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REVIEW ARTICLE

# Emerging treatment strategies for COVID-19 infection

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**Abstract**
The new type of coronavirus (COVID-19), SARS-CoV-2 originated from Wuhan, China and has led to a worldwide pandemic. COVID-19 is a novel emerging infectious disease caused by SARS-CoV-2 characterized as atypical pneumonia. As of July 1, 2020, more than 10 million people worldwide had been infected with SARS-CoV-2. The typical manifestations of COVID-19 include fever, sore throat, fatigue, cough, and dyspnoea combined with recent exposure. Most of the patients with COVID-19 have mild or moderate disease, however up to 5–10% present with severe and even life-threatening disease course. The mortality rates are approximately 2%. Therefore, there is an urgent need for effective and specific antiviral treatment. Currently, supportive care measures such as ventilation oxygenation and fluid management remain the standard of care. Several clinical trials are currently trying to identify the most potent drug or combination against the disease, and it is strongly recommended to enroll patients into ongoing trials. Antivirals can be proven as safe and effective only in the context of randomized clinical trials. Currently several agents such as chloroquine, hydroxychloroquine, favipiravir, monoclonal antibodies, antisense RNA, corticosteroids, convalescent plasma and vaccines are being evaluated. The large numbers of therapeutic interventions aim to define the most efficacious regimen. The aim of this article is to describe the treatment strategies that have been used for COVID-19 patients and review all the available literature.

**Keywords** SARS-CoV-2 · COVID-19 · Antivirals · Convalescent plasma · Remdesivir · Vaccines

## Introduction

The new type of coronavirus (COVID-19), SARS-CoV-2 originated from Wuhan, China and has led to a worldwide pandemic. The World Health Organization (WHO) has declared that COVID-19 has become a global health concern. The typical symptoms of COVID-19 include fever, sore throat, fatigue, cough, and dyspnoea combined with recent exposure. Due to interventions and control measures from the governments around the world and the changes in personal behaviors (such as masks wearing and social isolation), the number of new confirmed and suspected cases has been decreasing globally. However, the risk of transmission has not been eliminated yet and the COVID-19 outbreak remains a major challenge for clinicians. Most of the patients with COVID-19 have mild or moderate disease, however up to 5–10% present with severe and even life threatening disease course. The mortality rates are approximately 2%. Therefore, there is an urgent need for effective and specific antiviral treatment. Currently, supportive care measures such as ventilation oxygenation and fluid management remain the standard of care. Several clinical trials are currently trying to identify the most potent drug or combination against the disease and it is strongly recommended to enroll patients into ongoing trials. Antivirals can be proven as safe and effective if so, only in the context of randomized clinical trials. Currently several agents such as chloroquine,

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hydroxychloroquine, favipiravir, monoclonal antibodies, antisense RNA, corticosteroids, convalescent plasma and vaccines are being evaluated. The large numbers of therapeutic interventions aim to define the most efficacious regimen. The aim of this article is to describe the treatment strategies that have been used for COVID-19 patients and review all the available literature.

## Convalescent plasma

Plasma from patients that have been cured from COVID-19 infection, namely convalescent plasma, is a treatment with considerable historical background in other infectious diseases, but still explorative in the context of SARS-CoV-2. In a pandemic era, convalescent plasma could constitute an easily accessible source of antiviral antibodies.

Convalescent plasma may offer various beneficial actions in COVID-19 disease. First and foremost, the apparent mechanism pertains to the fact that antibodies from convalescent plasma can suppress viremia. Similarly to the strategies implemented in the SARS epidemic, theoretically, the administration of convalescent plasma at the early stage of the disease would be more effective [1]. Viremia peak is noted in the first week of infection in the majority of viral illnesses and a primary immune response of the host is usually developed by days 10–14 of infection [2] (beginning somewhat earlier according to other researchers) [3], signaling the clearance of the viruses. Other potential mechanisms include antibody-dependent cellular cytotoxicity, complement activation and phagocytosis (ADCP) [4]. Secondarily, the presence of non-neutralizing antibodies binding to the pathogens may also be helpful [5].

In any case, the administered antibody modifies inflammatory response and this can be optimally achieved during the early response, even at the asymptomatic stage [6]. It has also been suggested that, apart from the direct anti-viral properties, plasma components can provide other beneficial actions, such as restoring coagulation factors [7].

So far information on immune response to SARS-CoV-2 is rather limited. According to studies in process, a detailed analysis of 9 cases with mild upper respiratory tract symptoms revealed that seroconversion occurred 6–12 days after onset of symptoms, while antibodies were not detectable between day 3 and 6; after 2 weeks, all patients showed neutralizing antibodies. Seroconversion coincided with a slow but steady decline of sputum viral load [8]. In another study, the majority of PCR-confirmed SARS-CoV-2-infected persons seroconverted 2 weeks after disease onset [9]. A study on 173 COVID-19 patients showed that the presence of antibodies was less than 40% within the first week from disease onset, increasing to 94.3% for IgM and 79.8% for IgG on day 15 after onset; higher titer of total antibodies correlated with worse clinical classification [10]. To further assess the time for seroconversion and its correlation with disease severity and antibody titers, additional longitudinal studies evaluating large numbers of serum samples from COVID-19 patients, with a broad spectrum of clinical symptoms, are needed.

Until recently, case series were mostly reported (32 pts in total) with different disease severity [11–13]. Patients were administered other treatment regimens concurrently and 90% experienced positive outcomes [14]. The superimposition of effects mediated by other antiviral treatments, antibiotics and glucocorticoids administered concomitantly with convalescent plasma should be taken into consideration in the evaluation of these results.

