

Emerging therapies for COVID-19 pneumonia

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1. Emerging therapies for COVID-19, guidelines and recommendations

On 11 March 2020, the World Health Organization declared coronavirus disease 2019 (COVID-19) a pandemic. In recent months, with the spread of COVID-19, there has been growing interest in preventive and therapeutic treatments, but no effective strategies for either purpose have been confirmed by the available randomized controlled trials. Although future clinical trials may renew hope [1], management of COVID-19 is still exclusively supportive. The mechanism of infection with the causative agent of COVID-10, severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), requires binding of viral spike (S) proteins to the angiotensin-converting enzyme-2 (ACE2) receptor, a process facilitated by transmembrane serine protease 2 (TMPRSS2) and followed by down-regulation of ACE2 [2]. The viral membrane then fuses with the host cell, and the viral ribonucleic acid (RNA) is taken up via endocytosis. Within the host cell, it undergoes translation, proteolysis, RNA synthesis, new assembly, and exocytosis to further infect other cells. Secondary hemophagocytic lymphohistiocytosis, with a characteristic 'cytokine storm' component, has been recently proposed as a severe hyperinflammatory complication of COVID-19, driven by viral immune response [3]. Moreover, many COVID-19 patients suffer from abnormal coagulation, which has been associated with poor prognosis [4].

Recent advances have focused on a multi-target therapeutic strategy, taking inspiration from previous outbreaks of coronavirus infections, namely SARS and the Middle East respiratory syndrome (MERS). Nevertheless, no specific drugs are available for the treatment of either infection nor for COVID-19. With the current paucity of data, therapeutic choices that usually would never been adopted before appraising their utility is now being employed in an empiric and presumptive manner, moving away from core tenets of evidence-based medicine. Effective therapies are urgently needed.

Renin-angiotensin-aldosterone system (RAAS) inhibitors [2] have been proposed because they bind to ACE2. Data from Wuhan reported that a huge number of COVID-19 patients present with hypertension and RAAS inhibitors as daily antihypertensive therapy [2]. However, preclinical

studies demonstrated that RAAS inhibitors may upregulate ACE2 expression, raising concerns as to their efficacy in COVID-19 (Figure 1) [2]. Conversely, abrupt withdrawal of RAAS inhibitors in high-risk patients may result in clinical instability and adverse outcome. Clinical trials to determine whether the use of RAAS inhibitors may affect the outcome in COVID-19 are ongoing (Table 1) [2].

Chloroquine and hydroxychloroquine have broad-spectrum antiviral activity *in vitro* by inhibiting virus entry into host cells through the inhibition of glycosylation, proteolysis, and endosomal acidification of host receptors. Both drugs also have immunomodulatory effects, attenuating cytokine production [5]. Very recently, the U.S. Food and Drug Administration (FDA) reviewed the available literature, as well as data from the Adverse Event Reporting System Database and the American Association of Poison Control Centers National Poison Data System, suggesting that these drugs should be used only very cautiously in patients at increased risk of heart rhythm problems, especially considering the limitation of their use to hospital settings [5]. On May 7, an observational study of 1376 consecutive patients who received hydroxychloroquine was published in the *New England Journal of Medicine*. The study concluded that hydroxychloroquine did not influence the time from enrollment to intubation or death [6], implying limited clinical utility and perhaps detrimental effects. Thus, we recommend that physicians not use hydroxychloroquine until new data from randomized-controlled trials become available (Table 1).

Antiretroviral agents, such as protease inhibitors and integrase strand transfer inhibitors, have also been suggested for the treatment of COVID-19. The use of lopinavir/ritonavir did not result in benefits in terms of viral clearance or mortality rate in a randomized-controlled study of 199 COVID-19 patients. Randomization was made 13 days after symptom onset, suggesting that delayed initiation of therapy was probably associated with the inefficacy of treatment [7]. Other antiretrovirals are still undergoing preclinical testing, with possible evidence of efficacy against SARS-CoV-2 [1].

