin-angiotensin-system inhibitors and all-cause mortality in patients with COVID-19: a systematic review and meta-analysis of observational studies. *J Hypertens.* 2021; 39: 784-794. doi: 10.1097/HJH.0000000000002784.

2. Lee KCH, Sewa DW, Phua GC. Potential role of statins in COVID-19. *Int J Infect Dis.* 2020; 96: 615-617. doi: 10.1016/j.ijid.2020.05.115.

3. INSPIRATION-S Investigators. Atorvastatin versus placebo in patients with covid-19 in intensive care: randomized controlled trial. *BMJ* 2022; 376: e068407. doi: 10.1136/bmj-2021-068407.

4. Mather JF, Seip RL, McKay RG. Impact of famotidine use on clinical outcomes of hospitalized patients with COVID-19. *Am J Gastroenterol.* 2020;115: 1617-1623. doi: 10.14309/ajg.0000000000000832.

5. Remuzzi G, Schiaffino S, Santoro MG, FitzGerald GA, Melino G, Patrono C. Drugs for the prevention and treatment of COVID-19 and its complications: An update on what we learned in the past 2 years. *Front Pharmacol.* 2022;13:987816. doi: 10.3389/fphar.2022.987816.

6. Lopes RD, Macedo AVS, de Barros E Silva PGM, et al; BRACE CORONA Investigators. Effect of discontinuing vs continuing angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers on days alive and out of the hospital in patients admitted with COVID-19: a randomized clinical trial. *JAMA* 2021; 325: 254-264. doi: 10.1001/jama.2020.25864.

7. Cohen JB, Hanff TC, William P, et al. Continuation versus discontinuation of renin-angiotensin system inhibitors in patients admitted to hospital with COVID-19: a prospective, randomised, open-label trial. *Lancet Respir Med.* 2021; 9: 275-284. doi: 10.1016/S2213-2600(20)30558-0.

8. Bauer A, Schreinlechner M, Sappler N, et al.; ACEI-COVID Investigators. Discontinuation versus continuation of renin- angiotensin-system inhibitors in COVID-19 (ACEI-COVID): a prospective, parallel group, randomised, controlled, open-label trial. *Lancet Respir Med.* 2021; 9: 863-872. doi: 10.1016/S2213-2600(21)00214-9.

9. Bozkurt B, Kovacs R, Harrington B. Joint HFSA/ACC/AHA statement addresses concerns re: using RAAS antagonists in COVID-19. *J Card Fail.* 2020; 26: 370. doi: 10.1016/j.cardfail.2020.04.013.

10. Teixeira L, Temerozo JR, Pereira-Dutra FS, et al. Simvastatin downregulates the SARS-CoV-2-induced inflammatory response and impairs viral infection through disruption of lipid rafts. *Front. Immunol.* 2022; 13: 820131. doi: 10.3389/fimmu.2022.820131.

11. Wander PL, Lowy E, Beste LA, et al. Associations of statin use with 30-day adverse outcomes among 4 801 406 US Veterans with and without SARS- CoV-2: an observational cohort study. *BMJ Open* 2022; 12: e058363. doi: 10.1136/bmjopen-2021-058363.

12. Yousefifard M, Zali A, Zarghi A, Madani Neishaboori A, Hosseini M, Safari S. Non-steroidal anti-inflammatory drugs in management of COVID-19; a systematic review on current evidence. *Int J Clin Pract.* 2020;74: e13557. doi: 10.1111/ijcp.13557.

13. Food and Drug Administration. FDA advises patients on use of non-steroidal anti-inflammatory drugs (NSAIDs) for COVID-19. 2020. Available at: https://www.fda.gov/drugs/drug-safety-and-availability/fda-advises-patients-use-non-steroidal-anti-inflammatory-drugs-nsaids-covid-19. Accessed March 1, 2023.