In the first peer-reviewed study of convalescent plasma, 19 of 25 patients (76%) with severe COVID-19 who received convalescent plasma saw at least 1 point of clinical improvement based on WHO's ordinal scale measuring illness severity [15]. A randomized clinical trial of 103 patients with severe COVID-19 published in JAMA by researchers from China showed a nonsignificant clinical improvement in 51.9% of patients compared with improvement in 43.1% of patients who received standard treatment ($p=0.26$) [15]. However, the trial was halted early due to the decrease in COVID-19 patients in China during the study period, which could have contributed to the study being underpowered to detect a clinically significant result. A recent analysis of 5000 patients with severe or life threatening COVID-19 who received convalescent plasma according to the "US FDA Expanded Access Program for COVID-19 convalescent plasma" reported a 7-day mortality rate of 14.9% [16]. Donor selection according to the antibody titers or the potency of the neutralizing antibodies may further enhance the efficacy of convalescent plasma administration [17].

A pivotal, controversial point seems to pertain to the time of convalescent plasma administration in COVID-19, that should be as early as possible, to maximize efficacy, but at the same time oriented to severe cases. To this direction, the examination of risk markers, integrating clinical (gender, age, comorbidities), biochemical aspects in a comprehensive risk stratification, can provide a valuable tool about decision making, tracing promptly those patients with forthcoming poor prognosis, who would need most the early intervention with convalescent plasma. Emerging markers with such a potential, are lymphocytopenia, elevated procalcitonin, ferritin, D-dimer and C-reactive protein [18].

In line with the published case series concerning the optimal timing of convalescent plasma administration, a recent review by Tiberghien et al. [3] has presented a strategy of administration for high-risk patients (older than 70 or dependent on oxygen with a baseline oxygen saturation < 94%). According to preliminary remarks by the aforementioned research team, early treatment with convalescent plasma (not later than day 5) should be preferred, at any case

before seroconversion for SARS-CoV-2, which may occur on days 6–12. Another matched control study has suggested that the survival benefit of convalescent plasma may be more pronounced among non-intubated patients compared with those requiring mechanical ventilation [19]. The need for early administration is in line with observations in other diseases, such as pneumococcal pneumonia, where no benefit is noted if the antibody is administered after day 3 of the disease [4, 20].

Convalescent plasma administration seems to be a safe procedure, free from serious adverse effects. Meticulous selection of donors can minimize the risk of TRALI syndrome. Another potentially concerning phenomenon pertains to antibody-dependent enhancement (ADE) of coronavirus entry; this has been reported in viral diseases and refers to an enhancement of disease in the presence of certain antibodies [21]. A pertinent analysis of more than 5000 patients with severe or life threatening COVID-19 infection who received convalescent plasma showed that less than 1% of the patients experienced a serious adverse event in the first 4 h following the infusion. Severe adverse events with a potential, but not definitive, relation to convalescent plasma included mortality ($n=4$), transfusion-associated circulatory overload (TACO; $n=7$), transfusion-related acute lung injury (TRALI; $n=11$), and severe allergic transfusion reactions ($n=3$) [16]. Nevertheless, in view of the high titers of neutralizing antibodies that convalescent plasma includes against the same virus (SARS-Cov-2), as well as the previously documented, safe experience in SARS and MERS, the occurrence of ADE does not seem to represent a major problem, but surveillance is warranted [4].

## Remdesivir

Remdesivir is an RNA-dependent polymerase inhibitor tested for efficacy in treatment of SARS-CoV-2 infection and has demonstrated the most promising anti-viral therapeutic results. Unlike other nucleotide analogues, remdesivir is a phosphoramidate prodrug with broad-spectrum activity against many viruses, such as Filoviridae, Paramyxoviridae, Pneumoviridae, and Orthocoronavirinae (SARS-CoV and Middle East respiratory syndrome coronavirus [MERS-CoV]) [22, 23]. Remdesivir was initially developed by Gilead Sciences in 2017 as treatment for Ebola virus infection. Several phase 3 trials were initiated to evaluate the role of remdesivir for severe and moderate disease in the USA, South Korea and China. Recently the results of a double-blind, randomized, placebo-controlled trial of intravenous remdesivir in adults hospitalized with Covid-19 disease and evidence of lower respiratory tract involvement were reported (ACCT-1 trial). Patients were randomly assigned to receive either remdesivir (200 mg loading dose on day 1, followed by 100 mg daily for up to 9 additional days) or placebo for up to 10 days. The primary outcome was time to recovery, and this was defined as either discharge from the hospital or hospitalization only for infection-control. 1063 patients were randomized. The safety monitoring committee recommended early unblinding on the basis of data that showed shorter time to recovery in the remdesivir arm. 538 patients were assigned to remdesivir and 521 to placebo. The remdesivir group had a median recovery time of 11 days (95% CI 9 to 12), as compared with 15 days (95% CI 13 to 19) in those who received placebo (rate ratio for recovery, 1.32; 95% CI 1.12 to 1.55; $p<0.001$). The mortality estimation with Kaplan–Meier by 14 days was 7.1% with remdesivir and 11.9% with placebo (hazard ratio for death, 0.70; 95% CI 0.47 to 1.04). 114 of 541 patients in the remdesivir group reported serious adverse events [24]. Another ongoing phase 3 randomized, double-blind, placebo-controlled, multicenter study is evaluating the efficacy and safety of remdesivir in 452 hospitalized adult patients with severe respiratory disease [ClinicalTrials.gov Identifier: NCT04257656]. The results of this trial are anticipated. On May 1, 2020, The US FDA based on the results of the ACTT trial issued an EUA of remdesivir on April 29th 2020 to allow emergency use of the agent for severe COVID-19 (confirmed or suspected) in hospitalized adults and children. A phase 1b trial of an inhaled nebulized version was initiated in late June 2020 to determine if remdesivir can be used on an outpatient basis and at earlier stages of the disease. Post ACTT trial announcement the results from a smaller randomized trial which was conducted in China were reported. This was a randomized, double-blind, placebo-controlled, multicenter trial ($n=237$; 158 remdesivir and 79 placebo; 1 patient withdrew) which demonstrated that remdesivir was not associated with statistically significant clinical benefit, measured as time to clinical improvement, in adults hospitalized with severe disease. Although not statistically significant, patients receiving remdesivir had a numerically faster time to clinical improvement than those receiving placebo among patients with symptom duration of less than 10 days. The authors concluded this trend in reduction in time to clinical improvement in those treated earlier requires confirmation in larger studies [25]. The open-label phase 3 SIMPLE trial ($n=397$) in hospitalized patients with severe COVID-19 disease not requiring mechanical ventilation demonstrated similar improvement in clinical condition with the 5-day remdesivir regimen compared with the 10-day regimen on day 14 (OR: 0.75 [95% CI 0.51–1.12]). In this study, 65% of patients who received a 5-day course of remdesivir showed a clinical improvement of at least 2 points on the 7-point ordinal scale at day 14, compared with 54% of patients who received a 10-day course. The study demonstrates that possibly some patients could be treated with a 5-day regimen, which could significantly expand the number of patients who could be treated with the current supply of remdesivir. The trial is