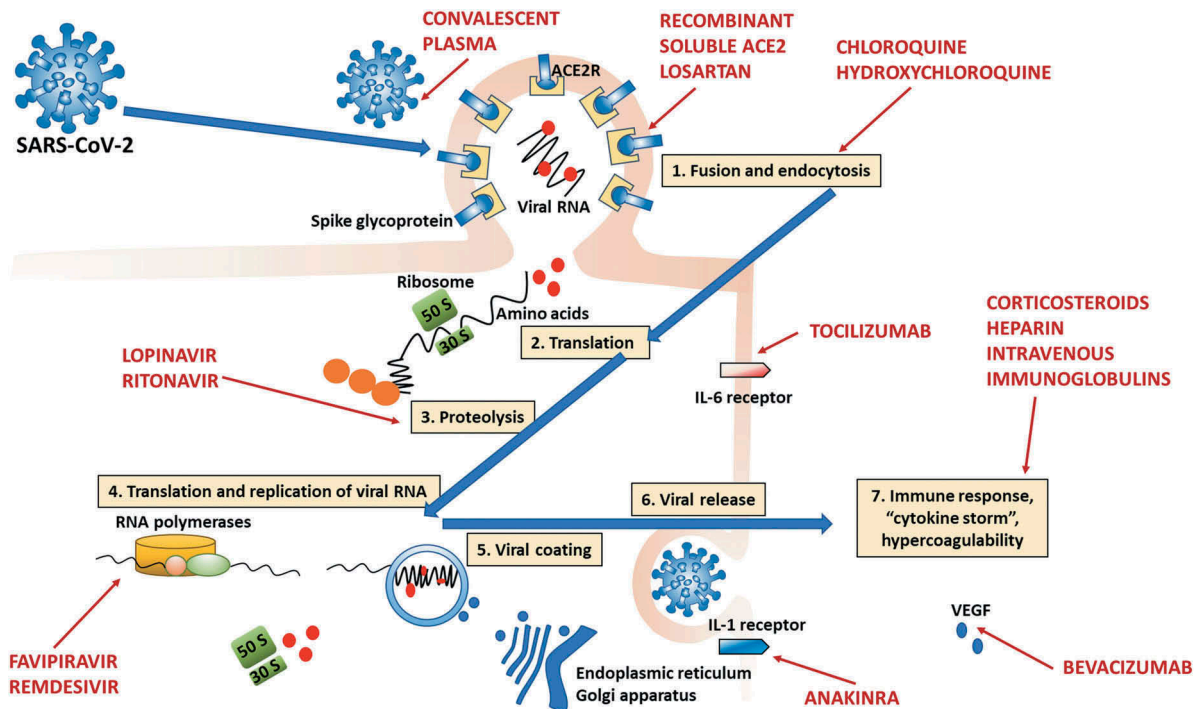


Figure 1. Main drugs tested to date in SARS-CoV-2 infection and their potential mechanisms and sites of action in the viral infection and replication cycle. 1. SARS-CoV-2 enters cells by binding the ACE2 receptor, with subsequent fusion and endocytosis (recombinant soluble ACE2 and losartan would act at this level). 2. Viral RNA is released, and transduction begins in the ribosome. 3. This is followed by proteolysis (may be inhibited by lopinavir/ritonavir), which in turn is followed by translation and replication (RNA polymerases; may be inhibited by favipiravir and remdesivir). 5. Then, the ribosome synthesizes nucleocapsid proteins, which will encase RNA, and the viral envelope is assembled with the aid of the endoplasmic reticulum and Golgi apparatus. The now-complete virions are released, ready to infect other cells. 7. In parallel, a local and systemic immune response is now activated, and hypercoagulability ensues (modulated by corticosteroids and heparin). The IL-6 receptor can be inhibited by tocilizumab, and the IL-1 receptor, by anakinra. Bevacizumab acts by inhibiting vascular endothelial growth factor (VEGF). Convalescent plasma reduces viral load.

