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IM - REVIEW

# COVID-19 treatment options: a difficult journey between failed attempts and experimental drugs

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**Abstract**
Since its outbreak in China in December 2019 a novel Coronavirus, named SARS-CoV-2, has spread worldwide causing many cases of severe pneumonia, referred to as COVID-19 disease, leading the World Health Organization to declare a pandemic emergency in March 2020. Up to now, no specific therapy against COVID-19 disease exists. This paper aims to review COVID-19 treatment options currently under investigation. We divided the studied drugs into three categories (antiviral, immunomodulatory and other drugs). For each molecule, we discussed the putative mechanisms by which the drug may act against SARS-CoV-2 or may affect COVID-19 pathogenesis and the main clinical studies performed so far. The published clinical studies suffer from methodological limitations due to the emergency setting in which they have been conducted. Nevertheless, it seems that the timing of administration of the diverse categories of drugs is crucial in determining clinical efficacy. Antiviral drugs, in particular Remdesivir, should be administered soon after symptoms onset, in the viraemic phase of the disease; whereas, immunomodulatory agents, such as tocilizumab, anakinra and steroids, may have better results if administered in pneumonia/hyperinflammatory phases. Low-molecular-weight heparin may also have a role when facing COVID-19-related coagulopathy. Up to now, treatment choices have been inferred from the experience with other coronaviruses or viral infection outbreaks. Hopefully, in the near future, new treatment strategies will be available thanks to increased knowledge on SARS-CoV2 virus and COVID-19 pathogenesis. In the meanwhile, further well-designed clinical trials are urgently needed to establish a standard of care in COVID-19 disease.

**Keywords** Antiviral drugs · Low-molecular-weight heparin · Remdesivir · SARS-CoV2 · Therapy · Tocilizumab

## Introduction

In December 2019, an outbreak of pneumonia cases of unknown origin, epidemiologically linked to the attendance of Wuhan Central Marketplace in Hubei province in China, was reported for the first time. The aetiological agent of this novel pneumonia has been named severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) and the name 2019 coronavirus disease (COVID-19) has been coined for the disease itself. Due to the rapid spread of the infection across all the continents, a pandemic emergency has been declared by the World Health Organization (WHO) in March 2020.

SARS-CoV-2 is an enveloped, non-segmented, positive-sense single-stranded ribonucleic-acid (RNA) β-coronavirus, which shares 80% sequence homology with SARS-CoV-1 and 50% sequence homology with Middle East Respiratory Syndrome (MERS)-CoV. Like SARS-CoV-1, SARS-CoV-2 cell entry depends on binding of the viral spike protein to Angiotensin-Converting Enzyme-2 (ACE-2) receptor of lower respiratory tract cells and on spike protein priming by the trans-membrane serine protease 2 (TMPRSS2).

Even if the majority of infected people develops smooth symptoms, a considerable number progresses to pneumonia with severe respiratory failure requiring hospitalization, and a minority develops life-threatening complications with poor prognosis. Older age and comorbidities (mainly arterial

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hypertension, followed by diabetes and ischemic heart disease) are the most significant risk factors associated with the worst outcomes [1]. To date, no specific therapy against COVID-19 disease exists; many trials are ongoing with different treatment options but further time will be necessary to develop an effective therapy.

COVID-19 disease's clinical course can be divided into three phases. The first one, the "viraemic phase", is characterized by fast viral replication and symptoms are caused by direct cytopathic effect. This phase lasts 7–8 days and is accompanied by mild and often non-specific symptoms, such as fever, malaise, myalgia, headache, dry cough, conjunctival congestion, anosmia and ageusia, abdominal pain and diarrhoea. In patients who are able to control the infection, prognosis and recovery are excellent. The second phase, named "acute or pneumonia phase", generally occurs after 7–8 days from the symptoms onset and is characterized by high fever, cough and dyspnoea, requiring close observation and management. At this stage, patients develop a frank viral pneumonia, with imaging showing bilateral infiltrates or ground-glass opacities, and blood tests revealing lymphocytopenia and increased lactate dehydrogenase (LDH) and C-reactive protein (CRP) levels. The patient's immune system begins to develop a reaction that, if balanced, leads to the final healing, if exaggerated, brings to the "phase of complications". This third phase, which is experienced by a minority of COVID-19 patients, is characterized by extrapulmonary systemic hyperinflammatory syndrome, also referred to as cytokine storm, due to an over-reaction of the immune system with over-production of pro-inflammatory cytokines and other mediators [1, 2]. Acute respiratory distress syndrome (ARDS) is the most frequent complication (20% of the patients) followed by acute heart failure, acute kidney injury (AKI), liver failure, shock, secondary bacterial infections, coagulopathies and disseminated intravascular coagulation (DIC). Notably, 20% of COVID-19 patients, and nearly 100% of the critical ones, develop coagulation disorders, in particular a hypercoagulable state which predisposes to DIC. Indeed, micro- and macro-thrombi in lungs and extrapulmonary districts have been described in several autopsy series, suggesting that thrombosis and inflammation are two processes that reinforce each other in the pathogenesis of COVID-19 complications [3].

Several innate immune signalling proteins are targeted by SARS-CoV-2 viral proteins, among all, Interferon (IFN) and Nf-Kb pathways. In particular, SARS-CoV-2 seems to be able to activate NLRP3 (NOD like receptor pyrin domain-containing 3) inflammasome causing the abnormal production of pro-inflammatory cytokines. The cytokine storm is induced by the activation of large numbers of white blood cells, including B cells, T cells, natural killer (NK) cells, macrophages, dendritic cells, neutrophils, monocytes, and resident tissue cells, such as epithelial and endothelial cells, which release high amounts of pro-inflammatory cytokines. Among the numerous molecules that increase in serum in a cytokine storm, complements, IFN-γ, interleukin (IL)-1β, IL-6, IL-12, and IL-17, and tumor necrosis factor-α (TNF-α) are of crucial importance. An Italian study found an increased capability of cluster of differentiation (CD)4 + or CD8 + T cells from COVID-19 patients with pneumonia to produce in vitro IL-17, that is able to strengthen the inflammation response and to activate neutrophils [4]. Pathway analysis of peripheral blood mononuclear cells transcriptome revealed that COVID-19 patients lymphopenia may be associated with activation of apoptosis and P53 signalling pathway in lymphocytes, in which SARS-CoV-2 seems to enter through the CD147 receptor. Virus-activated "cytokine storm syndrome" is a common feature of severe COVID-19 cases, a major reason for ARDS and multiorgan failure, and the main cause of mortality in COVID-19 disease [5].

Understanding COVID-19 disease pathogenesis is important to make the best treatment choices. For instance, antiviral drugs are likely more useful in the phases ruled by the direct cytopathic effect of SARS-CoV-2; whereas, at the later stages of COVID-19 disease, the treatment options to be theoretically considered more appropriate would be the ones which affect the immune response, such as corticosteroids and immunosuppressive/immunomodulatory agents [1, 2]. However, the pathogenic features of COVID-19 disease are yet far from being completely elucidated and no validated specific therapeutic options exist.

This paper intends to review COVID-19 treatment options which are currently under investigation. For each drug deemed to be potentially effective on COVID-19 disease, firstly we discuss the putative mechanisms by which the drug may act against SARS-CoV-2 or may affect COVID-19 pathogenesis, then we comment on the major clinical studies among COVID-19 patients involving the particular drug.

All the relevant studies were independently retrieved by two researchers by interrogating Pubmed and Google Scholar databases, using the following search strategies: "COVID-19 Treatment OR Therapy" and each single drug under investigation for COVID-19 treatment; attention has been paid to "cytokine storm in COVID-19" and "coagulation and COVID-19". The bibliographic research has been conducted until July 24, 2020.

## Antiviral drugs

The most important antiviral drugs tested in the COVID-19 pandemic are detailed below. Table 1 describes the main clinical studies on antiviral drugs for treating COVID-19 patients.

**Table 1** Main clinical studies testing antiviral drugs for the treatment of COVID-19 disease

| Drugs | References | Study type (ST), population (P) and dosage (D) | Results | Adverse effects (AE) | Limitations/comments |
|---|---|---|---|---|---|
| Lopinavir/ritonavir (LPN/r) | Cao et al. *NEJM* 2020 [6] | ST: randomized controlled open-label clinical trial<br>P: 199 severe COVID-19 pneumonia patients<br>D: 99 patients: LPN/r 400/100 mg twice daily for 14 days + supportive therapy vs. 100 patients: supportive therapy alone | Treatment with LPV/r was not associated with a better survival rate or with faster clinical improvement (HR 1.24, 95% CI 0.90–1.72). 28-day mortality and time to negative swabs were similar in the two groups | Gastro-intestinal symptoms (nausea, vomiting and diarrhoea). 13 patients discontinued the treatment due to AE | All the recruited patients had severe pneumonia and started antiviral therapy very late after symptoms onset (12–14 days) |
| | Liu and Xu. *International Journal of Infectious Disease* 2020 [7] | ST: retrospective, observational, single-centre study<br>P: 10 patients with moderate COVID-19 pneumonia<br>D: LPV 400 mg twice a day + nebulized Interferon α2b 5 mln UI twice a day | Positive effects on viral clearance, symptoms and imaging | Gastro-intestinal symptoms (nausea, vomiting, diarrhoea and hypokalemia). 3 patients discontinued the treatment | Small sample size, no case–control, short term follow-up<br>The drug seemed to be effective if administered early |
| | Deng et al. *Journal of Infection* 2020 [8] | ST: retrospective cohort study<br>P: 33 patients with moderate COVID-19 pneumonia<br>D: LPN/r 400/100 mg twice daily with or without Umifenovir (200 mg every 8 h) for 5–21 days | Superiority of the combination therapy (LPN/r + Umifenovir) in decreasing viral load with negative nasopharyngeal swabs after 7 days of therapy (75% vs. 35%, $p < 0.05$) and in improving CT imaging (69% vs. 29%, $p < 0.05$) | Increased levels of bilirubin; gastro-intestinal symptoms (nausea, vomiting and diarrhoea). No treatment discontinuation due to AE | Small sample size, no randomization, possible selection bias. Variable additional treatments in the two groups |
| | Hung et al. *Lancet* 2020 [9] | ST: multicentre, prospective, open-label, randomized phase 2 trial<br>P: 127 moderate COVID-19 pneumonia patients<br>D: 86 pts: LPN/r 400/100 mg for 14 days + ribavirin 400 mg twice daily for 14 days + Interferon β1b 8 mln UI on alternate days for 3 times maximum vs. 41 pts: LPN/r alone | The combination therapy group had a significantly shorter time to negative nasopharyngeal swabs (7 vs. 12 days; HR 4.37, 95% CI 1.86–10.24, $p = 0.001$). The significant viral response was also associated with clinical improvement | Nausea and diarrhea (no differences between treatment groups); mild and self-limited liver dysfunction. 1 patient in the control group discontinued LPN/r because of biochemical hepatitis. No deaths | Open-label trial without a placebo group; variable use of Interferon β1b according to time from symptoms onset<br>Patients were treated early after symptoms onset (5 days) |

**Table 1** (continued)

| Drugs | References | Study type (ST), population (P) and dosage (D) | Results | Adverse effects (AE) | Limitations/comments |
|---|---|---|---|---|---|
| Darunavir/cobicitstat (DAR/COB) | ClinicalTrials.gov Identifier: NCT04252274 | ST: ranzomized interventional clinical trial<br>P: 30 patients, still recruiting in China<br>D: Dar/Cob 800/150 mg once/day for 5 days + standard therapy vs. standard therapy alone | Outcomes: virological clearance rate of throat swabs, sputum, or lower respiratory tract secretions at day 7; adverse events and mortality at week 2 after end of treatment | Not available | Small sample size |
| Remdesivir | Wang et al. *Lancet* 2020 [11] | ST: Multicentre, randomized controlled double-blind clinical trial (ClinicalTrials.gov NCT04257656)<br>P: 237 patients with severe pneumonia<br>D: 158 patients: Remdesivir 200 mg IV day 1 and 100 mg IV from day 2 to day 10 + supportive therapy vs. 79 patients: placebo + supportive therapy | No significant differences in time to clinical improvement (HR 1.23, 95% CI 0.87–1.75) or 28-day mortality. Faster time to clinical improvement only among patients with symptoms duration of ten days or less (HR 1.52, 95% CI 0.95–2.43). No significant reduction of SARS-CoV2 RNA load or detectability in upper respiratory tract or sputum specimens | Treatment discontinuation because of AE (nausea, vomiting, increased liver enzymes, rash) more frequent in Remdesivir group (12% vs. 5%) | Target enrolment was not reached; patients were enrolled at late stages of disease |
| | Grein et al. *NEJM* 2020 [15] | ST: Case series on compassionate use of remdesivir<br>P: 61 patients with severe COVID-19 pneumonia<br>D: 200 mg IV day 1 and 100 mg IV from day 2 to day 10 | Improvement in oxygen support class (in ventilated patients) and in general conditions (in all classes of enrolled patients) | Increased liver enzymes, diarrhoea, rash, renal impairment and hypotension. 4 patients discontinued the therapy because of major AE | Treatment was started 12 (9–15) days after symptoms onset; patients enrolled were severe/critical with an overall mortality of 13% |
| | Holshue *NEJM* 2020 [16] | ST: Case report<br>P: a 35 years old male patient<br>D: Not available | Fast improvement of symptoms and imaging | No AE | The drug was started 7 days after symptoms onset |