continuing with an enrollment goal of 6000 patients [26]. Similarly, the phase 3 SIMPLE II trial in patients with moderate COVID-19 disease showed that 5 days of remdesivir treatment was 65% more likely to yield clinical improvement at day 11 than standard of care ($p=0.18$). These data show that early intervention with a 5-day treatment course can significantly improve clinical outcomes. The first published report regarding remdesivir compassionate use described clinical improvement in 36 of 53 hospitalized patients (68%) with severe COVID-19. At baseline, 30 patients (57%) were receiving ventilation and 4 (8%) extracorporeal membrane oxygenation (ECMO) [27]. Additional data for compassionate use of remdesivir were released on July 10, 2020 and demonstrated that remdesivir treatment was associated with significantly improved clinical recovery and a 62% reduction in the risk of mortality compared with standard of care. Findings from the comparative analysis showed that 74.4% of remdesivir-treated patients recovered by day 14 versus 59% of patients receiving standard of care. The mortality rate in patients treated with remdesivir in the analysis was 7.6% at day 14 compared with 12.5% among patients not taking remdesivir (adjusted OR 0.38; 95% CI 0.22–0.68, $p=0.001$).

## Hydroxychloroquine with or without azithromycin

Hydroxychloroquine is a well- known old-fashioned drug used for several decades for the treatment of rheumatoid arthritis, systemic lupus erythematosus and malaria prophylaxis [28, 29]. Hydroxychloroquine is a 4-aminoquinolone compound and the hydroxyl analogue of chloroquine. Hydroxychloroquine and chloroquine belong to the 4-aminoquinoline class and both have a basic side chain that distinguish these compounds from the 4-aminoquinoline core structure [28]. Although hydroxychloroquine has demonstrated an antiviral mechanism of action in vitro, there were limited data available regarding its potential efficacy in the clinical setting. In vitro studies have shown that hydroxychloroquine was active against SARS-CoV-2 with a multifactorial mechanism of action [30] and this became the rationale for further use in both treatment and prevention of COVID-19 infection. Another study had also reported that hydroxychloroquine was more potent against SARS-CoV-2 than chloroquine, and therefore, most studies were designed based on these results [30]. The past few months several studies investigated the role of hydroxychloroquine, with or without azithromycin, for the treatment of COVID-19 and these data have become recently available. A small, non-randomized study was conducted in France, which enrolled 20 patients with severe COVID-19 disease. All 20 patients were treated with hydroxychloroquine, with or without azithromycin. This study reported that hydroxychloroquine reduced SARS-CoV-2 load and the effect was enhanced by the addition of azithromycin [31]. This study was severely criticized due to several limitations and flaws [32]. The same study group based on these results designed another trial, non-randomized observational, which evaluated the role of hydroxychloroquine combined with azithromycin in 80 patients diagnosed with COVID-19 [33]. The study reported encouraging clinical outcomes and 83% of the patients achieved negative nasopharyngeal swab by day 7. As in the previous study there was no comparison group and again several limitations reduced the strength of the report significantly [33]. A different group from France reported that there was no benefit with the combination of hydroxychloroquine and azithromycin in 11 patients with COVID-19 [34]. In another retrospective observational French study 84 hospitalized patients were treated with hydroxychloroquine within the first 48 h of admission. 20% of these patients were also treated with azithromycin. The above-mentioned patients were compared with a control group of 89 patients. Hydroxychloroquine treatment with or without azithromycin did not reduce admissions to intensive care units or death at day 21 after hospital admission. Importantly, 7 patients treated with hydroxychloroquine developed QT prolongation and treatment discontinuation was required [35]. An open-label, randomized trial compared hydroxychloroquine plus the standard of care to standard of care alone in China. 150 patients were enrolled in total, 75 in each group. The hydroxychloroquine group showed a mild benefit in symptom resolution, however there was no benefit in negativity achievement of SARS-CoV-2 on molecular nasopharyngeal tests [35]. A large observational study was performed in New York, where the investigators compared the clinical outcomes in COVID-19 hospitalized patients who either received hydroxychloroquine or not. Most of the patients treated with hydroxychloroquine received the first dose within 48 h of admission. The multivariable analysis did not demonstrate significant differences for intubation or death among the two groups [36]. An international study published in The Lancet of 96,032 patients hospitalized for COVID-19 found a higher risk of mortality and de-novo clinically significant ventricular arrhythmias in patients who received hydroxychloroquine or chloroquine with or without azithromycin compared with no therapy. However, this study was retracted by The Lancet on June 4, 2020, due to questions raised about the clarity of the data. Finally, hydroxychloroquine was investigated as postexposure prophylaxis and was found not to be effective in preventing patients from developing COVID-19 after taking the drug within 4 days of a high-risk exposure [37]. All these reports raised several questions and controversy regarding the role of hydroxychloroquine in the COVID-19 setting. Most of these reports had significant limitations and were based on exceedingly small case series. Large, randomized, controlled clinical trials were more than necessary to address all these questions.