Favipiravir, a purine nucleoside leading to inaccurate viral RNA synthesis, has recently been approved for clinical trials to treat COVID-19 [8]. Remdesivir, a nucleotide analogue, although was developed as a therapeutic agent for Ebola and Marburg virus infections with interesting results, preliminary data in COVID-19 did not show clinical benefit [9]; phase III clinical trials are ongoing (Table 1). In addition to various antiviral drugs already on the market, there are also several small-molecule compounds currently in research and development which have shown significant inhibitory effects on many key proteins from similar coronaviruses, such as SARS-CoV and MERS-CoV. These drug candidates mostly inhibit viral enzymes, including proteases and components of RNA-dependent RNA polymerase (RdRp). Since the 3C-like proteinase (3CL^{pro}) has a high level of sequence homology between SARS-CoV and SARS-CoV-2, inhibitors tested against SARS-CoV 3CL^{pro} may also be active against SARS-CoV-2 [10]. Because of their ability to interfere with viral replication, interferons and interferon fusion proteins have been utilized as therapeutic agents for the treatment of viral infections for more than 20 years. Finally, several RNA therapies have been developed, and, in addition to directly targeting the virus, antisense oligonucleotides could be used to target disease-related proteins involved in the inflammatory process. Convalescent plasma has also been shown to reduce viral load and improve outcome in COVID-19 patients [11].

The use of corticosteroids to modulate the inflammatory response in COVID-19 remains controversial. Based on an expert consensus [12–14], their use should be carefully weighed, according to clinical evidence: in patients already taking corticosteroids for chronic diseases, increased use should be evaluated with caution, with doses not exceed 0.5–1 mg/kg/day (methylprednisolone or equivalent) and for no longer than 7 days. The safety and efficacy of methylprednisolone in COVID-19 patients have been investigated in the clinical setting (Table 1). Interleukin (IL)-6 plays a crucial role in the inflammatory response, showing both anti- and pro-inflammatory effects on cells that express the IL-6 receptor (IL-6R), such as macrophages, neutrophils, and T-cells. Tocilizumab, a recombinant humanized monoclonal antibody, is designed to bind IL-6R. In 2017, it was approved for the treatment of cytokine release syndrome, which has prompted its possible use for COVID-19 in patients with higher levels of IL-6; efficacy remains unclear and requires confirmation [3]. Anakinra, an IL-1 receptor inhibitor, may likewise induce a short-term reversible suppression of inflammation. However, both of these drugs might increase the risk of superinfections, making their clinical use for COVID-19 questionable [3]. Bevacizumab, a human monoclonal antibody against vascular endothelial growth factor, has been studied in COVID-19 patients to reduce lung edema [15].

Immunoglobulin therapy is considered another potential treatment for COVID-19 patients. Two small case series (five

Table 1. Main proposed drugs for the management of COVID-19 patients.

