COVID-19 Committee of the Lincei Academy

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

What we learned in the past two years

Executive Summary

The COVID-19 Committee of the Lincei Academy has updated the reviewed 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 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 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 pediatric patients at high risk. In addition to remdesivir, in the last months 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 reported.

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 randomized controlled trials 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 emergency authorization 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. Clinical trials involving several JAK inhibitors for the treatment of COVID-19 are ongoing, and their results will hopefully provide valuable information on the usefulness of these agents.

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. The results of ongoing phase II/III clinical trials will elucidate the benefit to risk profile of complement inhibitors, clarify the optimal target(s) in the complement cascade, and 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 heparin, unless contraindicated. Moreover, recent available evidence from RCTs supports the concept that therapeutic dose anticoagulation with low-molecular-weight heparin 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. There is not yet consensus on the role of extended thromboprophylaxis beyond the hospital stay. 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, and interferons.

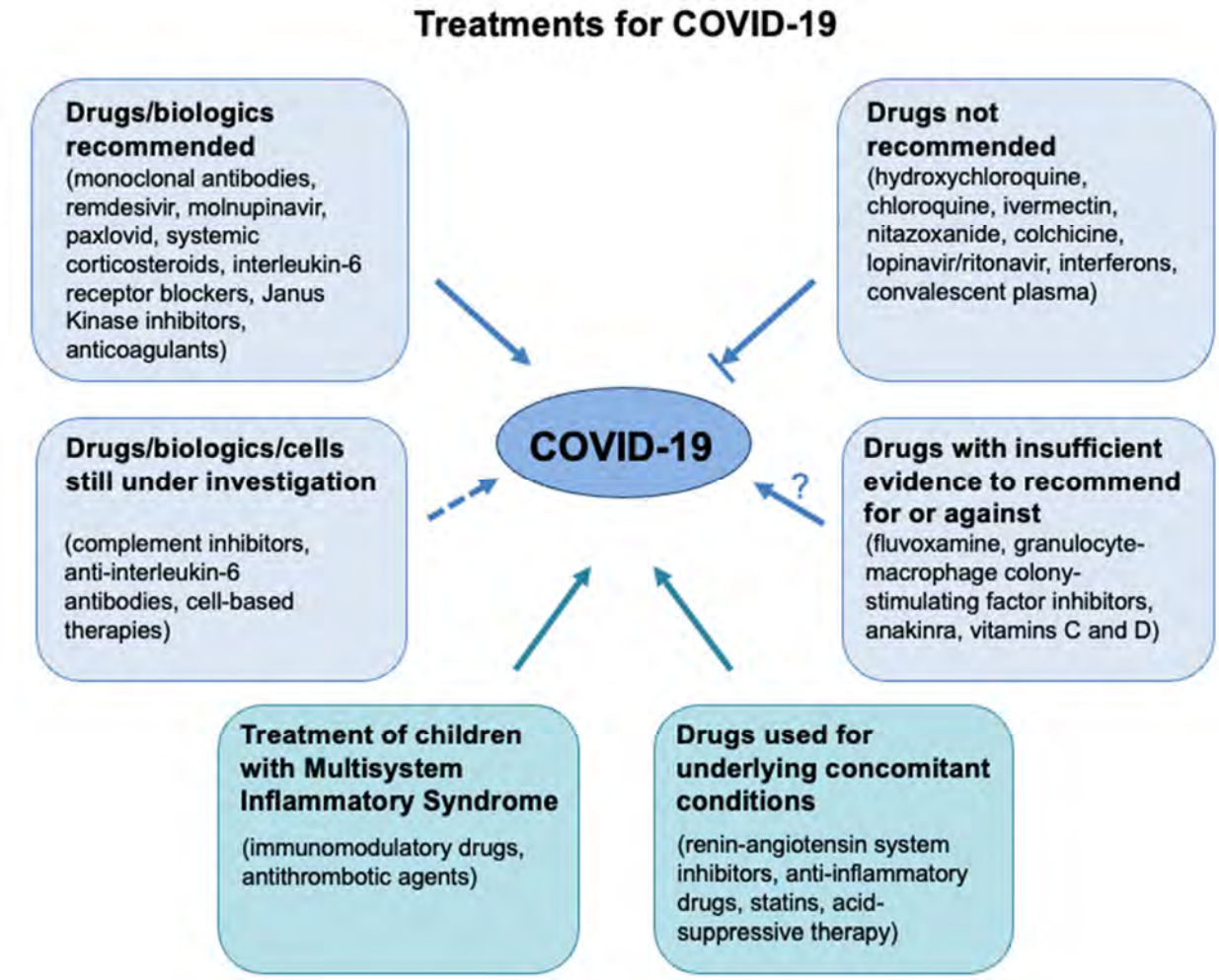
Several other drugs are still in clinical trials, and, considering the preliminary results, there is insufficient evidence to recommend for or against their use in COVID-19 patients. Among these, anakinra, an interleukin-1 receptor antagonist. While the SAVE-MORE trial in hospitalized patient with moderate 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 day 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 this 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 randomized control trials. 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 cardiovascular disease (or other indications) should continue these medications. Similar approach will 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 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 randomized clinical trial.

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 to 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 MIS-C children should also be given low-dose aspirin.



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).

At present, the newly discovered (2019) SARS-CoV-2 coronavirus continues to 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. Today (April 6, 2022) we dismayingly count 493,710,804 cases and 6,159,056 deaths (3). Given the unprecedented proportions of the pandemic in many countries, and the rise in the associated global death toll, over the past two 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 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. The documentation for this FDA authorisation, however, did not report or cite specific trials on which this decision was based, making it difficult to assess the scientific evidence for it. Nonetheless, physicians and healthcare providers interpreted the EUA for hydroxy-chloroquine as an instruction to incorporate this drug into therapeutic protocols for treating COVID-19 patients. However, on June 15th 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 (see below Section 9).

Indeed, it is necessary to conduct rigorous studies on COVID-19 drug candidates that will provide sufficient scientific data that can be evaluated meticulously, which will 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 review 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 now updated, reporting what we learned in the past two 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 review 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 reports the treatment management of children with Multi-system Inflammatory Syndrome (MSI-C), an illness temporally associated with preceding SARS-CoV-2 exposure.
This report does not intend to recommend any experimental drug, but to review the evidence supporting the efficacy and safety of these pharmacological treatments, highlight the official position of health authorities and panels of experts with regard to each drug or class of drugs considered, and briefly mention the ongoing trials registered with clinicaltrials.gov or the WHO register.

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

SARS-CoV-2 spike protein binds to its receptor, angiotensin-converting enzyme (ACE2), and is proteolytically activated by the transmembrane protease serine 2 (TMPRSS2), thus enabling the fusion of the virus with the cell membrane (1, 2). 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 (3), however DPP4 was unable to mediate virus entry in cells lacking ACE2 (1). Another tissue protease, the proprotein convertase furin, is involved in the cleavage of the spike protein, possibly promoting the subsequent cleavage by TMPRSS2 (4). However, furin inhibitors, unlike TMPRSS2 inhibitors, can interfere with important cell functions, thus furin is not an attractive drug target. Current approaches aimed at blocking SARS-CoV-2 cell entry are based on i) treatments inhibiting the SARS-CoV-2 spike-ACE2 interaction or ii) TMPRSS2 inhibitors.