**Table 1** (continued)

| Drugs | References | Study type (ST), population (P) and dosage (D) | Results | Adverse effects (AE) | Limitations/comments |
|---|---|---|---|---|---|
| | Beigel et al. *NEJM* 2020 [17] | ST: adaptive, randomized, double-blind, placebo-controlled trial; ADAPTIVE COVID-19 TREATMENT TRIAL (ACTT)<br>P: 1059 hospitalized patients affected by severe (88.7%) and moderate (11.3%) COVID-19 pneumonia<br>D: 538 patients: Remdesivir: 200 mg on day 1, followed by 100 mg daily for up to 9 additional days + standard of care vs. 521 patients: placebo for 10 days + standard of care | 31% faster time to recovery (11 vs. 15 days; HR 1.32, 95% CI 1.12–1.55; $p < 0.001$) with remdesivir. The benefit was most apparent in patients requiring oxygen. Mortality by 14 days were 7.1% with remdesivir and 11.9% with placebo (HR 0.70, 95% CI 0.47–1.04) | No significant differences in serious AE. Anemia, acute kidney injury, hyperglycemia and increased liver enzymes were the most common AE. No deaths related to treatment | Incomplete data: at the writing time 132 patients in the remdesivir group and 169 in the placebo group had not recovered and had not completed the day 29 follow-up visit<br>Treatment was started 9 (6–12) days after symptoms onset |
| | Goldman et al. *NEJM* 2020 [18] | ST: randomized, open- label, phase 3 trial<br>P: 397 hospitalized severe COVID-19 patients not requiring mechanical ventilation<br>D: 200 patients: Remdesivir IV 200 mg on day 1, then 100 mg/day for 5 days vs. 197 patients: Remdesivir IV 200 mg on day 1, then 100 mg/day for 10 days | Similar clinical status at day 14 in the two groups after adjustment for baseline features | Serious AE more common in 10-day group (21% Vs. 35%); acute respiratory failure was the most common serious AE. The most common AE were nausea, worsening of respiratory failure, elevation of liver enzymes and costipation | At baseline, patients assigned to the 10-day group had significantly worse clinical status than the 5-day group ($p = 0.02$). No placebo group; clinical heterogeneity of enrolled patients |
| Favipiravir | Chen *MedRxiv* 2020 [19] | ST: Prospective, multicentre, open-label, randomized clinical trial<br>P: 236 patients; 116 pts: standard therapy + Favipiravir; 120 patients: standard therapy + umifenovir<br>D: favipiravir: 1600 mg twice on day 1 and 600 mg twice daily from day 2 to day 14 for 7 days; Umifenovir: 200 mg 3 times daily for 7 days | Favipiravir slightly more effective in improving clinical signs | The most frequent AE due to favipiravir were psychiatric and gastro-intestinal symptoms, increased uric acid and liver enzymes | Concerns have been raised because of the study design, the inclusion criteria and the distribution of the stages of disease severity in the different treatment groups |

**Table 1** (continued)

| Drugs | References | Study type (ST), population (P) and dosage (D) | Results | Adverse effects (AE) | Limitations/comments |
|---|---|---|---|---|---|
| | Quingxiang et al. *Engineering* 2020 [21] | ST: Open-label control study<br>P: 80 patients with mild/moderate COVID-19 pneumonia. 35 patients: favipiravir + nebulized interferon alfa + standard therapy vs. 45 patients: LPN/r + nebulized interferon alfa + standard therapy<br>D: favipiravir 1600 mg twice on day 1 and 600 mg twice daily from day 2 to day 14; nebulized Interferon alfa 5 milions UI two times/day for 14 days; LPV/r 400/100 mg twice daily for 14 days | Favipiravir more effective in improving CT imaging (91.4% vs 62.2%) and viral clearance at 14 days | AE less common in favipiravir group (diarrhoea, liver enzymes alterations and weight loss) | Little sample size; not randomized and not double-blinded<br>Antiviral drugs were administered in 7 days from symptoms onset |
| Umifenovir | Lian et al. *Clinical Microbiology and Infection* (2020) [23] | ST: retrospective case–control study<br>P: 81 patients with moderate and severe COVID-19 disease<br>D: 45 patients: umifenovir + standard therapy vs. 36 patients: standard therapy alone | Umifenovir treatment was not associated with a faster virus clearance after 7 days from admission (73% Umifenovir vs 78% Controls, $p = 0.19$) nor with a faster symptoms' resolution (18 vs 16 days, $p = 0.42$). It was indeed associated with a longer hospital stay (13 vs 11 days, $p = 0.04$) | Digestive symptoms, including diarrhoea and nausea (not different from control group). No patients discontinued treatment because of AE. No severe impairment of liver and kidney function was observed | Little sample size; not randomized and not double-blinded |

### Lopinavir/Ritonavir (LPV/r)

Lopinavir is a protease inhibitor used for the treatment of Human Immunodeficiency Virus (HIV) infection. Lopinavir binds HIV protease and prevents the Gag/Pol polyprotein cleavage with the consequent production of immature non-infectious viral particles. Ritonavir enhances lopinavir plasmatic concentration by inhibiting cytochrome P450 3A isoform. The drug demonstrated inhibitory activity against SARS-CoV and MERS-CoV in vitro and in animal models. Moreover, a 2004 open-label study showed that the association LPV/r with ribavirin compared to ribavirin alone had a positive effect on ARDS development and mortality in patients with SARS-CoV severe pneumonia [6]. On this basis, LPV/r has been tested since the beginning of the pandemic. As shown in Table 1-LPN/r section, the studies on LPV/r carried out up to now present several criticisms, notably including small samples sizes and poor study design. Nevertheless, it is interesting to observe that in the single available randomized controlled clinical trial LPV/r therapy has not proven effectiveness in patients with severe disease and was not superior to standard therapy when started 12 days or later after symptoms onset. In the few studies evaluating LPN/r in association with other drugs, such as Umifenovir, Ribavirin and Interferons, combination therapies seem to perform better than LPN/r alone [6–9]. Further studies with a better study design on larger populations are urgently needed to establish if LPN/r could be an effective therapeutic option for COVID-19 disease and to evaluate the appropriate timing of administration of this drug.

### Darunavir/Cobicistat (DAR/COB)

Darunavir is a second-generation protease inhibitor used for HIV therapy. It is associated with cobicistat, which increases its plasma concentration by inhibiting cytochrome P450 3A4 isoform. DAR/COB is an alternative to treatment with LPV/r when the latter is not tolerated because of intestinal side effects [10]. In Table 1-DAR/COB section, we reported the design of a clinical trial testing DAR/COB in China.

### Remdesivir

Remdesivir is an adenosine analogue, which is included in nascent viral RNA chains resulting in premature termination. Formerly evaluated for the treatment of Ebola Virus infection, Remdesivir is a promising broad-spectrum antiviral drug active against a wide range of RNA viruses, including SARS-CoV and MERS-CoV in cultured cells, mice and non-human primate models. The drug seems to reduce viral load in lung tissue in SARS-CoV pneumonia in mice, leading to improvement in ventilatory function and healing of the damaged tissue [11]. It is worthy to acknowledge, however, that when the drug was administered after peak viral replication with airway epithelium damage already occurred, it did not improve survival and healing. This indicates that Remdesivir should be administered at the early stages of the disease [12]. Accordingly, Remdesivir reduced clinical signs, pulmonary lesions and viral replication if administered early after infection in a non-human primate model infected by SARS-CoV2 [11]. Since SARS-CoV and SARS-CoV2 RNA-dependent RNA polymerase (RdRp) share 96% sequence identity, it could be hypothesized that drugs targeting viral RdRp proteins of SARS-CoV can be effective on SARS-CoV2 too [13]. Indeed, Wang et al. demonstrated that, compared to other antiviral drugs, Remdesivir contrasts and controls SARS-CoV2 infection in vitro at lower micromolar concentrations with a very high selectivity index [14]. The Food and Drug Administration has authorized Remdesivir compassionate use for the treatment of adults and children with severe COVID-19 disease who do not respond to other treatments [15]. Holshue was first in reporting the case of a young man with COVID-19 disease, in which the treatment with Remdesivir started 7 days after symptoms onset, was effective in reducing radiological involvement and improving symptoms [16]. A case series of 61 patients treated with Remdesivir seemed to show drug effectiveness by improving oxygen support class and symptoms even when administered later after symptoms onset (9–12 days) in severe disease [17]. However, when tested in larger populations, the results seemed to be more conflicting. In a randomized double-blind trial no differences were noted in 28 days mortality and clinical improvement in patients treated with Remdesivir compared with subjects treated with placebo; it should be acknowledged that this study was performed on patients with advanced disease and didn't reach its enrolment target [13]. Conversely, a larger study on a population of 1059 hospitalized COVID-19 patients demonstrated that Remdesivir started at an advanced disease stage was superior compared to standard therapy in time to recovery and mortality; however, albeit statistically significant, the results of Remdesivir were only slightly better than placebo. Notably, since at the time of article submission a large amount of patients was still hospitalized, the data of this study were incomplete [17]. Finally, a study that compared 5-day versus 10-day Remdesivir administration failed to demonstrate better clinical outcomes depending on Remdesivir therapy duration; moreover, a higher burden of side effects was observed among patients in the 10-day therapy group [18]. These studies, taken together, seem to suggest that Remdesivir is the most effective antiviral drug available so far; for these reasons the Italian drug regulatory agency (AIFA) authorized Remdesivir administration in adult patients affected by severe COVID-19 disease. Since all the studies testing Remdesivir in COVID-19 disease up to now are based on patients with severe disease in an advanced

stage, it would be useful to evaluate the drug activity on moderate COVID-19 patients, administering the molecule soon after symptoms onset to observe if, in these categories of subjects, better results and an higher efficacy could be reached. The main studies are reported in Table 1-Remdesivir section.

### Favipiravir

Favipiravir is a new generation RdRp inhibitor active against a wide range of viruses. Its use, which is hampered by significant side effects (teratogenicity and suicide induction), is authorized in Japan, but not in Europe and USA, for influenza treatment when other antivirals are not effective [19]. Several studies with Favipiravir among COVID-19 patients have been authorized by regulatory agencies, many of which are still ongoing [20]. The available clinical trials are based on small populations, are not randomized and not double-blinded. Moreover, groups features were heterogeneous, making it hard to draw conclusions on its effect on COVID-19 disease. In the two studies reported in Table 1—Favipiravir section, it seemed slightly more effective than LPN/r and Umifenovir in improving imaging, viral clearance and clinical signs [19, 21].

### Umifenovir

Umifenovir is a small indole-derived molecule developed in Russia and approved in Russia and China for prophylaxis and treatment of influenza and other respiratory viral infections. It is able to block viral fusion with the cell membrane in Influenza A and B viruses. Umifenovir and its derivate Umifenovir mesylate had been reported to have antiviral activity against SARS-CoV in cell cultures [22]. A retrospective study based on a small population of patients affected by moderate and severe COVID-19 disease failed to observe significant differences in viral clearance and symptoms resolution between Umifenovir and standard therapy [23]. The limited evidence regarding the use of this drug on COVID-19 patients makes it impossible to draw conclusions.

## Immunomodulatory drugs

The main immunomodulatory drugs are reported below and the major studies on these molecules are detailed in Table 2.