More than 25 active hydroxychloroquine COVID-19 clinical trials were currently ongoing in the United States, including the large National Institutes of Health (NIH) clinical trial—the ORCHID Study [NCT04332991]. On March 28, 2020, the U.S. FDA approved chloroquine or hydroxychloroquine for emergency use to treat COVID-19. On April 24, 2020, the U.S. Food and Drug Administration issued a cautioning statement against use of hydroxychloroquine or chloroquine for COVID-19 patients outside of the hospital setting or a clinical trial due to risk of the induced arrythmias. On June 15, 2020, due to accumulating negative data, the FDA revoked the emergency authorization (EUA) use of chloroquine or hydroxychloroquine as a potential COVID-19 treatment. The agency reported that the legal criteria for issuing a EUA are no longer met. On June 20, 2020, the National Institute of Health announced the discontinuation of the ORCHID trial, which evaluated the efficacy and safety of the drug for hospitalized COVID-19 patients because the study failed to prove any clear benefit favoring the drug arm.

## Lopinavir-ritonavir and other anti-viral agents

Lopinavir-ritonavir is an HIV protease inhibitor and is indicated in combination with other antiretroviral products for the treatment of human immunodeficiency virus (HIV-1) infected adults, adolescents and children above the age of 2 years. In 2004, an open label study suggested that the addition of lopinavir–ritonavir to ribavirin reduced the risk of adverse clinical outcomes (defined as acute respiratory distress syndrome [ARDS] or death) as well as viral load among patients with SARS [38]. The comparison arm in this study was a historical control group that was treated only with ribavirin. The main limitations of the study were that it was an open label study without randomization design and patients were concurrently treated with glucocorticoids and ribavirin, making the pure effect of lopinavir-ritonavir difficult to assess. Lopinavir-ritonavir has also showed activity both in vitro and in animal models against Middle East respiratory syndrome and it is now studied in humans with MERS in combination with recombinant interferon 1b in a study that has completed accrual. The results of the study are anticipated.

In a phase 2 study recently published in 'The Lancet' [39], a total of 127 patients, with mild to moderate COVID-19 infection were randomized 2:1 to receive either a 14-day combination of lopinavir 400 mg and ritonavir 100 mg every 12 h, ribavirin 400 mg every 12 h, and three doses of 8 million international units of interferon beta-1b on alternate days (combination group) or 14 days of lopinavir 400 mg and ritonavir 100 mg every 12 h (control group). The primary endpoint was time to achieve negative nasopharyngeal swab for SARS-CoV-2 with RT-PCR. The median number of days from symptom onset to study enrolment and treatment initiation was 5 days. The results of this study showed that the combination therapy was more effective as the median time from treatment initiation to negative nasopharyngeal swab was 7 days [IQR 5–11] for the combination group and 12 days [8–15] for the control group; (hazard ratio 4.37 [95% CI 1.86–10.24], $p = 0.0010$). The authors suggested that early triple antiviral therapy might be effective in mild to moderate illness. The combination of lopinavir-ritonavir with or without ribavirin has been recommended as a treatment option for novel coronavirus, especially in countries that have been hit hard by the disease.

In a trial by Cao et al., patients with oxygen saturation of 94% or less (while they were breathing ambient air or a ratio of the partial pressure of oxygen to the fraction of inspired oxygen (Fio2) was less than 300 mm Hg) were randomly assigned in a 1:1 ratio to receive either lopinavir–ritonavir (400 mg and 100 mg, respectively) twice a day for 14 days, in addition to standard of care, or standard of care alone [2]. A total of 199 patients underwent randomization with 99 patients receiving the combination lopinavir–ritonavir and 100 receiving standard of care. The primary endpoint was time to clinical improvement and was not met in terms of the study. No benefit was observed since time to clinical improvement was same in both arms (16 days). Mortality at 28 days was similar in both groups as well as the percentages of patients with detectable viral RNA at various time points. The authors conclude that the combination showed no benefit in hospitalized patients with severe COVID-19 illness and future trials may help to confirm or exclude the clinical benefit of the combination. The study was not blinded, and this might have affected the assessment of clinical improvement. The study was also underpowered to highlight small effects.

In another study the efficacy and safety of the combination lopinavir/ritonavir was assessed in patients with mild/moderate COVID-19 in comparison to arbidol [40]. Arbidol is an anti-influenza drug targeting the viral hemagglutinin (HA) and can effectively block the fusion of influenza virus with its host cell. It has efficiently inhibited SARS-CoV-2 infection in vitro and therefore was used in the context of a clinical trial against COVID-19. The study was randomized and enrolled 86 patients with mild/moderate disease, 34 were randomly assigned to receive LPV/r, 35 to arbidol and 17 received no antiviral medication (control group). The primary endpoint was the rate of positive-to-negative conversion of SARS-CoV-2 with RT-PCR. There were no differences between the two groups both in primary and secondary endpoints, the rates of antipyresis, cough alleviation, or improvement of chest CT at days 7 or 14. Lopinavir-ritonavir or arbidol monotherapy failed to improve the clinical outcomes of hospitalized patients with mild/moderate COVID-19 infection versus supportive care. The study had quite a small sample size and this was the main limitation.

In a small randomized controlled trial held in China, chloroquine was compared to lopinavir/ritonavir in treating COVID-19 in 22 hospitalized patients, none of the patients enrolled was critically ill [41]. Among the 22 randomized patients, 10 received chloroquine and 12 received lopinavir/ritonavir. Both agents showed similar efficacy in terms of negative SARS-CoV-2 PCR test results at specific timepoints, CT scan improvement and days of hospitalization. The main limitations of the trial were the very small sample size and the participants fairly young age.

The NIH Panel for COVID-19 Treatment Guidelines that have been recently updated (16 Jun 2020) recommends against the use of lopinavir/ritonavir or other HIV protease inhibitors in COVID-19 infection. This is due to unfavorable pharmacodynamic data and mainly due to the fact that clinical trials have not demonstrated a clear clinical benefit in patients with COVID-19 [42].