14. COVID-19 Treatment Guidelines Panel. Coronavirus Disease 2019 (COVID-19) Treatment Guidelines. National Institutes of Health. Available at https://www.covid19treatmentguidelines.nih.gov/. Last updated May 31 2022.

13. Treatment management of children with Multisystem Inflammatory Syndrome (MIS-C)

In April 2020, during the peak of COVID-19 pandemic in Europe, multisystem inflammatory syndrome in children (MIS-C) was first described in reports of children presenting with a severe multisystem hyperinflammatory illness temporally associated with preceding SARS-CoV-2 infection or exposure (1,2). Since that time, cases have been reported worldwide. MIS-C represents a relatively small proportion of the total COVID-19 cases in children. According to the CDC, as for January 2023, there are over 9300 total cases with a median age of 9 years that meet the criteria of MIS-C and 76 reported deaths (estimated mortality 0.8%) (3). Compared to the predominance periods of the Alpha, Beta, and Delta variants of SARS-CoV-2, it is evident that MIS-C is significantly less frequent and less severe during the Omicron period (4). It is not known whether the observed difference is a consequence of some unique biological properties of variants, or simply a result of a gradually expanding population with immunity against SARS-CoV-2 (5). The incidence of MIS-C is higher in some ethnic groups, including Black, Hispanic, Latino, and Pacific Islander persons (5). Most MIS-C patients have serologic evidence of previous SARS-CoV-2 infection, but only a minority are RT-PCR positive for SARS-CoV-2 at presentation (6-7). In published case series many of the pediatric patients with this hyperinflammatory syndrome have had fever and mucocutaneous manifestations similar to those of Kawasaki's disease, a rare vasculitis of childhood that can cause coronary artery aneurysm (1,2,3,8). Some patients have presented with features of toxic shock syndrome, secondary hemophagocytic lymphohistiocystosis, or macrophage activation syndrome (9). Although the cause of Kawasaki's disease remains unknown, a preceding or active infection has been suspected (10). Like Kawasaki's disease, MIS-C is a syndrome with a range of clinical presentations and an absence of pathognomonic findings or diagnostic tests. Unlike Kawasaki's disease, however, MIS-C has been suggested in early reports to predominantly affect adolescents and children older than 5 years of age and to be associated with more frequent cardiovascular involvement (1,3,11). The current CDC case definition for MIS-C includes, i) an individual aged <21 years presenting with fever (>38°C), laboratory evidence of inflammation, and evidence of clinically severe illness that requires hospitalization with multisystem (>2) organ involvement; and ii) no alternative plausible diagnoses; and iii) positive for current or recent SARS-CoV-2 infection by RT-PCR, antigen test, or serology results; or COVID-19 exposure within the 4 weeks prior to the onset of symptoms (12). This CDC case definition slightly differs from that of WHO in the duration of the fever, as well as hospitalization requirement. Indeed, the CDC case definition requires that the child must have severe symptoms requiring hospitalization, whereas that of WHO does not (13).

Nonetheless, the pathogenesis of MIS-C is still being elucidated (14). Differences have been reported between MIS-C and typical Kawasaki's disease in terms of cytokine expression, and elevation of inflammatory markers. Moreover, differences in cytokine expression (tumor necrosis factor alpha and interleukin-10) have been shown between MIS-C and acute COVID-19 in children (15,16). Very recently, it has been hypothesized that monogenic inborn errors of immunity to SARS-CoV-2 may underline MIS-C in some children (17). In 1% of an international cohort of 558 subjects with MIS-C (aged 3 moths to 19 years), autosomal recessive deficiencies of OAS1 (2'-5'-oligoadenylate synthase 1), OAS2, or RNase (ribonuclease L) have been reported (17). Notably, the