### Chloroquine (CQ) and hydroxychloroquine (HCQ)

CQ and HCQ are used as antimalarial drugs. However, they have a very broad spectrum of action being effective against bacteria, fungi, protozoa and viruses. HCQ safety profile is better than that of CQ, therefore the former should be preferred as it gives the possibility to maintain higher doses for a longer time. CQ and HCQ are effective in vitro against diverse RNA and DNA viruses and inhibit in vitro replication of SARS-CoV229E in lung epithelial cells. CQ and HCQ have multiple mechanisms of action. Firstly, they are able to interfere with cell surface receptors. Specifically, HCQ inhibits sialic acid synthesis. Sialic acid is a monosaccharide located at the end of the sugar chains bound to transmembrane signaling proteins and is a critical component for ligand recognition. Avoiding this fundamental path, the drug interferes with the virus entry. Moreover, these drugs affect cell receptor glycosylation. In SARS-CoV in vitro studies, HCQ glycosylates ACE-2 receptor resulting in failure of recognition, binding and entry of the virus in the cell. HCQ also alkalizes the lysosomal pH. To facilitate the fusion of the virion with the cell, the lysosomal pH should be acid; an alkaline pH inhibits this path. Finally, HCQ can increase the intracellular pH and inhibit lysosomal activity in antigen-presenting cells preventing antigen processing and major histocompatibility complex (MHC) class II autoantigen presentation to T cells. As a consequence, the drug reduces T cell's activation, differentiation and expression of co-stimulatory proteins e.g. CD154 on CD4 + T cells and cytokines production from T and B cells (IL-1, IL-6 and TNF). Even the production of type I Interferons is attenuated [24].

Several studies have been performed to assess the efficacy of these molecules on COVID-19 patients and many other studies are still ongoing (see Table 2—CQ/HCQ section). Notably, CQ and HCQ have been included in the Guidelines for the Prevention, Diagnosis, and Treatment of Pneumonia Caused by SARS-CoV2 in China [25]. However, the results of CQ and HCQ for COVID-19 treatment are contradictory; some studies showed a clinically significant improvement in patients treated with CQ or HCQ with respect to untreated patients, while others did not show differences between the groups [25–33]. A group of French investigators tested the efficacy of HCQ in association with Azithromycin in mild-moderate COVID-19 patients soon after symptoms onset, in two different studies, showing a clear effectiveness of the above-cited molecules compared with standard therapy [26, 27]. The first study was severely criticized due to design faults and incomplete data [26]; the second one, even if followed by a detailed analysis, still had a small sample size [27]. A larger French clinical trial on a population of 1061 COVID-19 patients confirmed clinical improvement and faster viral clearance in patients treated with HCQ and Azithromycin. It should be highlighted that this study only enrolled patients with mild/moderate disease or asymptomatic subjects with a positive swab; moreover, treatment was started very early after symptoms onset [28]. After these studies, many others showed no clear benefits of HCQ and CQ therapy in COVID-19 patients. Some trials on

**Table 2** Main clinical studies testing immunomodulatory drugs for the treatment of COVID-19 disease

| Drugs | References | Study type (ST), population (P) and dosage (D) | Results | Adverse effects (AE) | Limitations/comments |
|---|---|---|---|---|---|
| Chloroquine (CQ)/ hydroxy-chloroquine (HCQ) | Gao et al. Letter to the editor, BioscienceTrend 2020 [25] | ST: case series<br>P: 100 Chinese patients with COVID-19 pneumonia<br>D: no data | CQ superior to supportive therapy alone in preventing pneumonia exacerbation, improving lung imaging and viral clearance and shortening the disease course | No significant side effects | No information about patients clinical conditions, disease severity and HCQ/CQ doses |
| | Chen et al. *Journal of Zhejiang university (Medical Science)* (2020) [29] | ST: pilot, randomized study<br>P: 30 patients with COVID-19 pneumonia<br>D: HCQ 400 mg/day for 5 days + supportive treatment vs supportive treatment alone | Inefficacy of HCQ in diminishing the viral load, fever and improving chest imaging | No data | Small sample size; heterogeneous population |
| | Gautret et al. *International Journal of Antimicrobial Agents* (2020) [26] | ST: open-label, non-randomized clinical trial<br>P: 35 patients with initial/moderate COVID-19<br>D: 20 patients: HCQ 200 mg 3 times/day for 10 days + azithromycin 500 mg on day 1, then 250 mg from day 2 to day 5 (in 6 patients) vs. 15 patients: control group treated only with supportive therapies | HCQ was associated with an increased viral clearance at day 6 (70% vs. 12.5%). All patients treated with HCQ + azithromycin underwent a complete viral clearance | No data | Small sample size; no intention to treat analysis; no analysis of clinical benefit; short term follow-up. 6 patients have been excluded from the treatment group for precipitation of clinical conditions |
| | Gautret *Travel Medicine and Infectious Disease* (2020): 101663 [27] | ST: non-controlled observational study<br>P: 80 relatively mild COVID-19 patients<br>D: HCQ 200 mg three times a day for 10 days and azithromycin 500 mg on day 1 and then 250 mg daily until day 5 | The majority of treated patients improved clinically. Fast viral clearance | Rare and minor AE: nausea, diarrhoea and blurred vision. Only 1 treatment discontinuation due to potential risk of interactions | Early treatment after symptoms onset (5 days)<br>Limitations: small sample size; no comparison with a placebo group |

**Table 2** (continued)

| Drugs | References | Study type (ST), population (P) and dosage (D) | Results | Adverse effects (AE) | Limitations/comments |
|---|---|---|---|---|---|
| | Million et al. *Travel Medicine and Infectious Disease* (2020): 101738 [28] | ST: retrospective report<br>P: 1061 COVID-19 patients (from asymptomatic to moderate disease)<br>D: HCQ 600 mg twice on day 1, then 400 mg daily for a median of 5 days + azithromycin 500 mg on day 1 and 250 mg from day 2 to 5 | HCQ and Azithromycin at early stage of COVID-19 disease resulted safe and associated with a very low fatality rate (0.9%). Good clinical outcome and virological cure were obtained in 91.7% of the patients within 10 days. Poor clinical outcomes associated with older age, severity of illness at presentation, low HCQ serum concentration, use of β-blockers and angiotensin II receptor blockers | Mild and rare AE: diarrhoea, abdominal pain, nausea, insomnia, transient blurred vision, urticaria and bollous rash. No deaths due to cardiac toxicity | Treatment started early after symptoms onset (6.4 ±3.8 days)<br>Some data were incomplete (CT scans and serum drug levels were not available for all patients); heterogeneous treatment duration |
| | Magagnoli et al. *Medrxiv* (2020) [30] | ST: retrospective analysis<br>P: 368 hospitalized patients with COVID-19 disease in all US Veterans Health administration medical centres. Three study arms: 97 patients treated with HCQ alone vs. 113 patients treated with HCQ + azithromycin vs. 158 patients treated with standard supportive therapy<br>D: no data about doses | Statistically significant increased mortality from any cause in HCQ group but not in HCQ + azithromycin group. No significant differences in the mechanical ventilation rate in the 3 groups | No data | Heterogeneous distribution of patients severity among treatment groups: severe/critical cases more frequently treated with HCQ or HCQ + azithromycin. Treatment was started late after symptoms onset |
| | Borba et al. *JAMA Network Open* 3.4 (2020): e208857 [31] | ST: parallel, double masked, randomized phase-2b clinical trial (CloroCOVID-19)<br>P: 81 patients hospitalized for severe/critical COVID-19 pneumonia<br>D: 40 patients: Low dose CQ (450 mg twice daily on day 1 and once daily for 4 days) vs. 41 patients: High dose CQ (600 mg twice daily for 10 days) | High-dose CQ arm presented more QTc > 500 ms (25%) and a trend toward higher lethality (17%) than low-dose CQ arm. Fatality rate was 13.5% (95% CI 6.9–23.0%), overlapping with historical data from similar patients not using CQ | In high-dose CQ group: CK and CK-MB elevation, QTc prolongation > 500 ms; 2 patients with ventricular tachycardia<br>In both groups: Hb reduction and creatinine elevation | Patients were included in the trial before laboratory confirmation, regardless of confirmed aetiology. Older age and heart disease were more prevalent in high-dose CQ group. The limited sample size did not allow the study to show any benefit regarding treatment efficacy |

**Table 2** (continued)

| Drugs | References | Study type (ST), population (P) and dosage (D) | Results | Adverse effects (AE) | Limitations/comments |
|---|---|---|---|---|---|
| | Geleris et al. *New England Journal of Medicine* (2020) [32] | ST: observational case–control study<br>P: 1376 patients hospitalized for severe COVID-19 pneumonia<br>D: 811 patients: HCQ 600 mg twice on day 1, then 400 mg daily for a median of 5 day vs. 565 patients: supportive and symptomathic therapies | No significant association between HCQ use and intubation or death (HR 1.04, 95% CI 0.82–1.32) | No data | HCQ-treated patients were more severely ill than untreated patients. All patients had an advanced disease and started therapy late after symptoms onset. Critically ill patients from both groups were also treated with remdesivir, tocilizumab and antibiotic agents |
| | Mehra et al. *Lancet* 2020 [33] | ST: multinational registry analysis<br>P: 96,032 patients hospitalized for COVID-19 pneumonia<br>D: 14,888 patients: treatment groups [3016 HCQ (median dose 596 mg for 4.2 day), 1868 CQ (765 mg for 6.6 days), 6221 HCQ + Macrolide (597 mg for 4.3 days), 3783 CQ + Macrolide (790 mg for 6.8 days)] vs. 81,144 patients: control group (standard of care) | Higher in-hospital mortality rates in each treatment group ($p < 0.0001$): control group (9.3%), HCQ (18%; HR 1.335, 95% CI 1.223–1.457), HCQ + macrolide (23.8%; 1.447, 1.368–1.531), CQ (16.4%; 1.365, 1.218–1.531), CQ + macrolide (22.2%; 1.368, 1.273–1.469) | All treatment groups were independently associated with an increased risk of de-novo ventricular arrhythmia during hospitalization | Observational study design. QT intervals were not measured and the arrhythmia pattern was not stratified. A drug dose–response analysis of the observed risks was not conducted |
| | Boulware et al. *New England Journal of Medicine* (2020) [36] | ST: Randomized, double-blind, placebo-controlled trial<br>P: 821 subjects with high-risk exposure to a confirmed COVID-19 contact<br>D: 414 subjects: HCQ 800 mg once, followed by 600 mg in 6 to 8 h, then 600 mg daily for 4 additional days vs. 407 subjects: placebo | Post-exposure prophylaxis with HCQ did not affect the incidence of new illness compatible with COVID-19 or confirmed infection (11.8% vs. 14.3%; − 2.4%, 95% CI − 7.0% to + 2.2%; $p = 0.35$) | Non-serious AE: nausea, loose stools, abdominal discomfort | Full adherence to the trial intervention differed according to trial group<br>Not all the symptomatic patients received a certainty diagnosis but the disease diagnosis was assessed with a clinical algorithm |

**Table 2** (continued)

| Drugs | References | Study type (ST), population (P) and dosage (D) | Results | Adverse effects (AE) | Limitations/comments |
|---|---|---|---|---|---|
| Corticosteroids | Fang et al. *The Journal of infection* (2020) [41] | ST: retrospective, case–control clinical trial<br>P: 78 patients with moderate/severe COVID-19 disease<br>D: 25 severe patients: methylprednisolone (median dose 40 mg daily) + standard therapy vs. 53 moderate patients: standard therapy | Corticosteroids use did not delay viral clearance | No data reported | 9 patients from the moderate disease group were also treated with corticosteroids. Small sample size and heterogeneous study population |
| | Wang et al. *MedRxiv* (2020) [42] | ST: Retrospective case–control clinical trial<br>P: 46 patients with severe COVID-19 pneumonia<br>D: 26 pts: methylprednisolone 1–2 mg/kg/day for 5–7 days; vs. 20 pts: no steroids | Corticosteroids treatment was associated with faster fever resolution, more rapid improvement in oxygen saturation and imaging alterations | No major AE | Non-randomized trial; small sample size. Possible selection bias and concomitant treatments |
| | Wu et al. *JAMA Internal Medicine* (2020) [43] | ST: retrospective cohort study<br>P: 201 severe/critical patients with COVID-19 pneumonia. 84 patients developed ARDS; 62 patients received methylprednisolone<br>D: not reported | Among the subgroup of patients with ARDS, steroid treatment correlated with a reduced mortality (HR 0.38; 95% CI 0.20–0.72; $p = 0.003$) | Not reported | Single-centre study. Study design not able to prove causality |
| | Horby et al. *NEJM* (2020) [44] | ST: randomized, controlled, open-label, adaptive, platform clinical trial (RECOVERY trial)<br>P: 6425 patients hospitalized with COVID-19<br>D: 2104 patients: dexamethasone 6 mg once per day (either by mouth or by intravenous injection) for ten days vs. 4321 patients: usual care alone | Dexamethasone determined a 33% reduction in mortality rate among patients receiving invasive mechanical ventilation (29.0% vs. 40.7%, RR 0.65 [95% CI 0.51–0.82]; $p = 0.0003$) and 20% reduction in patients requiring other respiratory support (0.80, 0.67–0.96; $p = 0.0021$). There was no benefit among those patients who did not require respiratory support | Not reported | Pre-print version not peer-reviewed |