	Proposed mechanism of action	Potential drawbacks	Supporting evidence	Ongoing trials (clinicaltrials.gov)
RAAS inhibitors				
Losartan	ACE2 receptor binding	Unclear effects on ACE2 expression in vivo	Preclinical studies [2]	NCT04312009 NCT04311177
Recombinant soluble ACE2	Restoring balance in the RAAS system and preventing organ injury	Limited data in vivo	In vitro [18]	NCT04287686
Aminoquinolines				
Chloroquine	Attenuation of cytokine production Inhibition of virus in host cells	Safety concerns arising from observational studies Several mild side-effects Hemolysis in G6PD-deficient patients Retinal damage QTc prolongation Similar to chloroquine, safety profile probably better	Preclinical studies [5,6]	NCT04328493 NCT04333628
Hydroxychloroquine	Same as chloroquine		Preclinical studies [5,6]	NCT04333654 NCT04328272 (with and without adjunctive azithromycin)
Antivirals				
Lopinavir/ritonavir	Inhibition of viral 3C-like protease	Several drug interactions Common side effects, including hepatotoxicity One negative trial in late moderate-severe patients [7]	Preclinical studies [7]	NCT04315948 NCT04252274
Darunavir/ritonavir	RNA polymerase inhibitor	Hepatotoxicity Nephrotoxicity One negative trial in severe patients [9]	Preclinical studies [9]	NCT04292899 NCT04257656
Darunavir/cobicistat	RNA polymerase inhibitor	Hyperuricemia Neutropenia Hepatotoxicity	Preclinical studies [9]	NCT04336904 NCT04319900
Remdesivir	Reduce viral load and improve outcome	Potential for pathogen transmission Antibody-dependent enhancement of infection	Pilot clinical study [11]	NCT04292340
Convalescent plasma from recovered donors containing SARS-CoV-2 neutralizing antibodies				
Immunomodulators				
Steroids (methylprednisolone and dexamethasone in particular)	Modulating the host inflammatory response and limiting organ damage [12]	Controversial effects on superinfections and viral clearance [12] Might increase superinfections Might increase superinfections Blocks C-reactive protein synthesis, limiting its usefulness in monitoring inflammation and bacterial superinfections	Observational data [19]	NCT04325061 NCT04323592
Tocilizumab	Persistent immune modulation through inhibition of IL6 receptor (3). Reduction of cytokine storm Short-term reversible suppression of inflammation through inhibition of IL1 receptor		Clinical rationale [3]	NCT04320615 NCT04317092
Anakinra	Reduce pulmonary edema and protein levels in bronchoalveolar lavage fluid through inhibition of vascular endothelial growth factor Immunomodulation Specific antiviral activity if extracted from recovered patients (hyperimmune plasma)	Might increase superinfections	Clinical rationale [1]	NCT04324021
Bevacizumab	Reduce pulmonary edema and protein levels in bronchoalveolar lavage fluid through inhibition of vascular endothelial growth factor Immunomodulation Specific antiviral activity if extracted from recovered patients (hyperimmune plasma)	Nosebleed, rectal bleeding, increased blood pressure, headache	Preclinical study [1]	NCT04275414
Intravenous immunoglobulins	Immunomodulation, reduction in inflammation and fibrosis	Unclear efficacy	Small case series [1]	NCT04321421
Mesenchymal stromal cells	Immunomodulation, reduction in inflammation and fibrosis	Unclear efficacy	Preclinical and clinical studies in ARDS [16]	NCT03042143, NCT04269525, NCT04252118, NCT04339660, NCT04288102, NCT04293692
Adjuvant therapy				
Unfractionated or low-molecular-weight heparin	Prophylaxis of thrombosis Protects endothelial cells from oxidative stress May modulate cytokine storm	Increased bleeding risk	Observational data [20]	

RAAS: renin-angiotensin-aldosterone system; ACE2: angiotensin-converting enzyme 2; G6PD: glucose-6-phosphate-dehydrogenase; IL6: interleukin 6; IL1: interleukin 1; HIV: human immunodeficiency virus.

and three, respectively) reported the use of immunoglobulins extracted from convalescent patients and the use of intravenous immunoglobulin for the treatment of SARS-CoV-2, respectively [1].

Mesenchymal stromal cells (MSCs) have demonstrated a good safety profile and possible efficacy in patients with acute respiratory distress syndrome (ARDS), but whether these therapies are effective for treating COVID-19 remain unknown. Few studies have been published [16] and several others are ongoing. COVID-19 patients with different degrees of severity were treated with MSCs or placebo, and no adverse events were observed. However, further studies on dose, dosing strategies, and timing of administration are needed.