Anti-spike antibodies and soluble ACE2 can block the interaction between the virus spike protein and ACE2. Passive immunization with convalescent plasma is presently used in different countries for the therapy of COVID-19 with the view that neutralizing antibodies could both inhibit the binding

of the virus to the cell and promote the clearance of the virus by immune cells. Neutralizing antibodies are thus promising candidates for prophylactic and therapeutic treatment of COVID-19. Previous 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 related to antibody-dependent enhancement of infection (ADE) should be considered (5). Randomized clinical trials are required to evaluate the efficacy and safety of anti-SARS-CoV-2 convalescent plasma, and at least two trials are ongoing (*EudraCT Number: 2020-001310-38; ChiCTR Number: ChiCTR2000030010*). Actually, the recently updated NIH guidelines (https://www.covid19treatmentguidelines.nih.gov/) recommend against the use of convalescent plasma for the treatment of 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 of them demonstrating efficacy (6,7), while others no benefits (8,9) on the incidence of disease progression and/or hospitalization. Notably, there is insufficient evidence in favor or against the use of high-titer convalescent plasma collected after the emergence of the Omicron variants. Monoclonal antibodies against SARS-CoV-2, some of which were derived from COVID-19 patients B-cells, were found to neutralize the virus in cultured cells (10, 11) and a recent study in a transgenic mouse model bearing human ACE2 confirmed that specific monoclonal antibodies can reduce virus titers in infected lungs (12). The latter report was complemented by a detailed structural analysis of the interaction between antibody, receptor binding domain (RBD) of the spike protein and ACE2, thus providing important information for 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 to 7 days after the onset of the illness (13-15). A serious problem with the use of neutralizing antibodies for the treatment of COVID-19 has been the rapid emergence and spread of mutations of the spike protein which are not recognized by available antibodies (16). The NIH Guidelines (https://www.covid19treatmentguidelines.nih.gov/) indicate that almost all available antibodies have reduced activities against the B.1.1.529 (Omicron) variant of concern (VOC). Moreover, Sotrovimab is no longer recommended as a treatment option for patients with COVID-19, since it has substantially reduced in vitro activity against the Omicron BA.2 subvariant, which has recently become one of the dominant subvariants in the United States and in Europe. Nonetheless, very recently the FDA has updated the emergency use authorization of Bebtelovimab, highlighting that this neutralizing monoclonal antibody retains activity to Omicron subvariants BA.2.12.1 and BA.4/BA.5.

An alternative approach to block the interaction between the SARS-Cov-2 and the ACE2 receptor is the use of picomolar miniprotein inhibitors that bind with high affinity 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 (17).

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*) (18,19). This rhsACE2 was recently found to inhibit SARS-CoV-2 infection in cultured cells and in human blood vessel and kidney organoids (20) and a clinical trial has been launched to use rhsACE2 as a treatment for patients with COVID-19 (*ClinicalTrials.gov identifier: NCT04335136*). This approach has been successfully applied to COVID-19 patients (21) and variations of this approach have been described. For example, engineering of ACE2 N-glycosylation by site-directed mutagenesis or glycosidase treatment resulted in enhanced binding affinities and improved virus neutralization (22). Treatment with recombinant human ACE2-Fc fusion protein (hACE2-Fc) effectively protected mice from 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 (23).

A completely new approach to block 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, including Omicron (24). A potential problem in the therapeutical utilization of MBL is the possibility that in advanced disease stages MBL may contribute to complement activation and uncontrolled inflammation.

TMPRSS2 protease inhibitors could be used to block a crucial step required for 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 (2). This drug is approved in Japan and Korea for use in chronic pancreatitis and has been repurposed in a clinical trial for COVID-19 (*ClinicalTrials.gov number NCT04353284*). Nafamostat mesylate, another drug used for many years in Japan for acute pancreatitis and disseminated intravascular coagulation (DIC), was recently reported to inhibit SARS-CoV-2 infection of Calu3 human lung cells in the nanomolar range, with 10-15 higher efficiency than camostat mesylate (25-27). The efficacy of nafamostat in COVID-19 patients is presently 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 mucolytic cough suppressant, could be used for the treatment of COVID-19 (28,29). Novel TMPRSS2 inhibitors have been recently identified through high-throughput screening (30), and a highly potent inhibitor has been recently well characterized (31). This compound, N-0385, is a small peptidemimetic which is active at low doses (nanomolar) at inhibiting 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 when delivered as nasal spray 12 hours after infection in mice expressing the human ACE2 receptor.

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 develop serious COVID-19 infection (32). This possibility is supported by epidemiological studies, showing that prostate cancer patients treated with anti-androgens are much less frequently affected by COVID-19 compared with those untreated (33). This study is

supported by new results from different labs and the effect of testosterone suppression in COVID-19 patients is investigated in clinical trials, including a trial using Degarelix, a FDA-approved drug for prostate cancer (*ClinicalTrials.gov identifier: NCT04397718*) (34). However, a recent randomized phase 2 clinical trial did not support a therapeutic effect of anti-androgen therapy in COVID-19 (35).

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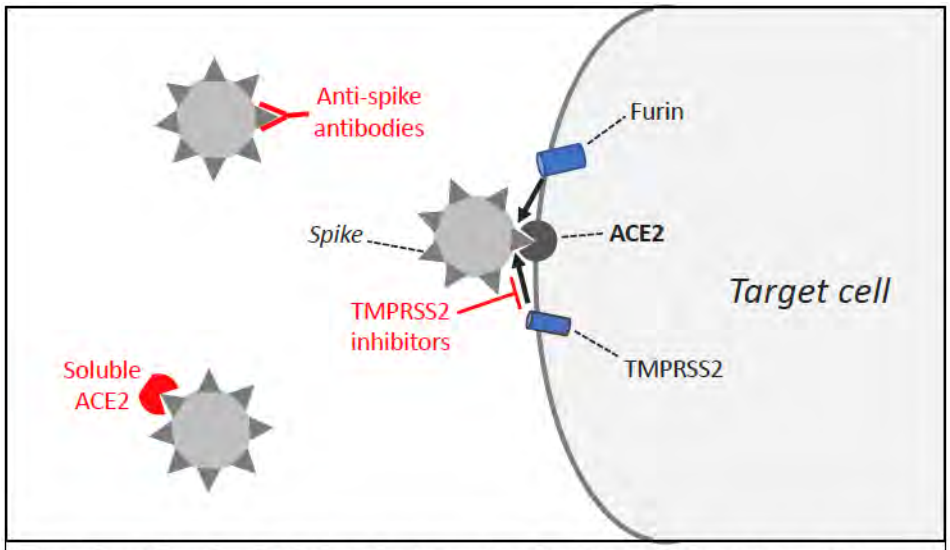

The entry of Sars-Cov-2 into target cells requires the binding of the spike protein present at the surface of the virus with cell membrane bound angiotensin converting enzyme 2 (ACE2), which acts as a receptor of the virus. Spike protein cleavage by the host cell proteases, transmembrane protease serine 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.