**Table 2** (continued)

| Drugs | References | Study type (ST), population (P) and dosage (D) | Results | Adverse effects (AE) | Limitations/comments |
|---|---|---|---|---|---|
| Tocilizumab (TCZ) | Xu et al. ChinaXiv: 20200300026. 2020 [45] | ST: observational clinical trial<br>P: 21 severe/critical patients with COVID-19 pneumonia<br>D: Tocilizumab 400 mg IV once | TCZ was effective with clinical, biochemical and radiological improvement in all patients | No AE | Incomplete final outcomes |
| | Colaneri et al. *Microorganisms* 8.5 (2020): 695 [46] | ST: retrospective cohort study<br>P: 112 critical COVID-19 patients<br>D: 21 patients: standard of care + TCZ 8 mg/kg IV up to a maximum of 800 mg with repetition after 12 h if needed vs. 91 patients: control group treated with standard of care alone (HCQ 200 mg bid, azithromycin 500 mg once, LMWH prophylactic and MTP 1 mg/kg/day up to a maximum of 80 mg/day) for ten days | TCZ did not significantly affect ICU admission and 7-day mortality rate when compared to standard of care | ALT elevation | Small sample size; observational study design; possible confounding effect of corticosteroids administered in both groups |
| | AIFA press release 17 June 2020 [47] | ST: randomized, controlled, multicentre, clinical trial<br>P: 252 patients with moderate COVID-19 disease<br>D: 126 patients received TCZ 8 mg/kg IV up to a maximum of 800 mg with repetition of the same dosage after 12 h + standard therapy vs. 126 patients who received standard therapy alone | Early trial interruption due to futility. The interim analysis showed that respiratory failure occurred in 28.3% of TCZ group vs. 27% of controls; no differences were seen in the ICU admission rates (10% vs. 7.9%) and mortality rates (3.3% vs. 3.2%) | No harmful AE | Press release |

**Table 2** (continued)

| Drugs | References | Study type (ST), population (P) and dosage (D) | Results | Adverse effects (AE) | Limitations/comments |
|---|---|---|---|---|---|
| | Guaraldi et al. *Lancet Rheumatology* (2020) [48] | ST: retrospective, observational cohort study<br>P: 544 patients with severe COVID-19 disease still not requiring ICU admission.<br>D: 179 patients: TCZ (8 mg/kg, maximum 800 mg administered twice 12 h apart IV or, if not available, 162 mg administered simultaneously in the thighs, for a total dose of 324 mg SC) + standard of care vs. 365 patients: standard therapy alone | After adjustment for age, sex, recruiting centre, duration of symptoms and SOFA score, TCZ resulted associated with a reduced risk of invasive mechanical ventilation or death (aHR 0.61, 95% CI 0.4–0.92; $p = 0.020$) | Higher rate of new infections with TCZ compared to control group (13% vs. 4%) | Open label study. Short follow-up |
| Sarilumab | ClinicalTrials.gov Identifier: NCT04327388 | ST: adaptive, phase 3, randomized, double-blind, placebo-controlled study<br>P: 400 hospitalized patients with severe/critical COVID-19 disease<br>D: Sarilumab 200 mg once or twice vs. placebo | Main outcomes: clinical improvement and 29-Day mortality | To be determined | Ongoing study |
| Anakinra and Emapalumab | ClinicalTrials.gov Identifier: NCT04324021 | ST: a phase 2/3, randomized, open-label, parallel group, 3-arm, multicentre study<br>D: anakinra i.v. infusion 4 times daily for 15 days (400 mg/day in total); emapalumab i.v. infusion every 3rd day for a total of 5 infusions. Day 1: 6 mg/kg. Days 4, 7, 10 and 13: 3 mg/kg vs. Standard of care | Major outcomes: clinical improvement, time to mechanical ventilation and overall survival | To be determined | Ongoing study |
| Anakinra | Pontali et al. *Journal of Allergy and Clinical Immunology* (2020) [50] | ST: case-reports<br>P: 5 patients with severe/moderate-COVID-19 disease with lung involvement<br>D: starting dose was 100 mg IV every 8 h for 24–48 h followed by tapering according to clinical response | Rapid resolution of systemic inflammation in all patients. Remarkable improvement in respiratory parameters with reduction of oxygen support requirement and early amelioration of imaging | No AE | Study design; small sample size; heterogeneity and concomitant treatment |

**Table 2** (continued)

| Drugs | References | Study type (ST), population (P) and dosage (D) | Results | Adverse effects (AE) | Limitations/comments |
|---|---|---|---|---|---|
| | Cavalli et al. *Lancet Rheumatology* (2020) [51] | ST: retrospective cohort study<br>P: 52 severe COVID-19 subjects with ARDS treated outside ICU with C-PAP<br>D: 29 patients: high dose anakinra IV (5 mg/kg twice a day until stable improvements) vs. 7 patients: low dose anakinra SC (100 mg twice a day interrupted after 7 days) vs. 16 patients: standard and supportive treatments alone (no steroids or other anti-inflammatory molecules) | High-dose anakinra was associated with reduction in serum CRP and progressive improvement in respiratory function. 21-day survival was 90% in the high-dose anakinra group and 56% in the standard treatment group ($p = 0.009$)<br>No significant effects of low-dose anakinra treatment | Bacteraemia and liver enzymes alterations | Retrospective nature; small sample size |
| Baricitinib | ClinicalTrials.gov Identifier: NCT04358614 | ST: open-label, non-randomized, clinical trial<br>P: 12 patients with moderate COVID-19 pneumonia<br>D: baricitinib oral tablets 4 mg/day + LPN/r 250 mg twice a day for 2 weeks vs. LPN/r 250 mg twice a day for 2 weeks | Outcomes: safety of Baricitinib combined with antiviral drugs, clinical outcome, ICU admission and discharge rate | To be determined | Ongoing study |
| Mavrilimumab | ClinicalTrials.gov Identifier: NCT04397497 | ST: randomized, double-blind, placebo-controlled trial (COMBAT-19 trial)<br>P: 50 patients with moderate COVID-19 pneumonia<br>D: Mavrilimumab 6 mg/kg IV once + standard therapy vs. PLACEBO 6 mg/kg IV once + standard therapy | Outcomes: safety and effectiveness of this treatment compared to standard therapy | To be determined | Ongoing study |
| Canakinumab | ClinicalTrials.gov Identifier: NCT04362813 | ST: phase 3, multicentre, randomized, double-blind, placebo-controlled trial<br>P: 150 patients with COVID-19 pneumonia<br>D: canakinumab 450 mg for body weight 40- 60 kg, 600 mg for 60–80 kg or 750 mg for > 80 kg IV single dose on Day 1 vs. standard therapy | Outcomes: efficacy and safety of Canakinumab on Cytokine Release Syndrome, clinical improvement, mortality and side effects | To be determined | Ongoing study |

**Table 2** (continued)

| Drugs | References | Study type (ST), population (P) and dosage (D) | Results | Adverse effects (AE) | Limitations/comments |
|---|---|---|---|---|---|
| Colchicine | Deftereos et al. *JAMA Network Open* 3.6 (2020): e2013136 [56] | S: prospective, randomized, open label, controlled trial (the GRECCO study)<br>P: 105 mild COVID-19 disease patients. D: 55 patients: colchicine 1.5 mg followed after 60 min by a second dose of 0.5 mg on the first day, then 0.5 mg twice a day for 3 weeks + standard therapy vs. 50 patients: standard therapy alone | Colchicine treatment was associated with significantly improved time to clinical deterioration. Colchicine was also associated with a non-significant attenuated D-dimer increase | Diarrhoea and abdominal pain. Only two patients in colchicine group had to discontinue therapy | Small sample size; open label design. The study aimed to assess whether colchicine administration could be linked to reduced myocardial events in COVID-19 disease; this could not be evaluated because of the relatively small number of cardiac events occurred during the study in both groups |
| Interferons | Hung et al. *Lancet* (2020) [9] | ST: multicentre, prospective, open-label, randomized phase 2 trial<br>P: 127 moderate COVID-19 pneumonia patients<br>D: 86 pts: LPN/r 400/100 mg for 14 days + ribavirin 400 mg twice daily for 14 days + interferon β1b 8 mln UI on alternate days for 3 times maximum vs. 41 pts: LPN/r alone | The combination therapy group had a significantly shorter time to negative nasopharyngeal swabs (7 vs. 12 days; HR 4.37, 95% CI 1.86–10.24, $p = 0.001$). The significant viral response was also associated with clinical improvement | Nausea and diarrhea (no differences between treatment groups); mild and self-limited liver dysfunction. 1 patient in the control group discontinued LPN/r because of biochemical hepatitis. No deaths | Open-label trial without a placebo group; variable use of Interferon β1b according to time from symptoms onset<br>Patients were treated early after symptoms onset (5 days) |
| | Zhou et al. *Frontiers in Immunology* 11 (2020): 1061 [61] | ST: retrospective cohort study<br>P: 77 patients with mild/moderate COVID-19 pneumonia<br>D: 7 patients: interferon-α2b (5 mU b.i.d. nebulized) vs. 24 patients: Umifenovir 200 mg tid vs. 46 patients: interferon-α2b + umifenovir | Interferon-α2b with or without Umifenovir significantly reduced the duration of detectable virus in the upper respiratory tract and significantly reduced blood levels of inflammatory markers (IL-6 and CRP) | Not reported | Non-randomized study; small sample size; unbalanced demographics between treatment arms |

small populations of severe hospitalized COVID-19 patients even showed an increased mortality in HCQ and CQ treated patients compared to untreated groups [29–33]. However, it is very difficult to draw definite conclusions since study design and heterogeneous populations were some of the many drawbacks of these studies. Recently, a multinational registry analysis on 96,032 severe/critical hospitalized COVID-19 patients was very welcomed by the scientific community since it was thought that a study on such a large population could be diriment and conclusive in understanding the real effectiveness of these two drugs. This analysis not only showed that HCQ and CQ alone and associated with macrolides were not effective in COVID-19 disease, but also that they determined an increased rate of cardiac side effects and an increased mortality rate, depicting an unsettling scenario on these drugs related to toxicity and danger [33]. The publication of this analysis was rapidly followed by the discontinuation of ongoing trials and by the prohibition of HCQ and CQ administration in COVID-19 disease patients by national regulatory agencies and WHO [34]. In a little time, many criticisms were raised against this trial, due to conflicting and missing data and doubts about veracity and accuracy of data collection; as a result, an investigation has been initiated and the study has been withdrawn [35]. Up to now, little can be affirmed with certainty from studies on HCQ and CQ; nevertheless, it seems that these molecules could be effective if administered early after symptoms onset. Further studies with a strong design and a large population need to be performed to understand the real effectiveness of these drugs in early disease stages. Recently, a clinical trial on HCQ used as prophylaxis of COVID-19 disease did not show differences between HCQ and placebo groups in preventing COVID-19 disease after a high-risk exposure [36]. Further studies are needed to understand if HCQ and CQ could be useful as pre-exposure prophylaxis in subjects at high risk (hospital staff, partners of COVID-19 positive patients, etc.).