COVID-19 patients may also develop severe coagulopathy. During SARS-CoV-2 infection, alveolar macrophages and T cells, among other immune cells, are recruited and activated in an uncontrolled immune response leading to a cytokine storm, characterized by elevated serum levels of interleukin-1 β , tumor necrosis factor (TNF)- α , and IL-6, which foster thrombin activity [4]. Heparin is commonly administered to these patients. IL-1 β and TNF- α target the glycocalyx via activation of metalloproteinases and heparanase, consequently reducing the content of proteoglycans (e.g., syndecan-1) and heparan sulfate, respectively. Glycocalyx shedding is linked to endothelial barrier rupture, which facilitates the increase of vascular permeability, leukocyte trafficking, and microthrombi. Heparin inhibits thrombin activity, protects endothelial cells from oxidative stress, and may modulate the cytokine storm [17].

Taken together, this evidence suggests that biologics have the potential to broaden the spectrum of treatment options for coronavirus-induced diseases. Prior knowledge and experience with the SARS and MERS outbreaks provide potential strategies for developing new target-specific therapeutic agents. However, COVID-19 has peculiar characteristics, and no strategies have demonstrated clear benefit to date; costs are also a concern.

Table 1 and Figure 1 summarize the current studies ongoing, and the mechanisms implicated in the treatment of COVID-19.

In conclusion, the main mechanism involved in the pathogenesis of COVID-19 appears to be activation of the inflammatory cascade with a massive pro-coagulable state, which can lead to rapid deterioration including potentially fatal secondary conditions (e.g., thromboembolic events, superinfections). In our opinion, 'common' drugs such as steroids and heparin should be used routinely to modulate inflammation and coagulability. The possibility of superinfection is a concern with corticosteroid therapy, while increased risk of bleeding may be seen with unfractionated or low-molecular-weight heparin. Strict monitoring of inflammatory (C-reactive protein, lymphocyte count, IL-6, ferritin, procalcitonin, etc.) and coagulation parameters (antithrombin III, prothrombin, D-dimer, fibrinogen, international normalized ratio, activated partial thromboplastin time, activated factor X) must be enforced and used to guide daily therapeutic management.

2. Expert opinion

The pandemic of novel coronavirus disease 2019 (COVID-19) has posed significant therapeutic challenges. High fatality rates

have been observed in COVID-19 patients who require mechanical ventilation. Therefore, there has been growing interest in the development of new therapies capable of improving outcomes. At present, there is no evidence from randomized-controlled trials to support any therapeutic strategy as effective for COVID-19. Extensive studies have evaluated different potential strategies; however, to date, no treatment had improved outcomes or reduced fatality rates. Several drugs, including RAAS inhibitors (losartan, recombinant soluble ACE2), aminoquinolines (hydroxychloroquine/chloroquine), antivirals (remdesivir, favipiravir), immunomodulators (steroids, anti-interleukin agents, intravenous immunoglobulin, mesenchymal stromal cells), and antithrombotics (heparin) have been studied. These studies have had several limitations, which seem to be associated with: 1) a lack of understanding of the pathophysiological mechanisms of SARS-CoV-2-induced pneumonia; 2) the difficulty of translating *in vitro* findings into clinical practice; and 3) the lack of proper clinical study designs to elucidate optimal dosage, timing, and population. SARS-CoV-2 is still a poorly known virus, and a better understanding of its transmission mechanisms, clinical spectrum of disease, diagnosis, and fatality rates is urgently needed. Future investigations should focus on new drugs targeting the pathophysiological pathways implicated in COVID-19. Moreover, several therapies which have been investigated may lead to more adverse effects than benefits. Evidence suggests there are different phenotypes of COVID-19, potentially distinguishable through distinct biomarkers, which may respond differently to treatments. Therefore, personalized approaches to COVID-19 management should be recommended, including the use of 'common' drugs such as heparin and steroids as part of daily clinical management. While we await the development of an effective vaccine, clinical trials should help define the best strategy to treat COVID-19 [18,19,20].

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Declaration of interest

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