3. Drugs inhibiting SARS-CoV-2 replication

Before the emergence of SARS-CoV-2 no specific antiviral treatment for coronavirus infection was available. Over the past two years considerable efforts have been directed towards the identification of antivirals effective against SARS-CoV-2 and, in particular, toward repurposing of FDA-approved drugs that have the potential to greatly accelerate clinical availability. So far, among a large number of antiviral drugs investigated in preclinical and clinical studies, three have been approved or have received emergency use authorization (EUA) for SARS-CoV-2 infections by health authorities in different countries [https://www.bio.org/policy/human-health/vaccines-biodefense/coronavirus/pipeline-tracker]. As for other antivirals used in the therapy of 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.

Remdesivir

Remdesivir, an adenosine analog prodrug originally developed for the treatment of 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 misintegrated into viral RNA by the viral RNA-dependent RNA polymerase (RdRp), resulting in chain termination (1). Remdesivir was identified early as a promising therapeutic candidate for 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 infection (reviewed in Eastman et al.) (1). These findings, along with the safety profile of remdesivir in the clinical trial assessment against EBOV (1), supported the evaluation of remdesivir as a potential therapeutic drug for repurposing against SARS-CoV-2 infections.

Based on initial observations of clinical improvement of a limited number of patients seriously ill with COVID-19 treated with remdesivir under compassionate use, over the last two years a large number of studies were launched to investigate the effectiveness of remdesivir, alone or in combination with other drugs, against COVID-19 (see *ClinicalTrials.gov*); however, these studies 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 the time to recovery in patients: a median of 10 days in hospital as compared with 15 days for those assigned to the placebo group (4).

Based on these findings, on May 1 2020 remdesivir (VEKLURY®) was made available in the US under an EUA for the treatment of adults and children with severe COVID-19 disease by the FDA

(https://www.fda.gov/news-events/press-announcements/coronavirus-covid-19-update-fda-issues-emergency-use-authorization-potential-covid-19-treatment), followed by the drug's authorization in the EU (*https://www.ema.europa.eu/en/medicines/human/EPAR/veklury#authorisation-details-section*). On October 22 2020, the FDA approved VEKLURY for use in adults and pediatric patients (12-years and older) requiring 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, even if lower in the patients treated with remdesivir, remained high: 11.4% as compared to 15.2% in the placebo group at day 29 after enrollment (4). Encouraging results relative to 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 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, remdesivir was reported to be less effective in patients hospitalized with moderate COVID-19 pneumonia in an open-label multinational study (NCT04292730) (6). 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 none of the four drugs produced any measurable benefit in 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 at 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 among 1282 patients admitted with COVID-19, in-hospital mortality of patients treated with remdesivir was lower than that of control patients: 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 resulted to be especially beneficial in 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 of the clinical trials of remdesivir in hospitalized patients described above likely contributed to increase uncertainty in the medical community about the effectiveness of drug; however, the fact that most of the studies indicated that treatment with remdesivir is most successful when started in early stages of infection, has opened new perspectives. It should be noted that, since remdesivir is given as an infusion, treatment was reserved, until recently, only for people hospitalized with COVID-19.

A recent placebo-controlled, randomized, double-blind trial that involved non-hospitalized COVID-19 patients who had 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 in non-hospitalized COVID-19 patients had an acceptable safety profile and resulted in an 87% lower risk of hospitalization or death than placebo (9). Based on the results of this study that came amidst a surge in COVID-19 cases and the reduced susceptibility to several anti-SARS-CoV-2 monoclonal antibodies due to the Omicron variant, on January 21 2022 the FDA authorized remdesivir for outpatient treatment of people at high risk of COVID-19 disease progression, and expanded the pediatric EUA to include treatment of non-hospitalized pediatric patients 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 the treatment of 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 for progression to severe COVID-19. Remdesivir is additionally authorized for these uses for pediatric patients less than 12 years of age weighing at least 3.5 kg. Detailed information can be found at the NIH COVID-19 Treatment Guidelines (https://www.covid19treatmentguidelines.nih.gov/antiviral-therapy/).

It should also be mentioned that remdesivir in combination with the Janus-kinase 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) (10). In a different study (ACTT-3 trial NCT04492475) 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 (11).

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 given as an infusion, the focus began to shift to oral drugs that could be used outside a hospital setting 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 pills.

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, Ebola virus and several zoonotic coronaviruses (reviewed in Tian et al.) (12). In the case of SARS-CoV-2, molnupiravir inhibits virus replication in human lung tissue (13), and blocks SARS-CoV-2 transmission in ferrets (14).

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 (15).

A series of preclinical and clinical studies indicated that molnupiravir is effective for the treatment of SARS-CoV-2 infection (12). After oral administration, molnupiravir is rapidly transformed into the active NHC metabolite in plasma, distributed in various organs, and converted into the NHC 5'-triphosphate by host kinases (12). There are several clinical trials for molnupiravir, 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, 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 after 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 on October 2021, found that the number of patients who died or needed to be hospitalized was about halved among those of the molnupiravir arm when compared with 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: participants receiving the drug had a risk of death or hospitalization through day 29 of 6.8% (48 of 709 participants), as compared with 9.7% (68 of 699 participants) in the placebo arm (difference, −3.0 percentage points; 95% CI, −5.9 to −0.1) (16). The proportion of patients experiencing adverse events were 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 that had not yet become dominant globally during the first half of the trial, lowered expectations and limited the initial enthusiasm for the drug (17). 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 (17,18).

In November 2021, MHRA (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 23 December 2021 the US 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). After FDA, the use of molnupiravir was authorized in other countries; however, the European Medicines Agency (EMA) is yet to grant conditional marketing authorization, while the Indian Council of Medical Research on 13 January 2022 excluded molnupiravir from its COVID-19 treatment guidelines over toxicity concerns (17). Finally, in March 2022 WHO recommended the use of molnupiravir only for non-severe COVID-19

patients with the highest risk of hospitalization, including older and unvaccinated people, and patients with immunodeficiencies or chronic disease; notably WHO recommends 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).

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/) currently recruiting a large number of patients. Also, in March 2022 a pharmacovigilance program was launched by WHO in low and middle income countries to provide further evidence on molnupiravir's safety in the general population (19).

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

Paxlovid

Differently from remdesivir and molnupiravir that 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 (20). Paxlovid is a co-packaged combination of nirmatrelvir (PF-07321332) and ritonavir tablets, developed for the treatment and post-exposure prophylaxis of COVID-19. 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 with maximal therapeutic benefit (21).

The SARS-CoV-2 genome encodes two polyproteins, pp1a and pp1ab, and four structural proteins (20). The polyproteins are cleaved by Mpro at multiple sites to generate a set of shorter, nonstructural proteins critical for viral RNA transcription and replication, including the RdRp complex (21). In addition to the Mpro key role in virus replication, the lack of closely related homologs in humans, identifies Mpro as an attractive antiviral drug target (21). On the basis of early studies on the small molecule protease inhibitor PF-00835231 investigated for the intravenous treatment of SARS-CoV-1 (22), 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 (20). Nirmatrelvir was also found to have good selectivity and safety profiles, as well as oral activity in a mouse-adapted SARS-CoV-2 model (20).