Favilavir/Avifavir or Avigan is an oral antiviral drug approved in Japan for influenza, also used for Ebola virus infection. In a randomized, open label trial conducted in China 240 patients were randomized to receive either Favipiravir (116 assessed) or Arbidol (120 assessed) [43]. Primary endpoint, defined as clinical recovery rate at Day 7, was not significantly different between the Favipiravir group (71/116) and the Arbidol group (62/120) ($p=0.1396$). There are several ongoing clinical trials evaluating safety and efficacy of favilavir against other antivirals in China, Japan, Canada and avifavir in Russia. Darunavir/Cobicistat and Darunavir/Ritonavir [44] have also been tested in a randomized controlled trial of 30 patients in China which showed that darunavir/cobicistat was not effective in the treatment of COVID-19. There are no data from clinical trials that support the use of other HIV protease inhibitors to treat COVID-19, such as Atazanavir.

Instead of administering a single drug, combination of antivirals with different mechanisms of action may be more effective. The adverse event profile of these drugs should not be underestimated.

## Thromboprophylaxis and fibrinolysis

Venous thromboembolism (VTE) and particularly pulmonary embolism (PE) have emerged as a significant risk associated with SARS-CoV-2 severe infection which is multi-fold higher compared to other viral pneumonias/acute respiratory distress syndromes. Reported incidence reaches 25–27% [45, 46]. Abnormal levels of hypercoagulability markers and poor scoring on standard VTE assessment risk-assessment tools is associated with worse prognosis [47, 48].Specific risk-stratification VTE assessment tools are not available yet and the current recommendation is to apply a universal pharmacological thromboprophylactic strategy for all hospitalized patients [49]. The preferred agent is low molecular weight heparin (LMWH) which should be withheld when there is active bleeding, when platelet count is less than $25\times10^9$/L, or fibrinogen less than 0.5 g/L [47, 50–52]. Unfractionated heparin or reduced dose LMWH should be administered when creatinine clearance less than 30 mL/minute and fondaparinux in patients with a history of heparin-induced thrombocytopenia [51]. A multimodal approach combining pharmacological and mechanical means should be applied in critically ill patients.

Direct oral anticoagulants (DOACs) and Vitamin K antagonists should be avoided as there might be unknown drug-drug interactions with investigational therapies and antivirals administered. Some groups opt for a stepped-up approach with intermediate dose LMWH in ICU-critically ill patients but data are currently insufficient to support such an approach outside the context of clinical trials [47, 53]. Therapeutic dose anticoagulation has also been proposed by some centers for the critically ill patients and others advocate stepping up from prophylactic or intermediate-dose to a full dose regimen in patients with deteriorating pulmonary status or ARDS but the data are very limited [46]. Ongoing randomized clinical trials (NCT04345848, Hep-COVID, and PROTECT COVID 19) aim to assess the efficacy and safety of more intense intermediate- to therapeutic-dose versus prophylactic-dose LMWH. Routine discharge on VTE prophylaxis is not currently required unless set criteria are met; regulatory approved regimens such as Direct oral anticoagulants (DOACs) can be used [51, 53]. Some early data suggest that LWMH or heparin thromboprophylaxis is associated with reduced mortality in critically ill SARS-COV-2 patients when D-dimer levels are more than 6 times the upper limit of normal [47, 50, 52, 54]. When VTE is suspected or confirmed patients should receive therapeutic dose anticoagulation for at least 3 months [55].

The rationale behind fibrinolysis in severely ill SARS-COV-2 infected patients lies in the coagulopathy observed and data that link fibrin deposition in the pulmonary vasculature is associated with ARDS development [52, 56]. In one case series alteplase administration followed by intravenous heparin in 3 critically ill patients with ARDS led to an improvement of the PaO2/FiO2 (P/F) ratio which was however only transient [56]. Other fibrinolytics including defibrotide are currently being evaluated but safety issues including bleeding remains a concern [56, 57].

## The potential role of antifibrotic therapy

The risk factors correlated with COVID-19 are shared with those of idiopathic pulmonary fibrosis suggesting that these patients might be at increased risk for severe disease. It is assumed that the pandemic will increase the fibrotic lung disease. Therefore, the available antifibrotics (nintedanib and

pirfenidone) might have a role in SARS-Cov-2 therapeutics (NCT04338802, NCT04282902).

## Interleukin-6 (IL-6) receptor antagonists and complement antagonists

The rationale behind the use of IL-6 receptor antagonists in the treatment of SARS-CoV-2-associated disease lies in the evidence in support of the role of cytokine release syndrome in the most severe manifestations of COVID-19 and the central part of IL-6 as a proinflammatory cytokine in this syndrome [58–60].

Use of IL-6 antagonists is not currently included in treatment guidelines for SARS-Co-V infection as there is a lack of robust clinical data in support for or against their use. A randomized, double-blind, placebo-controlled phase III trial is about to start to evaluate safety and efficacy of intravenous siltuximab (IL-6 monoclonal antibody) plus standard of care in patients with SARS-CoV-2-associated ARDS. Results of the observational cohort study in 188 patients (NCT04322188) were released online demonstrating a significantly lower 30d-day mortality rate in the siltuximab treated patients ($n=30$) versus controls ($n=188$) who received best supportive care. Some protocols include recommendations for the use of tocilizumab, another monoclonal IL-6 antibody. Two retrospective studies of 21 and 51 patients, respectively, in patients with SARS-Co-V and ARDS demonstrated some clinical benefit as adjunct to standard of care treatment [58, 61] Another single center study of 154 COVID-19 patients requiring mechanical ventilation evaluated efficacy and safety of tocilizumab and the drug was associated with lower mortality despite high occurrence of infections [62]. Multiple randomized, multicenter clinical trials are currently ongoing to assess its use either as a single agent or in combination treatments.

Based on the hypothesis that viral infections activate coagulation and complement cascades, triggering ARDS, antiCD5-a complement monoclonal antibody eculizumab, was combined with a JAK1/2 inhibitor, ruxolitinib in 17 patients and the combination demonstrated significant clinical benefit [63].