## Corticosteroids

Corticosteroids were largely used during SARS-CoV and MERS-CoV epidemics. During SARS-CoV epidemic in 2003, systemic glucocorticoids, even at a very high dosage (> 500 mg of methylprednisolone (MTP)/day), were widely used in infected patients who developed severe respiratory disease. A cohort study during SARS-CoV outbreak showed that the use of pulsed high-dose MTP was associated with clinical improvement; however, a previous retrospective study found that the use of pulsed steroids in severe SARS-CoV pneumonia patients was associated with higher 30-day mortality. A systematic review and meta-analysis on the use of corticosteroids in hospitalized patients with community-acquired pneumonia observed a reduction in mortality and need for mechanical ventilation in treated patients. However, a study on MERS-CoV pneumonia stated that the use of steroids was associated with a delayed viral clearance [37]. Against this background, WHO and the Center for Disease Control and prevention (CDC) did not recommend the administration of steroids in COVID-19 disease, at least not in the first disease phases [38]. A Chinese consensus conference proposed to use a low-medium steroid dosage (0.5–1 mg/kg/day of MTP) for 7 days, in patients with acute inflammatory response and dyspnoea [37]. Another Chinese group recommended MTP dosage of 40–80 mg/day (to be gradually reduced) in patients with severe COVID-19 pneumonia and 80–160 mg/day (to be gradually reduced) in critical patients [39]. On the one hand, steroids have an immunosuppressive activity that determines decreased viral clearance velocity, but on the other hand they show a potent anti-inflammatory activity. The latter action is now thought to be very useful in COVID-19 since the immune-system inflammatory response and cytokine storm drive the intermediate and advanced phases of the disease [40]. Pathological findings in COVID-19 pneumonia lungs consisted of pulmonary oedema with proteinaceous exudate and hyaline membrane formation, implying that the use of corticosteroids could be useful in severe patients [39]. Since trials on the use of corticosteroids among patients with respiratory complications from other infections yielded conflicting results, it is necessary to wait for the results of new ad hoc clinical trials to draw some conclusions on the use of systemic glucocorticoids for the treatment of COVID-19 disease (see Table 2—Corticosteroids section). Up to now, studies on the use of corticosteroids in COVID-19 disease were all conducted on very small populations of severe or critical patients and showed faster time in fever resolution, faster improvement in imaging and oxygen saturation and a better survival rate in ARDS patients treated with steroids compared to untreated patients without a delay in viral clearance [41–43]. In a very recent communication, Oxford University disclosed the preliminary results of the RECOVERY trial on the use of dexamethasone in severe and moderate COVID-19 disease. The study was conducted on a large population and showed the efficacy of dexamethasone in reducing deaths especially among ventilated patients and among those receiving oxygen supplementation; no benefit was found among patients not requiring respiratory support [44]. The results of this trial emphasize the importance of steroids as a potent weapon to be used without fear in intermediate and advanced COVID-19 disease phases. More studies are certainly needed to understand which molecule is more suitable, the dosage and the timing that may contribute to achieve the best results.

### Tocilizumab (TCZ)

TCZ is a humanized monoclonal antibody against IL-6 receptor approved in Europe for the treatment of rheumatoid arthritis and Takayasu arteritis. It is also effectively used in Chimeric Antigen Receptor (CAR)-T-cell cytokine release syndrome. TCZ acts as a cytokine storm blocker, reducing the systemic inflammatory response and macrophage activation. TCZ has been considered as one of the most promising drugs for COVID-19 disease based on the results of Chinese case reports showing a fast improvement in oxygen saturation, imaging and clinical conditions shortly after the molecule administration [45]. Nevertheless, results from Italian studies are more controversial. The partial results of a first Italian study seem to disprove this efficacy rate showing no differences in ICU admission and 7 days mortality rate among treated and untreated groups [46]. A recent press release of AIFA on the results of an Italian multicentre randomized clinical trial on the early administration of TCZ in non-severe COVID-19 disease patients reported that the study was interrupted due to futility since no differences in ICU admission, respiratory failure and mortality were seen among the treated and untreated patients [47]. Conversely, a retrospective observational Italian cohort study suggested that TCZ administration in severe COVID-19 disease resulted in a significantly reduced risk of death and mechanical ventilation [48]. These studies demonstrated that TCZ is probably not useful in preventing severe complications in patients with non-severe COVID-19 disease but may have beneficial effects in subjects with severe COVID-19 disease [47, 48]. Further randomized studies are needed to assess whether the drug could be useful in specific categories of patients. The main studies on the use of TCZ in COVID-19 disease are reported in Table 2—TCZ section.

### Sarilumab

Sarilumab is another IL-6 receptor antagonist. Several trials evaluating Sarilumab as a potential therapeutic option for COVID-19 treatment are ongoing [49]. Of note, AIFA authorized a clinical trial to evaluate the efficacy and safety of Sarilumab in severe/advanced disease (see Table 2—Sarilumab section).

### Anakinra

Anakinra is a recombinant antagonist of IL-1 receptor. IL-1 is another important cytokine involved in cytokine storm. Some studies have shown that MERS-CoV virus causes pyroptosis, a highly inflammatory form of programmed cell death, with a massive release of IL-1β. Since it seems that the same phenomenon happens in SARS-CoV2 infection, blocking this cytokine could be useful. Some case reports documented the efficacy of this drug in moderate/severe COVID-19 pneumonia [50]. Moreover, a retrospective, non-randomized, small sample size cohort study on a population of severe COVID-19 patients with ARDS, also showed Anakinra's efficacy in improving respiratory function and in reducing 21-day mortality [51]. Considering the encouraging results of these preliminary studies together with the drug mechanism of action and its remarkable safety profile, anakinra could theoretically be extremely useful in fighting COVID-19 disease. As such, many clinical trials testing this molecule are now ongoing (see Table 2—Anakinra section).

### Baricitinib

A group of approved drugs that can inhibit clathrin-mediated endocytosis and consequently virus entry into target cells was discovered through artificial intelligence. The selected drugs are inhibitors of members of the Numb-Associated-Kinase (NAK) family, including AP2 associated kinase 1 (AAK1) and cyclin G associated kinase (GAK), the inhibition of which has been shown to reduce viral infection in vitro. Baricitinib was identified as a NAK inhibitor, in particular, it showed high affinity for AAK. Also Fedratinib and Ruxolitinib have been found as potential therapeutic weapons belonging to this class. All these three drugs are potent Janus Kinase (JAK)-inhibitors approved for rheumatoid arthritis and myelofibrosis treatment and have powerful anti-inflammatory properties. A dosage study showed that the best option could be Baricitinib, due to the once a day oral administration, the acceptable safety profile and the double mechanism of action targeting JAK inhibition and clathrin-mediated endocytosis (i.e. inflammation and virus entry) at the tolerated dosage. Conversely, Fedratinib and Ruxolitinib are able to inhibit JAK but not the virus endocytosis at tolerated dosage. In addition, Baricitinib could be used in combination with other therapies due to its low plasma protein binding and minimal cytochrome interactions. However, there are also some concerns about Baricitinib use because the simultaneous inhibition of AAK and JAK can reduce Interferon-α levels determining a worsening of immune response with subsequent clinical deterioration [52]. For this reason, other authors suggested Fedratinib as the best drug option among this group because of its selective inhibition of JAK2. Fedratinib decreases IL-17 and IL-22 expression by T helper (Th)17 lymphocytes, suppresses GM-CSF function, but it does not compromise IL-21 mediated B cells function. As a consequence, JAK inhibitors would reduce the cytokine storm via multiple mechanisms [53]. More laboratory and clinical data are needed to clarify the use of these therapeutic options in COVID-19 disease. Table 2—Baricitinb section reports available studies on this topic.

### Colchicine

Colchicine is an old drug used in auto-inflammatory disorders and in gout. It counteracts the assembly of the NLRP3 inflammasome (the leading component in the development of ARDS), thereby reducing the release of IL-1b and an array of other interleukins, including IL-6, that are produced in response to danger signals. Colchicine also inhibits microtubule dynamics by binding to unpolymerized tubulin heterodimers. As such, it seems that colchicine could directly inhibit the virus entry phase in human cells due to interference with clathrin-mediated endocytosis via microtubules inhibition [54]. Indeed, in mice studies colchicine showed efficacy in inhibition of respiratory syncytial virus (RSV) replication and suppression of RSV-induced airway inflammation [55]. Moreover, colchicine was thought to be potentially useful for its double action on the heart and pericardium and on lungs due to a decrease in cytokine levels and interstitial cells activation [56]. Many trials in the early use of colchicine for COVID-19 are ongoing all over the world. A brief overview can be seen in Table 2—Colchicine section.

### Interferons

Interferons are a group of cytokines used for the communication between cells to trigger the defense mechanisms against pathogens. In particular, they have a critical role in the innate immune response, such as the activation of natural killer lymphocytes and macrophages. They also have several antiviral activities, including the induction of viral degradation, the alteration of RNA transcription and protein synthesis, and the promotion of cellular apoptosis. Interferons family is composed of type I, II and III interferons. As a member of type I interferons, Interferon-α is quickly produced during viral infections as part of the innate immune response. Its action leads to the inhibition of viral replication even in Coronaviruses infecting humans and animals. Interferon-β inhibits SARS-CoV virus replication in vitro while γ-Interferon doesn't. Interferon-α, and to a lesser extent Interferon β-1, were widely used in SARS-CoV and MERS-CoV epidemics, due to promising results demonstrated by in vitro and in animal studies. SARS-CoV virus produces proteins able to inhibit type I Interferon release causing a delayed production of type I Interferons and a delayed immune response. The rise in serum levels of type I Interferons at the advanced disease stage contributes to the development of cytokine storm with systemic hyperinflammation and accumulation of macrophages and monocytes in lung tissue. It seems that this mechanism also occurs in COVID-19 disease. In particular, SARS-CoV2 virus antagonizes STAT1, a key protein in the Interferon-mediated immune response. The importance of T cell-mediated immune response in respiratory Coronaviruses is well established. Type I Interferon response is crucial in T cell survival and T cells reduce cytokine storm by modulating the innate immune response. A combination of type I Interferon and γ-Interferon or λ-Interferon was shown to synergistically inhibit the virus replication in vitro [57]. Combining interferons gives the opportunity of lowering the dosage of each one to decrease side effects. The timing of Interferon administration is crucial. Type I Interferons effects are protective against virus infections in the first phases; in advanced disease high titers of α and β-Interferons are associated with worst outcomes because they sustain inflammatory response [58]. Up to date, evidence on the use of Interferons in COVID-19 pneumonia is lacking. Chinese national health service recommends α-Interferon 5 million IU twice a day nebulization as a coadjutant treatment option in COVID-19 infection; however, nebulization is a risky procedure due to the aerosol spread of the virus in the environment [59]. Some studies about the administration of Interferons in COVID-19 are reported in Table 2—Interferons section. These studies suggest better results in groups of COVID-19 patients treated with the association of antiviral drugs and Interferons (nebulized or sub-cutaneous administration) or Interferon alone compared to antiviral drugs alone [9, 60, 61]. However, up to date, well-designed studies on large populations are lacking.

### Emapalumab

Emapalumab is a human monoclonal antibody against Interferon-γ. It is used in the treatment of refractory primary hemophagocytic lymphohistiocytosis (HLH) [62]. The cytokine storm developed during advanced hyperinflammatory COVID-19 disease phase may resemble HLH [40]. Moreover, Interferon-γ levels are associated with an amplification of inflammation and generalized activation of immune response with subsequent hyper-inflammatory state and damage [57]. On this basis, researchers decided to begin clinical trials with Emapalumab as a potential therapeutic option for COVID-19 disease (see Table 2—Emapalumab section).

## Other drugs

In this section diverse drugs belonging to different classes with different mechanisms of action which may have potential therapeutic implications for COVID-19 disease are treated. The main studies on these molecules are reported in Table 3.