On December 14 2021 Pfizer announced that paxlovid significantly reduced hospitalization and death, based on an interim analysis of the Phase 2/3 EPIC-HR (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 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 April 14 2022 and confirmed the interim results (23). 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 at day 5 of treatment, and all deaths reported (13) occurred in the placebo group (23). 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 (23).

One important consideration on the use of paxlovid is that the concomitant use of nirmatrelvir plus ritonavir and certain drugs may result in potentially serious drug interactions; therefore paxlovid is contraindicated with use of certain drugs because of the risk of serious adverse events (24). It should also be noted that the EPIC-HR trial was restricted to unvaccinated persons; a separate, ongoing phase 2/3 trial of nirmatrelvir plus ritonavir, EPIC-SR (EPIC-Standard Risk, NCT05011513) includes vaccinated persons. A third phase 2/3 EPIC-PEP trial (NCT05047601), evaluating the efficacy and safety of paxlovid in the prevention of symptomatic SARS-CoV-2 infection in the adult household contacts of individuals with SARS-CoV-2 infection, is also currently ongoing.

Paxlovid received its first EUA on 22 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) at increased risk for progression to severe COVID-19. Paxlovid also received conditional authorization for the treatment of COVID-19 in the UK on 31 December 2021, and more recently in the EU (January 2022) (24).

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, received an EUA in different countries. Both drugs are available for oral use in non-hospitalized patients, however neither drug is a panacea: 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.

Actually a large number of antivirals targeting the SARS-CoV-2 polymerase or the main protease are currently under development; it is also expected that, as in the case of other viral diseases such as AIDS and Hepatitis C, combinations of antivirals targeting different viral or host proteins can boost their effectiveness and reduce the risk of developing drug resistance (25).

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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.

Minister Véran advised people to take paracetamol (acetaminophen) instead of NSAIDs to treat a fever (1). However, acetaminophen is an NSAID (2). The commonest 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 - inhibition of central PGE2 dependent activation of EPr3 (4). They are also analgesic through the same mechanism, reduction of PGE2 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 made a virtue of that 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 a 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 at low doses on the platelet, thereby minimizing its GI toxicity.

Aspirin is also an NSAID and given the thrombotic complications of COVID-19, it was suggested that aspirin might be beneficial in COVID. It appears that thrombotic events are 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 PGE2, PGD2, and prostacyclin (PGI2) 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, PGD2 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, and there is also evidence that PGE2 inhibits SARS coronavirus replication (14). Indomethacin, an NSAID, blocks coronavirus RNA synthesis, but independently of COX inhibition (15). By contrast, COX-2–dependent PGE2 attenuates the chronic antiviral lymphocyte response of unresolved viral infection (16). Thus, 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 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 deletion of an upstream biosynthetic enzyme, the phospholipase, PLA2G2D, prevented middle aged mice from lethal infection with SARS Cov-2 (18). While asapiprant is 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 is a feature of severe COVID-19 and roughly 30% of our patients have elevated d-dimers on hospitalization. As 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 evolution of a lipopolysaccharide (LPS) induced syndrome of ARDS in sheep (20). Unlike NSAIDs that 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 are precluded (21). Thus, serial analysis of PGs in patients with COVID-19 might suggest that modulation of individual PGs be considered for therapeutic intervention or to be biomarkers predictive of disease progression. Interestingly, studies of monocyte derived macrophages ex vivo, themselves strongly implicated in ARDS pathogenesis, suggest that even mild infections with COVID-19 inflict a lasting pro-inflammatory eicosanoid signature, still evident one month after infection (22).

Summary

So, if there is no clear evidence of risk from NSAIDs, should patients with clinically complicated SARS-CoV-2 infections be administered them as a treatment? No. There is no evidence of benefit either. If such a patient were also to have poor kidney function, maintenance of renal blood flow becomes critically dependent on vasodilator PGs, such as PGE2 and PGI2. Such a situation might also predispose the patient to the gastrointestinal and cardiovascular complications of NSAIDs. However, 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 reported.

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5. Corticosteroids

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 has 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.

A cautionary note is the possibility of harm amongst those patients who did not require respiratory support at the time of randomization. In these patients, mortality was higher in those receiving dexamethasone (17.8% vs 14.0%) although the difference did not attain statistical significance (RR, 1.19; 95% CI, 0.92 to 1.55).1

The usefulness of dexamethasone in patients with severe pulmonary complications of COVID-19 infection has been supported by further investigations (3,4). In particular, a subsequent meta-analysis of seven trials of glucocorticoids (dexamethasone, hydrocortisone, or methylprednisolone) for critically ill patients with COVID-19, including RECOVERY, has confirmed the findings of this trial (4).

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 (5,6).

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6. Immunomodulatory drugs

The Severe coronavirus disease 2019 (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. In particular, increased serum levels of IL-6 were found to predict adverse outcomes, especially need for mechanical ventilation and mortality (1,2). Thus, several observational studies and randomized controlled trials (RCTs) have been performed targeting IL-6 and its downstream signaling, such as the janus kinase (JAK) and signal transducer and activator of transcription (STAT) pathway.

To date, most of the therapeutic strategies have focused on the inhibition of IL-6 receptor by means of the monoclonal antibody tocilizumab, which was already been approved for the treatment of rheumatologic diseases and cytokine release syndrome induced by chimeric antigen receptor therapy. In observational studies performed in the United States and globally, tocilizumab appeared to improve clinical outcomes in hospitalized patients with COVID-19 pneumonia (3-5). Instead, the initial RCTs that examined this monoclonal antibody led to conflicting results (6-9). However, many of these clinical trials appear to be flawed by 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. Subsequently, in the setting of background corticosteroid therapy, the two largest RCTs evaluating tocilizumab, REMAP-CAP and RECOVERY, both reported a

survival benefit for tocilizumab in certain COVID-19 patients. In particular, the multiplatform, adaptive REMAP-CAP trial showed that in critically ill COVID-19 patients receiving organ support in the intensive care unit (ICU), treatment with tocilizumab markedly improved outcomes, including 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 4116 hospitalized COVID-19 patients with hypoxia and systemic inflammation, adding tocilizumab on top of standard care significantly reduced the primary outcome of 28 day 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 RCTs, on June 24 2021 the FDA issued an emergency authorization 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.

Sarilumab is the second monoclonal antibody blocking IL-6 receptor most commonly studied in COVID-19 patients. In parallel with encouraging data with tocilizumab, early uncontrolled studies that tested sarilumab also produced promising expectations (12,13), leading to the initiation of several RCTs. An adaptive design trial among 420 patients with severe or critical COVID-19 found that sarilumab did not meet its primary endpoint of time to improvement of two or more points on an ordinal seven-point clinical status scale compared to placebo (14). Likewise, the key secondary endpoint of the proportion of patients alive at day 29 was not significantly different between groups (14). A similar adaptive trial showed that in 298 critically ill COVID-19 patients requiring mechanical ventilation, sarilumab failed to show a benefit over placebo with regards to the primary endpoint of the proportion of patients with ≥ 1 point improvement in clinical status at day 22 (15). Among critical patients receiving mechanical ventilation and corticosteroids at baseline, there was a numerical, yet 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 showed a favorable effect on survival in patients with severe COVID-19 pneumonia in one RCT, while it was neutral in two other trials.