## Monoclonal antibodies

Several neutralizing SARS-CoV-2 monoclonal antibodies are currently evaluated in the setting of clinical trials. These antibodies target specific regions of the viral spike, are mainly IgG1 subtype and are characterized by long half-life. This indicates that could be administered in a single infusion. However, the bioavailability in tissues and organs affected by COVID-19 remains unknown [64].

## Non-steroidal anti-inflammatory drugs (NSAIDs)

Non-steroidal anti-inflammatory drugs (NSAIDs) are widely used as painkillers and antipyretic agents. Although NSAIDs are effective drugs for symptom relief during the course of viral and bacterial infections, their use has been linked to higher rates of cardiovascular events [65, 66]. Consequently, short-term administration of NSAID in respiratory tract infections remains questionable. The first concerns regarding NSAID use in patients with COVID-19 raised in March 2020 with a study showing that angiotensin converting enzyme 2 (ACE-2) activity can be increased by ibuprofen [67]. It is largely known that SARS-CoV-2 binds to target cells through the angiotensin converting enzyme 2 (ACE-2) receptor which is mainly expressed in epithelial cells of the respiratory tract, kidney and blood vessels. It has been hypothesized that increased expression of ACE-2 receptor linked to ibuprofen use may facilitate SARS-CoV-2 infection and that ACE-2 stimulating drugs such as NSAIDs may increase the risk of suffering from more severe COVID-19 [67, 68]. NSAIDs use has been also implicated in delaying the resolution of inflammation by inhibiting cyclo-oxygenases [69] and is associated with nephrotoxicity, bleedings and gastrointestinal complications [70]. Although several studies have linked ibuprofen with negative outcomes during the course of respiratory infections it is possible that they are subject to a number of biases [71]. Confounding by disease severity may serve as a potential risk of bias considering that patients with more severe disease are more likely to use NSAIDs for symptom relief compared to patients with mild symptoms. Nevertheless some authorities, have suggested that paracetamol should be considered first-line antipyretic agent, if not contraindicated, with ibuprofen reserved for individuals who are unable to tolerate paracetamol until ongoing trials further clarify harms and benefits of NSAIDs in people with COVID-19 [72]. LIBERATE Trial in COVID-19 (LIBERATE) is an ongoing randomized phase 4 double blinded controlled trial (ClinicalTrials.gov: NCT04334629) testing Lipid Ibuprofen Versus Standard of Care for Acute Hypoxemic Respiratory Failure Due to COVID-19. The study aims to evaluate the reduction in severity and progression of lung injury with three doses of lipid ibuprofen in patients with SARS-CoV-2 infections providing more pragmatic evidence of the role of ibuprofen use in COVID-19.

## Systemic corticosteroids

The efficacy of corticosteroids on inflammatory organ injury in viral pneumonias remains controversial [73]. SARS-CoV-2 infection often presents with a biphasic pattern: a first viremic phase lasting 7–10 days, followed in approximately 20% of patients by a second inflammatory phase

characterized by cytokine storm and respiratory failure [74]. Considering that host immune response plays a key role in the pathophysiological effects of organ failure in viral pneumonias it has been hypothesized that corticosteroids might have an effect on pulmonary and systemic inflammation.

Previous studies reported negative or neutral effects of corticosteroids on viral pneumonias caused by SARS-CoV, MERS-CoV or influenza [75–78]. Observational studies have shown more secondary infections, delayed viral clearance, increased mortality and more adverse effects such as psychosis, hyperglycaemia and avascular necrosis with steroid treatment [78]. However, the hyperinflammatory response frequently seen in COVID-19 has been not confirmed in other viral infections. In addition, the use of corticosteroids may have a role in acute respiratory distress syndrome (ARDS), a severe complication of SARS-CoV-2 infection.

Recently, a non-blinded randomized controlled trial of non-COVID-19 patients with ARDS under lung-protective mechanical ventilation reported a benefit of high-dose dexamethasone treatment ($n=139$) compared to routine intensive care ($n=138$). At 60 days, 21% of patients in the dexamethasone group and 36% of patients in the control group died (between-group difference −15.3% [−25.9 to −4.9]; $p=0.0047$) without significant differences in adverse events between the two groups [79]. A retrospective cohort analysis of patients with COVID-19 who developed ARDS (41.8% of patients) showed that treatment with methylprednisolone was associated with a decreased risk of death (HR 0.38; 95% CI 0.20–0.72) [80]. A single-center retrospective cohort study reported that hospitalized COVID-19 patients treated with steroids ($n=396$) had a lower mortality rate than those who did not receive steroids ($n=67$), 13.9% vs. 23.9% (HR 0.51, 95% CI 0.27–0.96, $p=0.044$) [81]. The recently published Randomized Evaluation of COVID-19 Therapy (RECOVERY) trial, an open label randomized controlled trial compering dexamethasone (6 mg daily for 10 days) versus standard of care, terminated enrollment early due to a mortality benefit with dexamethasone [82]. Hospitalized patients with COVID-19 randomized to dexamethasone arm ($n=2104$) had lower rates of 28-day mortality compared to the standard of care arm ($n=4321$) (RR 0.83, 95% CI 0.74–0.92, $p=0.0007$). Dexamethasone reduced deaths in ventilated patients (RR 0.65, 95% CI 0.48–0.88, $p=0.0003$) and patients receiving supplemental oxygen (RR 0.80, 95% CI 0.67–0.96, $p=0.0021$), but not in patients who did not require respiratory support (RR 1.22, 95% CI 0.86–1.75, $p=0.15$). The benefit of dexamethasone was apparent in patients being treated more than 7 days after symptom onset as inflammatory lung complications are likely to have been more common after the first week from symptom onset.

Based on current evidence low-dose systemic steroids for selected COVID-19 patients who are critically ill or require supplemental oxygen can be considered. However, routine corticosteroid use, especially in patients with mild symptoms or at the early stages of the disease may be avoided unless they are indicated for another reason such as exacerbation of asthma or chronic obstructive pulmonary disease (COPD), septic shock or ARDS in an individual basis.