**Table 3** Main clinical studies testing other drug classes for the treatment of COVID-19 disease

| Drugs | References | Study type (ST), population (P) and dosage (D) | Results | Adverse effects (AE) | Limitations/comments |
|---|---|---|---|---|---|
| Intravenous immuno-globulins (IVIGs) | ClinicalTrials.gov Identifier: NCT04381858 | ST: Randomized, controlled, clinical trial<br>P: 500 patients with severe COVID-19 pneumonia<br>D: 250 patients: convalescent plasma 400 ml IV (2 Units) vs. 250 patients: IVIGs 0.4 g/kg/day for 5 days | Outcomes: hospitalization time, oxygenation index evolution, rate of severe ARDS, rate and time to death, mean time with invasive mechanical ventilation and time to viral clearance | To be determined | Ongoing study |
| Hyperimmune plasma | Duan et al. *Proceedings of the National Academy of Sciences*, 2020 [66] | ST: case series<br>P: 10 severely-ill COVID-19 patients<br>D: 1 infusion of 200 ml of convalescent plasma with high titer neutralizing antibodies (> 1:640) at a median of 16.5 days from onset of symptoms | Significant clinical (fever, cough, shortness of breath, oxygen saturation), biochemical / virological (lymphocyte count, neutralizing antibody titer, SARS-CoV2 viral load) and radiological (CT scan) improvement in all 10 patients | No AE | Small case series without control population. Multiple concomitant treatments |
| | Zhang et al. *Chest,* 2020 [67] | ST: case reports<br>P: 4 critically-ill (ICU admitted) COVID-19 patients<br>D: 200–2400 ml of convalescent plasma ranging from day 11 to day 18 post-admission | Lung lesions resolution, decreased SARS-CoV2 viral load and clinical improvement; all 4 patients were discharged | No AE | Small case series without control population. Multiple concomitant treatments |
| | Shen et al. *JAMA,* 2020 [68] | ST: case reports<br>P: 5 critically-ill COVID-19 pneumonia patients (receiving mechanical ventilation)<br>D: convalescent plasma with antibody titer > 1:1000 and neutralizing antibody titer > 1:40 between day 10 and 22 from admission | 4 of 5 patients experienced increases in viral antibody titer, decreases in SARS-CoV2 viral loads, and resolution of fever and ARDS | No AE | Small case series without control population. Multiple concomitant treatments |

**Table 3** (continued)

| Drugs | References | Study type (ST), population (P) and dosage (D) | Results | Adverse effects (AE) | Limitations/comments |
|---|---|---|---|---|---|
| | Li et al. *JAMA* (2020) [69] | ST: open-label, multicentre, randomized clinical trial<br>P: 103 patients with severe or life threatening COVID-19 disease<br>D: 52 patients: Convalescent plasma + standard therapy vs. 51 patients: standard therapy alone | 28-day clinical improvement occurred in 51.9% of the convalescent plasma group vs 43.1% in the control group (HR 1.40, 95% CI 0.79–2.49; $p = 0.26$). There was no significant difference in 28-day mortality (15.7% vs 24.0%; OR 0.65, 95% CI 0.29–1.46; $p = 0.30$) or time from randomization to discharge (51.0% vs 36.0%; HR 1.61, 95% CI 0.88–2.93; $p = 0.12$). Convalescent plasma treatment was associated with a negative conversion rate of viral PCR at 72 h in 87.2% of the convalescent plasma group vs 37.5% of the control group (OR 11.39, 95% CI 3.91–33.18; $p < 0.001$) | Two patients in the convalescent plasma group experienced adverse events within hours after transfusion (fever and chills) that improved with supportive care (steroids) | Early termination of the trial, that did not reach its target sample size of 200 subjects. Median time from symptoms onset to randomization was 30 days |

**Table 3** (continued)

| Drugs | References | Study type (ST), population (P) and dosage (D) | Results | Adverse effects (AE) | Limitations/comments |
|---|---|---|---|---|---|
| Low molecular weight heparin (LMWH) and unfractioned heparin (UFH) | Tang et al. *Journal of Thrombosis and Haemostasis*, 2020 [3] | ST: retrospective cohort study<br>P: 449 patients with severe COVID-19 pneumonia<br>D: 99 patients receiving heparin (95% used LMWH 40–60 mg/day, 5% UHF 10,000–15,000 U/day, both for 7 days or more) vs. 350 controls | 28-day mortality of LMWH/UFH group was lower than control group in patients with SIC score > = 4 (40% vs 64.2%, $p=0.029$) or D-dimer sixfold higher than upper limit of normal (32.8% vs 52.4%, $p=0.017$) | Bleeding complications were unusual and commonly mild | Small sample size, non-randomized trial; presence of confounding factors (heterogeneous treatments among different groups) |
| | Ranucci et al. *Journal of Thrombosis and Haemostasis* (2020) [72] | ST: prospective observational study<br>P: 16 patients admitted to the ICU due to severe COVID-19 pneumonia with ARDS<br>D: at baseline all patients were treated with LMWH 4000UI twice/day; 10 patients received augmented dose LMWH 6000UI twice a day or 8000 twice a day if BMI > 35 and Clopidogrel 300 + 75 mg if PLT > 400,000/µl | Augmentation of LMWH was associated with adjustment of the coagulation parameters, in particular fibrinogen and D-dimer levels | No major bleedings were observed | No control group; small sample size; presence of confounding factors |
| | Fogarty et al. *British journal of Haematology* 2020 [73] | ST: retrospective cohort study<br>P: 83 patients with moderate/severe COVID-19 disease<br>D: enoxaparin 20 mg once/day if < 50 kg; 40 mg once/day if 50–100 kg; 40 mg bid if 101–150 kg and 60 mg bid if > 150 kg | None of the patients developed systemic DIC | Not reported | The effectiveness of LMWH in DIC prevention was not a study outcome but was extrapolated from results. Small sample size, non-randomized trial; presence of confounding factors |

## Intravenous immunoglobulins (IVIGs)

IVIGs are human immunoglobulin preparations derived from plasma, indicated for the treatment of diverse diseases such as autoimmune, inflammatory disorders and various immunodeficiencies [63]. During the SARS-CoV epidemic, many observational studies and case reports described IVIGs use in combination with anti-viral drugs for the treatment of critically ill patients. In a clinical review of different treatment protocols for SARS-CoV, the use of IVIGs combined with Interferon was described as ineffective [60]. Since the beginning of SARS-CoV2 infection in Wuhan, clinicians have used IVIGs in patients affected by COVID-19 pneumonia. Some authors, on the basis of the experience acquired with SARS-CoV pneumonia, proposed the use of IVIGs at the dosage of 0.3–0.5 g/kg/day for 5 days in the treatment of COVID-19 pneumonia. According to the authors' opinion, this treatment should be started as soon as possible in patients who present these features: leukopenia and lymphopenia (< 1000/ μL), D-dimer elevation above 4 times the upper limit of normal value (ULN) and cytokine increased levels (in particular IL-6). The rationale behind this approach is to try to reduce the cytokine storm developed in the most rapidly evolving patients [1]. Only a few case reports are available on the use of IVIGs in COVID-19 disease patients [64]. Because of the lack of evidence on IVIGs treatment in COVID-19 disease, physicians have proposed a number of trials to assess the efficacy of IVIGs compared to the standard care in severe patients (ClinicalTrials.gov Identifier: NCT04381858) (see Table 3—Intravenous immunoglobulins section).

## Hyperimmune plasma

Evidence shows that hyperimmune (or convalescent) plasma from patients who have recovered from various viral infections can be useful for the disease treatment without particular warnings. Hyperimmune (or convalescent) plasma has been already used as a last attempt in patients with critical SARS-CoV pneumonia not responding to maximal treatment [65]. Different studies on SARS-CoV pneumonia showed that hyperimmune plasma was effective in reducing hospitalization and mortality. A meta-analysis of 32 studies on SARS-CoV infection and severe influenza showed a statistically significant reduction in the pooled odds of mortality following convalescent plasma therapy compared with placebo or no therapy (OR 0.25; 95% CI 0.14–0.45) [66]. A protocol to encode the use of hyperimmune plasma also in patients affected by MERS-CoV pneumonia was established in 2015. Up to now, there are several case reports of COVID-19 patients treated with hyperimmune plasma that suggested a beneficial effect probably mediated by its antiviral activity. These case reports showed that COVID-19 patients treated with convalescent plasma had large reductions in serum viral loads and most were virus negative 3 days after infusion [66–68]. Recently the results of a Chinese randomized clinical trial have been published. This work, based on a population of 103 severely-ill or with life-threatening COVID-19 disease subjects, did not show statistically significant benefits of convalescent plasma compared to standard therapy in 28 days clinical improvement, mortality and time to discharge. In a subgroup analysis, severely-ill but not critical patients did show a faster clinical improvement when treated with convalescent plasma ($p = 0.03$). Of note, the study was underpowered, not reaching its target sample size of 200 patients, because enrolment was terminated prematurely due to control of infection spreading in China. It is important to highlight that conventionally convalescent plasma has the maximum efficacy in early viraemic stages of disease; whereas, in this study, it was administered very late after symptoms onset (median of 30 days) [69]. For these reasons, it would be useful to test hyperimmune plasma efficacy in early stages of COVID-19 disease to understand if a timelier administration could be associated with better outcomes. See Table 2—Hyperimmune plasma section.

## Low-molecular-weight heparin (LMWH) and unfractioned heparin (UFH)

The International Society of Thrombosis and Haemostasis (ISTH), based on the current literature, recommends measuring D-Dimer, prothrombin time and platelet count in all patients with COVID-19 disease. This strategy may help clinicians in stratifying patients who may need admission and close monitoring or not [70]. An adjunctive parameter to be considered is the serum fibrinogen, useful for the diagnosis of DIC, a condition highly prevalent in COVID-19 patients who did not survive the infection [7]. Due to the strong association between coagulopathy and mortality in COVID-19, the inhibition of thrombin generation may be beneficial. Moreover, it has been shown that heparin displays an anti-inflammatory action and various immunomodulatory properties. As such, prophylaxis dose LMWH has been proposed in all patients who require hospital admission for COVID-19 disease, in the absence of any contraindications [70]. The effectiveness of this approach is attested by Doctor Ning Tang et al. study; by stratifying patients according to the Sepsis-Induced Coagulopathy (SIC) score, the Authors showed a reduced mortality rate in patients treated with prophylactic LMWH or UFH compared to untreated patients among subjects with a SIC score higher than 4 [3]. Since the publication of this study, the awareness of the importance of anticoagulant therapy in COVID-19 grew exponentially. In his review on the immune changes caused by COVID-19 disease, Lin proposed to treat all severe and critical COVID-19

patients with D-Dimer value fourfold higher than normality with anticoagulant dose of LMWH (100 UI/kg every 12 h) for 3–5 days with close clinical monitoring [1]. A position paper from the Italian Society of Thrombosis and Haemostasis (SISET) expresses doubts about the administration of anticoagulant dose of LWMH in severe COVID-19 patients since no evidence currently supports this approach. The position paper also advises against the translatability of Chinese COVID-19 population D-dimer cut-off to the Italian population, since D-dimer levels are strongly correlated with age and Italian subjects with COVID-19 disease are much older than the Chinese ones. Further studies need to be conducted to establish a specific D-dimer cut-off for the Italian COVID-19 population. The final recommendations are to administer LMWH, UFH or fondaparinux at prophylaxis doses for venous thromboembolism (VTE) in all hospitalized COVID-19 patients, for the entire duration of the hospital stay. The use of intermediate-dose LMWH (i.e. Enoxaparin 4000 IU subcutaneously every 12 h) can be considered on an individual basis in patients with multiple risk factors for VTE [71]. An Italian group of ICU physicians highlighted the fact that since severe/critical COVID-19 disease is associated with an increased rate of DIC and VTE, augmented LMWH dosing could be useful in reducing the coagulopathy risk in mechanically ventilated patients. A study conducted in a northern Italy ICU department showed no increased mortality and no major bleeding after anticoagulant therapy augmentation (anticoagulant dosage of LMWH and UFH) indicating the need for further studies comparing the different doses of heparin and outcomes with a greater interest in mortality and adverse events [72]. The main studies on this topic can be found in Table 3—Low molecular weight heparin (LMWH) and unfractioned heparin (UFH) section [3, 72, 73].

## Azithromycin

Azithromycin is an antibiotic belonging to the macrolide class. It inhibits bacterial synthesis but also shows in vitro activity against viruses such as Influenza A H1N1 (interference with internalization process) and Zika (upregulation of virus-induced Interferons type I and III). Moreover, azithromycin anti-inflammatory effect on lung tissue, even during viral infections, is well known. It acts suppressing T-helper 1 and 2 lymphocyte-related cytokines (IL-1, IL-6, TNFα) and INF inducible protein 10 (IP-10)/macrophage derived chemokine (MDC) in monocytic cell line. Its use has been associated with decreased major respiratory complications during viral respiratory infections in clinical studies [74].