Siltuximab is a monoclonal antibody that prevents IL-6 from binding to its receptors. A non-peer reviewed study found that intravenous administration of siltuximab to 30 COVID-19 patients requiring ventilator support associated with a significantly lower mortality rate compared to that reported in control patients who received standard care alone (16).

Janus kinase (JAK) inhibitors exert immunomodulatory effects by inhibiting STAT-mediated signaling pathways of several cytokines. In particular, baricitinib is an orally administered inhibitor of JAK1 and JAK2 that blocks the intracellular signaling pathways of cytokines known to be elevated in severe COVID-19, including IL-2, IL-6, interferon-γ and granulocyte-macrophage colony-stimulating factor. Moreover, at variance with other JAK inhibitors, baricitinib was also postulated 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 in hospitalized patients with COVID-19 (18,19), prompted the initiation of RCTs. The ACTT-2 trial found that among 1033 patients with COVID-19 pneumonia receiving supplemental oxygen, high-flow oxygen or non invasive ventilation, the combination of baricitinib and remdesivir was superior to remdesivir alone with respect to the primary outcome of time to recovery, as measured on a 8-category ordinary scale (20). The combination treatment group also had 30% higher odds of improvement in clinical status at day 15 than the control group (20). The COV-BARRIER trial showed that among 1525 COVID-19 patients not requiring mechanical ventilation and with 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, non-invasive ventilation, invasive mechanical ventilation or mortality compared to standard of care alone (21). However, treatment with baricitinib reduced by 38.2% the key secondary outcome of mortality by 28 days (21). Taken together, the results of these trials suggest that baricitinib may have additive or even synergistic effects with other standard of care treatment modalities, including remdesivir and corticosteroids. Based on evidence from RCTs, baricitinib received emergency use authorization by 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 (22)

The results of a RCT that examined tofacitinib, a selective inhibitor of JAK1 and JAK3, in the setting of COVID-19 have also been reported. The STOP-COVID trial found that among 289 patients hospitalized with COVID-19 pneumonia, tofacitinib was superior to placebo in reducing the incidence of the primary endpoint of mortality or respiratory failure by 28 days (23). These effects were consistent regardless of duration of symptoms and use of glucocorticoids at baseline (23).

Clinical trials involving several JAK inhibitors for the treatment of COVID-19 are ongoing, and their results will hopefully provide valuable information on the usefulness of these agents.

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7. Complement inhibitors

The complement system is one of the host immune system's first lines of defence against invading pathogens (1). Nevertheless, its potential beneficial role in the immunity to SARS-CoV-2 has been overshadowed by multiple lines of evidence that implicate uncontrolled complement activation in the pathogenesis of severe coronavirus disease 2019 (COVID-19) (1-4). Mechanistically, SARS-CoV-2 can directly or indirectly activate all the three complement pathways (i.e., the classical, alternative and lectine pathways) (5-7). This process leads to the formation of several effectors, including the terminal products C5a and C5b-9, which contribute to lung inflammation and injury, endothelial damage and dysfunction, with eventual spreading of the inflammatory response to the circulation and other organs (4). Indeed, in patients with COVID-19 plasma levels of C5a and soluble C5b-9 increase in proportion to disease severity (7-8). Furthermore, extensive complement deposition has been reported in lung and kidney tissues of patients who died of COVID-19 (9-10).

Based on this background, several uncontrolled studies and some controlled clinical trials have been performed with different complement inhibitors in patients with severe COVID-19, while others are underway. To date, most of the strategies to target complement activation in COVID-19 have focused on C5 inhibition, in particular by means of the monoclonal antibody eculizumab, which was already approved for the treatment of paroxysmal nocturnal haemoglobinuria, atypical haemolytic uremic syndrome, myasthenia gravis and neuromyelitis optica spectrum disorder. Initial case series and explorative studies showed positive effects on clinical outcomes in patients with severe COVID-19 treated with eculizimab, even in combination with the JAK1/2 inhibitor ruxolitinib (11-14). A pilot study in five critically ill COVID-19 patients on mechanical ventilation who received eculizumab documented a mortality rate of 50%, compared to estimates as high as 97% for similar patients given standard care alone during the same period (15). In a non-randomized controlled study in 80 patients with severe COVID-19 admitted to an intensive care unit, treatment with eculizumab at more frequent and increased dosing compared to those indicated for atypical haemolytic 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 (16). More recently, another non-randomized controlled study showed that adding two 900 mg doses of eculizumab on top of standard therapy

in ten patients with severe COVID-19 who were receiving continuous positive airway pressure support for ≤ 24 hours safely improved respiratory dysfunction, and reduced the risk to reach the combined endpoint of mortality or discharge with chronic complications, compared to 65 contemporary similar controls who were given standard therapy alone (17).

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), in patients with severe COVID-19 requiring mechanical ventilation has been stopped after an iterim analysis revealed lack of efficacy (18).

Ongoing RCTs targeting C5 in severe COVID-19 are evaluating the monoclonal antibodies eculizumab (NCT04346797) and ravulizumab (NCT04570397 and NCT04390464), or peptide inhibitors of C5, such as zilucoplan (NCT04382755).

The impact of blocking the C5a-C5aR1 axis is also under clinical investigation. An initial phase II open-label RCT tested blockade of C5a with the monoclonal antibody vilobelimab in 30 patients with severe COVID-19. Although the primary endpoint of change in the ratio of arterial oxygen tension over fraction of inspired oxygen (PaO2/FiO2) on day five was not significantly different between patients randomized to vilobelimab and those allocated to standard of care alone, there was a trend for improved survival in the anti-C5a treatment group (19). This adaptive design trial has now progressed to a phase III placebo-controlled stage (NCT04333420) involving 399 patients receiving mechanical ventilation and using 28 day mortality as the primary endpoint. Similarly, a large phase II/III RCT (NCT04449588) testing the anti-C5a antibody BDB-001 in patients with severe COVID-19 is ongoing. Despite the promising results of C5aR1 blockade with the monoclonal antibody avdoralimab in a mouse model of acute lung injury (8), where it decreased pulmonary neutrophil and macrophage infiltration, in a double-blind RCT in patients with severe COVID-19 pneumonia, treatment with avdoralimab did not meet its primary endpoint of improving clinical status over placebo at days 14 or 28 (20).

Inhibition of C3, which is upstream of C5 in the complement cascade, does not appear to be a safe approach in patients with COVID-19, since it could reduce antiviral response and prevent immunity to additional infectious diseases. Nonetheless, the C3 inhibitor AMY-101 was initially reported in two case series for the treatment of four COVID-19 patients with acute respiratory distress syndrome, all of whom eventually recovered (13,21). A larger phase II RCT involving 144 patients with acute respiratory distress syndrome due to COVID-19 is planned (NCT04395456), but not yet recruiting.