## Bronchodilators/vasodilators

Wheezing has been not indicated as a common symptom of COVID-19 [83–85]. Bronchodilators should certainly be administered whenever indicated but should not be ordered as standard of care. Nebulizers are associated with aerosolization increasing the risk of SARS-CoV-2 transmission and should be avoided, especially in cases without an evidence-based benefit [86]. In patients with suspected or documented COVID-19, nebulized bronchodilator therapy using metered dose inhalers (MDIs) with spacer devices rather than nebulizers should be reserved for acute bronchospasm such as asthma [87] or exacerbation of chronic obstructive pulmonary disease [COPD] exacerbation. If nebulized therapy is used, patients should be in an airborne infection isolation room, and healthcare workers should use appropriate personal protection equipment (PPE). According to recent guidelines, aerosol-generating procedures on ICU patients with COVID-19 should be performed in a negative pressure room and the healthcare workers performing aerosol procedures should use fitted respirator masks (N95 respirators, FFP2, or equivalent), (best practice statement) [88].

Patients with severe hypoxemia may benefit from pulmonary vasodilators by improving ventilation-perfusion mismatch and decreasing pulmonary vascular pressure. Pulmonary vasodilators may be, especially useful for patients with decompensated or acute pulmonary arterial hypertension [88]. However, these agents do not reduce mortality in all-cause ARDS and should not be used instead of proved therapies. There is no evidence supporting the use of pulmonary vasodilators in COVID-19 patients. A meta-analysis of 13 RCTs (1243 patients) on inhaled nitric oxide (NO) in non-COVID-19 patients with ARDS failed to show significant effect on mortality (RR 1.04; 95% CI 0.9 to 1.19), and reported increased risk of acute kidney injury (RR 1.59; 95% CI 1.17 to 2.16). Although inhaled nitric oxide resulted in a transient improvement in oxygenation for the first 24 h, the positive effect disappeared beyond 24 h [89]. The most commonly used agents are inhaled nitric oxide gas and aerosolized epoprostenol, administered by continuous inhalation. Inhaled NO may be preferred because of less frequent need of filter changes, decreasing exposure of healthcare workers caring of COVID-19 patients. Inhaled vasodilators should only be administered through a closed system to reduce aerosolization. Improvement in oxygenation with inhaled vasodilators is typically seen within a few hours after initiation

of administration. Inhaled prostacyclins such as ilioprost have not been tested yet in severe ARDS. Based on very low quality of evidence, current guidelines suggest initiation (trial) of inhaled pulmonary vasodilators as a rescue therapy in mechanically ventilated adults with COVID-19, severe ARDS and hypoxemia, despite optimizing ventilation and other rescue strategies. In the absence of rapid improvement in oxygenation the treatment should be de-escalated [88]. Ongoing trials are under way (NOSARSCOVID; clinicaltrials.gov; NCT04290871) to provide more evidence on the effect of inhaled NO in severe acute respiratory syndrome in COVID-19 patients.

## Mechanical ventilation/high nasal flow/ECMO

Acute hypoxemic respiratory failure is one of the most common clinical manifestations that determine clinical outcome in COVID-19 patients. Although the majority of patients with COVID-19 infection have an asymptomatic or mild respiratory disease, a small but significant proportion of patients present acute respiratory distress syndrome (ARDS) requiring hospital and/or intensive care unit (ICU) admission and support with mechanical ventilation [90].

The value of noninvasive ventilation has not been fully clarified yet, but most COVID-19 centers use (at least as a trial) high flow nasal cannula (HFNC)/noninvasive positive pressure ventilation (NIPPV) in mild to moderate ARDS and reserve endotracheal intubation and mandatory mechanical ventilation for more severe ARDS patients. While there is a theoretical risk of healthcare personnel and patients contamination by using HFNC/NIPPV, there are substantial benefits mostly shown from previous meta-analysis [91, 92]. These meta-analyses have recently demonstrated that HFNC reduces the rate of intubation compared with conventional oxygen therapy but also compared with NIPPV in acute hypoxemic respiratory failure. Based on this evidence, the European Society of Intensive Care Medicine (ESICM) recommends its use in COVID-19 patients; however, there is strong recommendation of close monitoring for signs of respiratory status deterioration and early intubation in a controlled setting, when HNF/NIPPV are applied in COVID-19 patients [88].

The exact number of patients requiring mechanical ventilation is not clear yet; Recent observational data published from the Italian COVID-19 experience in Lombardy region and from New York City have shown that most critical ill COVID-19 positive patients admitted in ICU required invasive mechanical ventilation (> 80%) and presented a high hospital mortality rate (~ 50%) [93, 94].

Mechanical ventilation management includes protective ventilation and recruitability studies with lung mechanics assessment. The latter seems to have a central role in mechanical ventilation strategy as ARDS phenotypes in COVID19 have certain peculiarities. COVID-19 patients may present with a particular dissociation between the degree of hypoxemia and loss of lung volume (compliance) and response to positive end expiratory pressure (PEEP) [95] possibly due to the distinguished pattern of "endothelitis", vascular injury and microangiopathy [96]. However, COVID-19 can also induce refractory hypoxemia and/or hypercapnia, despite optimal management strategy (including muscle relaxation, prone position, and pulmonary vasodilators) with subsequent remarkable high in-hospital mortality.

In this specific group of critical ill COVID-19 patients, where there is no further treatment option, extracorporeal membrane oxygenation (ECMO) has been considered to have an important role as a rescue therapy in treatment strategy to increase survival. Results emerging from the ELSO registry demonstrate that ECMO is feasible and it has been applied with safety and efficacy in more than 1000 COVID-19 patients with severe refractory ARDS with a remarkable ICU/hospital discharge rate of more than 50% [43].