cytosolic OAS-RNase L pathway suppresses RIG-I (retinoic acid-inducible gene I)/MDA5 (melanoma differentiation-associated protein 5)-MAVS (mitochondrial antiviral-signaling protein)-mediated inflammation in double-stranded RNA (dsRNA)-stimulated mononuclear phagocytes. Thus, single-gene recessive inborn errors of the OAS-RNase L pathway unleash the production of SARS-CoV-2 triggered inflammatory cytokines by mononuclear phagocytes, thereby undelaying MIS-C. However, given the reported clinical similarity in MIS-C to Kawasaki's disease, the approach to treatment of MIS-C has been similar to that of Kawasaki's disease. Thus, in reported cohorts of children with MIS-C the most commonly used therapeutic approach is with intravenous immunoglobulin (IVIG) and glucocorticoids (1,2,6,7,11,18-21). The IVIG in combination with glucocorticoids is also the recommendation of the American College of Rheumatology for the first level treatment for most hospitalized children with MIS-C (22). Several non-randomized studies indicate that the front-line combination IVIG/glucocorticoids resulted in less treatment failure, faster recovery of cardiac function, shorter ICU stay, and decreased requirement for treatment escalation compared to IVIG monotherapy (11, 23-26). On this basis, it has been recommended using IVIG in combination with low-to-moderate dose glucocorticoids for children hospitalized with MIS-C, but not the routine use of IVIG monotherapy, unless glucocorticoid therapy is contraindicated (27). Indeed, there is uncertainty regarding the use of glucocorticoid monotherapy versus IVIG plus glucocorticoids as initial therapy for MIS-C because comparative studies evaluating these two treatment regimens are scanty (28). On the other hand, there is only a randomized trial that compared methylprednisolone for 3 days (n=38 patients) to a single dose of IVIG (n=38 patients). There was no difference in the primary outcome of length of hospital stay or deaths between the two study groups. However, in a secondary analysis, a significant difference as for the requirement of respiratory support was found between patients in the glucocorticoid group (27%) compared to those treated with IVIG (55%) (29). Thus, the NIH Panel recommends using glucocorticoid monotherapy in children with MIS-C only if IVIG is unavailable or contraindicated (27).

The combination of IVIG/glucocorticoid therapy usually results in a quick clinical improvement within the first 24 hours, characterized by the resolution of fever, improvement in organ function, and reduced levels of inflammatory markers, particularly C-reactive protein. Should MIS-C be refractory to the combined treatment (persistent fever, worsening organ dysfunction, and inflammatory marker increase), intensification therapy with higher-dose glucocorticoids (26,30) or the IL-1 receptor antagonist anakinra (7,19,20), or the monoclonal antibody anti-TNFα infliximab, have been proposed. However, comparative studies evaluating intensification therapies in children with refractory MIS-C are missing, precluding the determination of which of these agents is more effective in this setting. Actually, infliximab plus IVIG have been tested in a single-centre retrospective cohort study as initial immunomodulatory therapy in 72 children with MIS-C (31). The patients treated with infliximab plus IVIG compared to those treated with IVIG alone were less likely to require additional therapy, and had decreased ICU length of stay, decreased development of left ventricular dysfunction, and more rapid decline in C-reactive protein levels. Therefore, drug combination regimens may become a trend in future MIS-C treatment, but there is a need to determine whether a combination drug approach can maximize treatment efficacy while minimizing

adverse effects (32). Notably, children with MIS-C who receive multiple immunomodulatory agents are at risk for infection and need to be monitored carefully.

Similarly to children with Kawasaki's disease, it is expected that in those with MIS-C also platelet activation and endothelial dysfunction may occur (8). Thus, there is agreement that MIS-C children should also be given low-dose aspirin, if they are not at risk for bleeding. However, again based on evidence in Kawasaki's disease patients, MIS-C children who have large coronary artery aneurysms or with moderate-to-severe left ventricular dysfunction (at risk of intracardiac thrombosis) should receive therapeutic anticoagulation, unless contraindicated due to bleeding risk factors (27). Given the uncertainty of the benefit and the risk of major bleeding (33), prophylactic or therapeutic anticoagulation for children with MIS-C, but without large coronary artery aneurysms or left ventricular dysfunction, should be evaluated on the single case, considering the risk factors for thrombosis.