Other strategies aimed 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 of five patients with severe COVID-19 pneumonia, administration of the human recombinant C1 esterase inhibitor conestat alpha was safe and associated to clinical improvements, with all patients being discharged from hospital within 3 weeks (22). These encouraging results promoted the initiation of two similar phase II RCTs aimed at evaluating whether adding conestat alpha to standard therapy in hospitalized COVID-19 patients

could reduce the risk of progression to severe disease compared to standard therapy alone (NCT04530136 and NCT04414631). 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 (23). A phase II adaptive platform trial designed to rapidly evaluate promising investigational agents, including narsoplimab, for reducing the time to recovery or mortality risk in critically ill COVID-19 patients, is currently underway (NCT04488081).

Collectively, early clinical findings and emerging clinical trial evidence suggests the potential therapeutic benefits of some complement inhibitors in severe COVID-19. The results of ongoing phase II/III clinical trials will hopefully elucidate the benefit to risk profile of complement inhibitors and clarify the optimal target(s) in the complement cascade.

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8. Anticoagulants and other antithrombotic agents

Pathophysiologic and pharmacologic rationale

Microvascular and macrovascular thrombotic complications, including arterial but especially venous thromboembolism (VTE), appear to be common clinical features of COVID-19, particularly in hospitalized and critically ill patients (1,2). These thrombotic/thromboembolic events are promoted

by the inflammatory process underlying viral infections, such as SARS-CoV-2. In particular, inflammation induces excessive production of thrombin and a reduction in fibrinolysis caused by endothelial dysfunction due to the ongoing viral infection (3). Moreover, the hypoxia that characterizes SARS-CoV-2 infection also contributes to thrombosis by enhancing blood viscosity (3).

Heparin is a glycosaminoglycan with anticoagulant activity produced by basophils and mast cells in all mammals. It activates antithrombin III which, in turn, inhibits thrombin (Factor II), Factor X and other proteases involved in the blood coagulation cascade (4). Heparin and low-molecular-weight heparins (derived from unfractionated heparin by depolymerization) are commonly used prophylactically to prevent post-surgical venous thromboembolism, as well as in non-surgical patients with heart failure or acute respiratory failure, conditions characterized by reduced mobility. They are also used in the pharmacological treatment of deep vein thrombosis, pulmonary embolism and acute coronary syndromes.

Preclinical evidence of efficacy

Heparin also displays anti-inflammatory properties which could be valuable in the context of COVID-19. According to the immune-thrombosis model, the formation of thrombi inside blood vessels, in particular in microvessels, induces an innate immune response (5). Thus, blocking thrombin by means of heparin may dampen the inflammatory response. Heparin elicits anti-inflammatory functions also through mechanisms independent of its anticoagulant activity, which include binding to inflammatory cytokines, inhibiting neutrophil chemotaxis and leukocyte migration, neutralizing the positively charged peptide complement factor C5a, and sequestering acute phase proteins (6). In an animal model of acute lung injury, treatment with nebulized heparin reduced injury-mediated coagulation factors and inflammation in the alveolar space, without affecting systemic coagulation (7). Heparin also appears to protect the vascular endothelium. Apart from pathogens, histones released from damaged cells can also cause endothelial injury (8). Heparin can antagonize histones thereby protecting endothelial cells (9). This protective function seems to extend to the endothelial tight junctions as demonstrated in a sepsis animal model, where unfractionated heparin reduced lung edema and vascular leakage (10). Finally, experimental evidence suggests an antiviral potential for heparin. Indeed, heparin structure highly resembles heparan sulfate, a linear polyanionic polysaccharide used by a large number of human viruses, including coronaviruses, for attachment to target cells (11). A recent study has used spectroscopic techniques along with molecular modeling to show that the SARS-CoV-2 Spike S1 protein receptor binding domain interacts with heparin (12). This observation raises the intriguing possibility that heparin could compete with heparan sulfate for binding to SARS-CoV-2, thereby preventing virus entry into cells. Nevertheless, this hypothesis remains to be demonstrated (Figure). Also for the antiplatelet agent dipyridamole preclinical evidence of antiviral effects against SARS-CoV-2 has been documented. Indeed, dipyridamole was found to suppress SARS-CoV-2 replication in vitro at concentrations comparable to those reported in the blood of patients treated with this medication after ischemic stroke (13). These data suggest that the dosages of dipyridamole used to inhibit platelet aggregation could potentially suppress SARS-CoV-2 replication in infected patients.

Clinical evidence of efficacy in patients with COVID-19

A systematic review estimated the VTE event rate to be about 17% among COVID-19 inpatients, increasing to around 28% in those admitted to the intensive care units (ICUs) (14). Several organizations have released guidelines regarding the prevention and management of VTE in patients with COVID-19. All of them agree that hospitalized, non-pregnant patients with COVID-19 should receive, at a minimum, a prophylactic dose of anticoagulants to prevent VTE (15-19). Nevertheless, the optimal antithrombotic strategy across the spectrum of COVID-19 severity remains ill defined. Many randomized controlled trials (RCTs) have been performed and others are ongoing to evaluate the efficacy and safety of a variety of antithrombotic regimens in COVID-19 patients on all phases of illness, that is 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, proxylactic 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 (20). However, the study was terminated after enrollment of 9% of the planned total number of participants due to the lower than anticipated primary event rates. At present, routine administration of thromboprophylaxis is not recommended in ambulatory COVID-19 patients.

Several RCTs have evaluated the role of therapeutic doses of heparin in reducing VTE events, disease progression or mortality in hospitalized patients with COVID-19 not requiring 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, therapeutic dose anticoagulation with unfractionated or low-molecular-weight heparin was more effective than usual care thromboprophylaxis with regards to the primary outcome of survival free of organ support (21). Major bleeding occurred in 1.9% of patients receiving therapeutic dose anticoagulation and in 0.9% of those given thromboprophylaxis (21). The RAPID trial found that among 465 moderately ill COVID-19 patients with increased D-dimer levels, therapeutic compared to prophylactic anticoagulation 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 (22). 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 (22). 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%) not requiring 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 (23). There was no meaningful difference in major bleeding between groups, even though confidence intervals were wide (23).

Together, available evidence from RCTs supports the concept that therapeutic anticoagulation with low-molecular-weight heparin 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. 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, hospitalization duration, or oxygen use duration compared to prophylactic anticoagulation with heparin, but did increase the risk of bleeding (24). Therefore, use of therapeutic dose rivaroxiban or other direct oral anticoagulants is not recommended in hospitalized COVID-19 patients.

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 of organ support compared to usual care thromboprophylaxis, and was associated with more major bleeding events (25). 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 (26). Major bleeding occurred in 2.5% of patients in the intermediate dose group and in 1.4% of those in the standard dose group (26).

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 (27,28). The reasons why therapeutic doses of heparin appear to improve clinical outcomes in moderately ill, but not in critically ill, COVID-19 patients are unknown. It is conceivable that patients requiring ICU level care or organ support may have too advanced disease to benefit from therapeutic heparin, with organized thrombi quite resistant to the action of antithrombin III, the endogenous anticoagulant potentiated by heparin (29).