## Vaccines

Vaccine development is complex, lengthy and expensive process. Attrition rates are high and multiple candidates are required, with multiple steps, pauses for checks and data analysis to lead eventually to a licence production [97]. A new "pandemic paradigm" is required to include a platform that can provide scalable, technological flexibility and versatility with large scale production of a vaccine that is efficacious, safe and well-tolerated allowing fast starts and parallel step execution. The development cycle of a vaccine production against SARS-CoV-2 is moving remarkably fast given the major pandemic issue that has emerged and major international vaccine funding agencies are supporting the multitude of innovative ongoing efforts.

SARS-CoV-2 is composed of an single-stranded, positive-sense RNA enveloped molecule surrounded by functional and structural proteins which include protein E(envelope), protein S(spike), protein M (membrane), Protein N(nucleocapsid) [98]. The viral genome of SARS-CoV-2 was published only 4 weeks into the outbreak demonstrating tis genomic and phylogenetic similarity to SARS-CoV but also several bat coronaviruses [99].

Current approaches include the classical inactivated and attenuated vaccines, the viral protein S subunit, virus like particles (VLP), viral vector-based vaccines and the newer DNA- and RNA-based vaccines [42, 100]. Given the prior experience with SARS-CoV vaccine development in 2003 targeting the S subunit, some SARS-CoV-2 vaccines entered human clinical trials directly providing a head start [42, 101].

The first vaccine to enter clinical trials as early as February 2020, is a novel experimental RNA-based vaccine (mRNA-1273) which uses part of the S protein genetic code. It is being developed by Moderna Therapeutics (Cambridge, MA, USA), a pharmaceutical company working already on the SARS-CoV and MERS-CoV vaccines allowing the clinical development to skip certain animal testing based on earlier studies. The interim analysis results of the phase I clinical trial were released recently; the report included 45 participants (age 18–55) who received two intramuscular injections (at 28 days) of three dose levels (25, 100, 250 Âμgs). The 100 and 250 microgram doses lead to high levels of virus-neutralizing antibodies and the 100mcg dose had the maximum immunogenicity with minimum reactogenicity. The trial has expanded to include adults over 55 years of age and includes 120 participants [102]. The phase II clinical trial on safety, reactogenicity and immunogenicity of the two different doses began enrollment in May 2020 and a phase III trial is being prepared to launch soon (NCT04283461) [42].

Inovio Pharmaceuticals', already working on a novel DNA vaccine for MERS, (Plymouth Meeting, PA, USA) [42, 103, 104] has developed INO-4800 using the S gene. It has entered phase I clinical trials and is administered intradermally using electroporation. A phase II/III clinical trial is expected this summer. CanSino Biologics (Tianjin, China) have developed a vaccine based on their adenovirus vaccine platform built for Ebola vaccine and on the S subunit. Ad5-nCov is understudy in a phase I clinical trial [104]. (NCT04313127) The results of the phase I/II trial single-blind randomized trial of a chimpanzee adenovirus-vectored vaccine (ChAdOx1 nCoV-19) expressing the SARS-CoV-2 spike protein compared with a meningococcal conjugate vaccine (MenACWY) as a control were recently released. All participants in the non-randomized prime boost group ($n = 19$) at both dose levels had neutralizing activity following a booster dose and antibody responses correlated with ELISA assessed antibody levels. No serious adverse events were observed and the results for the randomized cohort are awaited [105].

A large number of different vaccines are currently in pre-clinical development. A new oral SARS-Cov-2 vaccine uses food-grade safe Saccharomyces cerevisiae as a carrier targeting the S protein (Tianjin University). mRNA-based vaccines at different stages of development are also underway; BTN162 (developed by BioTech, Mainz, Germany) encapsulates the nucleic acid in 80 nm ionizable, glycol-lipid nanoparticles and is expected to enter clinical trial testing soon [103]. Other mRNA vaccines are being developed by CureVac (Tübingen, Germany) and Pfizer (New York, NY, USA). Intranasal, recombinant adenovirus-based vaccines are being developed Altimmune Inc. (Gaithersburg, MD, USA) and Johnson and Johnson (New Brunswick, NJ, USA). A live-attenuated vaccine with swapped optimized codons with non-optimized codons is under development by Codagenix in collaboration with Serum institute in India. Antigen presenting cells modified by lentiviral vectors and acting as "minigenes" that express portions of SARS-Cov-2 based on dendritic cells, form the basis of two other vaccines under development by the Shenzhen Geno-Immune Medical Institute.

Which of these strategies will be most efficacious remain to be seen and despite immense efforts to rapidly develop a vaccine for large scale production, the clinical trial stages and regulatory hurdles are necessary to ensure short- and long-term safety and efficacy. Challenges in this process include determining which approach can induce the optimal immune response, potential exacerbation of lung disease and finally establishing that the protection inferred from the SARS and MERS experienced actually holds.

The H1N1 experience has made clear the need for novel development and manufacturing platforms that can be adapted to new emerging pathogens ("X"). Vaccine and biotech companies have been investing in such approaches in recent years. In addition financial instruments are required that can support pandemic vaccine development. To ensure herd immunity there is a need for a global instrument which is responsible for large-scale development, manufacturing and deployment of licenced vaccines [106].

## Conclusion

COVID-19 is a novel emerging infectious disease caused by SARS-CoV-2 characterized as atypical pneumonia. As of July 1, 2020, more than 10 million people worldwide had been infected with SARS-CoV-2. Advances in prevention and effective management of COVID-19 will require basic and clinical investigation and public health and clinical interventions. The pathogenesis of the new coronavirus is still not well defined. Most patients present with a self-limited course, however, a few will experience severe or even fatal disease. COVID-19 is defined as a multisystemic disease. The basic pathogenesis involves two descrete compounds; a severe lung inflammation and immune deficiency, both of which are related to an inappropriate immune response and increased production of cytokines. Thus, treatment approaches currently investigated include antiviral and anti-proinflammatory cytokines, anti-infectious and life support therapies, monoclonal antibodies and passive immunotherapy, especially in patients with severe disease. However, although the therapeutic strategy against the disease is of high importance the main way to prevent virus spread is the development of an effective and safe vaccine widely available.

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## Compliance with ethical standards

**Conflict of interest** The authors declare they have no conflict of interest.

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