## Doxycycline

Doxycycline is an antibiotic belonging to the tetracycline class. It interferes with bacterial protein synthesis and demonstrated in vitro activity against viral infections (i.e. Influenza and Dengue). Moreover, doxycycline presents documented anti-inflammatory effects by acting on Nf-Kb pathway and by inhibiting the production of pro-inflammatory cytokines (IL-1b, IL-6 and TNFα). An experimental study on mice showed that the use of doxycycline during severe influenza pneumonia was associated with a better outcome with less inflammatory lung lesions and ARDS prevention [75]. Another study suggested that the administration of doxycycline in patients with Dengue haemorrhagic fever reduced cytokines (IL-6 and TNFα) blood levels, mortality and discharge time [76]. Recent studies suggested that coronaviruses induce the proliferation of mast cells within the respiratory submucosa and that chemically-modified tetracyclines can induce apoptosis of mast cells and activation of protein-kinase C, thus decreasing levels of circulating inflammatory mediators [77]. For these reasons, some studies are testing doxycycline efficacy in COVID-19 pneumonia.

## Angiotensin receptor blockers (ARB) and ace-inhibitors

ACE2 is a monocarboxypeptidase that hydrolyses multiple peptides, including angiotensin; in particular, the enzyme forms angiotensin-(1–7) by cleaving angiotensin II. The former has a vasodilator, anti-inflammatory and anti-fibrotic effect, while the latter determines vasoconstriction and enhances inflammation. Since ARB and ACE inhibitors determine an increase in ACE2 expression, at the beginning of COVID-19 pandemic, it was thought that they could be harmful by facilitating SARS-CoV2 attachment and entry. However, it has been subsequently demonstrated that SARS-CoV2, once attached to ACE2, determines a down-regulation in its expression in the lungs and as a consequence a decrease in angiotensin-(1–7) and an increase in angiotensin II with possible acute lung injury and enhanced global inflammation. As such, it is now thought that these two drug classes may be beneficial in controlling lung damage and inflammation. ARB and ACE-inhibitors may act as a double-edged sword: on the one hand, these two drugs could enhance infectivity by inducing ACE2 over-expression; on the other hand, the subjects treated with ARB or ACE-inhibitors, once infected, exhibit a more balanced equilibrium between angiotensin II and angiotensin-(1–7) which may prevent systemic and lung inflammation and damage [78]. Some recent studies suggested that patients chronically treated with ARB and ACEi not only are not at increased

risk of SARS-CoV2 infection or worse COVID-19 outcomes but even may show a less severe disease course. Another possible therapeutic approach targeting this pathway consists in soluble recombinant ACE2 intravenous infusion; this strategy determines a reduction in viral load since the virus could bind the "false" receptor [79].

### TMPRSS2 inhibitor

TMPRSS2 is a serine protease located on the epithelial cell surface of specific tissues including the respiratory tract. This protease cleaves SARS-CoV2 spike protein and activates the virus internalization. Researchers have tried to find ways to reduce TMPRSS2 expression or to block its activity as a potential therapeutic option for COVID-19 disease. Two classes of drugs have been thought to be used so far: anti-androgens or oestrogens to reduce the TMPRSS2 expression, and Camostat Mesilate, a potent serine-protease inhibitor. In vitro studies have shown a potent inhibitory effect of Camostat Mesilate on SARS-CoV2 entry ability [80]. Five clinical studies to test Camostat Mesilate efficacy in COVID-19 disease are now ongoing.

## Conclusions

In this review, we described the main drugs tested until now or with a putative therapeutic role against SARS-CoV2 infection; in particular, we detailed the possible mechanisms of action of the selected molecules in COVID-19 disease and, when available, we described the results of the main clinical studies in COVID-19 patients. An important issue that is worth to highlight is the emergency setting in which these studies have been conducted so far. As such, the vast majority of the studies that have been analysed suffer from several methodological limitations, notably including a lack of randomized, double-blind design, a lack of control populations, small or underpowered sample size, and even a lack of peer review evaluations before publication. Moreover, in many cases, the compared groups of patients are heterogeneous in clinical features, disease severity and concurrent pharmacological treatment. Finally, the reported outcomes include a plethora of virologic, biochemical, radiological and clinical aspects that have poor consistency across studies and are limited in time of follow-up. Taking all these considerations in mind, it is difficult to draw some valid conclusions on drugs efficacy in COVID-19 disease treatment.

For what concerns antiviral drugs, the available pathophysiological knowledge on SARS-CoV2 infection and the clinical studies performed so far suggest that the timing of administration of this class of molecules is important. Indeed, they can be effective if dispensed in the first viraemic stage of infection, early after symptoms onset. Among these drugs, the most effective seems to be Remdesivir, even if further large, randomized, double-blind studies are eagerly awaited.

Among immunomodulatory drugs, CQ and HCQ are two controversial molecules. From early studies conducted in France it seems they can be effective if administered very early, at disease presentation. However, their use in hospitalized critical/severe COVID-19 patients do not seem to be associated with improved outcomes and there are concerns about their cardiotoxicity. Importance must be attributed to immunomodulatory drugs such as corticosteroids (in particular dexamethasone) and biological molecules (Tocilizumab, Anakinra and others) which seem to be valid and useful therapeutic options in the inflammatory phase of the disease. However, further studies are necessary to better understand if they can also be active in the early phases of the disease.

Among other classes of drugs, LMWH and UFH may also have a major therapeutic role when facing COVID-19-related coagulopathy, but future studies have to establish the correct dosing, timing and length of treatment.

In conclusion, COVID-19 is a novel, life-threatening disease, that does not have a cure able to arrest its progression yet. Up to now, treatment choices have been inferred from the experience with other coronaviruses or viral infection outbreaks. New treatment strategies will hopefully be available in the near future in parallel with the increased knowledge of SARS-CoV2 virus and COVID-19 pathogenesis. In the meantime, further well-designed clinical trials on homogeneous populations are urgently needed to establish a standard of care in COVID-19 disease.

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### Compliance with ethical standards

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## References

1. Lin L, Lu L, Cao W, Li T (2020) Hypothesis for potential pathogenesis of SARS-CoV-2 infection—a review of immune

changes in patients with viral pneumonia. Emerg Microbes Infect 9(1):727–732
2. Siddiqi HK, Mandeep RM (2020) COVID-19 illness in native and immunosuppressed states: a clinical-therapeutic staging proposal. J Heart Lung Transplant. 39(5):405–407
3. Tang N, Li D, Wang X, Sun Z (2020) Abnormal coagulation parameters are associated with poor prognosis in patients with novel coronavirus pneumonia. J Thromb Haemost 18(4):844–847
4. De Biasi S, Meschiari M, Gibellini L, Bellinazzi C, Borella R, Fidanza L, Paolini A (2020) Marked T cell activation, senescence, exhaustion and skewing towards TH17 in patients with COVID-19 pneumonia. Nat Commun 11(1):1–17
5. Azkur AK, Akdis M, Azkur D, Sokolowska M, van de Veen W, Brüggen MC, Akdis CA (2020) Immune response to SARS-CoV-2 and mechanisms of immunopathological changes in COVID-19. Allergy 75(7):1564–1581
6. Cao B, Wang Y, Wen D, Liu W, Wang J, Fan G, Li X (2020) A trial of lopinavir–ritonavir in adults hospitalized with severe COVID-19. N Engl J Med. 382(19):1787–1799
7. Liu F, Xu A, Zhang Y, Xuan W, Yan T, Pan K, Zhang J (2020) Patients of COVID-19 may benefit from sustained lopinavir-combined regimen and the increase of eosinophil may predict the outcome of COVID-19 progression. Int J Infect Dis. 95:183–191
8. Deng L, Li C, Zeng Q, Liu X, Li X, Zhang H, Xia J (2020) Arbidol combined with LPV/r versus LPV/r alone against Corona Virus Disease 2019: a retrospective cohort study. J Infect. 81(1):e1–e5
9. Hung IFN, Lung KC, Tso EYK, Liu R, Chung TWH, Chu MY, Shum HP (2020) Triple combination of interferon beta-1b, lopinavir–ritonavir, and ribavirin in the treatment of patients admitted to hospital with COVID-19: an open-label, randomised, phase 2 trial. Lancet 395(10238):1695–1704
10. AIFA-Darunavir/Cobicistat nella terapia dei pazienti adulti con COVID-19
11. Wang Y, Zhang D, Du G, Du R, Zhao J, Jin Y, Hu Y (2020) Remdesivir in adults with severe COVID-19: a randomised, double-blind, placebo-controlled, multicentre trial. Lancet. 395(10236):1569–1578
12. Sheahan TP, Sims AC, Graham RL, Menachery VD, Gralinski LE, Case JB, Bannister R (2017) Broad-spectrum antiviral GS-5734 inhibits both epidemic and zoonotic coronaviruses. Sci Transl Med 9(396):eaal3653
13. Agostini ML, Andres EL, Sims AC, Graham RL, Sheahan TP, Lu X, Ray AS (2018) Coronavirus susceptibility to the antiviral remdesivir (GS-5734) is mediated by the viral polymerase and the proofreading exoribonuclease. MBio 9(2):e00221–18
14. Wang M, Cao R, Zhang L, Yang X, Liu J, Xu M, Xiao G (2020) Remdesivir and chloroquine effectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. Cell Res 30(3):269–271
15. Grein J, Ohmagari N, Shin D, Diaz G, Asperges E, Castagna A, Nicastri E (2020) Compassionate use of remdesivir for patients with severe COVID-19. N Engl J Med 382(24):2327–2336
16. Holshue ML, DeBolt C, Lindquist S, Lofy KH, Wiesman J, Bruce H, Diaz G (2020) First case of 2019 novel coronavirus in the United States. N Engl J Med. 382(10):929–936
17. Beigel JH, Tomashek KM, Dodd LE, Mehta AK, Zingman BS, Kalil AC (2020) Lopez de Castilla D (2020) Remdesivir for the treatment of COVID-19—Final report. N Engl J Med. 383(19):1813–1826
18. Goldman JD, Lye DC, Hui DS, Marks KM, Bruno R, Montejano R, Chen YS (2020) Remdesivir for 5 or 10 days in patients with severe COVID-19. N Engl J Med. 383(19):1827–1837
19. Chen C, Huang J, Cheng Z, Wu J, Chen S, Zhang Y, Yin P (2020) Favipiravir versus arbidol for COVID-19: a randomized clinical trial. MedRxiv-pre-print version
20. Bollettino AIFA 20 Marzo (2020) AIFA precisa-Uso Favipiravir per COVID-19 non autorizzato in Europa e USA, scarse evidenze scientifiche sull'efficacia
21. Cai Q, Yang M, Liu D, Chen J, Shu D, Xia J, Shen C (2020) Experimental treatment with favipiravir for COVID-19: an open-label control study. Engineering. Epub ahead of print
22. Blaising J, Polyak SJ, Pécheur EI (2014) Arbidol as a broad-spectrum antiviral: an update. Antivir Res 107:84–94
23. Lian N, Xie H, Lin S, Huang J, Zhao J, Lin Q (2020) Umifenovir treatment is not associated with improved outcomes in patients with coronavirus disease 2019: a retrospective study. Clin Microbiol Infect. 26(7):917–921
24. Devaux CA, Rolain JM, Colson P, Raoult D (2020) New insights on the antiviral effects of chloroquine against coronavirus: what to expect for COVID-19? Int J Antimicrob Agents 55(5):105938
25. Gao J, Zhenxue T, Xu Y (2020) Breakthrough: chloroquine phosphate has shown apparent efficacy in treatment of COVID-19 associated pneumonia in clinical studies. Biosci Trends. 14(1):72–73
26. Gautret P, Lagier JC, Parola P, Meddeb L, Mailhe M, Doudier B, Honoré S (2020) Hydroxychloroquine and azithromycin as a treatment of COVID-19: results of an open-label non-randomized clinical trial. Int J Antimicrob Agents 56(1):105949
27. Gautret P, Lagier JC, Parola P, Meddeb L, Sevestre J, Mailhe M, Hocquart M (2020) Clinical and microbiological effect of a combination of hydroxychloroquine and azithromycin in 80 COVID-19 patients with at least a six-day follow up: a pilot observational study. Travel Med Infect Dis 34:101663
28. Million M, Lagier JC, Gautret P, Colson P, Fournier PE, Amrane S, Aubry C (2020) Full-length title: early treatment of COVID-19 patients with hydroxychloroquine and azithromycin: a retrospective analysis of 1061 cases in Marseille. France. Travel Med Infect Dis 35:101738
29. Chen J, Liu D, Liu L, Liu P, Xu Q, Xia L, Qian Z (2020) A pilot study of hydroxychloroquine in treatment of patients with common coronavirus disease-19 (COVID-19). J Zhejiang Univ (Medical Science) 49(1):215–219
30. Magagnoli J, Narendran S, Pereira F, Cummings TH, Hardin JW, Sutton SS, Ambati J (2020) Outcomes of hydroxychloroquine usage in United States veterans hospitalized with COVID-19. Med (N Y). Epub ahead of print
31. Borba MGS, Val FFA, Sampaio VS, Alexandre MAA, Melo GC, Brito M, Hajjar LA (2020) Effect of high vs low doses of chloroquine diphosphate as adjunctive therapy for patients hospitalized with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection: a randomized clinical trial. JAMA Netw Open 3(4):e208857–e208857
32. Geleris J, Sun Y, Platt J, Zucker J, Baldwin M, Hripcsak G, Sobieszczyk ME (2020) Observational study of hydroxychloroquine in hospitalized patients with COVID-19. N Engl J Med. 382(25):2411–2418
33. Mehra MR, Desai SS, Ruschitzka F, Patel AN (2020) Hydroxychloroquine or chloroquine with or without a macrolide for treatment of COVID-19: a multinational registry analysis. Lancet. S0140-6736(20)31180-6
34. https://www.aifa.gov.it/web/guest/-/aifa-sospende-l-autorizzazione-all-utilizzo-di-idrossiclorochina-per-il-trattamento-del-covid-19-al-di-fuori-degli-studi-clinici. Accessed 1 April 2020
35. RETRACTED: hydroxychloroquine or chloroquine with or without a macrolide for treatment of COVID-19: a multinational registry analysis. The Lancet 2020 (13 June). Vol 395
36. Boulware DR, Pullen MF, Bangdiwala AS, Pastick KA, Lofgren SM, Okafor EC, Engen NW (2020) A randomized trial of hydroxychloroquine as postexposure prophylaxis for COVID-19. N Engl J Med. 383(6):517–525