There is not yet consensus on the role of extended thromboprophylaxis beyond the hospital stay. Recently, the MICHELLE trial has shown that in 320 at high risk patients 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, either symptomatic or detecting using routine imaging tests, arterial thrombotic events and cardiovascular death, without increasing the risk of major bleeding (30).

Since platelets play a central role in the pathogenesis of COVID-19, the use of antiplatelet agents is also under clinical investigation. In particular, the platelet aggregation inhibitor dipyridamole may have additional antiviral properties (31), and three small RCTs are evaluating its effects in hospitalized patients with COVID-19 (NCT04391179, NCT04424901 and NCT04410328).

The results of ongoing clinical trials will hopefully clarify the place of pre-hospital and post-discharge antithrombotic prophylaxis in COVID-19 patients, and the potential benefits of other prophylactic and therapeutic agents, such as antiplatelet drugs.

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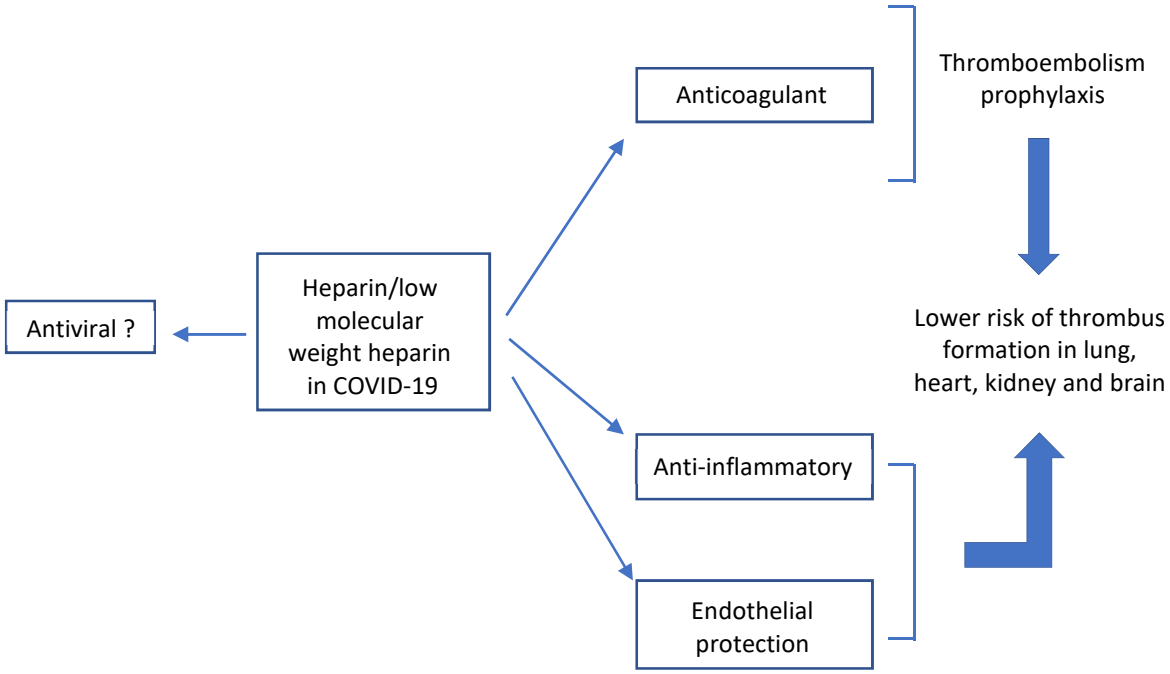

Figure. Possible effects of heparin and low molecular weight heparin in COVID-19

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

Hydroxychloroquine/chloroquine

Despite the in vitro evidence (1,2) and the results of preliminary anecdotal reports, according to NIH COVID-19 Treatment Guidelines (https://www.covid19treatmentguidelines.nih.gov/) (3), 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 (4-8). Similarly, the current trials on the use of hydroxychloroquine/chloroquine for the prophylaxis of SARS-CoV-2 infection (https://clinicaltrials.gov/ct2/results?cond=COVID19&term=preexposure+prophylaxis&cntry=&state=&city=&dist=) do not seem to show a conclusive support, and therefore the NIH Panel recommends against the use of hydroxychloroquine/chloroquine prophylaxis of COVID-19.

Ivermectin

This FDA-approved antiparasitic drug, has been shown to have 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). However, the NIH Panel indicates that there is insufficient evidence for the COVID-19 treatment, and recommended against its use (3).

Nitazoxanide

This broad-spectrum thiazolide antiparasitic agent FDA-approved, or its metabolite, tizoxanide, shows in vitro activity against a number of viral infections, including MERS-CoV, SARS-CoV, SARS-CoV-2 (14,15). On the basis of the early clinical trials (16), however, the NIH Panel recommends against its use for the treatment of COVID-19 (3).

Lopinavir/ritonavir

As other HIV protease inhibitors (darunavir/cobicistat), lopinavir/ritonavir have been used in clinical trials (5,17), but 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 in a variety of conditions (18), the NIH Panel guidelines recommend against its use to treat hospitalized and non-hospitalized COVID-19 patients (3). Indeed, in the large randomized RECOVERY trial in hospitalized COVID-19 patients, colchicine did not show benefits in term of 28-day mortality or other secondary outcomes (19). 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 (20).

Interferons

Approved to treat hepatitis B and hepatitis C virus infections, interferons are in current clinical trials. However, 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 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 clinical trial (3). These recommendations are supported by recent trials showing that interferon beta-1a was not of clinical benefit in the treatment of hospitalized patients with COVID-19 either alone or in combination to remdesivir or corticosteroids (5, 21).

Convalescent plasma

Plasma from patients that recovered from COVID-19 is not recommended from the NIH Panel (3). Whilst this is based on insufficient evidence of its efficacy, the treatment is 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. There is no evidence of efficacy against COVID-19 (22).

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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.

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 (2, 3), trials in humans are exploring the relevance of this drug in the setting of COVID-19. Several clinical studies (NCT04668950; NCT04718480; NCT04342663; NCT04510194) are still in progress, whilst the one completed failed to show a drastic conclusion. Indeed, the TOGETHER, an adaptive platform, double blind randomized placebo-controlled trial in non-hospitalized adults with COVID-19 and a known risk factor for severe illness, showed 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 (4). However, there was no significant difference in mortality rate between the two study groups.

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 (5). Anti-GM-CSF monoclonal antibodies may limit inflammation by minimizing the production of several pro-inflammatory mediators involved in COVID-19 (6). Several clinical trials have been completed (NCT04411680; NCT04326920; NCT04707664) or are still open (NCT04569877; NCT04341116), with incomplete results. Preliminary data published in pre-print format and a small randomized trial with anti-GM-CSF monoclonal antibodies provided conflicting results (7,8,9). Lenzilumab showed a significant improvement in the ventilator-free survival through day 28 of COVID-19 patients compared to placebo (8). Other studies, however, did not report a survival benefit for otilimab (7) or mavrilimumab (9) compared to placebo. Thus, the NIH COVID-19 Treatment Guidelines Panel recommends that there is insufficient evidence for the treatment of hospitalized COVID-19 patients (1).