References

1. Verdoni L, Mazza A, Gervasoni A, et al. An outbreak of severe Kawasaki-like disease at the Italian epicentre of the SARS-CoV-2 epidemic: an observational cohort study. *Lancet* 2020; 395: 1771–8.

2. Toubiana J, Poirault C, Corsia A, et al. Kawasaki-like multisystem inflammatory syndrome in children during the covid-19 pandemic in Paris, France: prospective observational study. *BMJ* 2020; 369: m2094.

3. Riphagen S, Gomez X, Gonzalez-Martinez C, Wilkinson N, Theocharis P. Hyperinflammatory shock in children during COVID-19 pandemic. *Lancet* 2020; 395: 1607–8.

4-. Cloete J, Kruger A, Masha M, et al. Paediatric hopitalisations due to COVID-19 during the first SARS-CoV-2 omicron (B.1.1.529) variant wave in South Afric a: a multicenter observational study. *Lancet Child Adolesc Health* 2022; 6: 294-302.

5. Lee P-I, Hsueh P-R. Multisystem inflammatory syndrome in children: A dysregulated autoimmune disorder following COVID-19. *J Microbiol Immunol and Infection* 2023; 56: 236-245.

6. Dufort EM, Koumans EH, Chow EJ, et al. Multisystem Inflammatory Syndrome in Children in New York State. *N Engl J Med.* 2020; 383: 347–58.

7. Feldstein LR, Rose EB, Horwitz SM, et al. Multisystem Inflammatory Syndrome in U.S. Children and Adolescents. *N Engl J Med.* 2020; 383: 334–46.

8. McCrindle BW, Rowley AH, Newburger JW, et al. Diagnosis, Treatment, and Long-Term Management of Kawasaki Disease: A Scientific Statement for Health Professionals From the American Heart Association. *Circulation* 2017; 135: e927–99.

9. Crayne C, Cron RQ. Pediatric macrophage activation syndrome, recognizing the tip of the iceberg. *Eur J Rheumatol.* 2019; 7(Suppl 1): 1–8.

10. Benseler SM, McCrindle BW, Silverman ED, Tyrrell PN, Wong J, Yeung RSM. Infections and Kawasaki disease: implications for coronary artery outcome. *Pediatrics* 2005; 116: e760-766.

11. Belhadjer Z, Méot M, Bajolle F, et al. Acute heart failure in multisystem inflammatory syndrome in children in the context of global SARS-CoV-2 pandemic. *Circulation* 2020; 142: 429–36.

12. Centers for Disease Control and Prevention. Information for healthcare providers about multisystem inflammatory syndrome in children (MIS-C). 2021. Available at: https://www.cdc.gov/mis/mis-c/hcp. Accessed February 15 2021.

13. BC Centre for Disease Control. Multi-system inflammatory syndrome in children and adolescents (MIS-C). BC Centre for Disease Control: Vancouver, BC, Canada, 2020.

14. Filippatos F, Tatsi E-B, Michos A. Immunology of multisystem inflammatory syndrome after COVID-19 in children: A review of current evidence. *Int J Mol Sci.* 2023; 24: 5711.

15. Rowley AH, Shulman ST, Arditi M. Immune pathogenesis of COVID-19-related multisystem inflammatory syndrome in children. *J Clin Invest.* 2020; 130: 5619–21.

16. Diorio C, Henrickson SE, Vella LA, et al. Multisystem inflammatory syndrome in children and COVID-19 are distinct presentations of SARS-CoV-2. *J Clin Invest.* 2020; 130: 5967–75.

17. Lee D, Le Pen J, Yatim A, et al. Inborn errors of OAS-RNase L in SARS-CoV-2-treated multisystem inflammatory syndrome in children. *Science* 2023; 379: 554. eabo3627.

18. Whittaker E, Bamford A, Kenny J, et al. Clinical characteristics of 58 children with a pediatric inflammatory multisystem syndrome temporally associated with SARS-CoV-2. *JAMA* 2020; 324: 259–69.