37. Shang L, Zhao J, Hu Y, Du R, Cao B (2020) On the use of corticosteroids for 2019-nCoV pneumonia. Lancet (London, England), 395(10225):683-684
38. Word Health Organisation. Clinical management of severe acute respiratory infection when novel coronavirus (nCov) infection is suspected: interim guidance. 2020-Centers for Disease Control and Prevention, Interim Clinical Guidance for management of patients with confirmed 2019 novel coronavirus (SARS-CoV-2) infection
39. Xu Z, Shi L, Wang Y, Zhang J, Huang L, Zhang C, Tai Y (2020) Pathological findings of COVID-19 associated with acute respiratory distress syndrome. Lancet Respir Med 8(4):420–422
40. Mehta P, McAuley DF, Brown M, Sanchez E, Tattersall RS, Manson JJ, HLH Across Speciality Collaboration (2020) COVID-19: consider cytokine storm syndromes and immunosuppression. Lancet (London, England). 395(10229):1033-1034
41. Fang X, Mei Q, Yang T, Li L, Wang Y, Tong F, Pan A (2020) Low-dose corticosteroid therapy does not delay viral clearance in patients with COVID-19. J Infect 81(1):147–178
42. Wang Y, Jiang W, He Q, Wang C, Wang B, Zhou P, Tong Q (2020) Early, low-dose and short-term application of corticosteroid treatment in patients with severe COVID-19 pneumonia: single-center experience from Wuhan, China. medRxiv
43. Wu C, Chen X, Cai Y, Zhou X, Xu S, Huang H, Song J (2020) Risk factors associated with acute respiratory distress syndrome and death in patients with coronavirus disease 2019 pneumonia in Wuhan. China. JAMA Intern Med. 180(7):934–943
44. RECOVERY Collaborative Group,Horby P, Lim WS, Emberson J, Mafham M, Bell J, Linsell L, Prudon B (2020). Dexamethasone in hospitalized patients with COVID-19: preliminary report. N Engl J Med. NEJMoa2021436
45. Xu X, Han M, Li T, Sun W, Wang D, Fu B, Zhang X (2020) Effective treatment of severe COVID-19 patients with tocilizumab. Proc Natl Acad Sci USA. 117(20):10970–10975
46. Colaneri M, Bogliolo L, Valsecchi P, Sacchi P, Zuccaro V, Brandolino F, Bruno R (2020) Tocilizumab for treatment of severe COVID-19 patients: preliminary results from SMAtteo COvid19 REgistry (SMACORE). Microorganisms 8(5):695
47. AIFA press release 17 June 2020: Studio randomizzato multicentrico in aperto sull'efficacia della somministrazione precoce del Tocilizumab in pazienti affetti da polmonite da COVID 19
48. Guaraldi G, Meschiari M, Cozzi-Lepri A, Milic J, Tonelli R, Menozzi M, Santoro A (2020) Tocilizumab in patients with severe COVID-19: a retrospective cohort study. Lancet Rheumatol
49. Comunicato AIFA 29/03/2020: COVID-19, AIFA autorizza tre nuovi studi per sperimentazioni di farmaci per il trattamento dell'infezione da nuovo coronavirus. Comunicato del 29 Marzo 2020
50. Pontali E, Volpi S, Antonucci G, Castellaneta M, Buzzi D, Tricerri F, Castagnola E (2020) Safety and efficacy of early high-dose IV anakinra in severe COVID-19 lung disease. J Allergy Clin Immunol. 146(1):213–215
51. Cavalli G, De Luca G, Campochiaro C, Della-Torre E, Ripa M, Canetti D, Tomelleri A (2020) Interleukin-1 blockade with high-dose anakinra in patients with COVID-19, acute respiratory distress syndrome, and hyperinflammation: a retrospective cohort study. Lancet Rheumatol. e325-e331
52. Zhang W, Zhao Y, Zhang F, Wang Q, Li T, Liu Z, Zeng X (2020) The use of anti-inflammatory drugs in the treatment of people with severe coronavirus disease 2019 (COVID-19): The experience of clinical immunologists from China. Clin Immunol. 214:108393
53. Wu D, Xuexian OY (2020) Th17 responses in cytokine storm of COVID-19: an emerging target of JAK2 inhibitor fedratinib. J Microbiol Immunol Infect. 53(3):368–370
54. Molad Y (2002) Update on colchicine and its mechanism of action. Curr Rheumatol Rep 4(3):252–256
55. Lu N, Yang Y, Liu H, Ding X, Ou Y, Xia J, Du Y (2019) Inhibition of respiratory syncytial virus replication and suppression of RSV-induced airway inflammation in neonatal rats by colchicine. 3 Biotech 9(11):392
56. Deftereos SG, Giannopoulos G, Vrachatis DA, Siasos GD, Giotaki SG, Gargalianos P, Dolianitis K (2020) Effect of colchicine vs standard care on cardiac and inflammatory biomarkers and clinical outcomes in patients hospitalized with coronavirus disease 2019: the GRECCO-19 randomized clinical trial. JAMA Netw Open 3(6):e2013136–e2013136
57. Cinatl J Jr, Michaelis M, Scholz M, Doerr HW (2004) Role of interferons in the treatment of severe acute respiratory syndrome. Expert Opin Biol Ther 4(6):827–836
58. Kuri T, Zhang X, Habjan M, Martínez-Sobrido L, García-Sastre A, Yuan Z, Weber F (2009) Interferon priming enables cells to partially overturn the SARS coronavirus-induced block in innate immune activation. J Gen Virol 90(Pt 11):2686
59. National health commission of the people's republic of China (2020) The diagnosis and treatment guide of COVID-19 pneumonia caused by new coronavirus infection 6th edition
60. Loutfy MR, Blatt LM, Siminovitch KA, Ward S, Wolff B, Lho H, Kain KC (2003) Interferon alfacon-1 plus corticosteroids in severe acute respiratory syndrome: a preliminary study. JAMA 290(24):3222–3228
61. Zhou Q, Chen V, Shannon CP, Wei XS, Xiang X, Wang X, Fish EN (2020) Interferon-α2b treatment for COVID-19. Front Immunol 11:1061
62. https://salute.regione.emilia-romagna.it/farmaci/covid-19/farmaci-e-studi-clinici/anakinra-emapalumab. Accessed 6 April 2020
63. Galeotti C, Kaveri SV, Bayry J (2017) IVIG-mediated effector functions in autoimmune and inflammatory diseases. Int Immunol 29(11):491–498
64. Cao W, Liu X, Bai T, Fan H, Hong K, Song H, Li T (2020) High-dose intravenous immunoglobulin as a therapeutic option for deteriorating patients with coronavirus disease 2019. In: Open forum infectious diseases, vol 7, no. 3. Oxford University Press, p ofaa102
65. Chen L, Xiong J, Bao L, Shi Y (2020) Convalescent plasma as a potential therapy for COVID-19. Lancet Infect Dis 20(4):398–400
66. Duan K, Liu B, Li C, Zhang H, Yu T, Qu J, Peng C (2020) Effectiveness of convalescent plasma therapy in severe COVID-19 patients. Proc Natl Acad Sci 117(17):9490–9496
67. Zhang B, Liu S, Tan T, Huang W, Dong Y, Chen L, Zou Y (2020) Treatment with convalescent plasma for critically ill patients with SARS-CoV-2 infection. Chest. 158(1):e9–e13
68. Shen C, Wang Z, Zhao F, Yang Y, Li J, Yuan J, Wei J (2020) Treatment of 5 critically ill patients with COVID-19 with convalescent plasma. JAMA 323(16):1582–1589
69. Li L, Zhang W, Hu Y, Tong X, Zheng S, Yang J, Hu C (2020) Effect of convalescent plasma therapy on time to clinical improvement in patients with severe and life-threatening COVID-19: a randomized clinical trial. JAMA. 324(5):460–470
70. Thachil J, Tang N, Gando S, Falanga A, Cattaneo M, Levi M, Iba T (2020) ISTH interim guidance on recognition and management of coagulopathy in COVID-19. J Thromb Haemost 18(5):1023–1026
71. Marietta M, Ageno W, Artoni A, De Candia E, Gresele P, Marchetti M, Tripodi A (2020) COVID-19 and haemostasis: a position paper from Italian Society on Thrombosis and Haemostasis (SISET). Blood Transfus 18(3):167
72. Ranucci M, Ballotta A, Di Dedda U, Bayshnikova E, Dei Poli M, Resta M, Menicanti L (2020) The procoagulant pattern of patients with COVID-19 acute respiratory distress syndrome. J Thromb Haemost. 18(7):1747–1751

73. Fogarty H, Townsend L, Ni CC, Bergin C, Martin-Loeches I, Browne P, Ryan K (2020) COVID19 coagulopathy in Caucasian patients. Br J Haematol. 189(6):1060–1061
74. Damle B, Vourvahis M, Wang E, Leaney J, Corrigan B (2020) Clinical pharmacology perspectives on the antiviral activity of azithromycin and use in COVID-19. Clin Pharmacol Ther. 108(2):201–211
75. Mohit S, Mahyar E Therapeutic potential for tetracyclines in the treatment of COVID-19 (**Accepted article**)
76. Fredeking MT, Zavala-Castro E, González-Martínez J, Moguel-Rodríguez P, Sanchez WC, Foster EJM, Diaz-Quijano AF (2015) Dengue patients treated with doxycycline showed lower mortality associated to a reduction in IL-6 and TNF levels. Recent Patents Antiinfect Drug Discov 10(1):51–58
77. Sandler C, Nurmi K, Lindstedt KA, Sorsa T, Golub LM, Kovanen PT, Eklund KK (2005) Chemically modified tetracyclines induce apoptosis in cultured mast cells. Int Immunopharmacol 5(11):1611–1621
78. Sommerstein R, Kochen MM, Messerli FH, Gräni C (2020) Coronavirus disease 2019 (COVID-19): do angiotensin-converting enzyme inhibitors/angiotensin receptor blockers have a biphasic effect? J Am Heart Assoc 9(7):e016509
79. Meng J, Xiao G, Zhang J, He X, Ou M, Bi J, Gao H (2020) Renin-angiotensin system inhibitors improve the clinical outcomes of COVID-19 patients with hypertension. Emerg Microb Infect 9(1):757–760
80. Stopsack KH, Mucci LA, Antonarakis ES, Nelson PS, Kantoff PW (2020) TMPRSS2 and COVID-19: serendipity or opportunity for intervention? Cancer Discov 10(6):779–782