Anakinra

Anakinra, interleukin-1 receptor antagonist, is used for the treatment of the Multisystem Inflammatory Syndrome in Children. 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. 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 (10), the REMAP-CAP, an open-label, adaptive platform, randomized controlled trial, showed no efficacy of anakinra in reducing the combined endpoint of in-hospital mortality and days of organ support (11).

Vitamin C and D

Vitamin C and D, have been used for the therapy or the prophylaxis of COVID-19. 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 of vitamin C in ambulatory or critically ill patients with COVID-19, and the available observational data are inconclusive (12, 13). Similarly, randomized clinical trials of vitamin D in patients with moderate to severe COVID-19 are very few, precluding robust conclusions about the effectiveness of this treatment on major outcomes of the disease (14).

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11. Cell-based therapy

Mesenchymal stromal cells and rational for use in COVID-19

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). Mesenchymal stromal cells (MSC) are pluripotent stem cells derived from the mesoderm and ectoderm with the capacity for self-renewal and differentiation into multiple cell types including osteoblasts, chondroblasts, adipocytes, and hepatocytes (1). They were first-discovered in the bone-marrow, and subsequently found and isolated from other tissues, such as the umbilical cord, placenta, and adipose tissue (1).

Compared with embryonic stem cells, MSC are easily available, more ethical, and easy to freeze and thaw under standard conditions in vitro, making clinical application more convenient and safer (2). MSC have low immunogenicity and homing properties, and have been shown to modulate overactive immune and hyperinflammatory processes, promote tissue repair, and secure antimicrobial molecules (1,3). These cells, with established safety profile when administered

intravenously (4), have been studied for treatment of autoimmune diseases (e.g. type 1 diabetes, systemic lupus erythematosus), inflammatory disorders, steroid-refractory graft-vs-host-disease (GvHD), acute and chronic kidney diseases, as well as in organ transplantation (5-7). MSC have been reported to limit inflammation and fibrosis in the lungs (8), and have generated variable yet promising results in acute respiratory distress syndrome (ARDS) of viral (9) and non-viral etiology (10,11).

When the immune system is over activated by viral infections, such as SARS-CoV-2, it will secrete a large amount of proinflammatory factors, resulting in cytokine storm production (12). In this setting, MSC exert their immunosuppressive properties to inhibit the occurrence and development of cytokine storms, through paracrine pathways or direct interaction with immune cells (13).

Together, these observations represented the rationale to hypothesize that MSC could reduce the acute lung injury and inhibit the cell-mediated inflammatory response induced by SARS-CoV-2. Notably, because MSC lack the ACE2 receptor for SARS-CoV-2 entry into the host cells, they are resistant to the infection (14).

Clinical evidence of efficacy in patients with COVID-19

In view of the current understanding of the pathophysiologic mechanisms of COVID-19 and the unique biological characteristics of MSC, the development of this 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 randomized control trials.

Initial pilot studies of intravenous infusion of bone marrow-derived- or umbilical cord-derived MSC (hUC-MSC) in 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 (15-17). Other investigators have then confirmed these promising results, albeit in very few patients (18). Indeed, in three out of five patients with severe ARDS caused by COVID-19, the infusion of allogenic hUC-MSC was safe, and demonstrated its anti-inflammatory capacity in the lungs, by improving the respiratory function, which allowed extubation on the ninth day post-infusion. Two patients died at 13 and 15 days after infusion. However, the interpretation of the findings of these pilot studies is limited by the small sample size and lack of randomization.

Notably, in a larger observational cohort of 210 severe/critically ill COVID-19 patients, significantly higher survival was documented in those who received hUC-MSC infusion before intubation (19).

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 hUC-MSC treatment (n=12, two cell infusions) or placebo (also two infusions of vehicle solution), both in addition to best standard of care (20). Inflammatory cytokines were significantly decreased in hUC-MSC treated subjects at day 6. Moreover, cell-treatment significantly improved patients' survival by day 31, compared to placebo group. Despite

the reported benefits of UC-MSC infusion in this study, again the interpretation of the results is limited by the small sample size and the change in an eligibility criterion from enrolling only individuals on invasive mechanical ventilation to including those 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 hUC-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 (21). Less encouraging are recent results of the STROMA-CoV-2 study, a multicenter, double-blind, randomized, placebo-controlled trial in adult patients with SARS-CoV-2-induced early mild-to-severe ARDS (22). Although the three hUC-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 (PaO2/FiO2)-ratio between baseline and day 7 post-infusion did not differ significantly in hUC-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 recently 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 (23). 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 achieves a long-term benefit in the recovery of lung lesions and symptoms in COVID-19 patients.

As of November 2021, according to Clinicaltrials.gov (https://www.clinicaltrials.gov), a total of 74 clinical trials were being assessing MSC for the treatment of COVID-19. Among these MSC clinical trials, 22 were using hUC-MSCs, 15 adipose tissue-derived MSCs (AD-MSCs) and 11 bone marrow-derived-MSC (BM-MSC).

In summary, while the clinical use of MSC to treat COVID-19 is still in the preliminary stage of investigation, promising results indicate that they could potentially be utilized in future treatments. Nonetheless, so far, no MSC products have been approved by the Food and Drug Administration (FDA) for the treatment of COVID-19. Therefore, the NIH COVID-19 Treatment Guidelines Panel recommends against the use of MSC for the treatment of COVID-19, except in an approved clinical (24).

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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 some cases, to present potential hazards.

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 (5). 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 (5-7). 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 (8).

Although simvastatin has been reported to downregulate the SARS-CoV-2-induced inflammatory response and to impair viral infection through disruption of lipid rafts (9), 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 (10).

Other agents, such as nonsteroidal anti-inflammatory drugs (NSAIDs), in particular ibuprofen, were postulated to have a negative impact without a clear mechanistic explanation (see section 4 of this document) (11). 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 (12).

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 (13).

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 (13).

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 (13).

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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 and by the end of June 2021, the Centers for Disease Control and Prevention (CDC) has reported 4196 confirmed MIS-C cases in United States, and 37 deaths (3-5). 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 (1,2,8,9). Some patients have presented with features of toxic shock syndrome, secondary hemophagocytic lymphohistiocystosis, or macrophage activation syndrome (10). Although the cause of Kawasaki's disease remains unknown, a preceding or active infection has been suspected (11). 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 (1,8,12). 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 (13).

Nonetheless, the pathogenesis of MIS-C is still being elucidated. 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 (14,15). 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 (1,2,6,7,12,16-19). 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 (20). 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 (12, 21-24). On this basis, it has been recommended using IVIG in combination with lo-to-moderate dose glucocorticoids for children hospitalized with MIS-C, but not the routine use of IVIG monotherapy, unless glucocorticoid therapy is contraindicated (25). 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 have not been performed. On the other hand, there is insufficient evidence to recommend either for or against the use of glucocorticoid monotherapy for children with MIS-C (25).

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 (24,26) or the IL-1 receptor antagonist anakinra (7,17,18), 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 (27). 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. 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 (9). 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 (25). Given the uncertainty of the benefit and the risk of major bleeding (28), 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.

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