19. Pouletty M, Borocco C, Ouldali N, et al. Paediatric multisystem inflammatory syndrome temporally associated with SARS-CoV-2 mimicking Kawasaki disease (Kawa-COVID-19): a multicentre cohort. *Ann Rheum Dis.* 2020; 79: 999–1006.

20. Lee PY, Day-Lewis M, Henderson LA, et al. Distinct clinical and immunological features of SARS-CoV-2-induced multisystem inflammatory syndrome in children. *J Clin Invest.* 2020; 130: 5942–50.

21. Feldstein LR, Tenforde MW, Friedman KG, et al. Characteristics and outcomes of US children and adolescents with multisystem inflammatory syndrome in children (MIS-C) Compared With Severe Acute COVID-19. *JAMA* 2021; 325: 1074–87.

22. Henderson LA, Canna SW, Friedman KG, et al. American College of Rheumatology Clinical Guidance for multisystem inflammatory syndrome in children associated with SARS-CoV-2 and hyperinflammation in pediatric COVID-19: Version 3. *Arthritis Rheumatol.* 2022; 74: e1–20.

23. Ouldali N, Toubiana J, Antona D, et al. Association of intravenous immunoglobulinspPlus methylprednisolone vs immunoglobulins alone with course of fever in multisystem inflammatory syndrome in children. *JAMA* 2021; 325: 855–64.

24. McArdle AJ, Vito O, Patel H, et al. Treatment of multisystem inflammatory syndrome in children. *N Engl J Med.* 2021; 385: 11–22.

25. Jonat B, Gorelik M, Boneparth A, et al. Multisystem inflammatory syndrome in children associated with coronavirus disease 2019 in a children's hospital in New York city: Patient characteristics and an institutional protocol for evaluation, management, and follow-up. *Pediatr Crit Care Med*. 2021; 22: e178–91.

26. Son MBF, Murray N, Friedman K, et al. Multisystem inflammatory syndrome in children - initial therapy and outcomes. *N Engl J Med.* 2021; 385: 23-34.

27. COVID-19 Treatment Guidelines Panel. Coronavirus Disease 2019 (COVID-19) Treatment Guidelines. National Institutes of Health. Available at: https://www.covid19treatmentguidelines.nih.gov/. Accessed 24 February 2022.

28. Channon-Wells S, Vito O, McArdle AJ, et al. Immunoglobulin, glucocorticoids, or combination therapy for multisystem inflammatory syndrome, in children: a propensity-weighted cohort study. *Lancet Rheumatol.* 2023; 5: e185-e189.

29. Welzel T, Atkinson A, Schoebi N et al., Methylprednisolone versus intravenous immunoglobulins in children with pediatric inflammatory multisystem syndrome temporally associated with SARS-CoV-2 (PIMS-TS): an open-label, multicentre, randomised trial. *Lancet Child Adolesc Health.* 2023; 7: 238-248.

30. Chiotos K, Bassiri H, Behrens EM, et al. Multisystem inflammatory syndrome in children during the coronavirus 2019 pandemic: A case series. *J Pediatric Infect Dis Soc*. 2020; 9: 393–8.

31. Cole LD, Osborne CM, Silveira LJ, et al. IVIG compared to IVIG plus infliximab in multisystem inflammatory syndrome in children. *Pediatrics* 2021: e2021052702.

32. McMurray JC, May JW, Cunningham MW, Jones OY. Multisystem inflammatory syndrome in children (MIS-C), a post-viral myocarditis and systemic vasculitis - a critical review of its pathogenesis and treatment. *Front Pediatr.* 2020; 8: 626182.

33. Whitworth H, Sartain SE, Kumar R, et al. Rate of thrombosis in children and adolescents hospitalized with COVID-19 or MIS-C. *Blood* 2021; 138: 190–8.

Rome, 4 August, 2023